WILSON AND GISVOLD'S

TEXTBOOK OF ORGANIC MEDICINAL AND PHARMACEUTICAL CHEMISTRY

TENTH EDITION

EDITED BY
JAIME N. DELGADO
WILLIAM A. REMERS

Lippincott - Raven

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PREFACE

For almost five decades *Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry* has been a standard in the literature of medicinal chemistry, and the recent editions edited by Professor Robert F. Doerge have continued to receive national and international acceptance. Generations of students and faculty have depended on this textbook not only for undergraduate courses in medicinal chemistry but also to supplement graduate studies. Moreover, students in other health sciences have found certain chapters useful at one time or another. It was therefore decided to revise the book, and the current editors and authors worked on the tenth edition with the objective of continuing the tradition of a modern textbook for undergraduate students and also for graduate students who need a general review of medicinal chemistry. Since the chapters include a blend of chemical and pharmacological principles necessary for understanding structure-activity relationships and molecular mechanisms of drug action, the book should be useful in supporting courses in medicinal chemistry and in complementing pharmacology courses.

The nature and breadth of medicinal chemistry are discussed in the introduction, and the chapters on physicochemical properties and on drug metabolism provide the fundamental theme of the conceptual approach to the study of medicinal chemistry. This theme continues as the basis for all the subsequent chapters on the classical types of therapeutic agents. In order to continue to integrate the basics of medicinal chemistry with recent advances that have led to contemporary phamacotherapeutics, the tenth edition has been designed to include new chapters on drug latentiation and prodrugs, immunizing biologicals, diagnostic imaging agents, and biotechnology. Moreover, the editors welcome the following new contributors to the tenth edition: Drs. Beale, Clark, DeRuiter, Hall, Hunter, Regan, Riley, Thomas, and Thurston. The chapters include summaries of current research trends that lead the reader to the original literature via bibliographies. Documentation and references continue to be an important feature of the book.

An important area of medicinal chemistry is the chemistry and biology of pharmaceutically important natural products, hence chapters include antibiotics, glycosides, and the study of alkaloids as prototypes that lead to synthetic medicinals. The table of contents reflects the important pharmacological types of therapeutic agents. To the extent possible each type is structurally characterized with mechanisms of action and therapeutic and pharmaceutical applications and limitations.

The editors extend thanks to all the authors who have effectively cooperated in the preparation of the current edition. Collectively, the authors represent many years of teaching and research experience in medicinal chemistry. The students also deserve acknowledgment of their critiques that directly and indirectly contributed to the quality of this edition.

Finally, we continue to be indebted to Professors Charles O. Wilson and Ole Gisvold, the originators of the book, and to the editors and authors who worked on and stimulated the continuation of the many revisions that have led to the current edition. They have significantly contributed to the education of countless pharmacists, medicinal chemists, and other pharmaceutical scientists.

Jaime N. Delgado
William A. Remers

WILSON AND GISVOLD'S

Textbook of Organic Medicinal and Pharmaceutical Chemistry

CHAPTER 1

Introduction

Jaime N. Delgado
William A. Remers

The discipline of medicinal chemistry is devoted to the discovery and development of new agents for treating diseases. Most of this activity is directed to new natural or synthetic organic compounds. Inorganic compounds continue to be important in therapy, for example, as antacids, mineral supplements, and radiopharmaceuticals, but organic molecules with increasingly specific pharmacological activities are clearly dominant. This book treats many aspects of organic medicinals: how they are discovered, how they act, and how they are developed into clinical agents. The process of establishing a new pharmaceutical is exceedingly complex and involves the talents of people from a variety of disciplines, including chemistry, biochemistry, molecular biology, physiology, pharmacology, pharmaceutics, and medicine. Medicinal chemistry is concerned mainly with the organic, analytical, and biochemical aspects of this process, but its scientists must interact productively with those in other disciplines. Thus, it occupies a strategic position at the interface of chemistry and biology.

The earliest drug discoveries were probably made by random sampling of higher plants. Crude plant drugs such as opium, belladonna, and ephedrine have been important for centuries. Knowledge of crude drugs was codified into the discipline of pharmacognosy, which has held a distinguished place in the pharmaceutical sciences. Pharmacognosy has changed with time, embracing microbiology when the antibiotics were discovered, and venturing into drug biosynthesis as biochemistry developed. In recent years, however, crude drug preparations have been largely replaced by their isolated and purified active components, making it unnecessary for pharmacists to verify the identity of natural materials. This factor, together with the increasing importance of synthetic organic compounds, has led to reduced emphasis on pharmacognosy in the pharmacy curriculum and often to its combination with medicinal chemistry. This textbook, while it does not presume to teach pharmacognosy, contains much valuable information and insight into the sources, isolation, and standardization of natural product drugs.

Hundreds of thousands of new organic chemicals are prepared annually throughout the world, and many of them are entered into pharmacological screens to determine whether they have useful biological activity. This process of random screening has been considered inefficient, but it has resulted in the identification of new lead compounds whose structures have been optimized to produce clinical agents. The antitubercular drug, ethambutol, was developed in this manner. More recently, automated high-throughput screening systems utilizing cell culture systems with linked enzyme assays and even receptor molecules derived from gene cloning have greatly increased the efficiency of random screening. It is now practical to screen enormous libraries of peptides and nucleic acids obtained from combinatorial chemistry procedures.

The opposite approach to high-volume screening, rational design, has also flourished recently. Significant advances in x-ray crystallography and nuclear magnetic resonance have made it possible to obtain detailed representations of enzymes and other drug receptors. Based on these representations, the techniques of molecular graphics and computational chemistry have provided novel chemical structures that have led to new drugs with potent medicinal activities. The newly introduced inhibitors of HIV protease were discovered in this way. Even if the receptor structure is not known in detail, rational approaches based on the physicochemical properties of lead compounds can provide new drugs. For example, the development of cimetidine as an antiulcer drug involved a careful study of the changes in antagonism of H_2 histamine receptors induced by varying the physical properties of structures based on histamine. Statistical methods, such as the Hansch analysis, which is based on the correlation of physicochemical properties with biological potency, are popular in attempts to optimize biological activity or at least to rationalize it.

Significant developments in medicinal chemistry have occurred since the 9th edition of this textbook was written.

They have made it desirable to add new chapters to this 10th edition. Thus, the strong focus on antiviral drug research, driven largely by the need to treat HIV infections, has prompted the creation of a separate chapter on antiviral agents. Dramatic progress in the application of molecular biology to the production of pharmaceutical agents has produced such important molecules as insulin, growth hormone, granulocyte colony-stimulating factor, erythropoietin, and clotting factor VIII, all products of cloned human genes. Molecular biology has provided for the definition of new drug receptor subtypes that may be useful in designing more selective therapeutic agents, and it has been important in the development of monoclonal antibodies for diagnosis and therapy. A new chapter on biotechnology describes these exciting applications and other possibilities for therapy, including antisense oligonucleotides and gene transfer methods. Biological agents are hardly new, but recent advances in understanding the immune system at the molecular level, including detailed chemical structures of immunoglobulins, make them especially interesting to medicinal chemistry. Techniques of genetic engineering now allow the preparation of pure surface antigens as vaccines while totally eliminating the pathogenic organisms from which they are derived. These advances are described in the new chapter on biologicals.

Examples of drug latentiation have been given throughout this textbook in previous editions, but this important concept has not been treated in an organized manner. A new chapter in this edition presents the theoretical basis of drug latentiation and provides a carefully organized set of examples in which the therapeutic value of drugs is significantly enhanced.

Another new chapter is devoted to agents for diagnostic imaging. It includes both radiopharmaceuticals (nuclear medicine) and contrast agents, mainly iodinated organic compounds, that absorb x-rays (radiology). Fundamental concepts of radiation and its biological effects are described, and numerous practical applications in diagnosis are provided.

All of the chapters in the 10th edition have been revised, and those in which significant new discoveries were made have been completely rewritten. Together with the new chapters, they provide an outline of medicinal chemistry that is contemporary and authoritative in its treatment of scientific concepts, as well as sufficiently specific about pharmaceutical products to be a valuable reference for the practicing pharmacist.

CHAPTER 2

Physicochemical Properties in Relation to Biological Action

John H. Block

Modern drug design, as compared with the classical approach—*Let's make a change on an existing compound or synthesize a new structure and see what happens*—has undergone a rapid evolution as an approach to solving a drug design problem. The increasing power and decreasing cost of desktop computing has had a major impact on solving drug design problems. While drug design increasingly is based on modern computational chemical techniques, it also utilizes sophisticated knowledge of disease mechanisms and receptor properties. A good understanding of how the drug is transported into the body, distributed throughout the body compartments, metabolically altered by the liver and other organs, and excreted from the patient is required along with the structural characteristics of the receptor. Acid-base chemistry is utilized to aid in formulation and biodistribution. Those structural attributes and substituent patterns responsible for optimum pharmacological activity can be predicted many times by statistical techniques such as regression analysis. Computerized conformational analysis permits the medicinal chemist to predict the drug's three-dimensional shape that is *seen* by the receptor. With the isolation and structural determination of specific receptors and the availability of computer software that can estimate the three-dimensional shape of the receptor, it is possible to design molecules that will show an optimum fit to the receptor.

OVERVIEW

A drug is a chemical molecule. Following introduction into the body, a drug must pass through many barriers, survive alternate sites of attachment and storage, and avoid significant metabolic destruction before it reaches the site of action, usually a receptor on or in a cell (Fig. 2-1). At the receptor, the following equilibrium (Rx. 2-1) usually holds:

$$\text{Drug} + \text{Receptor} \rightleftharpoons \text{Drug-Receptor Complex} \downarrow \text{Pharmacologic Response} \qquad \text{(Rx. 2-1)}$$

The ideal drug molecule will show favorable binding characteristics to the receptor such that the equilibrium lies to the right. At the same time, the drug will be expected to dissociate from the receptor and reenter systemic circulation to be excreted. Major exceptions include the alkylating agents used in cancer chemotherapy (see Chap. 4) and a few inhibitors of the enzyme acetylcholinesterase (see Chap. 17). Both of these subclasses of pharmacological agents form covalent bonds with the receptor. In these cases, the cell must destroy the receptor, or, in the case of the alkylating agents, the cell would be replaced, ideally with a normal cell. In other words, the usual use of drugs in medical treatment calls for the drug's effect to last for only a finite period of time. Then, if it is to be repeated, the drug will be administered again. If the patient does not tolerate the drug well, it is even more important that the agent dissociate from the receptor and be excreted from the body.

DRUG DISTRIBUTION

ORAL ADMINISTRATION

An examination of the *obstacle course* (Fig. 2-1) faced by the drug will give a better understanding of what is involved in developing a commercially feasible product. Assume that the drug is administered orally. The drug must go into solution in order for it to pass through the gastrointestinal mucosa. Even drugs administered as true solutions may not remain in solution as they enter the acidic stomach and then pass into the alkaline intestinal tract. (This will be explained further in the discussion on acid-base chemistry.) The ability

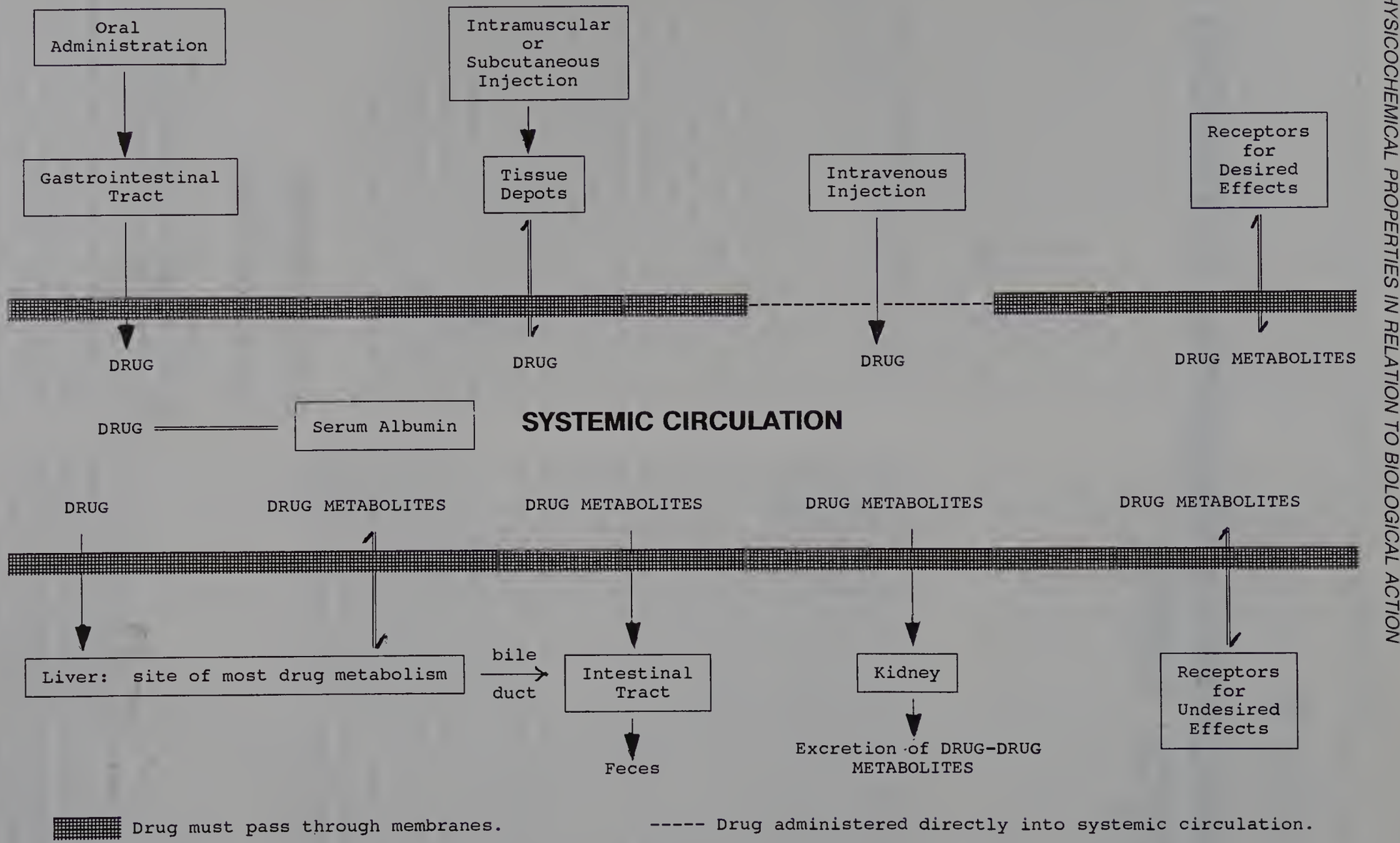
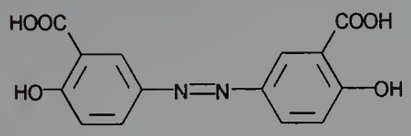


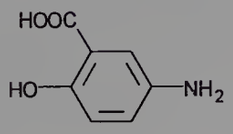
FIG. 2-1. *Summary of drug distribution.*

of the drug to dissolve is governed by several factors, including its chemical structure, variation in particle size and particle surface area, nature of the crystal form, type of tablet coating, and type of tablet matrix. By varying the dosage form and physical characteristics of the drug, it is possible to have a drug dissolve quickly or slowly, the latter being the situation for many of the sustained action products. An example is orally administered sodium phenytoin, where variation of both the crystal form and tablet adjuvants can significantly alter the bioavailability of this drug widely used in the treatment of epilepsy.

Chemical modification is also used, to a limited extent, to facilitate a drug reaching its desired target. An example is olsalazine, used in the treatment of ulcerative colitis. This drug is a dimer of the pharmacologically active mesalamine (5-aminosalicylic acid). The latter is not effective orally because it is metabolized to inactive forms before reaching the colon. The dimeric form passes through a significant portion of the intestinal tract before being cleaved by the intestinal bacteria to two equivalents of mesalamine.



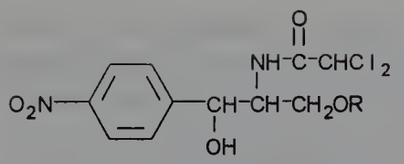
Olsalazine



Mesalamine

As illustrated by olsalazine, any compound passing through the gastrointestinal tract will encounter the large number and variety of digestive and bacterial enzymes, which, in theory, can degrade the drug molecule. In practice, when a new drug entity is under investigation, it will likely be dropped from further consideration if it is found to be unable to survive in the intestinal tract. An exception would be a drug for which there is no other effective product available or which provides a more effective treatment over existing products and can be administered by an alternate route, including parenteral, buccal, or transdermal.

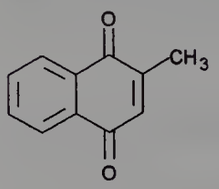
In contrast, these same digestive enzymes can be used to advantage. Chloramphenicol is water-soluble enough (2.5 mg/ml) that it comes in contact with the taste receptors on the tongue, producing an unpalatable bitterness. In order to mask this intense bitter taste, the palmitic acid moiety is added as an ester of the chloramphenicol's primary alcohol. This reduces the parent drug's water solubility (1.05 mg/ml) enough so that it can be formulated as a suspension that passes over the bitter taste receptors on the tongue. Once in the intestinal tract, the ester linkage is hydrolyzed by the digestive esterases to the active antibiotic chloramphenicol and the very common dietary fatty acid, palmitic acid.



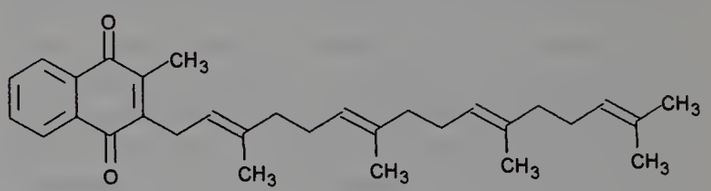
Chloramphenicol: R = H

Chloramphenicol Palmitate: R = $CO(CH_2)_{14}CH_3$

Olsalazine and chloramphenicol palmitate are examples of *prodrugs*. Most prodrugs are compounds that are inactive in their native form, but are easily metabolized to the active agent. Olsalazine and chloramphenicol palmitate are examples of prodrugs that are cleaved to smaller compounds, one of which will be the active drug. Others are metabolic precursors to the active form. An example of this type of prodrug is menadione, a simple naphthoquinone, which is converted in the liver to vitamin $K_{2(20)}$.

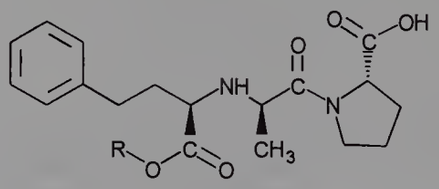


Menadione



Phytonadione (Vitamin $K_{2(20)}$)

Occasionally, the prodrug approach is used to enhance the absorption of a drug that is poorly absorbed from the gastrointestinal tract. Enalapril is the ethyl ester of enalaprilic acid, an active inhibitor of angiotensin converting enzyme (ACE). The ester prodrug is much more readily absorbed orally than the pharmacologically active carboxylic acid.



Enalapril: R = C_2H_5

Enalaprilic Acid: R = H

Unless the drug is intended to act locally in the gastrointestinal tract, it will have to pass through the gastrointestinal mucosa barrier into venous circulation in order to reach the site of the receptor. The drug's route involves distribution or partitioning between the aqueous environment of the gastrointestinal tract, the lipid bilayer cell membrane of the mucosa cells, possibly the aqueous interior of the mucosa cells, the lipid bilayer membranes on the venous side of the gastro-

intestinal tract, and the aqueous environment of venous circulation. Some very lipid-soluble drugs may follow the route of dietary lipids by becoming part of the mixed micelles, passing through the mucosa cells into the lymph ducts, servicing the intestines, and finally entering venous circulation via the thoracic duct.

The drug's passage through the mucosa cells can be passive or active. As will be discussed later in this chapter, the lipid membranes are very complex with a highly ordered structure. Part of this membrane is a series of channels or tunnels that form, disappear, and reform. There are receptors that move compounds into the cell by a process called "pinocytosis." Drugs that resemble a normal metabolic precursor or intermediate may be actively transported into the cell by the same system that transports the endogenous compound. On the other hand, most drug molecules are too large to enter the cell by an active transport mechanism through the passages.

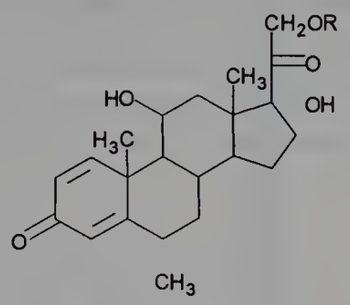
PARENTERAL ADMINISTRATION

Many times there will be therapeutic advantages to bypassing the intestinal barrier using parenteral (injectable) dosage forms. This is common in patients who, because of illness, cannot tolerate or are incapable of accepting drugs orally. Some drugs are so rapidly and completely metabolized to inactive products in the liver (first pass effect) that oral administration is precluded. But that does not mean that the drug administered by injection is not confronted by obstacles (Fig. 2-1). Intravenous administration places the drug directly into the circulatory system, where it will be rapidly distributed throughout the body, including tissue depots and the liver, where most biotransformations occur (see below), in addition to the receptors.

It is possible to inject the drug directly into specific organs or areas of the body. Intraspinal and intracerebral routes will place the drug directly into the spinal fluid or brain, respectively—bypassing a specialized epithelial tissue, the blood-brain barrier, which protects the brain from exposure to a large number of metabolites and chemicals. The blood-brain barrier is composed of membranes of tightly joined epithelial cells lining the cerebral capillaries. The net result is that the brain is not exposed to the same variety of compounds that other organs are. Local anesthetics are examples of administration of a drug directly onto the desired nerve. A spinal block is a form of anesthesia performed by injecting a local anesthetic directly into the spinal cord at a specific location in order to block transmission along specific neurons.

Most of the injections a patient will experience in a lifetime will be subcutaneous or intramuscular. These parenteral routes produce a depot in the tissues (Fig. 2-1), from which the drug must reach the blood or lymph. Once in systemic circulation, the drug will undergo the same distributive phenomena as orally and intravenously administered agents before reaching the target receptor. In general, the same factors that control the drug's passage through the gastrointestinal mucosa will also determine the rate of movement out of the tissue depot.

The already described prodrug approach also can be used to alter the solubility characteristics, which, in turn, can increase the flexibility in formulating dosage forms. The solubility of methylprednisolone can be altered from essentially water-insoluble methylprednisolone acetate to slightly water-insoluble methylprednisolone to water-soluble methylprednisolone sodium succinate. The water-soluble sodium succinate salt is used in oral, intravenous, and intramuscular dosage forms. Methylprednisolone itself is normally found in tablets. The acetate ester is found in topical ointments and sterile aqueous suspensions for intramuscular injection. Both the succinate and acetate esters are hydrolyzed to the active methylprednisolone by the patient's own systemic hydrolytic enzymes.



Methylprednisolone: R = H
Methylprednisolone Acetate: R = $C(=O)CH_3$
Methylprednisolone Sodium Succinate: R = $C(=O)CH_2CH_2COO^-$ Na^+

PROTEIN BINDING

Once the drug enters the systemic circulation (Fig. 2-1), it can undergo several events. It may stay in solution, but many drugs will be bound to the serum proteins, usually albumin (Rx. 2-2). Thus, a new equilibrium must be considered. Depending on the equilibrium constant, the drug can remain in systemic circulation bound to albumin for a considerable period of time and not be available to the sites of biotransformation, the pharmacological receptors, and excretion.

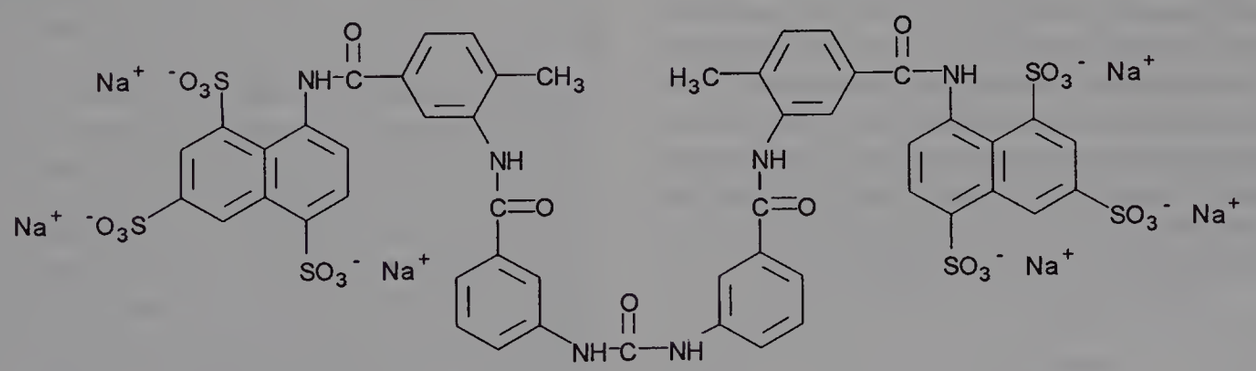
Drug + Albumin ⇌ Drug-Albumin Complex (Rx. 2-2)

The effect of protein binding can have a profound effect on the drug's effective solubility, biodistribution, half-life in the body, and interaction with other drugs. A drug with such poor water solubility that therapeutic concentrations of the unbound (active) drug normally cannot be maintained still can be a very effective agent. The albumin-drug complex acts as a reservoir by providing concentrations of free drug large enough to cause a pharmacological response at the receptor.

Protein binding may also limit access to certain body compartments. The placenta is able to block passage of proteins from maternal to fetal circulation. Thus, drugs that normally would be expected to cross the placental barrier and possibly harm the fetus are retained in the maternal circulation, bound to the mother's serum proteins.

Protein binding also can prolong the drug's duration of action. The drug-protein complex is too large to pass through the renal glomerular membranes, preventing rapid excretion of the drug. Protein binding limits the amount of drug available for biotransformation (see below and Chap. 3) and for interaction with specific receptor sites. For example, the large, polar trypanocide suramin remains in the body in the protein-bound form for as long as 3 months ($t_{1/2}$ = 50 days). The maintenance dose for this drug is based on weekly administration. At first, this might seem to be an advantage to the patient. It can be, but it also means that, should the patient have serious adverse reactions, a significant length of time will be required before the concentration of drug falls below toxic levels.

The drug-protein binding phenomenon can lead to some interesting drug-drug interactions resulting when one drug displaces another from the binding site on albumin. A large number of drugs can displace the anticoagulant warfarin from its albumin binding sites. This increases the effective concentration of warfarin at the receptor, leading to an increased prothrombin time (increased time for clot formation) and potential hemorrhage.



Suramin Sodium

TISSUE DEPOTS

The drug can also be stored in tissue depots. Neutral fat constitutes some 20% to 50% of body weight and constitutes a depot of considerable importance. The more lipophilic the drug, the more likely it will concentrate in these pharmacologically inert depots. The short-acting, lipophilic barbiturate thiopental disappears into tissue protein, redistributes into body fat, and then slowly diffuses back out of the tissue depots but in concentrations too low for a pharmacological response. Thus, only the initially administered thiopental is present in high enough concentrations to combine with its receptors. The remaining thiopental diffuses out of the tissue depots into systemic circulation in concentrations too small to be effective (Fig. 2-1), is metabolized in the liver, and is excreted.

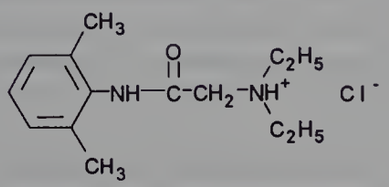
In general, structural changes in the barbiturate series (see Chap. 14) that favor partitioning into the lipid tissue stores decrease duration of action but increase central nervous system depression. Conversely, the barbiturates with the slowest onset of action and longest duration of action contain the more polar side chains. This latter group of barbiturates both enters and leaves the central nervous system very slowly as compared to the more lipophilic thiopental.

DRUG METABOLISM

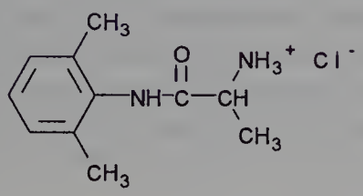
All substances, including drugs, metabolites, and nutrients that are in the circulatory system, will pass through the liver. Most molecules absorbed from the gastrointestinal tract will enter the portal vein and initially be transported to the liver. A significant proportion of a drug will partition or be transported into the hepatocyte, where it may be metabolized by hepatic enzymes to inactive chemicals during the initial trip through the liver by what is known as the first-pass effect.

Lidocaine is a classic example of the significance of the first-pass effect. Over 60% of this local anesthetic-antiarrhythmic agent is metabolized during its initial passage through the liver, resulting in it being impractical to administer orally. When used for cardiac arrhythmias, it is administered intravenously. This rapid metabolism of lidocaine is used to advantage when stabilizing a patient with cardiac arrhythmias. Should too much lidocaine be administered intravenously, toxic responses will tend to decrease because of rapid biotransformation to inactive metabolites. An understanding of the metabolic labile site on lidocaine

led to the development of the primary amine analog tocainide. In contrast to lidocaine's half-life of <2 hr, tocainide's half-life is ~15 hr with 40% of the drug excreted unchanged. The development of orally active antiarrhythmic agents is discussed in more detail in Chap. 19.

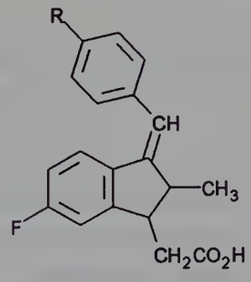


Lidocaine



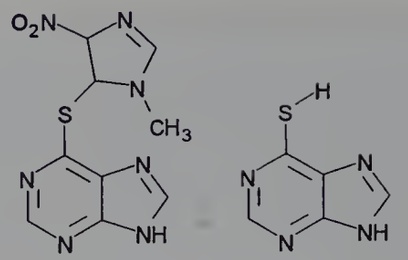
Tocainide

A study of the metabolic fate of a drug is a requirement for all new products. Many times it is found that the metabolites are also active. Indeed, sometimes the metabolite is the pharmacologically active molecule. These drug metabolites can provide the leads for additional investigations of potentially new products. Examples of where an inactive parent drug is converted to an active metabolite include the nonsteroidal anti-inflammatory agent sulindac being reduced to the active sulfide metabolite; the immunosuppressant azathioprine being cleaved to the purine antimetabolite 6-mercaptopurine; and purine and pyrimidine antimetabolites and antiviral agents being conjugated to their nucleotide form (acyclovir phosphorylated to acyclovir triphosphate). Many times both the parent drug and its metabolite are active, which has led to additional commercial products, instead of just one being marketed. About 75% to 80% of phenacetin (now withdrawn from the U. S. market) is converted to acetaminophen. In the tricyclic antidepressant series (see Chap. 14) imipramine and amitriptyline are N-demethylated to desipramine and nortriptyline, respectively. All four compounds have been marketed in the United States. The topic of drug metabolism is discussed more fully in Chap. 13.

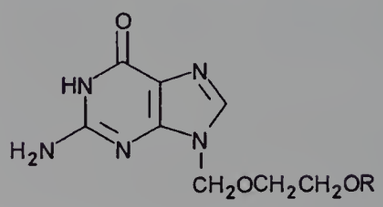


Sulindac: R = $CH_3S(=O)$

Active Sulfide Metabolite: R = CH_3S

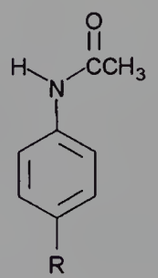


Azathioprine 6-Mercaptopurine



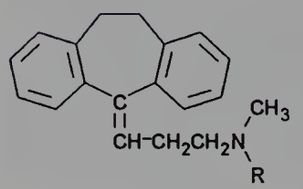
Acyclovir: R = H

Acyclovir triphosphate: R = O-P-O-P-O-P



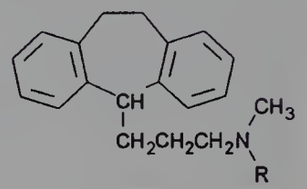
Phenacetin: R = OC_2H_5

Acetoaminophen: R = OH



Amitriptyline: R = CH_3

Nortriptyline: R = H



Imipramine: R = CH_3

Desipramine: R = H

Although a drug's metabolism can be a source of frustration for the medicinal chemist, pharmacist, and physician and lead to inconvenience and compliance problems with the patient, it is fortunate that the body has the ability to metabolize foreign molecules (xenobiotics). Otherwise, many of these substances could remain in the body for years. This has been the complaint against certain lipophilic chemical pollutants, including the once very popular insecticide DDT. After entering the body, these chemicals reside in body tissues, slowly diffusing out of the depots and potentially harming the individual on a chronic basis for several years. They can also reside in tissues of commercial food animals who have been slaughtered before the drug has "washed out" of the body.

EXCRETION

The main route of excretion of a drug and its metabolites is through the kidney. For some drugs, enterohepatic circulation (Fig. 2-1), where the drug reenters the intestinal tract

from the liver through the bile duct, can be an important part of the agent's distribution in the body and route of excretion. Either the drug or drug metabolite can reenter systemic circulation by passing once again through the intestinal mucosa. A portion of either also may be excreted in the feces. Nursing mothers must be concerned because drugs and their metabolites can be excreted in human milk and be ingested by the nursing infant.

It is important to keep a sense of perspective when learning about drug metabolism. As will be explained in Chap. 3, drug metabolism can be conceptualized as occurring in two stages or phases. Intermediate metabolites that are pharmacologically active usually are produced by Phase I reactions. The products from the Phase I chemistry are converted into inactive, usually water-soluble end products by the Phase II reactions. The latter, commonly called *conjugation* reactions, can be thought of as synthetic reactions that involve addition of water-soluble substituents. Examples are glucuronic acid, sulfate, and glutathione. Obviously, drugs that are bound to serum protein or show favorable partitioning into tissue depots are going to be metabolized and excreted more slowly for the reasons already discussed.

This does not mean that for drugs that remain in the body for longer periods of time lower doses can be administered or the drug be taken fewer times per day by the patient. Several variables determine dosing regimens, of which the affinity of the drug for the receptor is crucial. Reexamine Rx. 2-1 and Fig. 2-1. If the equilibrium does not favor formation of the drug-receptor complex, higher and usually more frequent doses will have to be administered. If the partitioning into tissue stores, metabolic degradation, and/or excretion is favored, it will take more of the drug and usually more frequent administration in order to maintain therapeutic concentrations at the receptor.

RECEPTOR

With the possible exception of general anesthetics (see Chap. 14), the working model for a pharmacological response consists of a drug binding to a specific receptor. Many drug receptors actually are used by endogenously produced ligands. Cholinergic agents interact with the same receptors as the neurotransmitter acetylcholine. Synthetic corticosteroids bind to the same receptors as cortisone and hydrocortisone. Many times receptors for the same ligand will be found in a variety of tissues throughout the body. The nonsteroidal anti-inflammatory agents (see Chap. 22) inhibit the prostaglandin-forming enzyme cyclooxygenase, which is found in nearly every tissue. This class of drugs has a long list of side effects with many patient complaints. Note in Fig. 2-1 that, depending on which receptors contain bound drug, there may be desired or undesired effects. This is because there are a variety of receptors with similar structural requirements found in several organs and tissues. Thus, the nonsteroidal anti-inflammatory drugs combine with the desired cyclooxygenase receptors at the site of the inflammation and the undesired receptors in the gastrointestinal mucosa, causing severe discomfort and sometimes ulceration. One of the ''second-generation'' antihistamines, terfenadine, is claimed to cause less sedation because it does not readily penetrate the blood-brain barrier. The rationale is that less of this antihistamine is available for the receptors in the central nervous system, which are responsible for the sedation response characteristic of antihistamines. In contrast, some antihistamines are used for their central nervous system depressant activity, which would imply that a considerable proportion of the administered dose is crossing the blood-brain barrier relative to binding to the histamine$_1$ receptors in the periphery.

Although it is normal to think of side effects as undesirable, they sometimes can be beneficial and lead to new products. The successful development of oral hypoglycemic agents used in the treatment of diabetes began when it was found that certain sulfonamides had a hypoglycemic effect (see Chap. 19). Nevertheless, a real problem in drug therapy is patient compliance in taking the drug as directed. Drugs that cause serious problems and discomfort tend to be avoided by the patient.

SUMMARY

One of the goals is to design drugs that will interact with receptors at specific tissues. There are several ways to do this, including (a) altering the molecule, which, in turn, can change the biodistribution, (b) searching for structures that show increased specificity for the target receptor that will produce the desired pharmacological response while decreasing the affinity for undesired receptors that produce adverse responses, and (c) the still experimental approach of attaching the drug to a monoclonal antibody that will bind to a specific tissue antigenic for the antibody. Alteration of biodistribution can be achieved by changing the drug's solubility, enhancing its ability to resist being metabolized (usually in the liver), altering the formulation or physical characteristics of the drug, and changing the route of administration. If a drug molecule can be designed in such a way that its binding to the desired receptor is enhanced relative to the undesired receptor, and biodistribution remains favorable, smaller doses of the drug can be administered. This, in turn, reduces the amount of drug available for binding to those receptors responsible for its side effects.

The medicinal chemist is confronted with several challenges in designing a bioactive molecule. A good fit to a specific receptor is desirable, but the drug would normally be expected eventually to dissociate from the receptor. The specificity for the receptor would be such that side effects would be minimal. The drug would be expected to clear the body within a reasonable time. Its rate of metabolic degradation should allow reasonable dosing schedules and, ideally, oral administration. Many times, the drug chosen for commercial sales has been selected from the hundreds of com-

pounds that have been screened. It usually is a compromise product that meets a medical need while demonstrating good patient acceptance.

ACID-BASE PROPERTIES

The majority of drugs used today can be classified as acids or bases. As will be noted shortly, a large number of drugs can behave as either acids or bases as they begin their journey into the patient in different dosage forms and end up in systemic circulation. A drug's acid-base properties can influence greatly its biodistribution and partitioning characteristics.

Over the years, at least four major definitions of acids/bases have been developed. The model commonly used in pharmacy and biochemistry was developed independently by Lowry and Brönsted. In their definition an *acid* is defined as a proton donor and a *base* as a proton acceptor. Notice that for a base, *there is no mention of the hydroxide ion.*

ACID-CONJUGATE BASE

Representative examples of pharmaceutically important acidic drugs are listed in Table 2-1. Each acid, or proton donor, yields a *conjugate base.* The latter is the product produced after the proton is lost from the acid. They range from the chloride ion (reaction *a*), which does not accept a proton in aqueous media, to ephedrine (reaction *h*), which is an excellent proton acceptor.

Notice the diversity in structure of these proton donors. They include the classical hydrochloric acid (reaction *a*), the weakly acidic dihydrogen phosphate anion (reaction *b*), the ammonium cation such as is found in ammonium chloride (reaction *c*), the carboxylic acetic acid (reaction *d*), the enolic form of phenobarbital (reaction *e*), the carboxylic acid moiety of indomethacin (reaction *f*), the imide structure of saccharin (reaction *g*), and the protonated amine of ephedrine (reaction *h*). Because all are proton donors, they must be treated as acids when calculating *p*Hs of a solution or percent ionization of the drug. At the same time, as will be noted shortly, there are important differences in the pharmaceutical properties of ephedrine hydrochloride (an acid salt of an amine) as compared with indomethacin, phenobarbital, or saccharin.

BASE-CONJUGATE ACID

The Brönsted-Lowry theory defines a *base* as a molecule that accepts a proton. The product resulting from the addition of a proton to the base is the *conjugate acid.* Pharmaceutically important bases are listed in Table 2-2. Again, there are a variety of structures, including the easily recognizable base, sodium hydroxide (reaction *a*), the basic component of an important physiological buffer, sodium monohydrogen phosphate which is also the conjugate base of dihydrogen phosphate (reaction *b*), ammonia which is also the conjugate base of the ammonium cation (reaction *c*), sodium acetate which is also the conjugate base of acetic acid (reaction *d*), the enolate form of phenobarbital which is also the conjugate base of phenobarbital (reaction *e*), the carboxylate form of indomethacin which is also the conjugate base of indomethacin (reaction *f*), the imidate form of saccharin which is also the conjugate base of saccharin (reaction *g*), and the amine ephedrine which is also the conjugate base of ephedrine hydrochloride (reaction *h*). Notice that the conjugate acid products in Table 2-2 are the reactant acids in Table 2-1. Conversely, most of the conjugate base products in Table 2-1 are the reactant bases in Table 2-2. Also, notice that, whereas phenobarbital, indomethacin, and saccharin are un-ionized in the protonated form, the protonated (acidic) forms of ammonia and ephedrine are ionized salts (Table 2-1). The opposite is true when examining the basic forms of these drugs. The basic forms of phenobarbital, indomethacin, and saccharin are anions, whereas ammonia and ephedrine are electrically neutral (Table 2-2).

It is important to realize that each of the examples in Tables 2-1 and 2-2 can function as either a proton donor (acid) or proton acceptor (base). This can be best understood by emphasizing the concept of conjugate acid–conjugate base pairing. Complicated as it first may seem, conjugate acids and conjugate bases are nothing more than the products of an acid-base reaction. In other words, they will appear to the right of the reaction arrows. Examples from Tables 2-1 and 2-2 are rewritten in Table 2-3 as complete acid-base reactions.

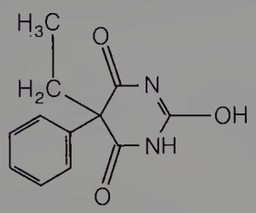
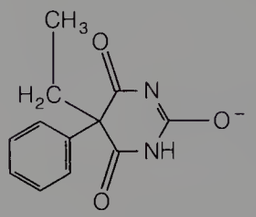
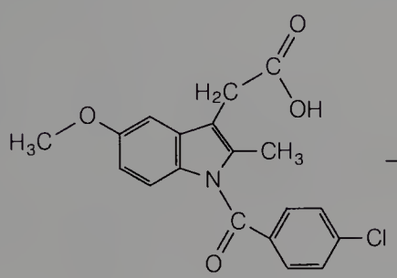
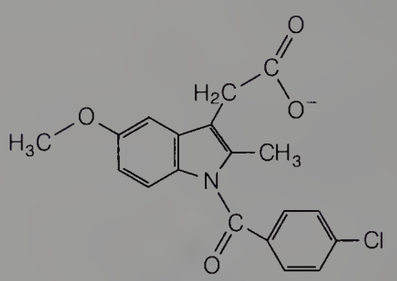
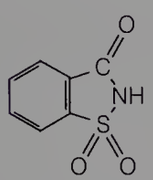
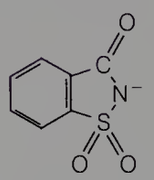
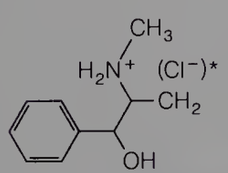
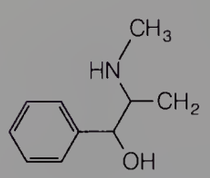
Careful study of Table 2-3 will show water functioning as a proton acceptor (base) in reactions *a,c,e,g,i,k,m* and proton donor (base) in reactions *b,d,f,h,j,l,n*. Hence, water is known as an amphoteric substance. Water can either be a weak base accepting a proton to form the strongly acidic hydrated proton or hydronium ion H_3O^+ (reactions *a,c,e,g,i,k,m*), or a weak acid donating a proton to form the strongly basic hydroxide anion OH^- (reactions *b,d,f,h,j,l,n*).

ACID STRENGTH

While any acid-base reaction can be written as an equilibrium reaction, an attempt has been made in Table 2-3 to indicate which sequences are unidirectional or only show a small reversal. For hydrochloric acid, the conjugate base, Cl^-, is so weak a base that it essentially does not function as a proton acceptor. That is why the chloride anion was not included as a base in Table 2-2. In a similar manner, water is such a weak conjugate acid that there is little reverse reaction involving water donating a proton to the hydroxide anion of sodium hydroxide.

TABLE 2-1

EXAMPLES OF ACIDS

Acid	⟶	H^+	+	Conjugate Base
(a) Hydrochloric acid HCl	⟶	H^+	+	Cl^-*
(b) Sodium dihydrogen phosphate (monobasic sodium phosphate) NaH_2PO_4 (Na^+, H_2PO_4)*	⟶	H^+	+	$NaHPO_4^{2-}$ (Na^+*, HPO_4^{2-})
(c) Ammonium chloride NH_4Cl (NH_4^+, Cl^-)*	⟶	H^+	+	NH_3 (Cl^-)*
(d) Acetic acid CH_3COOH	⟶	H^+	+	CH_3COO^-
(e) Phenobarbital		⟶ H^+ +		
(f) Indomethacin		⟶ H^+ +		
(g) Saccharin		⟶ H^+ +		
(h) Ephedrine hydrochloride		⟶ H^+ (Cl^-*) +		

* The sodium cation and chloride anion do not take part in these reactions.

Two logical questions to ask at this point are how one predicts in which direction an acid-base reaction lies and to what extent the reaction goes to completion. The common physical chemical measurement that contains this information is known as the pK_a. The pK_a is the negative logarithm of the modified equilibrium constant for an acid-base reaction written such that water will be the base or proton acceptor. It can be derived as follows:

Assume that a weak acid, HA, reacts with water.

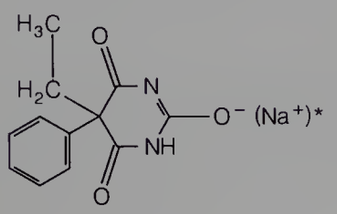
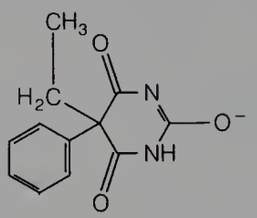
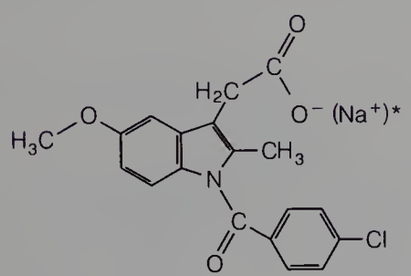
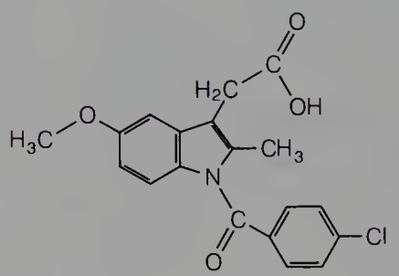
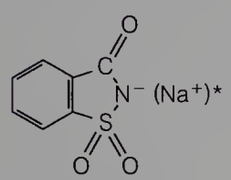
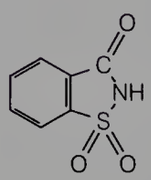
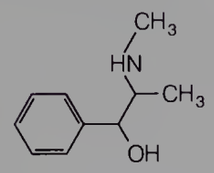
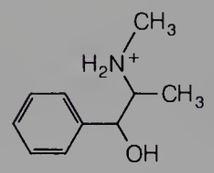
Acid	Base		Conj. Acid		Conj. Base	
HA	+ H_2O	⇌	H_3O^+	+	A^-	(Rx. 2-3)

The equilibrium constant, K_{eq}, for Rx. 2-3 is:

$$K_{eq} = \frac{[H_3O^-][A^-]}{[HA][H_2O]} = \frac{[\text{conj. acid}][\text{conj. base}]}{[\text{acid}][\text{base}]} \qquad \text{(Eq. 2-1)}$$

TABLE 2-2

EXAMPLES OF BASES

Base	+	H^+	⟶	Conjugate Acid		
(a) Sodium hydroxide $NaOH$ (Na^{+*}, OH^-)	+	H^+	⟶	H_2O	+	Na^{+*}
(b) Sodium monohydrogen phosphate (dibasic sodium phosphate) Na_2HPO_4 ($2\,Na^{+*}$, HPO_4^{2-})	+	H^+	⟶	$H_2PO_4^-$	+	$2\,Na^{+*}$
(c) Ammonia NH_3	+	H^+	⟶	NH_4^+		
(d) Sodium acetate CH_3COONa (CH_3COO^-, Na^{+*})	+	H^+	⟶	CH_3COOH	+	Na^{+*}
(e) Phenobarbital sodium						
	+	H^+	⟶		+	Na^{+*}
(f) Indomethacin sodium						
	+	H^+	⟶		+	Na^{+*}
(g) Saccharin sodium						
	+	H^+	⟶		+	Na^{+*}
(h) Ephedrine						
	+	H^+	⟶			

* The sodium cation is present only to maintain charge balance. It plays no direct acid-base role.

It turns out that in a dilute solution of a weak acid, the molar concentration of water can be treated as a constant 55.5 M. This number is based on the density of water equaling 1. Therefore, 1 L of water weighs, 1000 g. With a molecular weight of 18, the molar concentration of water in 1 L of water will be:

$$[H_2O] = \frac{\text{Weight}_{H_2O}}{MW_{H_2O}} = \frac{1{,}000\text{ g}}{18\text{ g}} = 55.5\text{ M}$$

Thus, with $[H_2O] = 55.5$, Eq. 2-1 can be simplified to

$$K_a = K_{eq}[H_2O] = K_{eq}(55.5) = \frac{[H_3O^+][A^-]}{[HA]} = \frac{[\text{conj. acid}][\text{conj. base}]}{[\text{acid}]} \qquad \text{(Eq. 2-2)}$$

By definition

$$pK_a = -\log K_a \qquad \text{(Eq. 2-3)}$$

and

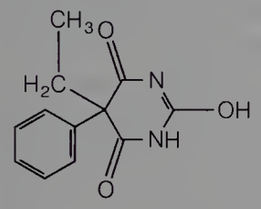
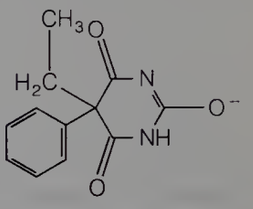
$$p\text{H} = -\log [H_3O^+] \qquad \text{(Eq. 2-4)}$$

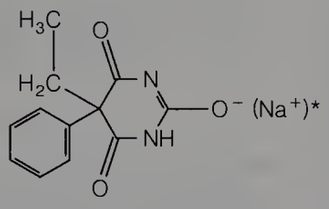
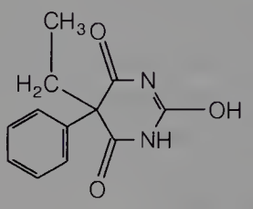
TABLE 2-3

EXAMPLES OF ACID-BASE REACTIONS (WITH THE EXCEPTION OF HYDROCHLORIC ACID, WHOSE CONJUGATE BASE (Cl^-) HAS NO BASIC PROPERTIES IN WATER, AND SODIUM HYDROXIDE, WHICH GENERATES HYDROXIDE, THE REACTION OF THE CONJUGATE BASE IN WATER IS SHOWN FOR EACH ACID)

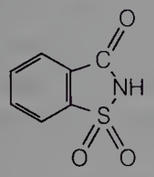
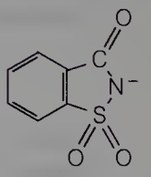
Acid	+	*Base*	⇌	*Conjugate Acid*	+	*Conjugate Base*
Hydrochloric acid						
(a) HCl	+	H_2O	⟶	H_3O^+	+	Cl^-
Sodium hydroxide						
(b) H_2O	+	$NaOH$	⟶	H_2O	+	$OH^-(Na^+)$*
Sodium dihydrogen phosphate and its conjugate base, sodium monohydrogen phosphate						
(c) $H_2PO_4^-(Na^+)$*	+	H_2O	⇌	H_3O^+	+	$HPO_4^{2-}(Na^+)$*
(d) H_2O	+	$HPO_4^{2-}(2Na^+)$*	⇌	$H_2PO_4^{2-}(Na^+)$*	+	$OH^-(Na^+)$*
Ammonium chloride and its conjugate base, ammonia						
(e) $NH_4^+(Cl^-)$*	+	H_2O	⇌	$H_3O^+(Cl^-)$*	+	NH_3
(f) H_2O	+	NH_3	⇌	NH_4^+	+	OH^-
Acetic acid and its conjugate base, sodium acetate						
(g) CH_3COOH	+	H_2O	⇌	H_3O^+	+	CH_3COO^-
(h) H_2O	+	$CH_3COO^-(Na^+)$*	⇌	CH_3COOH	+	$OH^-(Na^+)$*

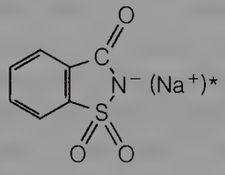
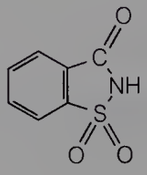
Indomethacin and its conjugate base, indomethacin sodium, will show the identical acid–base chemistry as acetic acid and sodium acetate, respectively. Phenobarbital and its conjugate base, phenobarbital sodium

(i)  + H_2O ⇌ H_3O^+ + 

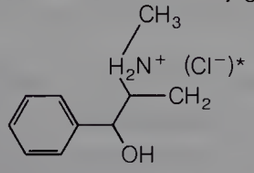
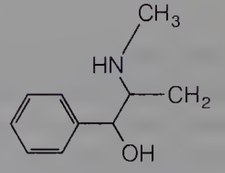
(j) H_2O +  ⇌  + $OH^-(Na^+)$*

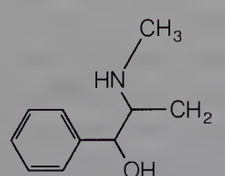
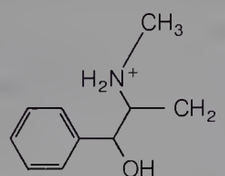
Saccharin and its conjugate base, saccharin sodium

(k)  + H_2O ⇌ H_3O^+ + 

(l) H_2O +  ⇌  + $OH^-(Na^+)$*

Ephedrine HCl and its conjugate base, ephedrine

(m)  + H_2O ⇌ $H_3O^+(Cl^-)$* + 

(n) H_2O +  ⇌  + OH^-

* The chloride anion and sodium cation are present only to maintain charge balance. These anions play no other acid-base role.

The modified equilibrium constant, K_a, is customarily converted to pK_a (the negative logarithm) in order to put it on the same scale as *p*H. Therefore, rewriting Eq. 2-2 in logarithmic form produces

$$\log K_a = \log [H_3O^+] + \log [A^-] - \log [HA]$$

$$= \log [H_3O^+] + \log [\text{conj. base}] - \log [\text{acid}] \qquad \text{(Eq. 2-5)}$$

Rearranging Eq. 2-5 gives

$$-\log [H_3O^+] = -\log K_a + \log [A^-] - \log [HA]$$

$$= -\log K_a + \log [\text{conj. base}] - \log [\text{acid}] \qquad \text{(Eq. 2-6)}$$

Substituting Eqs. 2-3 and 2-4 into Eq. 2-6 produces

$$p\text{H} = \text{p}K_a + \log \frac{[A^-]}{[HA]} = \text{p}K_a + \log \frac{[\text{conj. base}]}{[\text{acid}]} \qquad \text{(Eq. 2-7)}$$

Equation 2-7 is more commonly called the Henderson-Hasselbalch equation and is the basis for most calculations involving weak acids and bases. It is used to calculate the *p*H of solutions of weak acids, weak bases, and buffers consisting of weak acids and their conjugate bases or weak bases and their conjugate acids. Because the pK_a is a modified equilibrium constant, it corrects for the fact that weak acids do *not* completely react with water.

A very similar set of equations would be obtained from the reaction of a protonated amine, BH^+, in water. The reaction would be:

		Conj.	Conj.	
Acid	Base	Acid	Base	
$BH^+ + H_2O$	$\rightleftharpoons$	$H_3O^+ +$	B	(Rx. 2-4)

The equilibrium constant, K_{eq}, is defined as:

$$K_{eq} = \frac{[H_3O^+][B]}{[BH^+][H_2O]} = \frac{[\text{conj. acid}][\text{conj. base}]}{[\text{acid}][\text{base}]} \qquad \text{(Eq. 2-8)}$$

Notice that Eq. 2-8 is identical to Eq. 2-1 when the general [conj. acid][conj. base] representation is used. Therefore, using the same simplifying assumption that water will remain at a constant concentration of 55.5 M in dilute solutions, Eq. 2-8 can be rewritten as:

$$K_a = K_{eq}(55.5) = \frac{[H_3O^+][B]}{[BH^+]} = \frac{[\text{conj. acid}][\text{conj. base}]}{[\text{acid}]} \qquad \text{(Eq. 2-9)}$$

Rearranging Eq. 2-9 into logarithmic form and substituting the relationships expressed in Eqs. 2-3 and 2-4 yields the same Henderson-Hasselbalch equation (Eq. 2-10).

$$p\text{H} = \text{p}K_a + \log \frac{[B]}{[BH^+]} = \text{p}K_a + \log \frac{[\text{conj. base}]}{[\text{acid}]} \qquad \text{(Eq. 2-10)}$$

Rather than trying to remember the specific form of the Henderson-Hasselbalch equation for an HA or BH^+ acid, it is simpler to use the general form of the equation (Eq. 2-11) expressed in both Eqs. 2-7 and 2-10.

$$p\text{H} = \text{p}K_a + \log \frac{[\text{conj. base}]}{[\text{acid}]} \qquad \text{(Eq. 2-11)}$$

With this version of the equation, there is no need to remember whether the species in the numerator/denominator is ionized or un-ionized. The molar concentration of the proton acceptor is the term in the numerator and the molar concentration of the proton donator is the denominator term.

What about weak bases such as amines? In aqueous solutions, water will function as the proton donor or acid (Rx. 2-5), producing the familiar hydroxide anion (conjugate base). Traditionally, a modified equilibrium constant called the pK_b was derived following the same steps that produced Eq. 2-2. It is now more common to express the basicity of a chemical in terms of the pK_a using the relationship in Eq. 2-12.

		Conj.	Conj.	
Acid	Base	Acid	Base	
$H_2O +$	B	$\rightleftharpoons BH^+ +$	OH^-	(Rx. 2-5)

$$\text{p}K_a = \text{p}K_b - 14 \qquad \text{(Eq. 2-12)}$$

Warning! It is *important* to recognize that a pK_a for a base is in reality the pK_a of the conjugate acid (acid donor or protonated form) of the base. The pK_a for ephedrine is listed in the Appendix as 9.6 and for ammonia 9.3. In reality, this is the pK_a of the protonated form such as ephedrine hydrochloride (reaction *m* in Table 2-3) and ammonium chloride (reaction *e* in Table 2-3), respectively. This is confusing to students, pharmacists, clinicians, and experienced scientists. It is crucial that the chemistry of the drug be understood when interpreting a pK_a value. When reading tables of pK_a values, such as those found in the Appendix, it is essential to realize that the listed value is for the proton donor form of the molecule, no matter what form is indicated by the name. See Table 2-4 for several worked examples as to how the pK_a is used to calculate *p*Hs of solutions, required ratios of [conjugate base]/[acid], and percent ionization at specific *p*Hs.

Just how strong, or weak, are the acids whose reactions in water are illustrated in Table 2-3? Bear in mind that the K_as or pK_as are modified equilibrium constants that will indicate whether the acid's reaction in water (proton donor or base) tends to go to completion, resulting in the formation of large amounts of conjugate acid and conjugate base, or whether the base accepts few protons from water, forming little conjugate acid and conjugate base.

Refer back to Eq. 2-2 and, using the K_a values in Table 2-5, substitute the K_a term for each of the acids. For hydrochloric

TABLE 2-4

EXAMPLES OF CALCULATIONS REQUIRING THE pK_a

1. What is the ratio of ephedrine to ephedrine HCl (pK_a 9.6) in the intestinal tract at pH 8.0? Use Equation 2-11.

$$8.0 = 9.6 + \log \frac{[\text{ephedrine}]}{[\text{ephedrine HCl}]} = -1.6$$

$$\frac{[\text{ephedrine}]}{[\text{ephedrine HCl}]} = 0.025$$

The number whose log is −1.6 is 0.025, meaning that there are 25 parts ephedrine for every 1000 parts ephedrine HCl in the intestinal tract whose environment is *p*H 8.0.

2. What is the *p*H of a buffer containing 0.1 *M* acetic acid (pK_a 4.8) and 0.08 *M* sodium acetate? Use Equation 2-11.

$$p\text{H} = 4.8 + \log \frac{0.08}{0.1} = 4.7$$

3. What is the *p*H of a 0.1 *M* acetic acid solution? Use the following equation for calculating the *p*H of a solution containing either an HA or BH^+ acid.

$$p\text{H} = \frac{pK_a - \log [\text{acid}]}{2} = 2.9$$

4. What is the *p*H of a 0.08 *M* sodium acetate solution? Remember, even though this is the conjugate base of acetic acid, the pK_a will still be used. The pK_w term in the following equation corrects for the fact that a proton acceptor (acetate anion) is present in the solution. The equation for calculating the *p*H of a solution containing either an A^- or B base is

$$p\text{H} = \frac{pK_w + pK_a + \log [\text{base}]}{2} = 8.9$$

5. What is the *p*H of an ammonium acetate solution? The pK_a of the ammonium (NH_4^+) cation is 9.3. Always bear in mind that the pK_a refers to the ability of the proton donor form to release the proton into water to form H_3O^+. Since this is the salt of a weak acid (NH_4^+) and the conjugate base of a weak acid (acetate anion), the following equation is used. Note that molar concentration is not a variable in this calculation.

$$p\text{H} = \frac{pK_{a_1} + pK_{a_2}}{2} = 7.1$$

6. What is the percentage ionization of ephedrine HCl (pK_a 9.6) in an intestinal tract buffered at *p*H 8.0 (see example 1)? Use Equation 2-14 because this is a BH^+ acid.

$$\%\ \text{ionization} = \frac{100}{1 + 10^{(8.0-9.6)}} = 97.6\%$$

Only 2.4% of ephedrine is present as the un-ionized conjugate base.

7. What is the percentage ionization of indomethacin (pK_a 4.5) in an intestinal tract buffered at *p*H 8.0? Use Equation 2-13 because this is an HA acid.

$$\%\ \text{ionization} = \frac{100}{1 + 10^{(4.5-8.0)}} = 99.97\%$$

For all practical purposes indomethacin is present only as the anionic conjugate base in that region of the intestine buffered at *p*H 8.0.

acid, it should be obvious that a K_a of 1.26×10^6 means that the numerator term containing the concentrations of the conjugate acid and conjugate base products is huge relative to the denominator term representing the concentration of the reactants. In other words, there essentially is no unreacted HCl left in an aqueous solution of hydrochloric acid. At the other extreme is ephedrine HCl with a pK_a of 9.6 or K_a of 2.51×10^{-10}. Here, the denominator representing the concentration of ephedrine HCl greatly predominates over that of the products, which, in this example, would be ephedrine (conjugate base) and H_3O^+ (conjugate acid). In other words, the protonated form of ephedrine is a very poor proton donor. It holds onto the proton. Indeed, free ephedrine (the conjugate base in this reaction) is an excellent proton acceptor.

A general rule for determining whether a chemical is strong or weak acid or base is

pK_a <2: strong acid; essentially no basic properties in water
pK_a 4–6: weak acid; very weak conjugate base
pK_a 8–10: very weak acid; weak conjugate base
pK_a >12: essentially no acidic properties in water; strong conjugate base

It is important to realize that this delineation is only approximate. Other properties also become important when considering cautions in handling acids and bases. Phenol has a pK_a of 9.9, slightly less than that of ephedrine HCl. Why is phenol considered corrosive to the skin, whereas ephedrine HCl or free ephedrine is considered innocuous when applied to the skin? Phenol has the ability to partition through the normally protective lipid layers of the skin. Due to this property, this extremely weak acid has carried the name carbolic acid. Thus, the pK_a simply tells a person the acid properties of the protonated form of the chemical. It does not represent anything else concerning other potential toxicities.

PERCENT IONIZATION

Using the drug's pK_a, the formulation pharmacist can adjust the *p*H in order to insure maximum water solubility (ionic form of the drug) or maximum solubility in nonpolar media (nonionic form). This is where the understanding of the drug's acid-base chemistry becomes important. Note Rxs. 2-6 and 2-7:

TABLE 2-5

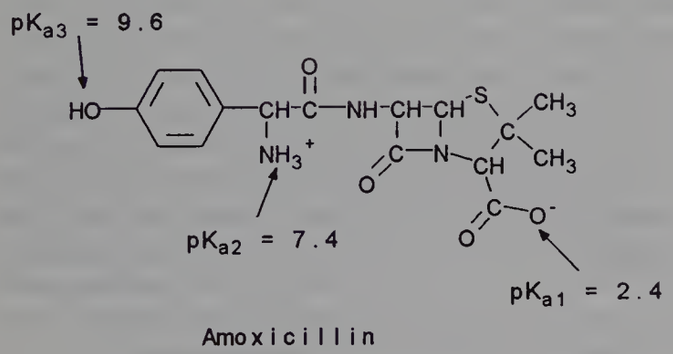
REPRESENTATIVE K_a AND pK_a VALUES FROM THE REACTIONS LISTED IN TABLE 2-3 (SEE THE APPENDIX)

Hydrochloric acid	1.26×10^6	−6.1
Dihydrogen phosphate	6.31×10^{-8}	7.2
Ammonia (ammonium)	5.01×10^{-10}	9.3
Acetic acid	1.58×10^{-5}	4.8
Phenobarbital	3.16×10^{-8}	7.5
Saccharin	2.51×10^{-2}	1.6
Indomethacin	3.16×10^{-5}	4.5
Ephedrine (as the HCl salt)	2.51×10^{-10}	9.6

Acid	Base	Conj. Acid	Conj. Base	
$HA_{(un\text{-}ionized)}$	+ H_2O ⇌	H_3O^+	+ $A^-_{(ionized)}$	(Rx. 2-6)

Acid	Base	Conj. Acid	Conj. Base	
$BH^+_{(ionized)}$	+ H_2O ⇌	H_3O^+	+ $B_{(un\text{-}ionized)}$	(Rx. 2-7)

Acids can be divided into two types, HA and BH^+, based on the ionic form of the acid (or conjugate base). HA acids go from nonionized acids to ionized conjugate bases (Rx. 2-6). In contrast, BH^+ acids go from an ionized (polar) acid to nonionized (nonpolar) conjugate base (Rx. 2-7). In general, pharmaceutically important HA acids include the inorganic acids (e.g., HCl, H_2SO_4), enols (e.g., barbiturates, hydantoins), carboxylic acids (e.g., low molecular weight organic acids, arylacetic acids, *N*-aryl anthranilic acids, salicylic acids), and amides and imides (e.g., saccharin, sulfonamides). The chemistry is simpler for the pharmaceutically important BH^+ acids: they are all protonated amines. A polyfunctional drug can have several pK_as (e.g., amoxicillin). The latter's ionic state is based on amoxicillin's ionic state at physiological *p*H 7.4 (see the following discussion on percent ionization).



Amoxicillin

The percent ionization of a drug is calculated using Eqs. 2-13 for HA acids and 2-14 for BH^+ acids.

$$\% \text{ ionization} = \frac{100}{1 + 10^{(pK_a - pH)}} \quad \text{(Eq. 2-13)}$$

$$\% \text{ ionization} = \frac{100}{1 + 10^{(pH - pK_a)}} \quad \text{(Eq. 2-14)}$$

A plot of percent ionization versus *p*H is illustrative of how the degree of ionization can be shifted significantly with small changes in *p*H. The curves for an HA acid (indomethacin) and BH^+ (protonated ephedrine) are shown in Fig. 2-2. First, note that when *p*H = pK_a, the compound is 50% ionized (or 50% un-ionized). In other words, when the pK_a is equal to the *p*H, the molar concentration of the acid equals the molar concentration of its conjugate base. In the Henderson-Hasselbalch equation, the pK_a = *p*H when log [conj. base]/[acid] = 1. An increase of 1 *p*H unit from the pK_a (increase in alkalinity) causes an HA acid (indomethacin) to become 90.9% in the ionized conjugate base form but results in a BH^+ acid (ephedrine HCl) decreasing its percent ionization to only 9.1%. An increase of 2 *p*H units

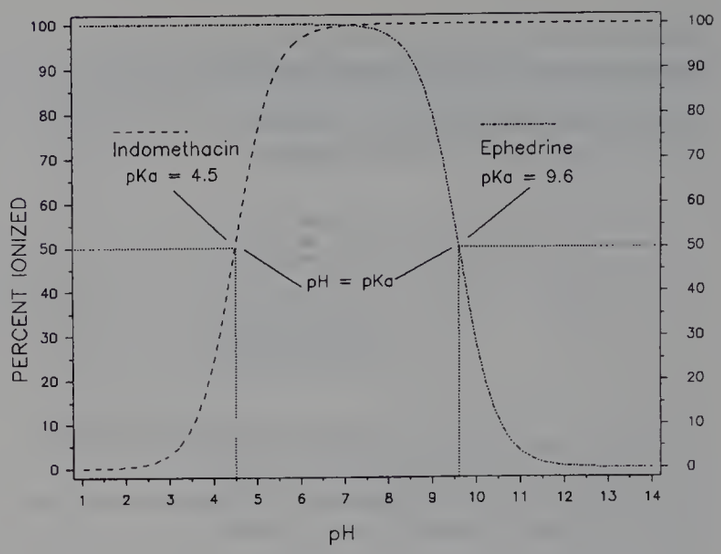


FIG. 2-2. *Percentage ionized versus* p*H for indomethacin (*pK_a *4.5) and ephedrine (*pK_a *9.6).*

essentially shifts an HA acid to complete ionization (99%) and a BH^+ acid to the nonionic conjugate base form (0.99%).

Just the opposite is seen when the media is made more acidic relative to the drug's pK_a value. Increasing the hydrogen ion concentration (decreasing the *p*H) will shift the equilibrium to the left, thereby increasing the concentration of the acid and decreasing the concentration of conjugate base. In the case of indomethacin, a decrease of 1 *p*H unit below the pK_a will increase the concentration of un-ionized (protonated) indomethacin to 9.1%. Similarly, a decrease of 2 *p*H units results in only 0.99% of the indomethacin being present in the ionized conjugate base form. The opposite is seen for the BH^+ acids. The percent of ephedrine present as the ionized (protonated) acid at 1 *p*H unit below the pK_a is 90.9% and 2 *p*H units below the pK_a is 99.0%. These results are summarized in Table 2-6.

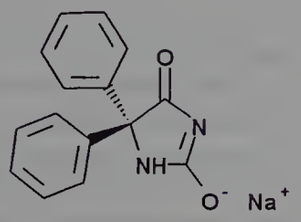
With this knowledge in mind, return to the drawing of amoxicillin. At physiological *p*H, the carboxylic acid (HA acid; pK_{a1} 2.4) will be in the ionized carboxylate form, the primary amine (BH^+ acid; pK_{a2} 7.4) will be 50% protonated and 50% in the free amine forms, and the phenol (HA acid; pK_{a3} 9.6) will be in the nonionized protonated form. A knowledge of percent ionization makes it easier to explain and predict why problems and discomfort due to *p*H ex-

TABLE 2-6

PERCENTAGE IONIZATION RELATIVE TO THE pK_a

	Ionization (%)	
	HA Acids	***BH⁺ Acids***
pK_a − 2 *p*H units	0.99	99.0
pK_a − 1 *p*H unit	9.1	90.9
pK_a = *p*H	50.0	50.0
pK_a + 1 *p*H unit	90.9	9.1
pK_a + 2 *p*H units	99.0	0.99

tremes can occur with the use of some preparations. Phenytoin (HA acid; pK_a 8.3) injection must be adjusted to *p*H 12 with sodium hydroxide in order to insure complete ionization and maximize water solubility. In theory, a *p*H of 10.3 will result in 99.0% of the drug being in the anionic water-soluble conjugate base. To reduce even further that insoluble 1% in the acid form and maintain excess alkalinity, the *p*H is raised to 12 to obtain 99.98% of the drug in the ionized form. Even then, a cosolvent system of 40% propylene glycol, 10% ethyl alcohol, and 50% water for injection is used to insure a complete solution. This highly alkaline solution is irritating to the patient and generally cannot be administered as an admixture with other intravenous fluids that are buffered more closely at physiological *p*H 7.4. This decrease in *p*H will result in the parent un-ionized phenytoin precipitating out of solution.



Phenytoin Sodium

Adjustments in *p*H to maintain water solubility can sometimes lead to chemical stability problems. An example is indomethacin (HA acid; pK_a 4.5), which is unstable in alkaline media. Therefore, the preferred oral liquid dosage form is a suspension buffered at *p*H 4 to 5. Because this is near the drug's pK_a, only 50% will be in the water-soluble form. There is a medical indication requiring the intravenous administration of indomethacin to premature infants. The intravenous dosage form is the lyophilized (freeze-dried) sodium salt, which is reconstituted just prior to use.

DRUG DISTRIBUTION AND pK_a

The pK_a can have a pronounced effect on the pharmacokinetics of the drug. As has already been discussed, drugs will be transported in the aqueous environment of the blood. Those drugs that are in an ionized form will tend to distribute throughout the body more rapidly than un-ionized (nonpolar) molecules. With few exceptions, the drug must leave the polar environment of the plasma in order to reach the site of action. In general, drugs pass through the nonpolar membranes of capillary walls, cell membranes, and the blood-brain barrier in the un-ionized (nonpolar) form. For HA acids, it will be the parent acid that will readily cross these membranes (Fig. 2-3). The situation is just the opposite for the BH^+ acids. The un-ionized conjugate base (free amine) will be the species most readily crossing the nonpolar membranes (Fig. 2-4).

Consider the changing *p*H environment experienced by the drug molecule when orally administered. The drug will

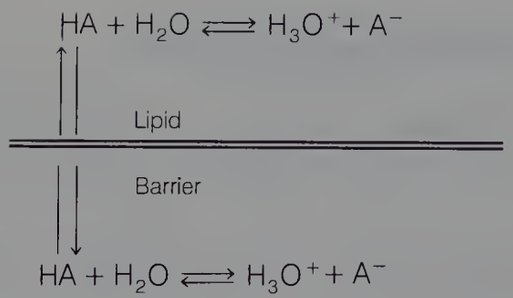


FIG. 2-3. *Passage of HA acids through lipid barriers.*

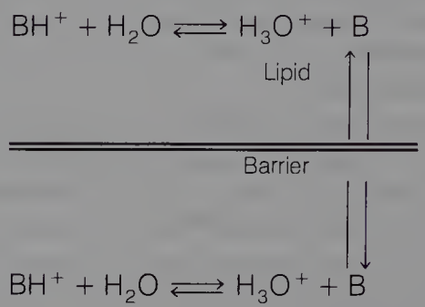


FIG. 2-4. *Passage of BH^+ acids through the lipid barrier.*

first encounter the acidic stomach, where the *p*H can range from 2 to 6 depending on the presence of food. HA acids with pK_as of 4 to 5 will tend to be nonionic and be absorbed partially through the gastric mucosa. (The main reason most acidic drugs are absorbed from the intestinal tract rather than the stomach is that the microvilli of the intestinal mucosa provide a huge surface area relative to what is found in the gastric mucosa of the stomach.) In contrast, amines (pK_a 9 to 10) will be protonated (BH^+ acids) and usually will not be absorbed until reaching the mildly alkaline intestinal tract (*p*H ≈ 8). Even here only a portion of the amine-containing drugs will be in their nonpolar conjugate base form. Whenever a nonpolar form of either an HA acid (as the acid) or a BH^+ acid (as the conjugate base) passes the lipid barrier, the equilibrium conjugate base/acid (as determined by the *p*H of the environment) must be maintained.

For example, once in systemic circulation, the plasma *p*H of 7.4 will be one of the determinants as to whether the drug will tend to remain in the aqueous environment of the blood or partition across lipid membranes into hepatic tissue to be metabolized, into the kidney for excretion, into tissue depots, or to the receptor tissue. A useful exercise is to calculate the [conj. base]/[acid] ratio using the Henderson-Hasselbalch equation (Eq. 2-11) or percent ionization for ephedrine (pK_a 9.6; Eq. 2-14) and indomethacin (pK_a 4.5; Eq. 2-13) at *p*H 3.5 (stomach), *p*H 8.0 (intestine), and *p*H 7.4 (plasma) (see examples 1, 6, and 7 in Table 2-4). Of course, the previously discussed effect of protein binding can greatly alter any prediction of biodistribution based solely on pK_a.

STATISTICAL PREDICTION OF PHARMACOLOGICAL ACTIVITY

Just as mathematical modeling is used to explain and model many chemical processes, it has been the goal of medicinal chemists to quantify the effect of a structural change on a defined pharmacological response. This would meet three goals in drug design: to (a) predict biological activity in untested compounds; (b) define the structural requirements required for a good fit between the drug molecule and the receptor; and (c) design a test set of compounds in order to maximize the amount of information concerning structural requirements for activity from a minimum number of compounds tested. This aspect of medicinal chemistry commonly is referred to as quantitative structure activity relationships (QSAR).

The goals of QSAR studies were first proposed around 1865 to 1870 by Crum-Brown and Fraser, who showed that the gradual chemical modification in the molecular structure of a series of poisons produced some important differences in their action.[1] They postulated that the physiological action, Φ, of a molecule is some function of its chemical constitution, C. This can be expressed as follows:

$$\Phi = f(C) \qquad \text{(Eq. 2-15)}$$

Equation 2-15 states that a defined change in chemical structure would result in a predictable change in physiological action. The problem now becomes one of numerically defining chemical structure. It still is a fertile area of research. What has been found is that biological response can be predicted from physical chemical properties such as vapor pressure, water solubility, electronic parameters, steric descriptors, and partition coefficient (Eq. 2-16). Today, the partition coefficient has become the single most important physical chemical measurement for QSAR studies. Note that Eq. 2-16 is the equation for a straight line ($Y = mx + b$).

$$\text{Log } BR = a(\text{physical chemical property}) + c \qquad \text{(Eq. 2-16)}$$

where

BR = a defined pharmacological response usually expressed in millimoles such as the effective dose in 50% of the subjects (ED_{50}), the lethal dose in 50% of the subjects (LD_{50}), or the minimum inhibitory concentration (MIC). It is common to express the biological response as a reciprocal, $1/BR$ or $1/C$

a = the regression coefficient or slope of the straight line

c = the intercept term on the Y axis (when the physical chemical property equals zero)

The log value of the dependent variable (concentration necessary to obtain a defined biological response) is used to linearize the data. As will be shown shortly, QSARs are not always linear. Nevertheless, using logarithms is an acceptable statistical technique. Now, why is the biological response usually expressed as a reciprocal? Sometimes, one obtains a statistically more valid relationship. More importantly, expressing the biological response as a reciprocal usually produces a positive slope (Fig. 2-6). Let us examine a hypothetical example. Assume that BR is the ED_{50}.

Compound	Physicochemical property	BR (mmole)	1/BR (mmole)
A	0.10	0.4	0.25
B	0.15	0.3	0.33
C	0.20	0.2	0.5
D	0.25	0.1	1.0

Notice that it requires four times as much compound A relative to compound D to produce the same defined biological response in 50% of the test subjects. Also notice that the physicochemical property is increasing as the potency is decreasing. In other words, there is an inverse relationship between physicochemical property and biological potency. In contrast, there is a positive relationship between the reciprocal of the biological response ($1/BR$) and physicochemical property because $1/BR$ is increasing as the physicochemical property is increasing.

As has been emphasized previously, the drug will go through a series of partitioning steps: (a) leaving the aqueous extracellular fluids, (b) passing through lipid membranes, and (c) entering other aqueous environments before reaching the receptor. In this sense, a drug is undergoing the same partitioning phenomena that happens to any chemical in a separatory funnel containing water and a nonpolar solvent such as hexane, chloroform, or ether. The difference between the separatory funnel model and what actually occurs in the body is that the partitioning in the funnel will reach an equilibrium where the rate of chemical leaving the aqueous phase and entering the organic phase will equal the rate of chemical moving from the organic phase to the aqueous phase. This is not the physiological situation. Refer to Fig. 2-1, and note that dynamic changes are occurring to the drug such as it being metabolized, bound to serum albumin, excreted from the body, and bound to receptors. The circumstance for the drug is not static. Upon administration, the drug will be *pushed* through the membranes because of the high concentration of drug in the extracellular fluids relative to the concentration in the intracellular compartments. In an attempt to maintain equilibrium ratios, the flow of the drug will be from systemic circulation through the membranes onto the receptors. As the drug is metabolized and excreted from the body, it will be *pulled* back across the membranes and the concentration of drug at the receptors will decrease.

Because much of the time the drug's movement across

membranes is a partitioning process, the partition coefficient has become the most common physicochemical property. The question that now must be asked is what immiscible nonpolar solvent system best mimics the aqueous-lipid membrane barriers found in the body? It is now realized that the n-octanol/water system is an excellent estimator of drug partitioning in biological systems. Indeed, one could argue that it was fortuitous that n-octanol was available in reasonable purity for the early partition coefficient determinations. To appreciate why this is so, it is necessary to understand the chemical nature of the lipid membranes.

These membranes are not anhydrous fatty or oily structures. As a first approximation, they can be considered as bilayers composed of lipids consisting of a polar cap and large hydrophobic tail. Phosphoglycerides are major components of lipid bilayers. Other groups of bifunctional lipids include the sphingomyelins, galactocerebrosides, and plasmologens. The hydrophobic portion is composed largely of unsaturated fatty acids, mostly with *cis* double bonds. In addition, there is a considerable amount of cholesterol esters, protein, and charged mucopolysaccharides in the lipid membranes. The final result is that these membranes are highly organized structures composed of channels for transport of important molecules such as metabolites, chemical regulators (hormones), amino acids, glucose, and fatty acids into the cell and removal of waste products and biochemically produced products out of the cell. The cellular membranes are dynamic, with the channels forming and disappearing depending on the cell's and body's needs.

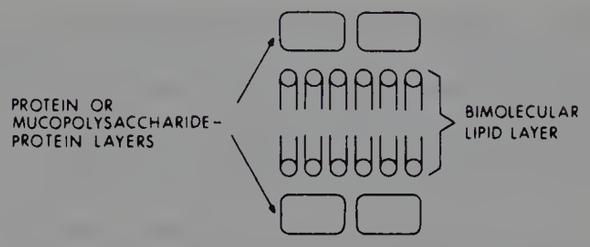


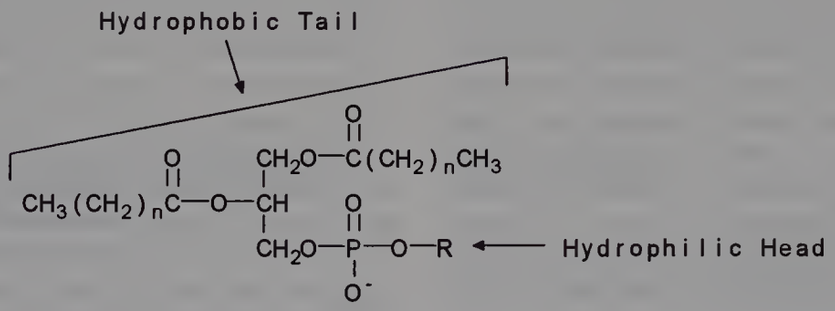
FIG. 2-5. *Schematic representation of the cell membrane.*

In addition, the membranes on the surface of nucleated cells have specific antigenic markers, major histocompatibility complex (MHC), by which the immune system monitors the cell's status. There are receptors on the cell surface where hormones such as epinephrine and insulin bind, setting off a series of biochemical events within the cell. Some of these receptors are used by viruses to gain entrance into the cells where the virus reproduces. As newer instrumental techniques are developed, and genetic cloning permits isolation of the genetic material responsible for forming and regulating the structures on the cell surface, the image of a passive lipid membrane has disappeared to be replaced by a very complex, highly organized, dynamically functioning structure.

For purposes of the partitioning phenomenon, picture the cellular membranes as two layers of lipids (Fig. 2-5). The two outer layers, one facing the interior of the cell and the other the exterior, will consist of the polar ends of the bifunctional lipids. Keep in mind that these surfaces are exposed to an aqueous polar environment. The polar ends of the charged phospholipids and other bifunctional lipids will be solvated by the water molecules. There also will be considerable amounts of charged proteins and mucopolysaccharides present on the surface. In contrast, the interior of the membrane will be populated by the hydrophobic aliphatic chains from the fatty acid esters.

PARTITION COEFFICIENT

With this representation in mind, a partial explanation can be presented as to why the *n*-octanol/water partitioning system seems to mimic the lipid membranes/water systems found in the body. It turns out that *n*-octanol is not as nonpolar as initially might be predicted. Water-saturated octanol contains 2.3 M water, whereas *n*-octanol–saturated water contains little of the organic phase. The water in the *n*-octanol phase apparently approximates the polar properties of the lipid bilayer, whereas the lack of octanol in the water phase mimics the physiological aqueous compartments, which are relatively free of nonpolar components. In contrast, partitioning systems such as hexane/water and chloroform/water contain so little water in the organic phase that they are poor



models for the lipid bilayer/water system found in the body. At the same time, it must be remembered that the n-octanol/water system is only an approximation of the actual environment found in the interface between the cellular membranes and the extracellular/intracellular fluids.

The basic procedure for obtaining a partition coefficient is to shake a weighed amount of chemical in a flask containing a measured amount of water-saturated octanol and octanol-saturated water. Many times, the aqueous phase will be buffered with a phosphate buffer at *p*H 7.4 to reflect physiological *p*H. This corrects for the ratio of [conjugate base]/[acid] found in vivo. The amount of chemical in one or both of the phases is determined by an appropriate analytical technique and the partition coefficient calculated from Eq. 2-17. The octanol/water partition coefficient has been determined for thousands of compounds, including drugs, agricultural chemicals, biochemical intermediates and metabolites, and common chemicals. Many of these determinations have been obtained in several other organic solvent/aqueous systems such as ether, chloroform, triolein, and hexane.

$$P = \frac{[\text{chemical}]_{\text{oct}}}{[\text{chemical}]_{\text{aq}}} \qquad \text{(Eq. 2-17)}$$

The determination of partition coefficients is tedious and time consuming. Some chemicals are too unstable and either degrade during the procedure, which can take several hours, or cannot be obtained in sufficient purity for an accurate determination. This has led to attempts at approximating the partition coefficient. Perhaps the most popular approach has been high-performance liquid chromatography (HPLC) or thin-layer chromatography (TLC). In each case, the support phase is nonpolar, either by permanent bonding (usually octadecylsilane) or a coating of octanol, mineral oils, or related materials. The mobile phase usually contains some water miscible organic solvent in order to hold enough of the chemical, whose partition coefficient is being determined, in solution. Sometimes the partition coefficient is calculated from the retention data by regression analysis using Eq. 2-18. The "*a*" and "*c*" terms have the same uses as in Eq. 2-16.

$$\log P = a(\log \text{retention}) + c \qquad \text{(Eq. 2-18)}$$

This model has at least two limitations. First, in order to obtain valid numerical values for "*a*" and "*c*" in Eq. 2-16, partition coefficients must be obtained initially by the classical shake flask method, their retention times obtained in the identical chromatographic system that will be used for the new compounds, and the results analyzed using Eq. 2-18. Second, chromatographic determinations of the partition coefficients usually only work when determining the retention times of chemicals of the same chemical class and similar substitution patterns. Because of these limitations, it is common to use directly the retention data in the prediction of biological response (Eq. 2-19). A chemical's retention on a chromatographic support is the result of a combination of its partitioning, steric, and electronic properties. Because these same physical chemical properties are important variables in determining a drug's biological response, excellent correlations have been obtained between chromatographic retention parameters and biological response. While the model represented by Eq. 2-19 is useful in predicting biological response, it is not as definitive as the models presented below (Eqs. 2-20 to 2-22) because the precise physical chemical properties are combined into one chromatographic retention term. In other words, it is not possible to determine the relative importance of lipophilicity, electronic effects, or steric influence on the biological response when using Eq. 2-19.

$$\text{Log } BR = a(\text{log retention}) + c \qquad \text{(Eq. 2-19)}$$

Most recently, there has been a concentrated effort to calculate the partition coefficient based on the atomic components of the molecule. Each atom type is assumed to contribute a fixed amount to the chemical's partition coefficient. Because this assumption breaks down quickly, several correction factors are used. Cyclohexene will serve as an example.

$$\begin{aligned}\text{Log } P &= 6(\text{carbon atoms}) + 12(\text{hydrogen atoms}) \\ &\quad + (n - 1)\text{bonds} + \text{double bond correction} \\ \text{Log } P &= 6(0.20) + 12(0.23) + 5(-0.09) + (-0.55) \\ &= 2.96\end{aligned}$$

For purposes of comparison, the observed octanol/water partition coefficient (expressed as a logarithm) is 2.86. Because of the correction factors, these calculations become so complex that they must be done by a computer program that analyzes the structure and identifies those structural attributes requiring correction factors. Convenient as the calculation method may be, it must be realized that its accuracy is dependent on first determining experimental partition coefficients of chemicals exhibiting very similar chemistry. It is from these experimentally determined partition coefficients that the correction factors are derived.

There are several commercial drug design software packages that contains modules that estimate a chemical's partition coefficient. Some use the method described in the previous paragraph. Others use quantum chemical parameters. In all cases, it is important that the algorithm be validated against test sets of diverse chemical structures whose partition coefficients have been determined by the classical shake flask method.

There are simpler methods for estimating lipophilicity that will give reasonably correct results. These are based on the additive effect on the partition coefficient that is seen when varying a series substituents on the same molecule. Over the years, fairly extensive tables have been developed that contain the contribution (π) of a wide variety of substituents to the partition coefficient. The method can be illustrated for chlorobenzene. The log P for benzene is 2.13 and for chlorobenzene is 2.84. The π value for the chlorine substitu-

ent is obtained by subtracting the log P values for benzene and chlorobenzene.

$$\pi_{Cl} = \log P_{chlorobenzene} - \log P_{benzene}$$

$$\pi_{Cl} = 2.84 - 2.13 = 0.71$$

While the π substituent method has its limitations, particularly when there are significant resonance and inductive effects due to the presence of multiple substituents, it can work well for a series of compounds that have similar substitution patterns.

OTHER PHYSICOCHEMICAL PARAMETERS

There is a series of other constants that measure the contribution by substituents to the molecule's total physicochemical properties. These include Hammett's σ constant; Taft's steric parameter, E_s; Charton's steric parameter, ν; Verloop's multidimensional steric parameters, L, B_1, B_5; and molar refractivity, *MR*. The latter has become the second most useful physicochemical parameter used in classical QSAR modeling. It is a complex term based on the molecule's refractive index, molecular weight, and density, and can be considered a measure of the molecule's bulk and electronic character. One reason for its popularity is that it is easy to calculate from tables of atoms using a minimum of correction factors. Of the listed physicochemical parameters group, it is most easy to locate values for π, σ, E_s, and *MR*. A representative list can be found in Table 2-7.

Table 2-7 is illustrative of several items that must be kept in mind when selecting substituents to be evaluated in terms of the type of factors that influence a biological response. For electronic parameters such as σ, the location on an aromatic ring is important due to resonance versus inductive effects. Notice the twofold differences seen between σ_{meta} and σ_{para} for the three aliphatic substituents and iodo, and severalfold difference for methoxy, amino, fluoro, and phenolic hydroxyl.

Selection of substituents from a certain chemical class may not really test the influence of a parameter on biological activity. There is little numerical difference among the σ_{meta} or σ_{para} values for the four aliphatic groups or the four halogens. It is not uncommon to go to the tables and find missing parameters such as the E_s values for acetyl and *N*-acyl.

Nevertheless, medicinal chemists can use information from extensive tables of physicochemical parameters in order to minimize the number of substituents required to find out if the biological response is sensitive to electronic, steric, and/or partitioning effects.[2] This is done by selecting substituents in each of the numerical ranges for the different parameters. In Table 2-7, there are three ranges of π values, (-1.23 to -0.55), (-0.28 to 0.56), and (0.71 to 1.55); three ranges of *MR* values, (0.92 to 2.85), (5.02 to 8.88), and (10.30 to 14.96); and two main clusters of σ values, one for the aliphatic substituents and the other for the halogens. In the ideal situation, substituents will be selected from each of the clusters in order to determine the dependence of the biological response over the largest possible variable space. Depending on the biological responses obtained from testing the new compounds, it is possible to determine if lipophilicity (partitioning), steric bulk (molar refraction), or electron withdrawing/donating properties are important determinants of the desired biological response.

TABLE 2-7

SAMPLING OF PHYSICOCHEMICAL PARAMETERS USED IN QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIPS INVESTIGATIONS

Substituent Group	π	σ_{meta}	σ_{para}	E_s	*MR*
—H	0.00	0.00	0.00	0.00	1.03
—CH_3	0.56	−0.07	−0.17	−1.24	5.65
—CH_2CH_3	1.02	−0.07	−0.15	−1.31	10.30
—$CH_2CH_2CH_3$	1.55	−0.07	−0.13	−1.60	14.96
—$C(CH_3)_2$	1.53	−0.07	−0.15	−1.71	14.96
—OCH_3	−0.02	0.12	−0.27	−0.55	7.87
—NH_2	−1.23	−0.16	−0.66	−0.61	5.42
—F	0.14	0.34	0.06	−0.46	0.92
—Cl	0.71	0.37	0.23	−0.97	6.03
—Br	0.86	0.39	0.23	−1.16	8.88
—I	1.12	0.35	0.18	−1.40	13.94
—CF_3	0.88	0.43	0.54	−2.40	5.02
—OH	−0.67	0.12	−0.37	−0.55	2.85
—$COCH_3$	−0.55	0.38	0.50		11.18
—$NHCOCH_3$	−0.97	0.21	0.00		14.93
—NO_2	−0.8	0.71	0.78	−2.52	7.36
—CN	−0.57	0.56	0.66	−0.51	6.33

From Hansch, C., Leo, A. J.: Substituent Constants for Correlation Analysis in Chemistry and Biology. New York, John Wiley & Sons, 1979.[2]

QSAR MODELS

Currently, there are three models or equations seen in QSAR analysis using physicochemical parameters, represented by Eqs. 2-20, 2-21, and 2-22. These three equations are illustrated (Fig. 2-6) using the logarithm of the partition coefficient (log P) as the physical chemical parameter. First there is the linear model (Eq. 2-20). Because plots indicated a nonlinear relationship between biological response and the partition coefficient, a parabolic model was developed (Eq. 2-21). Examination of Fig. 2-6 shows an optimum log *P* (log P_o), where maximum biological activity will be obtained before a decrease in activity is seen. One explanation for this phenomenon is that hydrophilic drugs will tend to stay in the aqueous phase whereas lipophilic chemicals will prefer the lipid bilayer. In both cases, less drug is being transported to the receptor resulting in the actual concentration of receptor bound drug being decreased. In other words, the equilibrium seen in Rx. 2-1 shifts to the left. There will be a group of drugs whose log P will place them near the top of the parabola such that their lipophilic-hydrophilic balance

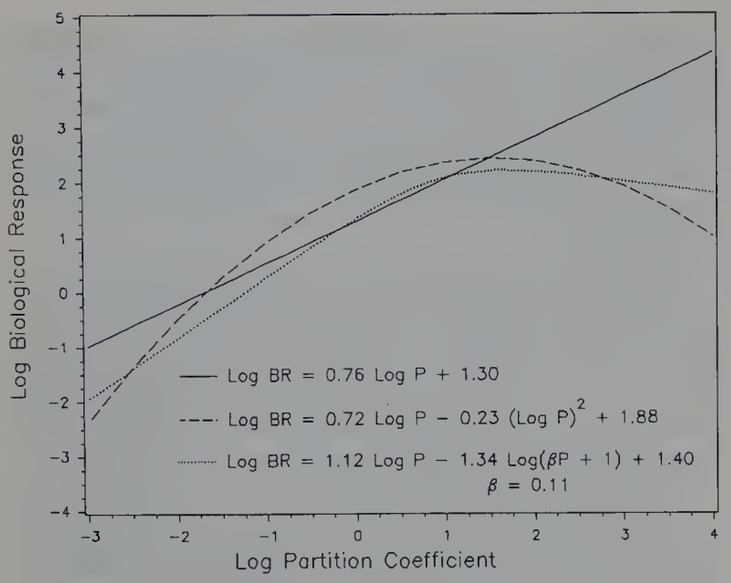


FIG. 2-6. *Log biologic response versus log partition coefficient using linear, parabolic, and bilinear models.*

will permit them to penetrate both aqueous and lipid barriers and reach the receptor.

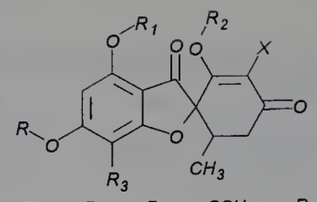
$$\text{Log } 1/C = a\,(\log P) + c \qquad \text{(Eq. 2-20)}$$

$$\text{Log } 1/C = a\,(\log P) - b\,(\log P)^2 + c \qquad \text{(Eq. 2-21)}$$

$$\text{Log } 1/C = a\,(\log P) - b\log(\beta P + 1) + c \qquad \text{(Eq. 2-22)}$$

The third QSAR equation in current use is the bilinear model (Eq. 2-22). It consists of two straight lines: one ascending and one descending. The β-term connects the two lines. There are several interpretations for the β term. One explanation is based on the ratio of the rate constant for diffusion out of the octanol layer into the aqueous environment being different from the rate of diffusion out of the aqueous layer into the octanol. In other words, what may be simulated with the bilinear model is recognition that the rate of diffusion from the extracellular fluids into the lipid bilayer is different from the rate of diffusion out of the lipid bilayer into the intracellular environment. Another interpretation is recognition that the kinetics of partitioning through the lipid bilayer will be different from the kinetics of binding to the receptor. A third explanation takes into account the different volumes of the aqueous and lipid bilayers in the biological system.

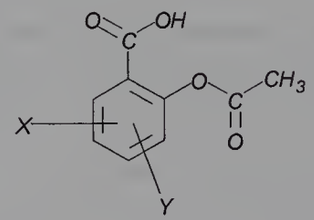
With this background in mind, three examples of QSAR equations taken from the medicinal chemistry literature will be presented. One will show a linear relationship (Eq. 2-23) and the others a parabolic (Eq. 2-24) and a bilinear (Eq. 2-25) correlation. A study of a group of griseofulvin analogues showed that there is a linear relationship (Eq. 2-23) between the biological response and both lipophilicity (log P) and electronic character (σ).[3] It was suggested that the antibiotic activity may depend on the enone system facilitating the addition of griseofulvin to a nucleophilic group such as the SH moiety in a fungal enzyme.



Griseofulvin: $R = R_1 = R_2 = R_3 = OCH_3$; $R_3 = Cl$; $X = H$

$$\text{Log } BR = (0.56)\log P + (2.19)\sigma_x - 1.32 \qquad \text{(Eq. 2-23)}$$

A parabolic relationship (Eq. 2-24) was reported for a series of substituted acetylated salicylates (substituted aspirins) tested for anti-inflammatory activity.[4] There is a nonlinear relationship between the biological response and lipophilicity and a significant detrimental steric effect seen with substituents at position 4. The two sterimol parameters used in this equation were L, defined as the length of the substituent along the axis of the bond between the first atom of the substituent and the parent molecule and B_2, defined as a width parameter. Steric effects were not considered significant at position 3 because the sterimol parameters for substituents at position 3 are not part of Eq. 2-24. The optimal partition coefficient (Log P_o) for the substituted aspirins in this assay was 2.6.



Aspirin: $X = Y = H$

$$\text{Log } 1/\text{ED}_{50} = 1.03 \log P - 0.20(\log P)^2 - 0.05\,L_{(4)} - 0.24\,B_{2(4)} + 2.29 \qquad \text{(Eq. 2-24)}$$

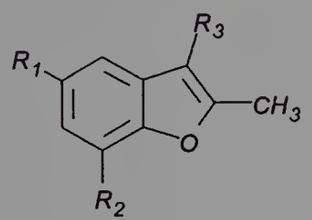
Related to these mathematical QSAR models based on biological responses is QSAR used to analyze pharmacokinetic activity. One example of this (Eq. 2-25) is a simulation of barbiturate absorption, which leads to the bilinear model.[5,6]

$$\text{Log } k_{\text{DIFF}} = 0.949 \log P - 1.238 \log(\beta P + 1) - 3.131 \qquad \text{(Eq. 2-25)}$$

where log $\beta = -1.271$; log $P_o = 1.79$; and k_{DIFF} = diffusion rate constant. At this point, it is appropriate to ask the question: Are all the determinations of partition coefficients and compilation of physical chemical parameters useful only where a statistically valid QSAR model is obtained? The answer is a firm "no." One of the most useful spinoffs from the field of QSAR has been the application of experimental design to the selection of new compounds to be synthesized and tested. Let's assume that a new series of drug molecules is to be synthesized based on the following structure. The goal is to test the effect of the 16 substituents in Table 2-7

at each of the three positions on our new series. The number of possible analogues is equal to 16^3, or 4,096, compounds, assuming that all three positions will always be substituted with one of the substituents from Table 2-7. If hydrogen is included when a position is not substituted, there are 17^3, or 4,913, different combinations. The problem is to select a small number of substituents that will represent the different ranges or clusters of values for lipophilicity, electronic influence, and bulk. An initial design set could include the methyl and propyl from the aliphatic, fluorine and chlorine from the halogens, N-acetyl and phenol from the substituents showing hydrophilicity, and a range of electronic and bulk values. Including hydrogen, there will be 7^3, or 343, different combinations. Obviously, that is too many for an initial evaluation. Instead, certain rules have been devised to maximize the information obtained from a minimum number of compounds. These include the following:

1. Each substituent must occur more than once at each position on which it is found.
2. The number of times that each substituent at a particular position appears should be approximately equal.
3. No two substituents should be present in a constant combination.
4. When combinations of substituents are a necessity, they should not occur more frequently than any other combination.



Following these guidelines, the initial test set can be reduced to 24 to 26 compounds. Depending on the precision of the biological tests, it will be possible to see if the data will fit a QSAR model. Even an approximate model usually will indicate the types of substituents to test further and what positions on the molecules are sensitive to substitution and, if sensitive, to what degree variation in lipophilic, electronic, or bulk character are important. Just to be assured that the model is valid, it is a good idea to synthesize a couple of compounds that the model predicts would be inactive. As each group of new compounds is tested, the QSAR model is refined until the investigators have a pretty good idea as to what substituent patterns are important for desired activity. It must be emphasized that these same techniques used to develop potent compounds with desired activity also can be used to evaluate the influence of substituent patterns on undesired toxic effects and pharmacokinetic properties.

In their *pure form*, the rules listed above can be used to select a minimum number of compounds for a test set using what are known as identity variables. No physicochemical parameters are required. In its simplest form, the equation takes the form outlined in Eq. 2-26. This approach has been known as a Free-Wilson analysis.[7]

$$\text{Log } BR = \sum (\text{substituent contributions}) + \text{contribution from the base molecule} \quad \text{(Eq. 2-26)}$$

An example is a small set of phosphorous containing acetylcholinesterase inhibitors that were selected using the rules for designing a test set and evaluated as possible insecticides.[8] The result is a complex equation that produces a coefficient for each substituent. They are summarized in Table 2-8.

Examination of this table shows that ethyl and ethoxy at R_1, ethoxy and isopropoxy at R_2, and oxo at R_4 have minimal influence on biological activity. In contrast, methyl and isopropoxy at R_1, methoxy, propoxy, and butoxy at R_2, all three nitrophenoxy substituents at R_3, and thio at R_4 significantly influence the biological response. The predicted Log 1/*BR* for the compound, where R_1 = methyl, R_2 = propoxy, R_3 = 4-nitrophenoxy, and R_4 = thio, would be calculated from Eq. 2-26:

$$\begin{aligned}\text{Log } BR &= R_1 + R_2 + R_3 + R_4 + \text{Base Molecule} \\ &= 0.729 + 0.543 + 0.611 - 1.673 + 5.143 \\ &= 5.353\end{aligned}$$

One of the newer QSAR methods combines statistical techniques with molecular modeling and has been referred to as three-dimensional QSAR (3D-QSAR). The independent variables include spatial distances among and between pharmacophores and physicochemical parameters located at specific distances from the molecule. Because 3D-QSAR is dependent on the molecular modeling algorithms, it will be discussed later in the molecular modeling section of this chapter.

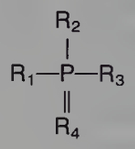
CLASSIFICATION METHODS

There are other statistical techniques besides regression analysis that are used in drug design. These fit under the classification of multivariate statistics and include discriminant analysis, principal component analysis, and pattern recognition. The latter can consist of a mixture of statistical and nonstatistical methodologies. The goal usually is to try to ascertain those physicochemical parameters and structural attributes that contribute to a class or type of biological activity. Then the chemicals are classified into groupings such as carcinogenic/noncarcinogenic, sweet/bitter, active/inactive, and depressant/stimulant.

The term "multivariate" is used because of the wide variety and number of independent or descriptor variables that may be used. The same physicochemical parameters seen in QSAR analyses are used, but, in addition, the software in

TABLE 2-8

COEFFICIENTS FOR SUBSTITUENTS IN A SET OF ACETYLCHOLINESTERASE INHIBITORS (8)



R_1 = CH_3, C_2H_5, OCH_3, OC_2H_5, OC_3H_7, $OC(CH_3)_2$
R_2 = OCH_3, OC_2H_5, OC_3H_7, $OC(CH_3)_2$, OC_4H_9
R_3 = —$O(C_6H_4)(2—NO_2)$, —$O(C_6H_4)(3—NO_2)$, —$O(C_6H_4)(4—NO_2)$
R_4 = O, S

R_1		R_2		R_3		R_4	
Methyl	0.729	Methoxy	−0.598	2-Nitrophenoxy	0.856	Oxo	0.052
Ethyl	−0.167	Ethoxy	0.186	3-Nitrophenoxy	−1.134	Thio	−1.673
Ethoxy	−0.168	Propoxy	0.543	4-Nitrophenoxy	0.611		
Propoxy	−0.405	Isopropoxy	−0.164				
Isopropoxy	−1.267	Butoxy	0.786				

Contribution from the base molecule = 5.143.

the computer programs "breaks" the molecule down into substructures. These structural fragments also become variables. Examples of the typical substructures used include carbonyls, enones, conjugation, rings of different sizes and types, *N*-substitution patterns, and aliphatic substitution patterns such as 1,3- or 1,2-disubstituted. The end result is that for even a moderate size molecule typical of most drugs, there can be 50 to 100 variables.

The technique is to develop a large set of chemicals well characterized in terms of the biological activity that is going to be predicted. This is known as the training set. Ideally, it should contain hundreds, if not thousands, of compounds, divided into active and inactive types. In reality, sets smaller than 100 are studied. Most of these investigations are retrospective ones in which the investigator locates large data sets from several sources. This means that the biological testing likely followed different protocols. That is why classification techniques tend to avoid using continuous variables such as ED_{50}, LD_{50}, and MIC. Instead, arbitrary endpoints such as active or inactive, stimulant or depressant, sweet or sour, etc. are used.

Once the training set is established, the multivariate technique is carried out. The algorithms are designed to group the underlying commonalities and select those variables having the greatest influence on biological activity. The predictive ability is then tested with a test set of compounds that have been put through the same biological tests used for the training set. For the classification model to be valid, it is important for the investigator to select data sets whose results are not intuitively obvious and could not be classified by a trained medicinal chemist. Properly done, classification methods can identify structural and physicochemical descriptors that can be powerful predictors and determinants of biological activity.

There are several examples of successful applications of this technique.[9] One study consisted of a diverse group of 140 tranquilizers and 79 sedatives subjected to a two-way classification study (tranquilizers versus sedatives). The ring types included phenothiazines, indoles, benzodiazepines, barbiturates, diphenylmethanes, and a variety of heterocycles. Sixty-nine descriptors were used initially to characterize the molecules. Eleven of these descriptors were crucial to the classification, 54 had intermediate use and were dependent on the composition of the training set, and four descriptors were of little use. The overall range of prediction accuracy was 88% to 92%. The results with the 54 descriptors indicate an important limitation when large numbers of descriptors are used. The inclusion or exclusion of descriptors and parameters can be dependent on the composition of the training set. It is important that the training set be representative of the population of chemicals that are going to be evaluated. Indeed, repeating the study on different randomly selected training sets is important.

Classification techniques lend themselves to studies lacking quantitative data. An interesting classification problem involved olfactory stimulants in which the goal was to select chemicals that had a musk odor. A group of 300 unique compounds was selected from a group of odorants that included 60 musk odorants plus 49 camphor, 44 floral, 32 ethereal, 41 mint, 51 pungent, and 23 putrid odorants. Initially, 68 descriptors were evaluated. Depending on the approach, the number of descriptors was reduced to 11 to 16, consisting mostly of bond types. Using this small number, the 60 musk odorants could be selected from the remaining 240 compounds, with an accuracy of 95% to 97%.

The use of classification techniques in medicinal chemistry has matured over years of general use. The types of descriptors have expanded to spatial measurements in three-dimensional space similar to those used in 3D-QSAR (see below). Increasingly, databases of existing compounds are scanned looking for molecules that possess what appear to be the desired parameters. If successful, compounds that are

predicted to be active provide the starting point for synthesizing new compounds for testing. One can see parallels between the search of chemical databases and screening plant, animal, and microbial sources for new compounds. Although the statistical and pattern recognition methodologies have been in use for a very long time, there still needs to be considerable research into their proper use, and further testing of their predictive power is needed. The goal of scanning databases of already synthesized compounds in order to select compounds for pharmacological evaluation will require considerable additional development in the various multivariate techniques.

COMBINATORIAL CHEMISTRY

Elegant as the statistical techniques described above are, the goal remains to synthesize large numbers of compounds in order not to miss promising marketable products. At the same time, traditional synthetic and biological testing are very costly. This has led to the technique called ''combinatorial chemistry.'' The latter employs libraries of chemical moieties that react with a parent or base molecule in a small number of defined synthetic steps. Return to the two examples presented with the discussion of the Free-Wilson analysis (see above). As the number of different substituents is considered, literally >10,000 compounds are possible. Remember that the medicinal chemist can select subsets of substituents that vary in lipophilicity, steric bulk, induction, and resonance effects, and use the four rules for the placing and utilization of the substituents. Properly done, a relatively small number of compounds will be obtained that will show the dual importance of each of the physicochemical parameters being evaluated at each position on the molecule and the effect of specific moieties at each position. This ''rational'' approach to drug design assumes that there is some understanding of the target receptor and that there is a lead molecule, commonly called the ''prototype molecule.'' A classic example is the dihydrofolate reductase inhibitor, methotrexate, which has been one of the prototypes that laboratories have used to synthesize and test new inhibitors. Another example is the benzodiazepine structure. It has a defined structure whose activity varies with the substituents.

What about the situation where little is known about the mechanisms causing the disease process? Until recently, this has been the normal situation when searching for new molecules with the desired pharmacological response. With the discovery of penicillin came the realization that microbial organisms produced ''antibiotics.'' This started screenings of microbial products looking for new antibiotics. In a similar manner, thousands of synthetic compounds and plant extracts have been screened for anticancer activity. Some have called this ''irrational'' drug design, but it has produced most of the drugs currently prescribed. This approach also is very expensive, particularly when one realizes the cost to synthesize, isolate, and test each new compound plus the time and expense it takes to take an active compound through the efficacy and safety testing before its release to the general public is approved by government regulatory agencies.

Combinatorial chemistry is one method of reducing the cost of drug discovery in which the goal is to find new leads or prototype compounds or optimizing and refining the structure activity relationships.[10,11] Libraries of ''reactive'' chemical moieties provide the chemical diversity of products that will be screened for activity. The chemistry is elegant, but relatively simple in that the same few reactions are required to make thousands of compounds in a particular series. The reactions must be clean and reproducible and have high yields. Often, solid-state synthetic methods are used in which compounds are ''grown'' onto polymer support. Robotics can be used to reduce further the cost of synthesis. Biological testing can also be automated in a process called ''high through put screening,'' which can test tens to hundreds of structures at a time. Many times it is possible to take advantage of gene cloning techniques, clone the desired receptor, and measure the binding of the newly synthesized compounds to the cloned receptor.

In order to maintain some focus on the needed structures, information theory has been used to construct the libraries of substituents. These libraries tend to maximize chemical diversity in terms of physicochemical parameters. Many of these libraries are sold commercially by firms specializing in this technique. The synthetic methodologies cover the spectrum of producing thousands of relatively pure compounds to mixtures of compounds that are tested as mixtures. Of course, mixtures can be difficult to classify and it can be difficult to determine which products in the mixture are active and which ones are inactive. Elegant methods have been developed that chemically ''tag'' each compound with a small peptide, nucleotide, or other small molecule that is pharmacologically inert. When mixtures of products are obtained, they are screened for activity. Only those mixtures that are biologically active are retained. In a process called ''deconvolution,'' the synthesis is repeated in an iterative manner producing smaller and sometimes overlapping mixtures. The screening is repeated until the active compounds are identified. Examine Table 2-9. This simplified outline shows how four steps will identify the three active components in a 20-compound investigation. (Keep in mind that the actual combinatorial process will produce hundreds or thousands of compounds for testing.)

Assume that the project calls for synthesizing 20 compounds A to T. Rather than carry out 20 distinct syntheses followed by 20 separate screening experiments, all of which can take weeks, four combinatorial syntheses are carried out such that four mixtures containing five compounds each are obtained. Only the three mixtures that test positive in the screening assay are retained. The synthesis is repeated producing five mixtures of three components each and the testing is repeated. Six more syntheses are carried out this time producing overlapping two-component mixtures, and the assays are repeated. It is now possible to determine that com-

TABLE 2-9

SIMPLIFIED DECONVOLUTION SCHEME FOR A 20-COMPOUND COMBINATORIAL CHEMISTRY SCREEN

A	B*	C	D	E	F	G	H*	I	J	K	L	M	N*	O	P	Q	R	S	T
Carry out the synthesis producing four five-component mixtures. Screen the mixtures.																			
AB*CDE					FGH*IJ					KLM*NO					PQRST				
Retain only the three mixtures containing active components. Repeat the synthesis producing three-component mixtures and repeat the screening.																			
AB*C				DEF				GH*I				JKL				MN*O			
Discard the inactive mixtures. Repeat the synthesis producing overlapping two-component products and repeat the screening.																			
AB*			B*C				GH*				H*I			MN*				N*O	
Only compounds B, H, and N need to be chemically characterized.																			

* Active compound.

pounds B, H, and N are active. Instead of 20 syntheses and 20 assays, only 15 were required. Further, time-consuming purification of each mixture was not required. This process is very similar to that carried out by natural-product chemists. The microbial, plant, or animal tissue is extracted with a variety of solvents, beginning with nonpolar hydrocarbons and ending with an alcohol or water, and the fractions are screened for activity. Only the active fractions are retained. The latter are more carefully fractionated using biological assays to follow the purifications. In either combinatorial synthesis or natural product isolation, once active compounds are identified, larger-scale, more-focused syntheses can be done using QSAR-derived experimental design and/or molecular modeling (see below) to yield compounds different from those produced from the combinatorial library of chemical fragments.

Other methods that are used commonly in combinatorial chemistry include attaching structures of known composition to polystyrene beads (one compound per bead) or synthesizing structures onto a microchip-sized matrix where a compound's location gives its identity. The latter is called "spatially addressable synthesis."

MOLECULAR MODELING (COMPUTER-AIDED DRUG DESIGN)

The low cost of powerful desktop computers gives the medicinal chemist the ability to "design" the molecule based on an estimated fit onto a receptor or have similar spatial characteristics found in the prototypical lead compound. Of course, this assumes that the molecular structure of the receptor is known in enough detail that a reasonable estimation of its three-dimensional shape can be made. Where a good understanding of the geometry of the active site is known, databases containing the three-dimensional coordinates of the chemicals in the database can be searched rapidly by computer programs that select candidates likely to fit in the active site. As will be seen, there have been some dramatic successes using this approach. Before proceeding further, it is important to have an understanding of ligand (drug)-receptor interactions and conformational analysis.

DRUG-RECEPTOR INTERACTIONS

At this point, let us assume that the drug has entered systemic circulation (Fig. 2-1), passed through the lipid barriers, and is now going to make contact with the receptor. As was illustrated in Rx. 2-1, this is an equilibrium process. A good ability to fit the receptor will favor binding and the desired pharmacological response. In contrast, a poor fit will favor the reverse reaction. With only a small amount of drug bound to the receptor, there will be a much smaller pharmacological effect. Indeed, if the amount of drug bound to the receptor is too small, there may be no discernable response observed. There are many variables that contribute to a drug's binding to the receptor. These include the structural class, the three-dimensional shape of the molecule, and types of chemical bonding involved in the binding of the drug to the receptor.

Most drugs that belong to the same pharmacological class have certain structural features in common. The barbiturates act on specific CNS receptors, causing depressant effects; hydantoins act on CNS receptors, producing an anticonvulsant response; benzodiazepines combine with the GABA receptors, with resulting anxiolytic activity; steroids can be divided into such classes as corticosteroids, anabolic steroids, progestogens, and estrogens, each acting on specific receptors; nonsteroidal anti-inflammatory agents inhibit enzymes required for the prostaglandin cascade; penicillins and cephalosporins inhibit enzymes required to construct the bacterial cell wall; and tetracyclines act on bacterial ribosomes.

RECEPTOR

With the isolation and characterization of receptors becoming a common occurrence, it is hard to realize that the concept of receptors began as a postulation. It early had been realized that molecules with certain structural features would elucidate a specific biological response. Very slight changes in structure could cause significant changes in biological activity. These structural variations could increase or decrease activity or change an agonist into an antagonist. This early and fundamentally correct interpretation called for the drug (ligand) to fit onto some surface (the receptor) that had fairly strict structural requirements if proper binding of the drug was to occur. The initial receptor model was based on a rigid lock-and-key concept with the drug (key) fitting into a receptor (lock). It has been used to explain why certain structural attributes produce a predictable pharmacological action. This model still is useful, although it is important to realize that both the drug and the receptor can have considerable flexibility. Molecular graphics, using programs that calculate the preferred conformations of drug and receptor, show that the receptor can undergo an adjustment in three-dimensional structure when the drug makes contact. Using current space-age language, the drug docks with the receptor.

More complex receptors now are being isolated, characterized, and cloned. The first receptors to be isolated and characterized were the reactive and regulatory sites on enzymes. Acetylcholinesterase, dihydrofolate reductase, angiotensin, and HIV protease converting enzyme are examples of enzymes whose active sites (the receptors) have been modeled. Most drug receptors probably are receptors for natural ligands used to regulate cellular biochemistry and function and communicate between cells. Receptors include a relatively small region of a macromolecule, which may be an isolatable enzyme, a structural and functional component of a cell membrane, or a specific intracellular substance, such as a protein or a nucleic acid. Specific regions of these macromolecules are visualized as being oriented in space in a manner that permits their functional groups to interact with the complementary functional groups of the drug. This interaction initiates changes in structure and function of the macromolecule, which lead ultimately to the observable biological response. The concept of spatially oriented functional areas forming a receptor leads directly to specific structural requirements for functional groups of a drug, which must be complementary to the receptor.

It now is possible to isolate membrane-bound receptors, although it still is difficult to elucidate their structural chemistry, because, once separated from the cell membranes, these receptors may lose their native shape. This is because the membrane is required to hold the receptor in its correct shape. One method of receptor isolation is affinity chromatography. In this technique, a ligand, many times an altered drug molecule known to combine with the receptor, is attached to a chromatographic support phase. A solution containing the desired receptor is passed over this column. The receptor will combine with the ligand. It is common to add a chemically reactive grouping to the drug, resulting in the receptor and drug covalently binding with each other. The drug-receptor complex is washed from the column, where it is then characterized further.

A more recent technique uses recombinant DNA. The gene for the receptor is located and cloned. It is transferred into a bacterium, yeast, or animal, which then produces the receptor in large enough quantities to permit further study. Further, it sometimes is possible to determine the DNA sequence of the cloned gene. Using the genetic code for amino acids, the amino acid sequence of the protein component of the receptor can be determined and the receptor then modeled, producing an estimated three-dimensional shape. The model for the receptor becomes the template for designing new ligands.

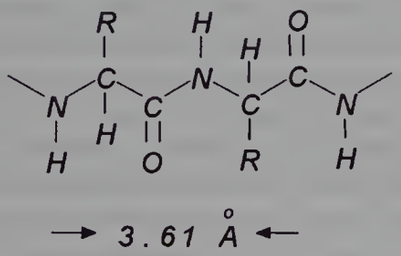
The preceding discussion in this chapter emphasized that the cell membrane is a highly organized, dynamic structure which interacts with small molecules in specific ways. The previous focus was on the lipid bilayer component of this complex structure. The receptor components of the membranes appear to be mainly protein in nature. They constitute a highly organized region of the cell membrane. The same type of molecular specificity seen in such proteins as enzymes and antibodies is also a property of the drug receptor. The nature of the amide link in proteins provides a unique opportunity for the formation of multiple internal hydrogen bonds, as well as internal formation of hydrophobic, van der Waals', and ionic bonds by side chain groups, leading to such organized structures as the α-helix, which contains about four amino acid residues for each turn of the helix. An organized protein structure would hold the amino acid side chains at relatively fixed positions in space and available for specific interactions with a small molecule.

Proteins have the potential to adopt many different conformations in space without breaking their covalent amide linkages. They may shift from highly coiled structures to partially disorganized structures, with parts of the molecule existing in random chain, or to folded sheet structures, contingent on the environment. In the monolayer of a cell membrane, the interaction of a small foreign molecule with an organized protein may lead to a significant change in the structural and physical properties of the membrane. Such changes could well be the initiating events in the tissue or organ response to a drug, such as the ion-translocating effects produced by interaction of acetylcholine and the cholinergic receptor.

The large body of information now available on relationships between chemical structure and biologic activity strongly supports the concept of flexible receptors. The fit of drugs onto or into macromolecules is only rarely an all-or-none process as pictured by the earlier lock-and-key concept of a receptor. Rather, the binding or partial insertion of groups of moderate size onto or into a macromolecular pouch appears to be a continuous process, at least over a limited range, as indicated by the frequently occurring regular in-

crease and decrease in biologic activity as one ascends a homologous series of drugs. A range of productive associations between drug and receptor may be pictured, which leads to agonist responses, such as those produced by cholinergic drugs. Similarly, strong associations may lead to unproductive changes in the configuration of the macromolecule, leading to an antagonistic or blocking response, such as that produced by anticholinergic agents. The fundamental structural unit of the drug receptor is generally considered to be protein, although this may be supplemented by its associations with other units, such as mucopolysaccharides and nucleic acids.

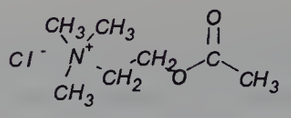
In the maximally extended protein, the distance between peptide bonds ("identity distance") is 3.61 Å. For many types of biologic activity, the distance between functional groups leading to maximal activity approximates this identity distance or some whole-number multiple of it. Many parasympathomimetic (acetylcholine-like) and parasympatholytic (cholinergic-blocking) agents have a separation of 7.2 Å (2 × 3.6 Å) between the ester carbonyl group and nitrogen.[12] This distance is doubled between quaternary nitrogens of curare-like drugs; 14.5 Å (4 × 3.61 Å).[13] The preferred separation of hydrogen-bonding groups in estrogenic compounds (e.g., hydroxyls of diethylstilbestrol) is 14.5 Å (4 × 3.61 Å).[14]



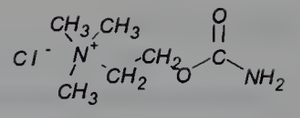
Identity Distance in Extended Protein

A related spacing of 5.5 Å, which corresponds to two turns of the α-helical structure common to proteins, is found between functional groups of many drugs. The most frequently occurring of these is the R-X-CH_2-CH_2-NR'_2 (X = N; X = O, or X = C) structure that is present in local anesthetics, antihistamines, adrenergic-blocking agents, and others.[15]

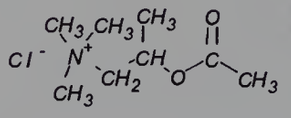
Studies involving the relative effectiveness of various molecules of well-defined structural and functional types have contributed to an understanding of the stereochemical and physicochemical properties of their biologic receptors. Pfeiffer concluded that parasympathomimetic stimulant action depends on two adjacent oxygen atoms at distances of approximately 5.0 Å and 7.0 Å from a methyl group or groups attached to nitrogen.[12] Because these compounds (acetylcholine, methacholine, carbachol, and bethanechol) do not have rigid structures, the actual distance between the oxygen and the methyl groups varies; however, the more extended conformations would be favored in solution.



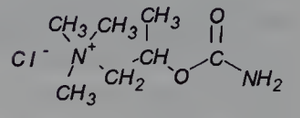
Acetylcholine Chloride



Carbachol Chloride



Methacholine Chloride



Bethanechol Chloride

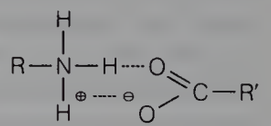
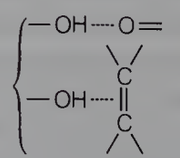
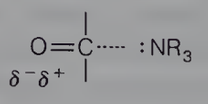
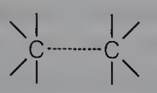
DRUG-RECEPTOR INTERACTION: FORCES INVOLVED

A biologic response is produced by the interaction of a drug with a functional or organized group of molecules, which may be called the biologic receptor site. This interaction would be expected to take place by utilizing the same bonding forces involved as those when simple molecules interact. These, together with typical examples, are collected in Table 2-10.

Most drugs do not possess functional groups of a type that

TABLE 2-10

TYPES OF CHEMICAL BONDS

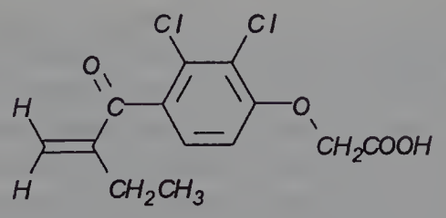
Bond Type	*Bond Strength (kcal/mol)*	*Example*
Covalent	40–140	$CH_3—OH$
Reinforced ionic	10	
Ionic	5	$R_4N^{\oplus}\cdots\cdots{}^{\ominus}I$
Hydrogen	1–7	
Ion-dipole	1–7	$R_4N^{\oplus}\cdots\cdots :NR_3$
Dipole-dipole	1–7	
van der Waals'	0.5–1	
Hydrophobic	1	See Text

Adapted from a table in Albert, A. Selective Toxicity. New York, John Wiley & Sons, 1986, p. 183.

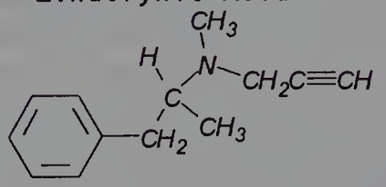
would lead to ready formation of the strong and essentially irreversible covalent bonds between drug and biologic receptors. In most cases, it is desirable that the drug leave the receptor site when the concentration decreases in the extracellular fluids. Therefore, most useful drugs are held to their receptors by ionic or weaker bonds. When relatively long-lasting or irreversible effects are desired (e.g., antibacterial, anticancer), drugs that form covalent bonds with the receptor are effective and useful.

The alkylating agents, such as the nitrogen mustards (e.g., mechlorethamine) used in cancer chemotherapy, furnish an example of drugs that act by formation of covalent bonds (Fig. 2-7). These are believed to form the reactive immonium ion intermediates, which alkylate and thereby link together proteins or nucleic acids, preventing their normal participation in cell division.

Covalent bond formation between drug and receptor is the basis of Baker's concept of *active-site-directed irreversible inhibition.*[16] Considerable experimental evidence on the nature of enzyme inhibitors has supported this concept. Compounds studied possess appropriate structural features for reversible and highly selective association with an enzyme. If, in addition, the compounds carry reactive groups capable of forming covalent bonds, the substrate may be irreversibly bound to the drug-receptor complex by covalent bond formation with reactive groups adjacent to the active site. The diuretic drug, ethacrynic acid (see Chap. 18) is an α,β-unsaturated ketone, thought to act by covalent bond formation with sulfhydryl groups of ion transport systems in the renal tubules. Another example of a drug which covalently binds to the receptor is selegiline (see Chap. 15), an inhibitor of monoamine oxidase-B. Other examples of covalent bond formation between drug and biologic receptor site include the reaction of arsenicals and mercurials with cysteine sulfhydryl groups, the acylation of bacterial cell wall constituents by penicillin, and the phosphorylation of the serine hydroxyl moiety at the active site of cholinesterase by organic phosphates.



Ethacrynic Acid



Selegiline

Keep in mind that it is desirable that most drug effects be reversible. For this to occur, relatively weak forces must be involved in the drug-receptor complex, yet be strong enough that other binding sites will not competitively deplete the site of action. Compounds with a high degree of structural specificity may orient several weak-binding groups, such that the summation of their interactions with specifically oriented complementary groups on the receptor will provide a total bond strength sufficient for a stable combination. Consequently, for drugs acting by virtue of their structural specificity, binding to the receptor site will be carried out by hydrogen bonds, ionic bonds, ion-dipole and dipole-dipole interactions, and van der Waals' and hydrophobic forces.

Considering the wide variety of functional groups found on a drug molecule and receptor, there will be a variety of secondary bonding forces. Ionization at physiologic pH would normally occur with the carboxyl, sulfonamido, and aliphatic amino groups, as well as the quaternary ammonium group at any pH. These sources of potential ionic bonds are frequently found in active drugs. Differences in electronegativity between carbon and other atoms, such as oxygen and nitrogen, lead to an unsymmetric distribution of electrons (dipoles) that are also capable of forming weak bonds with

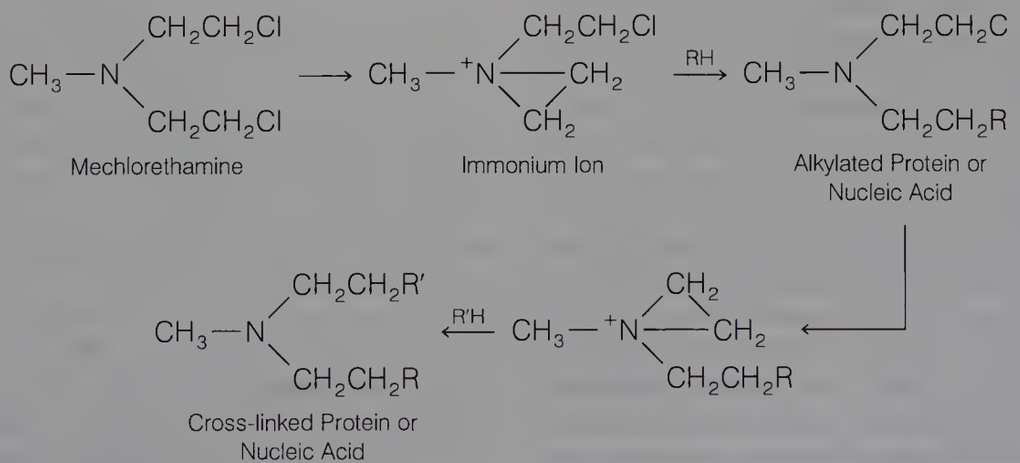


FIG. 2-7. *Formation of the immonium cation and its alkylation of protein or nucleic acid: R,R' = free amino groups of proteins, adenyl, or phosphate groups of nucleic acids.*

regions of high or low electron density, such as ions or other dipoles. Carbonyl, ester, amide, ether, nitrile, and related groups that contain such dipolar functions are frequently found in equivalent locations in structurally specific drugs.

The relative importance of the *hydrogen bond* in the formation of a drug-receptor complex is difficult to assess. Many drugs possess groups, such as carbonyl, hydroxyl, amino, and imino, with the structural capabilities of acting as acceptors or donors in the formation of hydrogen bonds. However, such groups would usually be solvated by water, as would the corresponding groups on a biologic receptor. Relatively little net change in free energy would be expected in exchanging a hydrogen bond with a water molecule for one between drug and receptor. However, in a drug-receptor combination, several forces could be involved, including the hydrogen bond, which would contribute to the stability of the interaction. Where multiple hydrogen bonds may be formed, the total effect may be sizeable, such as that demonstrated by the stability of the protein α-helix, and by the stabilizing influence of hydrogen bonds between specific base pairs in the double helical structure of DNA.

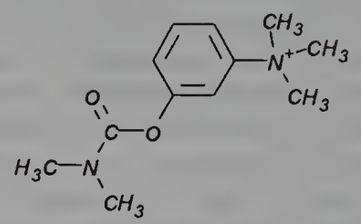
Van der Waals' forces are attractive forces created by the polarizability of molecules and are exerted when any two uncharged atoms approach each other very closely. Their strength is inversely proportional to the seventh power of the distance. Although individually weak, the summation of their forces provides a significant bonding factor in higher-molecular-weight compounds. For example, it is not possible to distill normal alkanes with >80 carbon atoms, because the energy of ~80 kcal/mol required to separate the molecules is approximately equal to the energy required to break a carbon-carbon covalent bond. Flat structures, such as aromatic rings, permit close approach of atoms. With van der Waals' forces of ~0.5 to 1.0 kcal/mol for each atom, about six carbons (a benzene ring) would be necessary to match the strength of a hydrogen bond. The aromatic ring is frequently found in active drugs, and a reasonable explanation for its requirement for many types of biologic activity may be derived from the contributions of this flat surface to van der Waals' binding to a correspondingly flat receptor area.

The *hydrophobic bond* is a concept used to explain attractive interactions between nonpolar regions of the receptor and the drug. Explanations such as the "isopropyl moiety of the drug fits into a hydrophobic cleft on the receptor composed of the hydrocarbon side chains of the amino acids valine, isoleucine, and leucine" are commonly used to explain why a nonpolar substituent at a particular position on the drug molecule is important for activity. Over the years, the concept of hydrophobic bonds has developed. There has been considerable controversy over whether or not the bond actually exists. Thermodynamic arguments on the gain in entropy (decrease in ordered state) when hydrophobic groups cause a partial collapse of the ordered water structure on the surface of the receptor have been proposed to validate a hydrophobic bonding model. There are two problems with this concept. First, the term *hydrophobic* implies repulsion. The term for attraction is *hydrophilicity*. Second and, perhaps, more important, there is no truly water-free region on the receptor. This is true, even in the areas populated by the nonpolar amino acid side chains. An alternate approach is to consider only the concept of hydrophilicity and lipophilicity. The predominating water molecules solvate polar moieties, effectively squeezing the nonpolar residues toward each other.

STERIC FEATURES OF DRUGS

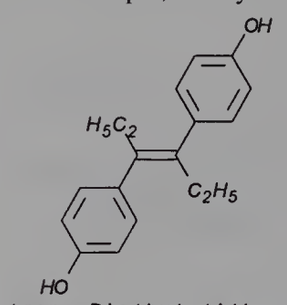
Regardless of the ultimate mechanism by which the drug and the receptor interact, the drug must approach the receptor and fit closely to its surface. Steric factors determined by the stereochemistry of the receptor site surface and that of the drug molecules are, therefore, of primary importance in determining the nature and the efficiency of the drug-receptor interaction. With the possible exception of the general anesthetics, such drugs must possess a high degree of structural specificity to initiate a response at a particular receptor.

Some structural features contribute a high degree of structural rigidity to the molecule. For example, aromatic rings are planar, and the atoms attached directly to these rings are held in the plane of the aromatic ring. Hence, the quaternary nitrogen and carbamate oxygen attached directly to the benzene ring in the cholinesterase inhibitor, neostigmine, are restricted to the plane of the ring and, consequently, the spatial arrangement of at least these atoms is established.

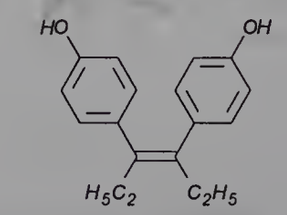


Neostigmine

The relative positions of atoms attached directly to multiple bonds are also fixed. For the double bond, *cis* and *trans* isomers result. For example, diethylstilbestrol exists in two



trans-Diethylstilbesterol



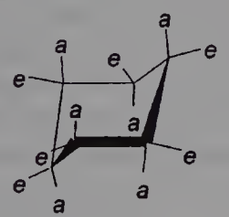
cis-Diethylstilbesterol

fixed stereoisomeric forms. *trans*-Diethylstilbestrol is estrogenic, whereas the *cis*-isomer is only 7% as active. In *trans*-diethylstilbestrol, resonance interactions and minimal steric interference tend to hold the two aromatic rings and connecting ethylene carbon atoms in the same plane.

Geometric isomers, such as the *cis* and the *trans* isomers, hold structural features at different relative positions in space. These isomers also have significantly different physical and chemical properties. Therefore, their distributions in the biologic medium are different, as are their capabilities for interacting with a biologic receptor in a structurally specific manner.

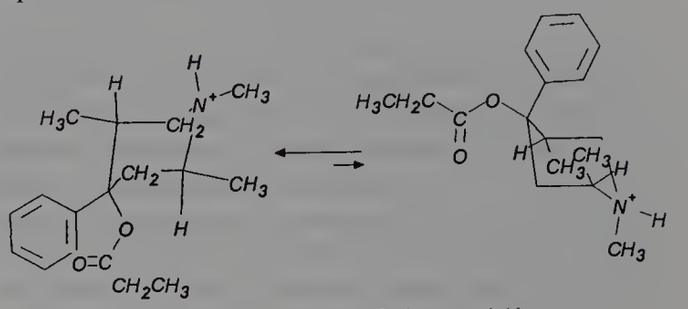
More subtle differences exist for *conformational* isomers. Similarly to geometric isomers, these exist as different arrangements in space for the atoms or groups in a single classic structure. Rotation about bonds allows interconversion of conformational isomers. However, an energy barrier between isomers is often sufficiently high for their independent existence and reaction. Differences in reactivity of functional groups, or interaction with biologic receptors, may be due to differences in steric requirements of the receptors. In certain semirigid ring systems, such as the steroids, conformational isomers show significant differences in biologic activities (see Chap. 23). Methods for calculating these energy barriers are discussed later.

The principles of conformational analysis have established some generalizations about the more stable structures for saturated (nonaromatic) ring systems. In the cyclohexane derivatives, bulky groups tend to be held approximately in the plane of the ring, the *equatorial* position. Substituents attached to bonds perpendicular to the general plane of the ring (*axial* position) are particularly susceptible to steric crowding. Thus, 1,3-diaxial substituents larger than hydrogen may repel each other, twisting the flexible ring and placing the substituents in the less crowded equatorial conformation.

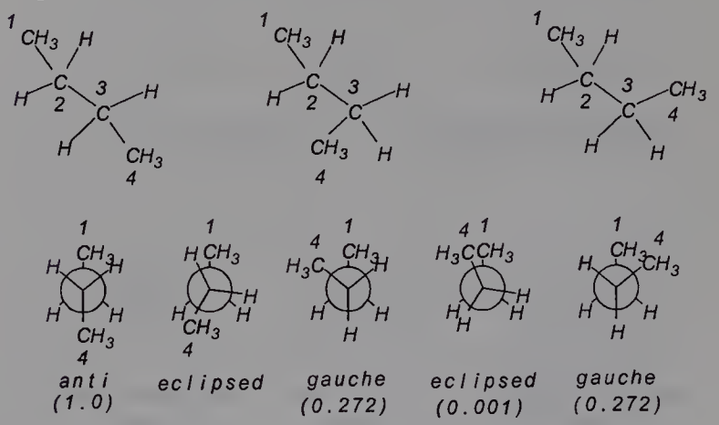


Equitorial (e) and axial (a) substitution in the chair form of cyclohexane.

Similar calculations may be made for saturated heterocyclic ring systems, such as substituted piperidines. Generally, an equilibrium mixture of conformers may exist. For example, the potent analgesic trimeperidine (see Chap. 22) has been calculated to exist largely in the form in which the bulky phenyl group is in the equatorial position, this form being favored by 7 kcal/mol over the axial species. The ability of a molecule to produce potent analgesia has been related to the relative spatial positioning of a flat aromatic nucleus, a connecting aliphatic or alicyclic chain, and a nitrogen atom, which exists largely in the ionized form at physiologic pH.[17] It might be expected that one of the conformers would be responsible for the analgesic activity; however, here, it appears that both the axially and the equatorially oriented phenyl group may contribute. In structurally related isomers, the conformations of which are fixed by the fusion of an additional ring, both the compound in which the phenyl group is in the axial position and the isomer in which the phenyl group is in the equatorial position have equal analgesic potency.[18] It must be remembered that this work was published years before the natural ligands for the opiate receptor were discovered.

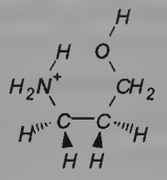


Open chains of atoms, which form an important part of many drug molecules, are not equally free to assume all possible conformations, there being some that are sterically preferred. Energy barriers to free rotation of the chains are present, owing to interactions of nonbonded atoms. For example, the atoms tend to position themselves in space such that they occupy staggered positions, with no two atoms directly facing each other (eclipsed). Thus, for butane at 37°, the calculated relative probabilities for four possible conformations show that the maximally extended *anti* form is favored 3.6:1 over the two equivalent *gauche* forms. As the two substituted carbons 2 and 3 rotate about the bond connecting them, the 1- and 4-methyl groups will pass. As the two substituted carbons 2 and 3 rotate about the bond connecting them, the 1- and 4-methyl groups will pass by the hydrogens on carbons 2 and 3. These are lower-energy *eclipsed* forms relative to when the 1- and 4-methyl groups pass each other, producing a higher energy *eclipsed* result. At normal temperatures, only ~1:1,000 molecules may be expected to be in this conformation.



Nonbonded interactions in polymethylene chains tend to favor the most extended *anti* conformations, although some of the partially extended *gauche* conformations also exist. Intramolecular bonding between substituent groups can

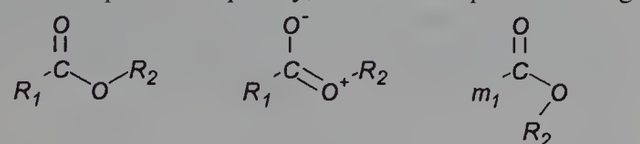
make what might first appear to be an unfavorable conformation favorable. Consider 1-amino-pentane-3-ol. Because of intramolecular hydrogen bonding, the eclipsed form will be a stable conformation.



eclipsed

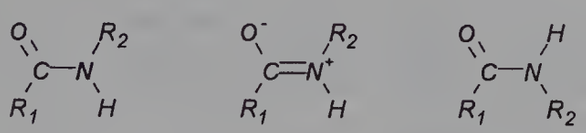
As pointed out with butaine-1,2-diol, the introduction of atoms other than carbon into a chain strongly influences the conformation of the chain. Because of resonance contributions of forms in which a double bond occupies the central bonds of esters and amides, a planar configuration is favored in which minimal steric interference of bulky substituents occurs. Hence, an ester is mainly in the *anti*, rather than the *gauche* form. For the same reason, the amide linkage is essentially planar, with the more bulky substituents occupying the *anti* position. Therefore, ester and amide linkages in a chain tend to hold bulky groups in a plane and to separate them as far as possible. As components of the side chains of drugs, ester and amide groups favor fully extended chains and, also, add polar character to that segment of the chain. The foregoing considerations make it clear that the ester linkages in succinyl choline provide both a polar segment, which is readily hydrolyzed by plasma cholinesterase (see Chap. 17), and additional stabilization to the fully extended form. This form is also favored by repulsion of the positive charges at the ends of the chain.

The conformations favored by stereochemical considerations may be further influenced by *intramolecular interactions* between specific groups in the molecule. *Electrostatic forces*, involving attractions by groups of opposite charge, or repulsion by groups of like charge, may alter molecular size and shape. Consequently, the terminal positive charges



anti-planar form | *resonance form* | *gauche-planar form*

Stabilizing planar structure of esters



anti-planar form | *resonance form* | *gauche-planar form*

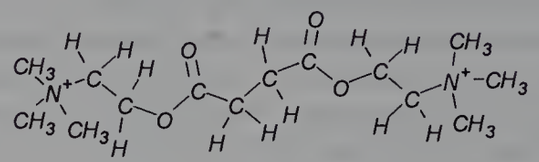
Stabilizing planar structure of amides

on the polymethylene bis-quaternary ganglionic blocking agent hexamethonium, and the neuromuscular-blocking agent decamethonium, make it most likely that the ends of these molecules are maximally separated in solution.

$(CH_3)_3N^+\text{-}(CH_2)_n\text{-}N^+(CH_3)_3$

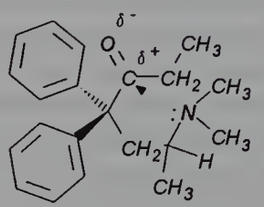
Hexamethonium $n = 6$

Decamethonium $n = 10$



Extended form of succinyl choline

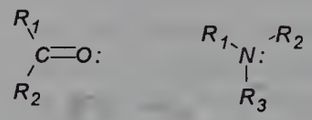
In some cases *dipole-dipole interactions* appear to influence structure in solution. Methadone may exist partially in a cyclic form in solution, because of dipolar attractive forces between the basic nitrogen and carbonyl group.[19] In such a conformation, it closely resembles the conformationally more rigid potent analgesics, morphine, meperidine, and their analogues (see Chap. 22), and it may be this form that interacts with the analgesic receptor.



Conformation of methadone stabilized by dipolar interactions

An intramolecular *hydrogen bond*, usually formed between donor hydroxy and amino groups and acceptor oxygen and nitrogen atoms, might be expected to add stability to a particular conformation of a drug in solution. However, in aqueous solution donor and acceptor groups tend to be bonded to water, and little gain in free energy would be achieved by the formation of an intramolecular hydrogen bond, particularly if unfavorable steric factors involving nonbonded interactions were introduced in the process. Therefore, it is likely that internal hydrogen bonds play only a secondary role to steric factors in determining the conformational distribution of flexible drug molecules.

Hydrogen bonding donor groups



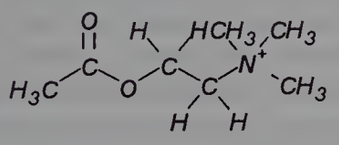
Hydrogen bonding acceptor groups

Conformational Flexibility and Multiple Modes of Action

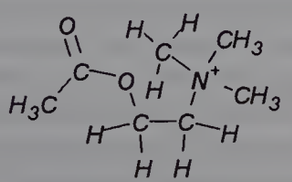
It has been proposed that the conformational flexibility of most open-chain neurohormones, such as acetylcholine, epi-

nephrine, serotonin, histamine, and related physiologically active biomolecules, permits multiple biologic effects to be produced by each molecule, by virtue of their ability to interact in a different and unique conformation with different biologic receptors. Thus, it has been suggested that acetylcholine may interact with the muscarinic receptor of postganglionic parasympathetic nerves and with acetylcholinesterase in the fully extended conformation and, in a different, more-folded structure, with the nicotinic receptors at ganglia and at neuromuscular junctions.[20,21] Acetylcholine bromide exists in a quasi-ring form in the crystal, with an *N*-methyl hydrogen atom close to, and perhaps forming a hydrogen bond with, the backbone oxygen.[22] In solution, however, it is able to assume a continuous series of conformations, some of which are energetically favored over others.[21]

Conformationally rigid acetylcholine-like molecules have been used to study the relationships between these various possible conformations of acetylcholine and their biologic effects. (+)-*trans*-2-Acetoxycyclopropyl trimethylammonium iodide, in which the quaternary nitrogen atom and acetoxyl groups are held apart in a conformation approximating that of the extended conformation of acetylcholine, was about five times more active than acetylcholine in its muscarinic effect on dog blood pressure, and equally active to acetylcholine in its muscarinic effect on the guinea pig ileum.[23] The (+)-*trans*-isomer was hydrolyzed by acetylcholinesterase at a rate equal to the rate of hydrolysis of acetylcholine. It was inactive as a nicotinic agonist. In contrast, the (−)-*trans*-isomer and the mixed (±)-*cis*-isomers were 1/500 and 1/10,000, respectively, as active as acetylcholine in muscarinic tests on guinea pig ileum and were inactive as nicotinic agonists. Similarly, the *trans*-diaxial relationship between the quaternary nitrogen and acetoxyl group led to maximal muscarinic response and rate of hydrolysis by true acetylcholinesterase in a series of isomeric 3-trimethylammonium-2-acetoxydecalins.[24] These results could be interpreted as either that acetylcholine was acting in a *trans* conformation at the muscarinic receptor, and was not acting in a *cisoid* conformation at the nicotinic receptor, or that the nicotinic response is highly sensitive to steric effects of substituents being used to orient the molecule.



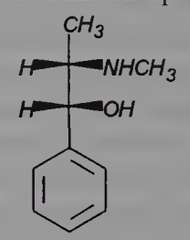
Acetylcholine - extended conformation



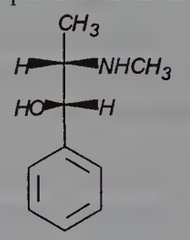
Acetylcholine - quasi-ring conformation

OPTICAL ISOMERISM AND BIOLOGICAL ACTIVITY

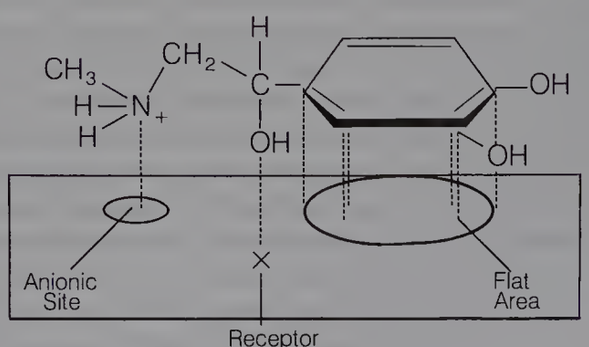
The widespread occurrence of differences in biological activities for *optical activities* has been of particular importance in the development of theories on the nature of drug-receptor interactions. Most commercial drugs are asymmetric, meaning that they cannot be divided into symmetrical halves. While D, L isomers will have the same physical properties, a large number of drugs are *diastereomeric*, meaning that have two or more asymmetric centers. Diastereomers will have different physical properties. Examples are the diastereomers ephedrine and pseudoephedrine. The former has a melting point of 79° and is soluble in water, whereas pseudoephedrine's melting point is 118° and it is only sparingly soluble in water. Keep in mind that receptors will be asymmetric because they are mostly protein, meaning that they are constructed from L-amino acids. Note Fig. 2-10. A ligand fitting that hypothetical receptor will have to have a positive charged moiety in the upper left corner and a hydrophobic region in the upper right. Therefore, one would predict that optical isomers will also have different biological properties. Well-known examples of this phenomenon include (−)-hyoscyamine, exhibiting 15 to 20 times more mydriatic activity than (+)-hyoscyamine, and (−)-ephedrine, showing three times more pressor activity than (+)-ephedrine, five times more pressor activity than (+)-pseudoephedrine, and 36 times more pressor activity than (−)-pseudoephedrine. All of ascorbic acid's antiscorbutic properties reside in the (+)-isomer. A postulated fit to epinephrine's receptor can explain why (−)-epinephrine exhibits 12 to 15 times more vasoconstrictor activity than (+)-epinephrine. This is the classical three-point attachment model. For epinephrine, the benzene ring, benzylic hydroxyl, and protonated amine must have the stereochemistry seen with the (−)-isomer to match up with the hydrophobic or aromatic



Ephedrine
(Erythro configuration)



Pseudoephedrine
(Threo configuration)



(−)-Epinephrine — more active

region, anionic site, and a hydrogen bonding center on the receptor. The (+)-isomer (the mirror image) will not align properly on the receptor.

In addition to the fact that most receptors are asymmetric, there are other reasons that stereoisomers show different biological responses. Active transport mechanisms involve asymmetric carrier molecules, which means that there will be preferential binding of one stereoisomer over others. When differences in physical properties exist, the distribution of isomers between body fluids and tissues where the receptors are located will differ. The enzymes responsible for drug metabolism are asymmetric, which means that biological half-lives will be different among possible stereoisomers of the same molecule. The latter may be a very important variable because the metabolite actually may be the active molecule.

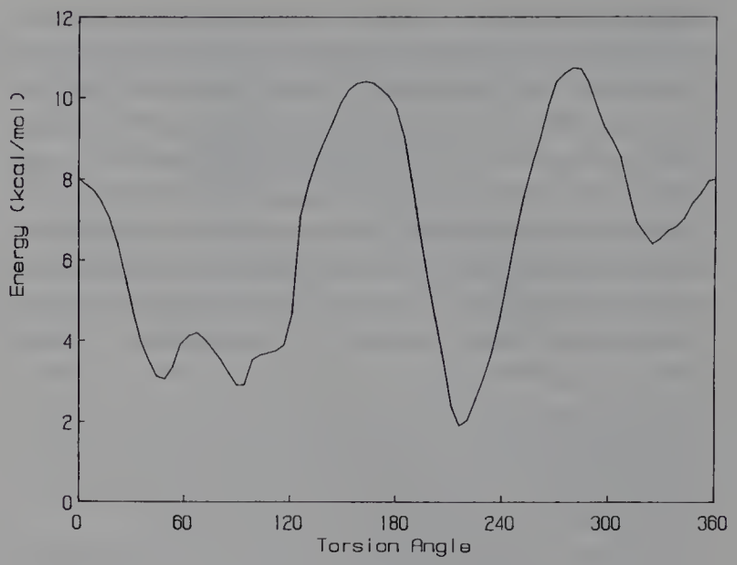


FIG. 2-8. *Diagram showing the energy maxima and minima as two substituted carbons connected by a single bond are rotated 360° relative to each other.*

CALCULATED CONFORMATIONS

It should now be obvious that it is important for medicinal chemists to obtain an accurate understanding of the active conformation of the drug molecule. Originally, molecular models were constructed from kits containing a variety of atoms of different valence and oxidation states. Thus, there would be carbons suitable for carbon-carbon single, double, and triple bonds, carbon-oxygen bonds for alcohols or ethers and the carbonyl moiety, carbon-nitrogen bonds for amines, amides, imines, and nitriles, and carbons for three-, four-, five-, and larger-member rings. More complete sets include a variety of heteroatoms including nitrogen, oxygen, and sulfur of various oxidation states. These kits might be ball and stick, stick or wire only, or space filling. The latter contained attempts at realistically visualizing the effect of a larger atom such as sulfur relative to the smaller oxygen. The diameters of the atoms in these kits are proportional to the van der Waal radii, usually corrected for overlap effects. In contrast, the wire models usually depict accurate intraatomic distances between atoms. A skilled chemist using these kits usually can obtain a reasonably accurate three-dimensional representation. This is particularly true if it is a moderately simple molecule with considerable rigidity. An extreme example is a steroid with the relatively inflexible fused-ring system. In contrast, molecules with chains consisting of several atoms can assume many shapes. Yet, only one shape or conformation can be expected to fit onto the receptor. The number of conformers can be estimated from Eq. 2-27. Calculating the "global minimum," the lowest energy conformation, can be a difficult computational problem. Assume that there are three carbon–carbon-free rotatable single bonds that are rotated in 10° increments. Equation 2-27 states that there 46,656 different conformations. A typical energy diagram is shown in Fig. 2-8. Notice that some of the minima are nearly equivalent, and it is easy to move from one minimum to another. From energy diagrams, it is difficult to answer the question: Which of the ligand's low or moderately low conformations fits onto the receptor? This question can be answered partially by assuming that lower energy conformations are more highly populated and, thus, more likely to interact with the receptor. Nevertheless, specific interactions like hydrogen bond formation can affect the energy levels of different conformations. Therefore, it is seldom true that the bound conformation of a drug is its lowest energy conformation.

$$\text{Number of conformers} = \left(\frac{360}{\text{angle increment}}\right)^{\text{No. rotatable bonds}} \qquad \text{(Eq. 2-27)}$$

There are three common quantitative ways to obtain estimations of preferred molecular shapes required for a good fit at the receptor. The first, which is the oldest and is considered to be the most accurate, is x-ray crystallography. When properly done, resolution down to a few angstrom units can be obtained. This permits an accurate mathematical description of the molecule, providing atomic coordinates in three-dimensional space that can be drawn using a chemical graphics program. A serious limitation of this technique is the requirement for a carefully grown crystal. Some chemicals will not form crystals. Others form crystals with mixed symmetries. Nevertheless, with the newer computational techniques, including high-speed computers, large databases of x-ray crystallographic data are now available. These databases can be searched looking for structures, including substructures, similar to the molecule of interest. Depending on how close is the match, it is possible to obtain a pretty good idea of the low-energy conformation of the drug molecule.

There also is the "debate" that asks if the conformation found in the crystal represents the conformation "seen" by the receptor. For rigid molecules, it probably is. The question is very difficult to answer for flexible molecules. A common

technique is to accurately determine the crystal structure of a protein and then *soak* the crystal in a nonaqueous solution of the drug. This allows the drug molecules to diffuse into the active site. The resulting crystal is re-analyzed, using different techniques, and the bound conformation of the drug can be rapidly determined without redoing the entire protein. Many times the structure of a bound drug can be determined in a day or less.

Because of the drawbacks to x-ray crystallography, two purely computational methods that require only a knowledge of the molecular structure are utilized. The two approaches are known as quantum mechanics and molecular mechanics. Both are based on assumptions that (a) a molecule's three-dimensional geometry is a function of the forces acting on the molecule and (b) these forces can be expressed by a set of equations that pertain to all molecules. For the most part, both computational techniques assume that the molecule is in an isolated system. Solvation effects from water, which are common to any biological system, tend to be ignored, although this is changing with increased computational power. Calculations now can include limited numbers of water molecules, the number dependent on the amount of available computer time. Interestingly, many crystals grown for x-ray analysis can contain water in the crystal lattice. High-resolution nuclear magnetic resonance (NMR) provides another means of obtaining the structures of macromolecules and drugs in solution.

There are fundamental differences in approach between the quantum and molecular mechanics approaches. They illustrate the dilemma that can confront the medicinal chemist. Quantum mechanics is derived from basic theoretical principles at the atomic level. The model, itself, is exact, but the equations used in the technique are only approximate. The molecular properties are derived from the electronic structure of the molecule. The assumption is made that the distribution of electrons within a molecule can be described by a linear sum of functions that represent an atomic orbital. (For carbon, this would be s, p_x, p_y, etc.) Quantum mechanics is computation intensive with the calculation time for obtaining an approximate solution approximately increasing by N^4 times, where N is the number of such functions. Until the advent of the high-speed supercomputers, quantum mechanics in its *pure* form was restricted to small molecules. In other words, it was not practical to conduct a quantum mechanical analysis of a drug molecule.

In order to make the technique more practical, simplifying techniques have been developed. While the computing time is decreased, the accuracy of the outcome is also lessened. In general, quantum mechanics type of calculations in medicinal chemistry is a method that is *still waiting to happen.* It is being used by those laboratories with access to large-scale computing, but there is considerable debate as to its utility because so many simplifying approximations need to be made for larger molecules.

In contrast, medicinal chemists are embracing molecular mechanics. This approach is derived from empirical observations. In contrast to quantum mechanics, the equations in molecular mechanics have exact solutions. At the same time, it must be realized that the parameters that are used in these equations are adjusted in order to insure that the outcome fits experimental observations. In place of the fundamental electronic structure used in quantum mechanics, molecular mechanics uses a model consisting of balls (the atoms) connected by springs (the bonds). The total energy of a molecule consists of the sum of the following energy terms:

E_c: stretching and compressing of the bonds (springs)
E_b: bending about a central atom
E_t: rotation about bonds
E_v: van der Wals interactions
E_u: electrostatic interactions

Each atom is defined (parameterized) in terms of these energy terms. What this means is that the validity of molecular mechanics is dependent on the accuracy of the parameterization process. From an historical point, saturated hydrocarbons have proved easy to parameterize, followed by selective heteroatoms such as ether oxygens and amines. Unsaturated systems, including aromaticity, caused problems due to the delocalization of the electrons, but this seems to have been solved. Charged atoms such as the carboxylate anion and protonated amine can prove to be a real problem, particularly if the charge is delocalized. Nevertheless, molecular mechanics increasingly is being used by medicinal chemists to gain a better understanding of the preferred conformation of drug molecules and the macromolecules that comprise a receptor. The computer programs are readily available and run on relatively inexpensive, but powerful desktop computers.

In summary, quantum mechanics attempts to model the position or distribution of the electrons or bonds while molecular mechanics attempts to model the positions of the nuclei or atoms. A common use of quantum mechanics calculations is to generate or verify molecular mechanics parameters. Larger structures can be studied using molecular mechanics, and using simulation techniques such as molecular dynamics, the behavior of drugs in solution or even in passage through bilayer membranes can be studied.

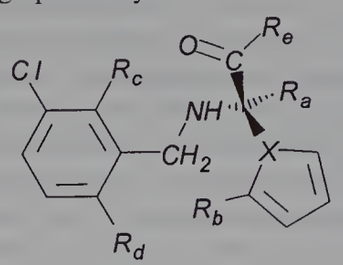
The only way to test the validity of the outcome from either quantum or molecular mechanics calculations is to compare the calculated structure or property with actual experimental data. Obviously, crystallographic data provides a reliable measure of the accuracy of at least one of the low-energy conformers. Since that is not always feasible, other physical chemical measurements are used for comparison. These include comparing calculated vibrational energies, heats of formation, dipole moments, and relative conformational energies with measured values. Where results are inconsistent, the parameter values are adjusted. This readjustment of the parameters is analogous to the fragment approach for calculating octanol/water partition coefficients. The values for the fragments and the accompanying correction factors are determined by comparing calculated partition

coefficients with a large population of experimentally determined partition coefficients.

THREE-DIMENSIONAL QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIPS

With molecular modeling becoming more common, the QSAR paradigm that traditionally used physicochemical descriptors on a two-dimensional molecule can be adapted to three-dimensional space. Essentially, the method requires knowledge of the three-dimensional shape of the molecule. Indeed, accurate modeling of the molecule is crucial. A reference, possibly the prototype molecule, or shape is selected against which all other molecules are compared. The original method called for overlapping the test molecules with the reference molecule and minimizing the differences in overlap. Then distances are calculated between arbitrary locations on the molecule. These distances were used as variables in QSAR regression equations. While overlapping rigid ring systems such as tetracyclines, steroids, and penicillins is relatively easy, flexible molecules can prove challenging. Examine the following hypothetical molecule. Depending on the size of the various *R* groups and the type of atom represented by "*X*," a family of compounds represented by this molecule could have a variety of conformations. Even when the conformations might be known with reasonable certainty, the reference points crucial for activity must be identified. Is the overlap involving the tetrahedral carbon important for activity? Or should the five-membered ring provide the reference points, and which way should it be rotated? Assuming that R_b is an important part of the pharmacophore, should the five-membered ring be rotated so that R_b is pointed down or up? These are not trivial questions, and successful 3D-QSAR studies have been dependent on just how the investigator positions the molecules relative to each other. There are several instances where apparently very similar structures have been shown to bind to a given receptor in different orientations. The pteridine rings on dihydrofolate and methotrexate bind differently to dihydrofolate reductase.

There are a variety of algorithms for measuring degree of conformational and shape similarities, including molecular shape analysis (MSA),[25] distance geometry[26], and molecular similarity matrices.[27,28] Many of the algorithms use graph theory, in which the bonds that connect the atoms of a molecule can be thought of as paths between specific points on the molecule. Molecular connectivity is a commonly used application of graph theory.[29–31]



Besides comparing how well a family of molecules overlaps with a reference molecule, there are sophisticated software packages that determine the physical chemical parameters located at specific distances from the surface of the molecule. An example of this approach is Comparative Molecular Field Analysis (CoMFA).[32,33] Examine Fig. 2-9. The hypothetical molecule is placed in a field and its surface sampled at a specified distance. The parameter types include steric, Lennard-Jones potentials and other quantum chemical parameters, electrostatic and steric parameters, and partition coefficients. This produces thousands of independent variables. Standard regression analysis requires that the dimensionality be reduced and rigorous tests of validity be used. Partial least squares (PLS) has been the most common statistical method used. Elegant as the CoMFA algorithm is for explaining ligand-receptor interactions for a set of molecules, that method alone does not readily point the investigator toward the next molecule that should be synthesized.

SEARCHING THREE-DIMENSIONAL DATABASES

As pointed out earlier in this chapter, receptors are being isolated and cloned. This means that it is possible to determine their structures. Most are proteins, which means determining their amino acid sequence. Also, keep in mind that many enzymes become receptors when the goal is to alter their activity. Examples of the latter include acetylcholine, monoamine oxidase, HIV-protease, rennin, angiotensin-converting enzyme, and tetrahydrofolate reductase. Because enzymes are easy to isolate relative to membrane-bound receptors, most searching for ligands contained in chemical databases is based on information obtained from enzyme active sites.

The first step is to convert the traditional or historical two-dimensional molecules into three-dimensional structures whose intramolecular distances are known. Keeping in mind

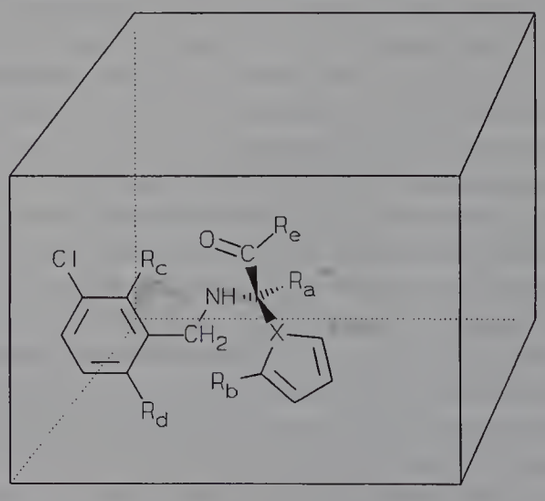


FIG. 2-9. *Molecule situated in a CoMFA grid.*

the problems of finding the "correct" conformation for flexible molecule, false hits and misses might result from the search. Next, the dimensions of the active site must be determined. Ideally, the receptor has been crystallized, and, from the coordinates, the intramolecular distances between what are assumed to be key locations are obtained. If the receptor cannot be crystallized, there are methods for estimating the three-dimensional shape.

Fortunately, the crystal structures of literally thousands of proteins have been determined and their structures stored in the Brookhaven Protein Databank. It is now known that proteins with similar functions will have similar amino acid sequences in various regions of the protein. These sequences tend to show the same shapes in terms of α-helix, parallel and anti-parallel β-pleated forms, turns in the chain, etc. Using this information plus molecular mechanics parameters, the shape of the protein and the dimensions of the active site can be estimated. Figure 2-10 contains the significant components of a hypothetical active site. Notice that there are four amino acid residues at positions 25, 73, 102, and 147 that have been identified as important for either binding the ligand to the site or for the receptor's intrinsic activity. Keep in mind that Fig. 2-10 is a two-dimensional representation of a three-dimensional image. Therefore, the distances between amino acid residues must take into account each residue being above or below the planes of the other three residues. For an artificial ligand to "dock" or fit into the site, six distances must be considered, as follows: A: Lys–Glu, B: Glu–Phe, C: Phe–Ser, D: Ser–Lys, E: Glu–Phe, and F: Lys–Phe. In reality, not all six distances may be important. In selecting potential ligands, candidates might include a positive charged residue (protonated amine), aromatic ring, hydrogen bond donor or acceptor (hydroxy, phenol, amine, nitro), and hydrogen bond acceptor or a negative charged residue (carboxylate) that will interact with the aspartate, phenylalanine, serine, and lysine residues, respectively. A template containing the appropriate residues at the proper distances with correct geometries is constructed and the chemical database searched looking for molecules that fit the template. A degree of fit or match is obtained for each "hit." Their biological responses are obtained and the model for the receptor further refined. New, better defined ligands may be synthesized.

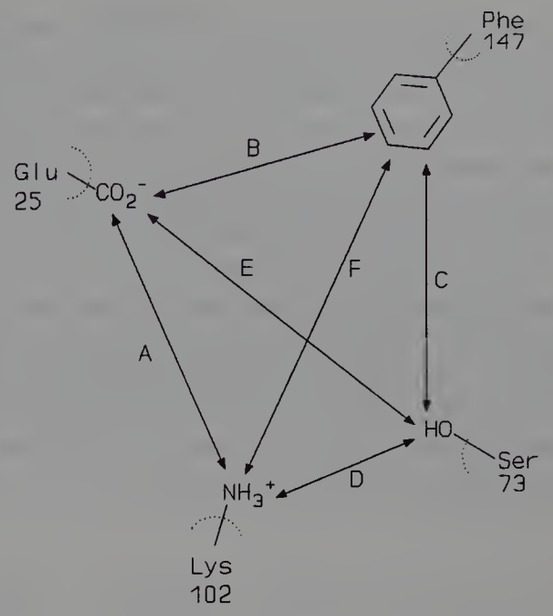


FIG. 2-10. *Diagram of a hypothetical receptor site showing distances between functional groups.*

ISOSTERISM

The term *isosterism* has been widely used to describe the selection of structural components, the steric, electronic, and solubility characteristics, which make them interchangeable in drugs of the same pharmacologic class. The concept of isosterism has evolved and changed significantly in the years since its introduction by Langmuir in 1919.[34] Langmuir, while seeking a correlation that would explain similarities in physical properties for nonisomeric molecules, defined *isosteres* as compounds or groups of atoms having the same number and arrangement of electrons. Those isosteres that were isoelectric (i.e., with the same total charge as well as same number of electrons) would possess similar physical properties. For example, the molecules N_2 and CO, both possessing 14 total electrons and no charge, show similar physical properties. Related examples described by Langmuir were CO_2, N_2O, N_3^-, and NCO^- (Table 2-11).

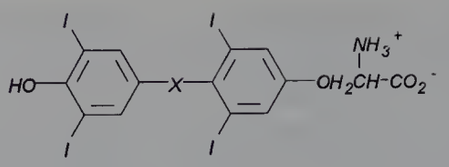
With increased understanding of the structures of molecules, less emphasis has been placed on the number of electrons involved because variations in hybridization during bond formation may lead to considerable differences in the angles, the lengths, and the polarities of bonds formed by atoms with the same number of peripheral electrons. Even the same atom may vary widely in its structural and electronic characteristics when it forms a part of a different functional group. Thus, nitrogen is part of a planar structure in the nitro group, but forms the apex of a pyramidal structure in ammonia and amines.

TABLE 2-11

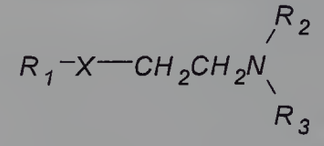
COMMONLY USED ALICYCLIC CHEMICAL ISOSTERES[35]

A.	Univalent atoms and groups	
	(1)	—CH_3 —NH_2 —OH —F —Cl
	(2)	—Cl —SH
	(3)	—Br —*i*—Pr
B.	Bivalent atoms and groups	
	(1)	—CH_2— —NH— —O— —S—
	(2)	—$COCH_2R$ —CONHR
	(3)	—CO_2R —COSR
C.	Trivalent atoms and groups	
	(1)	—CH═ —N═

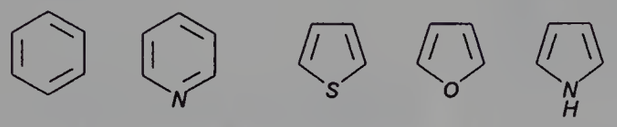
Groups of atoms that impart similar physical or chemical properties to a molecule, because of similarities in size, electronegativity, or stereochemistry, are now frequently referred to under the general term of *isostere*. The early recognition that benzene and thiophene were alike in many of their properties led to the term "ring equivalents" for the vinylene group (—CH=CH—) and divalent sulfur (—S—). This concept has led to replacement of the sulfur atom in the phenothiazine ring system of tranquilizing agents with the vinylene group to produce the dibenzodiazepine class of antidepressant drugs (see Chap. 15). The vinylene group in an aromatic ring system may be replaced by other atoms isosteric to sulfur, such as oxygen (furan) or NH (pyrrole); however, in such cases, aromatic character is significantly decreased.



Thyroid Hormone Analogs: X = O, S, CH_2



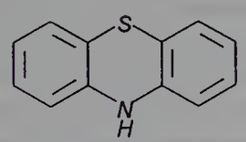
Antihistamines: X = O, NH, CH_2
Cholinergic Blocking Agents: X = $-CO_2-$, $-CONH-$, $-COS-$



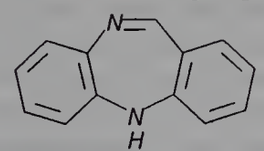
benzene *pyridine* *thiophene* *furan* *pyrrole*

cyclopentane *tetrahydrothiophene* *tetrahydrofuran* *pyrrolidine*

Examples of isosteric pairs that possess similar steric and electronic configurations are the carboxylate (COO^-) and sulfonamide (SO_2NR^-) ions, ketone (C=O) and sulfone (O=S=O) groups, chloride (Cl^-) and trifluoromethyl (CF_3) groups. Divalent ether (—O—), sulfide (—S—), amine (—NH—), and methylene ($—CH_2—$) groups, al-



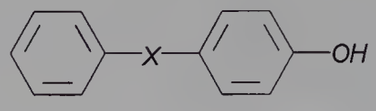
phenothiazine



dibenzodiazepine

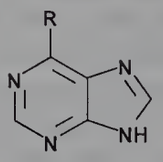
though dissimilar electronically, are sufficiently alike in their steric nature to be frequently interchangeable in designing new drugs.

Compounds may be altered by isosteric replacements of atoms or groups, to develop analogues with select biologic effects, or to act as antagonists to normal metabolites. Each series of compounds showing a specific biologic effect must be considered separately, for there are no general rules that will predict whether biologic activity will be increased or decreased. Some examples of this type are as follows:



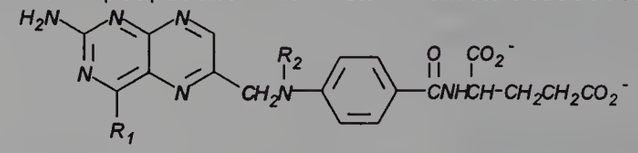
Antibacterial: X = S, Se, O, NH, CH_2

When a group is present in a part of a molecule in which it may be involved in an essential interaction or may influence the reactions of neighboring groups, isosteric replacement sometimes produces analogues that act as antagonists. Some examples from the field of cancer chemotherapy are as follows:



Adenine R = NH_2]
Hypoxanthine R = OH] Metabolites

6-Mercaptopurine R = SH Antimetabolite



Folic Acid R_1 = OH; R_2 = H *Metabolite*

Aminopterin R_1 = NH_2; R_2 = H]
Methotrexate R_1 = NH_2; R_2 = CH_3] *Antimetabolites*

The 6-NH_2 and 6-OH groups appear to play essential roles in the hydrogen-bonding interactions of base pairs during nucleic acid replication in cells. The substitution of the significantly weaker hydrogen-bonding isosteric sulfhydryl groups results in a partial blockage of this interaction and a decrease in the rate of cellular synthesis.

In a similar fashion, replacement of the hydroxyl group of pteroylglutamic acid (folic acid) by the amino group leads to aminopterin, a folate antimetabolite. Addition of the methyl group to the p-aminobenzoate nitrogen produced methotrexate, which is used in cancer chemotherapy, psoriasis, and as an immunosuppressant in rheumatoid arthritis.

As a better understanding develops of the nature of the interactions between drug-metabolizing enzymes and biologic receptors, selection of isosteric groups with particular electronic, solubility, and steric properties should permit the rational preparation of more selectively acting drugs. At the same time, results obtained by the systematic application of the principles of isosteric replacement are aiding in the understanding of the nature of these receptors.

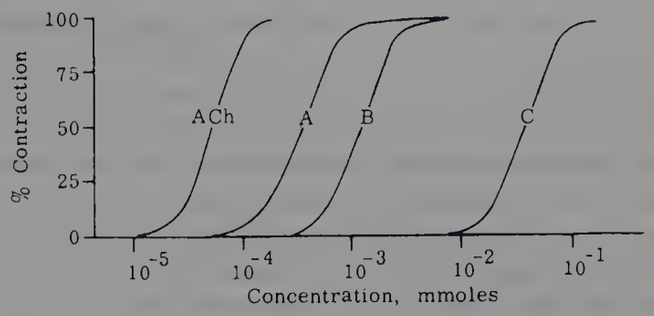


FIG. 2-11. *Dose-response curves for contraction produced by acetylcholine (ACh) and alkytrimethylammonium salts on the rat jejunum.*

A. $CH_3CH_2OCH_2CH_2\overset{+}{N}Me_3$

B. $CH_3CH_2CH_2CH_2CH_2\overset{+}{N}Me_3$

C. $CH_3CH_2CH_2\overset{+}{N}Me_3$

(Modified from Ariëns, E. J., Simonis, A. M.: J. Pharm. Pharmacol. 16:137, 289, 1964.)

DRUG-RECEPTOR INTERACTIONS AND SUBSEQUENT EVENTS

Once bound at the receptor site, drugs may act either to initiate a response (*stimulant* or *agonist* action) or to decrease the activity potential of that receptor (*antagonist* action) by blocking access to it by active molecules. The chain of events leading to an observable biologic response must be initiated in some fashion by either the process of formation or the nature of the drug-receptor complex. Current theories on the mechanism of action of drugs at the receptor level are based primarily on the studies of Clark[36] and Gaddum,[37] whose work supports the assumption that the tissue response is proportional to the number of receptors occupied. The "occupancy theory" of drug action has been modified by Ariëns[38] and Stephenson,[39] who have divided the drug-receptor interaction into two steps: (a) combination of drug and receptor and (b) production of effect. Thus, any drug may have structural features that contribute independently to the *affinity* for the receptor and to the efficiency with which the drug-receptor combination initiates the response (*intrinsic activity* or *efficacy*). The Ariëns-Stephenson concept retains the assumption that the response is related to the number of drug-receptor complexes.

In the Ariëns-Stephenson theory, both agonist and antagonist molecules possess structural features that would enable formation of a drug-receptor complex (strong affinity). However, only the agonist possesses the ability to cause a stimulant action (i.e., possesses intrinsic activity). The affinity of a drug may be estimated by comparison of the dose required to produce a pharmacologic response with the dose required by a reference standard drug or the natural ligand for that receptor. Thus, acetylcholine produces a normal S-shaped curve if the logarithm of the dose is plotted against the percentage contraction of the rat jejunum (a segment of the small intestine). A series of related alkyl trimethylammonium salts (ethoxyethyl trimethylammonium, pentyl trimethylammonium, propyl trimethylammonium) (Fig. 2-11) is able to produce the same degree of contraction of the tissue as does acetylcholine, but higher doses are required. The shape of the dose-response curve is the same, but the series of parallel curves is shifted to higher dose levels. Therefore, the alkyl trimethylammonium compounds are said to possess the same intrinsic activity as acetylcholine, being able to produce the same maximal response, but to show a lower affinity for the receptor, as larger amounts of the drug are required.

By contrast, structural change of a molecule can lead to a gradual decline in the maximal height and slope of the log dose-response curves (Fig. 2-12) in which situation the loss in activity may be attributed to a decline in intrinsic activity. For example, pentyl trimethylammonium ion is able to produce a full acetylcholine-like contraction. Successive substitution of methyl by ethyl groups (pentylethyl dimethylammonium, pentyldiethyl methylammonium, pentyl triethylammonium) leads to successive decreases in the maximal effect obtainable, with pentyl triethylammonium ion producing no observable contraction. The loss in acetylcholine-like activity for pentyl triethylammonium ion is apparently caused by a loss in intrinsic activity, without a significant decrease in the affinity for the receptor because the compound acts as a competitive inhibitor (antagonist) for active derivatives of the same series.

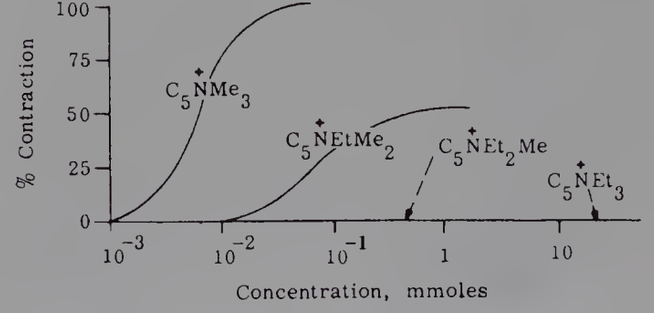


FIG. 2-12. *Dose-response curves for contraction produced by pentyl trialkylammonium slts on the rat jejunum. (Modified from Ariëns, E. J., Simonis, A. M.: J. Pharm. Pharmacol. 16:137, 189, 1964).*

For an antagonist, it is desirable to have high affinity and low or zero intrinsic activity (i.e., to bind firmly to the receptor, but to be devoid of activity). Many examples are available for which structural modifications of an agonist molecule lead consecutively to compounds with decreasing agonist and increasing antagonist activity. Such modifications on acetylcholine-like structures, usually by addition of bulky nonpolar groups to either end (or both ends) of the molecule, may lead to the complete antagonistic activity found in the parasympatholytic compounds (e.g., atropine) discussed in Chap. 17.

In contrast with the occupancy theory, Croxatto[40] and Paton[41] have proposed that excitation by a stimulant drug is proportional to the *rate* of drug-receptor combination, rather than to the number of receptors occupied. The *rate theory* of drug action proposes that the rate of association and dissociation of an agonist is rapid, and this leads to the production of numerous impulses per unit time. An antagonist, with strong receptor-binding properties, would have a high rate of association but a low rate of dissociation. The occupancy of receptors by antagonists, assumed to be a nonproductive situation, prevents the productive events of association by other molecules. This concept is supported by the fact that even blocking molecules are known to cause a brief stimulatory effect before blocking action develops. During the initial period of drug-receptor contact when few receptors are occupied, the rate of association would be at a maximum. When a substantial number of sites are occupied, the rate of association would fall below the level necessary to evoke a biologic response.

The *occupation* and the *rate* theories of drug action do not provide specific models at the molecular level to account for a drug acting as agonist or antagonist. The *induced-fit* theory of enzyme-substrate interaction,[42] in which combination with the substrate induces a change in conformation of the enzyme, leading to an enzymatically active orientation of groups, provides the basis for similar explanations of mechanisms of drug action at receptors. Assuming that protein constituents of membranes play a role in regulating ion flow, it has been proposed[43] that acetylcholine may interact with the protein and alter the normal forces that stabilize the structure of the protein, thereby producing a transient rearrangement in the membrane structure and a consequent change in its ion-regulating properties. If the structural change of the protein led to a configuration in which the stimulant drug was bound less firmly and dissociated, the conditions of the *rate* theory would be satisfied. A drug-protein combination that did not lead to a structural change would result in a stable binding of the drug and a blocking action.

A related hypothesis (the *macromolecular perturbation theory*) of the mode of acetylcholine action at the muscarinic (postganglionic parasympathetic) receptor has been ad-

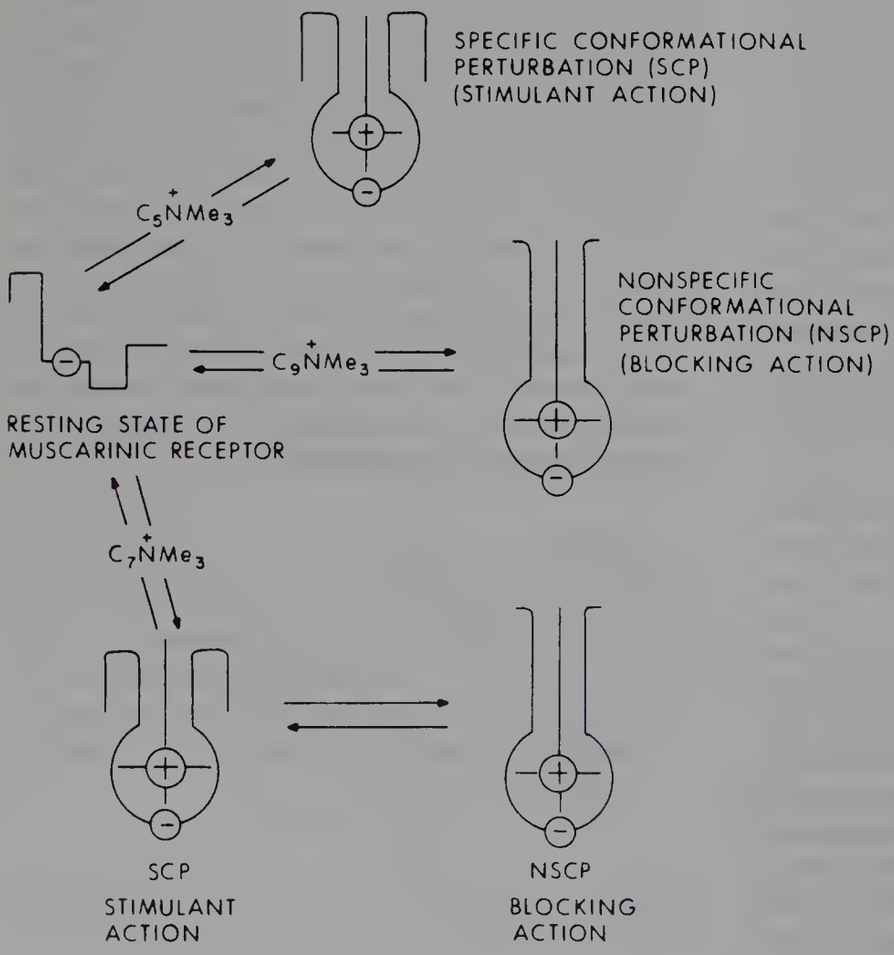


FIG. 2-13. *Schematic representation of alkyl trimethylammonium ions reacting with the muscarinic receptor. (Modified from Belleau, B.: J. Med. Chem. 7:776, 1964.)*

vanced by Belleau.[44] It is proposed that interaction of small molecules (substrate or drug) with a macromolecule (such as the protein of a drug receptor) may lead either to *specific conformational perturbations* (SCP) or to *nonspecific conformational perturbations* (NSCP). A SCP (specific change in structure or conformation of a protein molecule) would result in the specific response of an agonist (i.e., the drug receptor would possess intrinsic activity). If a NSCP occurs, no stimulant response would be obtained, and an antagonistic or blocking action may be produced. If a drug possesses features that contribute to formation of both a SCP and a NSCP, an equilibrium mixture of the two complexes may result, which would account for a partial stimulant action.

The alkyl trimethylammonium ions, $R—N^+(CH_3)_3$, in which the alkyl group, R, is varied from 1 to 12 carbon atoms, provide a homologous series of muscarinic drugs that serve as models for the macromolecular perturbation theory of events that may occur at the drug receptor. With these simple analogues, hydrophobic forces, in addition to ion-pair formation, are considered to be the most important in contributing to receptor binding. Lower alkyl trimethylammonium ions (C_1 to C_6) stimulate the muscarinic receptor and are considered to possess a chain length that is able to form a hydrophobic bond with nonpolar regions of the receptor, altering receptor structure in a specific perturbation, e.g., $C_5N^+(CH_3)_3$ (Fig. 2-13). With a chain of 8 to 12 carbon atoms, the antagonistic action observed is considered to result from a NSCP of a network of nonpolar residues at the periphery of the catalytic surface, e.g., $C_9N^+(CH_3)_3$ (Fig. 2-13). The intermediate heptyl and octyl derivatives act as partial agonists, and it is considered that they may form an equilibrium mixture of drug-receptor combinations, with both active SCP forms and inactive NSCP forms present, e.g., $C_7N^+(CH_3)_3$ (Fig. 2-13).

SELECTED WEB PAGES

The field of drug design, particularly those aspects that are computer intensive, is increasingly being featured on Web pages. Faculty and students might find it instructive to search the Web at regular intervals. Many university chemistry departments have organized Web pages that provide excellent linkages. Listed below are a small number of representative sites that feature drug design linkages. Some have excellent illustrations. These listings should not be considered any type of endorsement by the author, editors, or publisher. Indeed, some of these sites are still under development, and some may disappear.

http://fox.pomona.claremont.edu/chem/
http://www.nih.gov/
http://hackberry.chem.niu.edu/cheminf.html
http://www.awod.com/netsci
http://www.pharma.ethz.ch/qsar/
http://www.scamag.com/links/default.html

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CHAPTER 3

Metabolic Changes of Drugs and Related Organic Compounds

Lawrence K. Low

Metabolism plays a central role in the elimination of drugs and other foreign compounds (*xenobiotic*) from the body. Most organic compounds entering the body are relatively lipid-soluble (*lipophilic*). Therefore, to be absorbed, they must traverse the lipoprotein membranes of the lumen walls of the gastrointestinal (GI) tract. Once in the bloodstream, these molecules can diffuse passively through other membranes to reach various target organs to effect their pharmacologic actions. Owing to their reabsorption in the renal tubules, lipophilic compounds are not excreted to any substantial extent in the urine.

If lipophilic drugs or xenobiotics were not metabolized to polar, water-soluble products that are readily excretable, they would remain indefinitely in the body, eliciting their biologic effects. Thus, the formation of water-soluble metabolites not only enhances drug elimination, but also leads to compounds that are generally pharmacologically inactive and relatively nontoxic. Consequently, drug metabolism reactions traditionally have been regarded as *detoxication* (or *detoxification*) processes.[1] However, it is incorrect to assume that drug metabolism reactions are always detoxifying. Many drugs are biotransformed to pharmacologically active metabolites. Some metabolites have significant activity that contributes substantially to the pharmacologic effect ascribed to the parent drug. Occasionally, the parent compound is inactive and must be converted to a biologically active metabolite.[2,3] In addition, it is becoming increasingly clear that not all metabolites are nontoxic. Indeed, many toxic side effects (e.g., tissue necrosis, carcinogenicity, teratogenicity) of drugs and environmental contaminants can be attributed directly to the formation of chemically reactive metabolites that are highly detrimental to the body.[4–6]

GENERAL PATHWAYS OF DRUG METABOLISM

Drug metabolism reactions have been divided into two categories: *phase I* (*functionalization*) and *phase II* (*conjugation*) reactions.[1,7] Phase I, or functionalization reactions, include oxidative, reductive, and hydrolytic biotransformationals (Box 3-1).[8] The purpose of these reactions is to introduce a polar functional group (e.g., OH, COOH, NH_2, SH) into the xenobiotic molecule. This can be achieved by direct introduction of the functional group (e.g., aromatic and aliphatic hydroxylation) or by modifying or ''unmasking'' existing functionalities (e.g., reduction of ketones and aldehydes to alcohols; oxidation of alcohols to acids; hydrolysis of ester and amides to yield COOH, NH_2, and OH groups; reduction of azo and nitro compounds to give NH_2 moieties; oxidative *N*-, *O*-, and *S*-dealkylation to give NH_2, OH, and SH groups). Although phase I reactions may not produce sufficiently hydrophilic or inactive metabolites, they generally tend to provide a functional group or ''handle'' in the molecule that can undergo subsequent phase II reactions.

The purpose of phase II reactions is to attach small, polar, and ionizable endogenous compounds such as glucuronic acid, sulfate, glycine, and other amino acids to the functional handles of phase I metabolites to form water-soluble conjugated products. Parent compounds that already have existing functional groups, such as OH, COOH, and NH_2, often are conjugated directly by phase II enzymes. Conjugated metabolites are readily excreted in the urine and are generally devoid of pharmacologic activity and toxicity. Other phase II pathways, such as methylation and acetylation, terminate or attenuate biologic activity, whereas glutathione (GSH) conjugation protects the body against chemically reactive compounds or metabolites. Thus, phase I and phase II reactions complement one another in detoxifying and facilitating the elimination of drugs and xenobiotics.

To illustrate, consider the principal psychoactive constituent of marijuana, Δ^1-tetrahydrocannabinol (Δ^1-THC). This lipophilic molecule (octanol/water partition coefficient ~ 6000)[9] undergoes allylic hydroxylation to give 7-hydroxy-Δ^1-THC in humans.[10] More polar than its parent compound, the 7-hydroxy metabolite is further oxidized to the corresponding carboxylic acid derivative Δ^1-THC-7-oic acid,

BOX 3-1. GENERAL SUMMARY OF PHASE I AND PHASE II METABOLIC PATHWAYS

PHASE I OR FUNCTIONALIZATION REACTIONS

Oxidative Reactions

Oxidation of aromatic moieties
Oxidation of olefins
Oxidation at benzylic, allylic carbon atoms, and carbon atoms α to carbonyl and imines
Oxidation at aliphatic and alicyclic carbon atoms
Oxidation involving carbon–heteroatom systems:
- Carbon–nitrogen systems (aliphatic and aromatic amines; includes *N*-dealkylation, oxidative deamination, *N*-oxide formation, *N*-hydroxylation)
- Carbon–oxygen systems (*O*-dealkylation)
- Carbon–sulfur systems (*S*-dealkylation, *S*-oxidation, and desulfuration)

Oxidation of alcohols and aldehydes
Other miscellaneous oxidative reactions

Reductive Reactions

Reduction of aldehydes and ketones
Reduction of nitro and azo compounds
Miscellaneous reductive reactions

Hydrolytic Reactions

Hydrolysis of esters and amides
Hydration of epoxides and arene oxides by epoxide hydrase

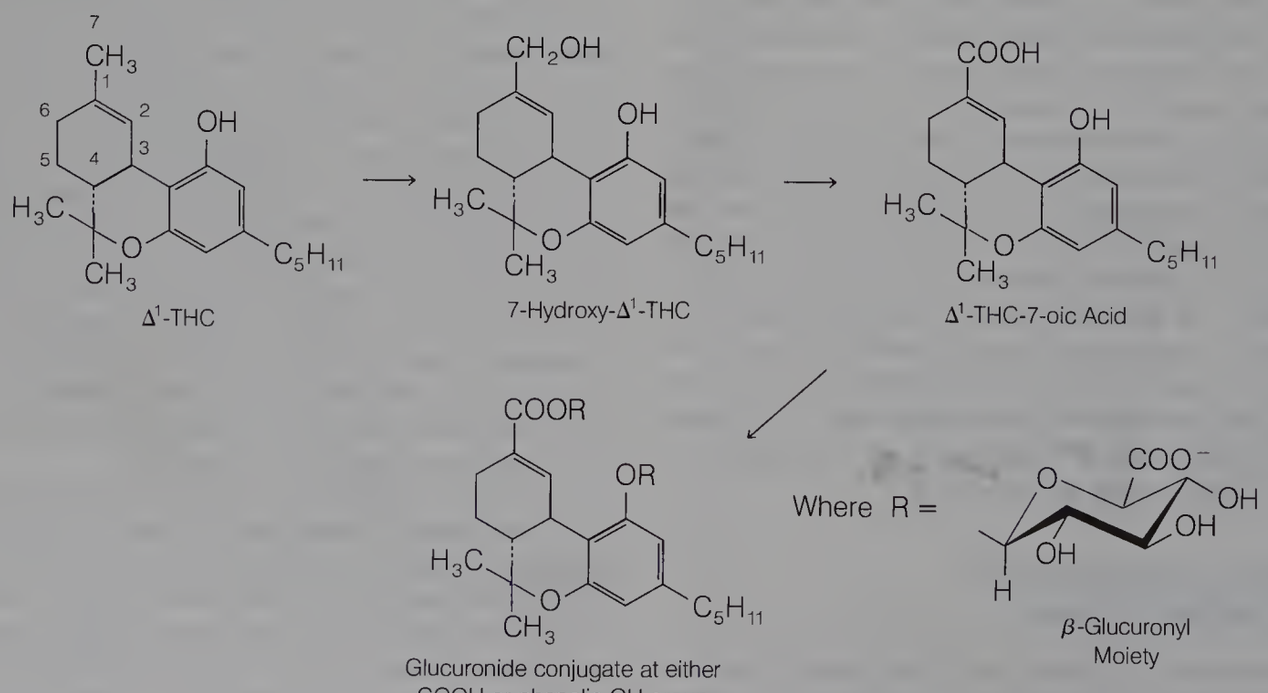
PHASE II OR CONJUGATION REACTIONS

Glucuronic acid conjugation
Sulfate conjugation
Conjugation with glycine, glutamine, and other amino acids
Glutathione or mercapturic acid conjugation
Acetylation
Methylation

which is ionized (pK_a COOH ~ 5) at physiologic *p*H. Subsequent conjugation of this metabolite (either at the COOH or phenolic OH) with glucuronic acid leads to water-soluble products that are readily eliminated in the urine.[11]

In the foregoing series of biotransformations, the parent Δ^1-THC molecule is made increasingly more polar, ionizable, and hydrophilic. The attachment of the glucuronyl moiety (with its ionized carboxylate group and three polar hydroxyl groups; see structure) to the Δ^1-THC metabolites notably favors partitioning of the conjugated metabolites into an aqueous medium.

The purpose of this chapter is to provide the student with a broad overview of drug metabolism. Various phase I and phase II biotransformation pathways (see Box 3-1) will be outlined. Representative drug examples for each pathway will be presented. Drug metabolism examples in humans will be emphasized, although discussion of metabolism in other mammalian systems is necessary. The central role of the cytochrome P-450 monooxygenase system in oxidative drug biotransformation will be elaborated. Discussion of other enzyme systems involved in phase I and phase II reactions will be presented in their respective sections. In addition to stereochemical factors that may affect drug metabolism, biologic factors such as age, sex, heredity, disease state, and species variation will be considered. The effects of enzyme induction and inhibition on drug metabolism as well as a section on pharmacologically active metabolites will be included.



SITES OF DRUG BIOTRANSFORMATION

Although biotransformation reactions may occur in many tissues, the liver is, by far, the most important organ in drug metabolism.[12] It is particularly rich in almost all of the drug-metabolizing enzymes to be discussed in this chapter. The liver is a well-perfused organ and plays a paramount role in the detoxification and metabolism of endogenous and exogenous compounds present in the bloodstream. Orally administered drugs that are absorbed through the GI tract must first pass through the liver. Therefore, they are susceptible to hepatic metabolism (first-pass effect) before reaching the systemic circulation. Dependent on the drug, this metabolism can sometimes be quite significant and, as a result, decrease oral bioavailability. For example, in humans several drugs are metabolized extensively by the first-pass effect.[13] The following list includes some of those drugs:

Isoproterenol
Lidocaine
Meperidine
Morphine
Nitroglycerin
Pentazocine
Propoxyphene
Propranolol
Salicylamide

Some drugs (e.g., lidocaine) are removed so effectively by first-pass metabolism that they are ineffective when given orally.[14] Thus, one can appreciate the enormous metabolizing capability of the liver.

Because most drugs are administered orally, the intestine appears to play an important role in the extrahepatic metabolism of xenobiotics. For example, in humans orally administered isoproterenol undergoes considerable sulfate conjugation in the intestinal wall.[15] Several other drugs (e.g., levodopa, chlorpromazine, and diethylstilbestrol)[16] also have been reported to be metabolized in the GI tract. Esterases and lipases present in the intestine may be particularly[17] important in carrying out hydrolysis of many ester prodrugs (see ''Hydrolytic Reactions,'' below). Bacterial flora present in the intestine and colon appear to play an important role in the reduction of many aromatic azo and nitro drugs (e.g., sulfasalazine).[18] Intestinal β-glucuronidase enzymes are capable of hydrolyzing glucuronide conjugates excreted in the bile, thereby liberating the free drug or its metabolite for possible reabsorption (enterohepatic circulation or recycling).[19]

Although other tissues, such as kidney, lungs, adrenal glands, placenta, brain, and skin, have some degree of drug-metabolizing capability, the biotransformations that they carry out often are more substrate-selective and more limited to particular types of reaction (e.g., oxidation, glucuronidation).[20] In many instances, the full metabolic capabilities of these tissues have not been explored fully.

ROLE OF CYTOCHROME P-450 MONOOXYGENASES IN OXIDATIVE BIOTRANSFORMATIONS

Of the various phase I reactions that will be considered, oxidative biotransformation processes are, by far, the most common and important in drug metabolism. The general stoichiometry that describes the oxidation of many xenobiotics (R—H) to their corresponding oxidized metabolites (R—OH) is given by the following equation:[21]

$$RH + NADPH + O_2 + H^+ \rightarrow ROH + NADP^+ + H_2O$$

The enzyme systems carrying out this biotransformation are referred to as *mixed function oxidases* or *monooxygenases*.[22,23] The reaction requires both molecular oxygen and the reducing agent NADPH (reduced form of nicotinamide adenosine dinucleotide phosphate). It should be emphasized that during this oxidative process, one atom of molecular oxygen (O_2) is introduced into the substrate R—H to form R—OH and the other oxygen atom is incorporated into water. The mixed function oxidase system[24] is actually made up of several components, the most important being an enzyme called cytochrome P-450, which is responsible for transferring *an oxygen atom* to the substrate R—H. Other important components of this system include the NADPH-dependent cytochrome P-450 reductase and the NADH-linked cytochrome b_5. The latter two components, along with the cofactors NADPH and NADH, supply the reducing equivalents (electrons) needed in the overall metabolic oxidation of foreign compounds. The proposed mechanistic scheme by which the cytochrome P-450 monooxygenase system catalyzes the conversion of molecular oxygen to an ''activated oxygen'' species will be elaborated later.

The cytochrome P-450 enzyme is a heme–protein.[25] The heme portion is an iron-containing porphyrin called protoporphyrin IX, and the protein portion is called the apoprotein. Cytochrome P-450 is found in high concentrations in the liver, the major organ involved in the metabolism of xenobiotics. The presence of this enzyme in many other tissues (e.g., lung, kidney, intestine, skin, placenta, adrenal cortex) shows that these tissues have drug-oxidizing capability too. The name ''cytochrome P-450'' is derived from the fact that the reduced (Fe^{2+}) form of this enzyme binds with carbon monoxide to form a complex that has a distinguishing spectroscopic absorption maximum at 450 nm.[26]

An important feature of the hepatic cytochrome P-450 mixed function oxidase system is its ability to metabolize an almost unlimited number of diverse substrates by a variety of oxidative transformations.[27] This versatility is believed to be attributable to the substrate nonspecificity of cytochrome P-450 as well as to the presence of multiple forms of the enzyme.[28]* Some of these P-450 enzymes

* Cytochrome 450 (phenobarbital-inducible) presently falls in the CYP 2 and CYP 3 families of isozymes, while cytochrome P-448 (polyaromatic hydrocarbon-inducible and TCDD-inducible) falls in the CYP 1 family of isozymes.

are selectively inducible by various chemicals (e.g., phenobarbital, benzo[*a*]pyrene, 3-methylcholanthrene).[29] One of these inducible forms of the enzyme (cytochrome P-448)[30] is of particular interest and will be discussed later.

The cytochrome P-450 monooxygenases are located in the endoplasmic reticulum, a highly organized and complex network of intracellular membranes that is particularly abundant in tissues such as the liver.[30] When these tissues are disrupted by homogenization, the endoplasmic reticulum loses its structure and is converted into small vesicular bodies known as microsomes.

Microsomes isolated from hepatic tissue appear to retain all of the mixed function oxidase capabilities of intact hepatocytes; because of this, microsomal preparations (with the necessary cofactors, e.g., NADPH, Mg^{2+}) are utilized frequently for in vitro drug metabolism studies. Because of its membrane-bound nature, the cytochrome P-450 monooxygenase system appears to be housed in a lipoidal environment. This may explain, in part, why lipophilic xenobiotics are generally good substrates for the monooxygenase system.[31]

The catalytic role that the cytochrome P-450 monooxygenase system plays in the oxidation of xenobiotics is summarized in the cycle shown in Fig. 3-1.[32,33] The initial step of this catalytic reaction cycle starts with the binding of the substrate to the oxidized (Fe^{3+}) resting state of cytochrome P-450 to form a P-450–substrate complex. The next step involves the transfer of one electron from NADPH-dependent cytochrome P-450 reductase to the P-450–substrate complex. This one-electron transfer reduces Fe^{3+} to Fe^{2+}. It is this reduced (Fe^{2+}) P-450–substrate complex that is capable of binding dioxygen (O_2). The dioxygen–P-450–substrate complex which is formed then undergoes another one-electron reduction (by cytochrome P-450 reductase–NADPH and/or cytochrome b_5 reductase–NADH) to yield what is believed to be a peroxide dianion–P-450 (Fe^{3+})–substrate complex. Water (containing one of the oxygen atoms from the original dioxygen molecule) is released from the latter intermediate to form an activated oxygen–P-450–substrate complex (Fig. 3-2). The activated oxygen $[FeO]^{3+}$ in this complex is highly electron-deficient and a potent oxidizing agent. The activated oxygen is transferred to the substrate (RH), and the oxidized substrate product (ROH) is released from the enzyme complex to regenerate the oxidized form of cytochrome P-450.

It is important to recognize that the key sequence of events appears to center around the alteration of a dioxygen–P-450–substrate complex to an activated oxygen–P-450–substrate complex, which is then capable of effecting the critical transfer of oxygen from P-450 to the substrate.[34,35] In view of the potent oxidizing nature of the activated oxygen being transferred, it is not surprising that numerous substrates are

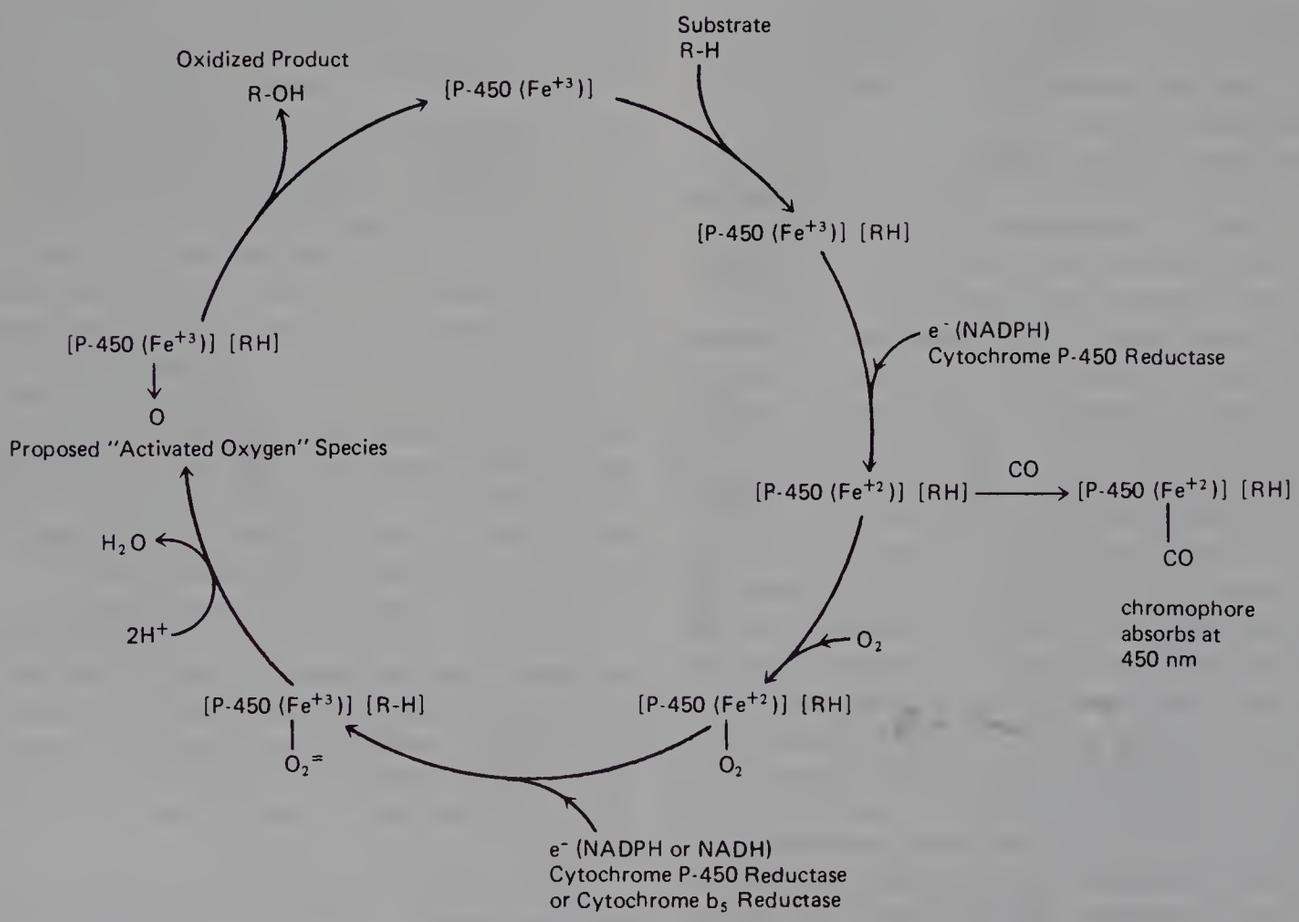


FIG. 3-1. *Proposed catalytic reaction cycle involving cytochrome P-450 in the oxidation of xenobiotics.*

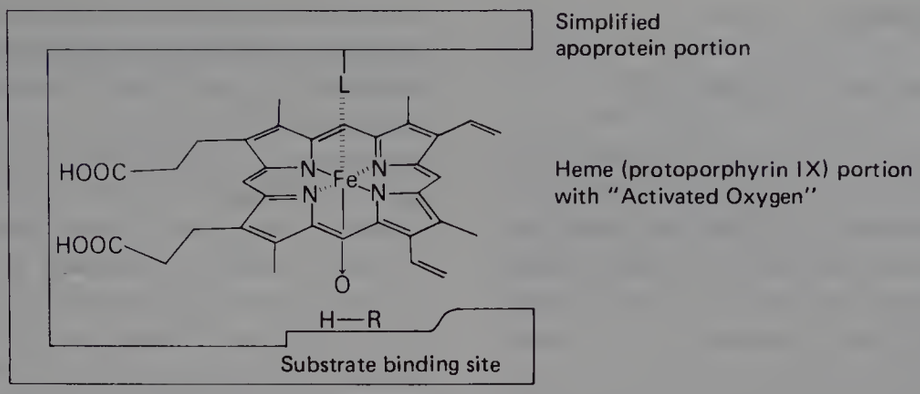


FIG. 3-2. *A simplified depiction of the proposed activated oxygen–cytochrome P-450–substrate complex. Note the simplified apoprotein portion and the heme (protoporphyrin IX) portion of cytochrome P-450 and the close proximity of the substrate RH undergoing oxidation.*

capable of being oxidized by cytochrome P-450. The mechanistic details of oxygen activation and transfer in cytochrome P-450-catalyzed reactions continue to be an active area of research in drug metabolism.[32]

The many types of oxidative reaction carried out by cytochrome P-450 will be enumerated in the sections to follow. Many of these oxidative pathways are summarized schematically in Fig. 3-3 (see also Box 3-1).[34]

The versatility of cytochrome P-450 in carrying out a variety of oxidation reactions on a multitude of substrates may be attributable to the multiple forms of the enzyme. Consequently, it is important for the student to realize that the biotransformation of a parent xenobiotic to several oxidized metabolites is carried out not just by one form of P-450 but, more likely, by several different forms.[36] Extensive studies indicate that the apoprotein portions of various cytochrome P-450s differ from one another in their tertiary three-dimensional structure (owing to differences in amino acid sequence or the makeup of the polypeptide chain).[25,28] Because the apoprotein portion is important in substrate binding and catalytic transfer of activated oxygen, these structural differences may account for some substrates being preferentially or more efficiently oxidized by one particular form of cytochrome P-450.

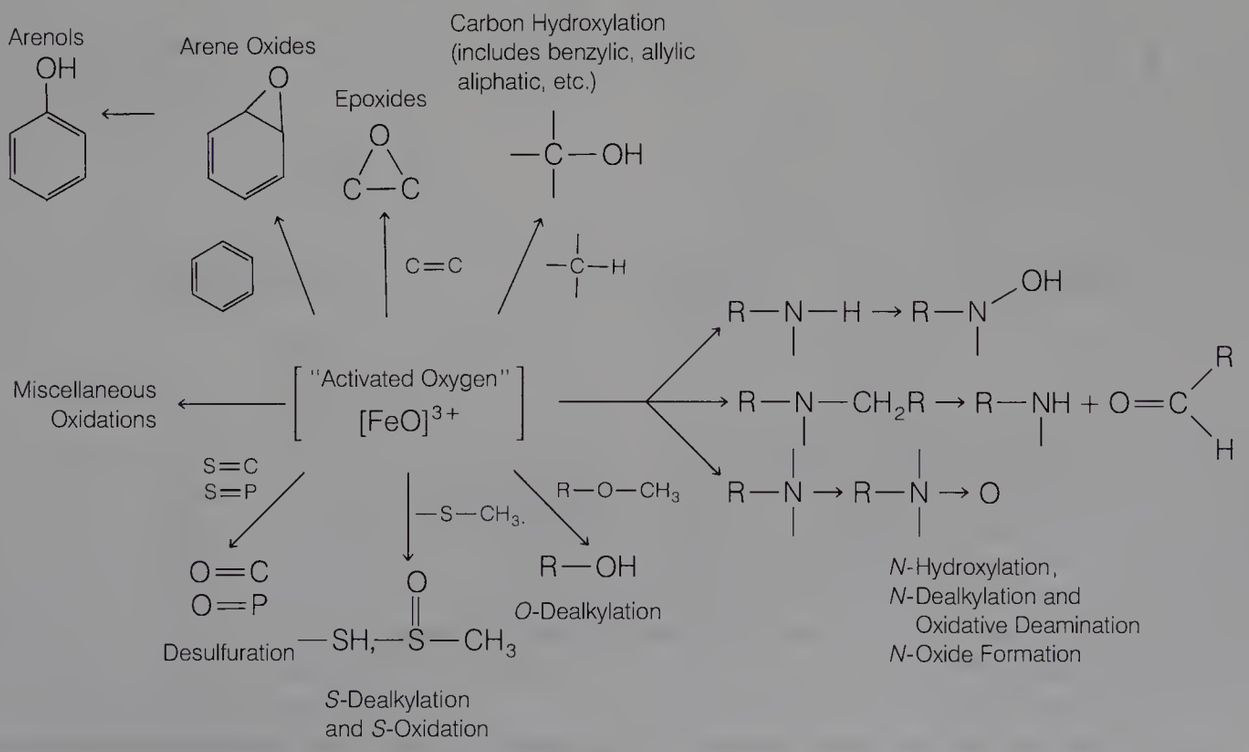
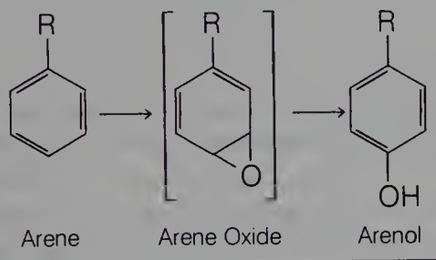


FIG. 3-3. *Schematic summary of cytochrome P-450-catalyzed oxidation reactions. (Adapted from Ullrich, V.: Top. Curr. Chem. 83:68, 1979.)*

OXIDATIVE REACTIONS

OXIDATION OF AROMATIC MOIETIES

Aromatic hydroxylation refers to the mixed function oxidation of aromatic compounds (*arenes*) to their corresponding phenolic metabolites (*arenols*).[37] Almost all aromatic hydroxylation reactions are believed to proceed initially through an epoxide intermediate called an "arene oxide," which rearranges rapidly and spontaneously to the arenol product in most instances. The importance of arene oxides in the formation of arenols and in other metabolic and toxicologic reactions will be discussed shortly.[38] Attention will now focus on the aromatic hydroxylation of several drugs and xenobiotics.



Most foreign compounds containing aromatic moieties are susceptible to aromatic oxidation. In humans, aromatic hydroxylation is a major route of metabolism for many drugs containing phenyl groups. Important therapeutic agents such as propranolol,[39] phenobarbital,[40] phenytoin,[41] phenylbutazone,[42] phenformin,[43] 17α-ethinylestradiol,[44] and (*S*)(−)-warfarin,[45] among others, undergo extensive aromatic oxidation (Fig. 3-4 shows structure and site of hydroxylation). In most of the drugs just mentioned, hydroxylation occurs at the *para*-position.[46] Most phenolic metabolites formed from aromatic oxidation undergo further conversion to polar and water-soluble glucuronide or sulfate conjugates, which are readily excreted in the urine. For example, the major urinary metabolite of phenytoin found in humans is the *O*-glucuronide conjugate of *p*-hydroxyphenytoin.[41] Interestingly, the *para*-hydroxylated metabolite of phenylbutazone, oxyphenbutazone, is pharmacologically active and has been marketed itself as an anti-inflammatory agent (Tandearil, Oxalid).[42] Of the two enantiomeric forms of the oral anticoagulant warfarin (Coumadin), only the more active *S*(−)-enantiomer has been shown to undergo substantial aromatic hydroxylation to 7-hydroxywarfarin in humans.[45]

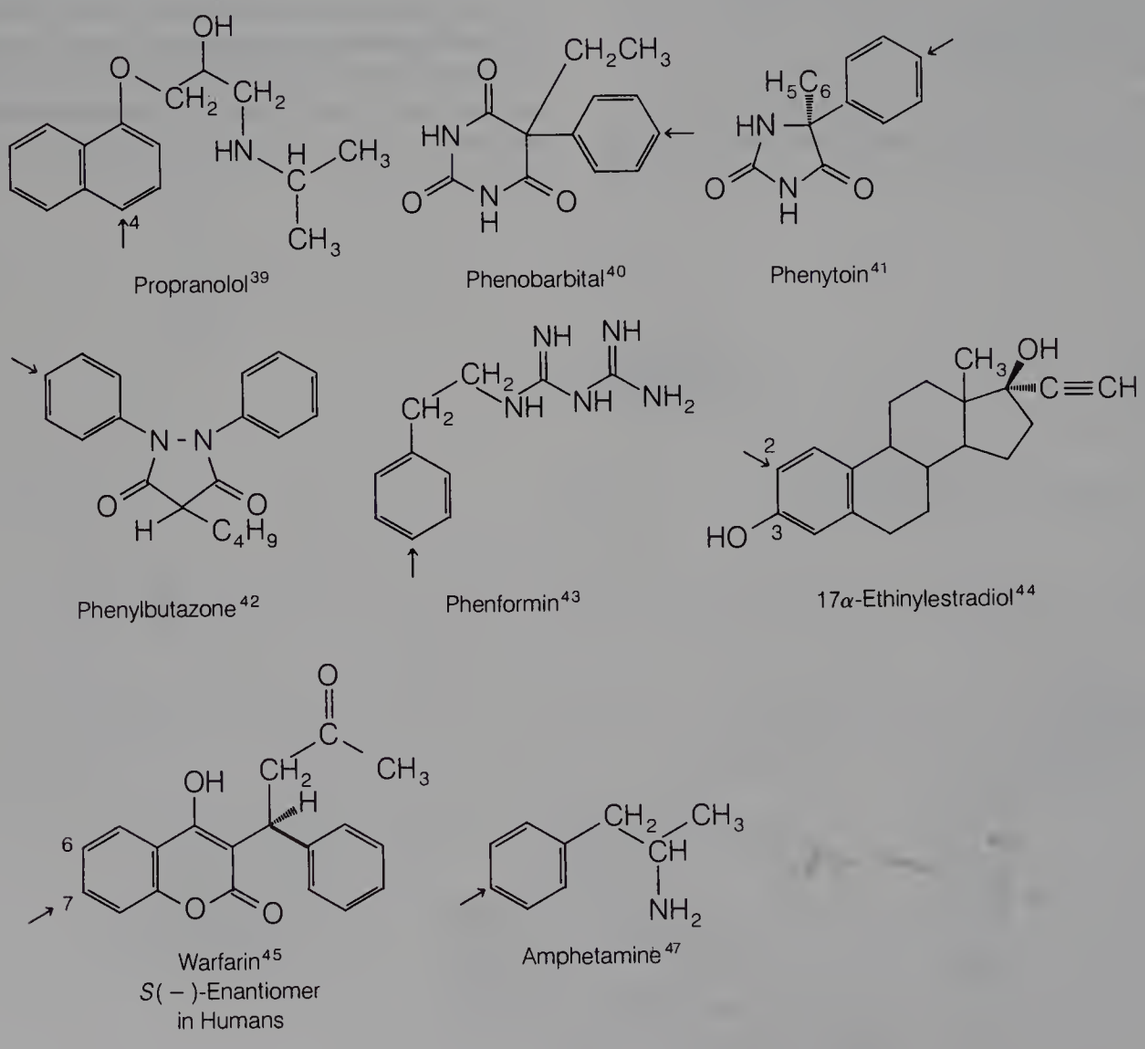
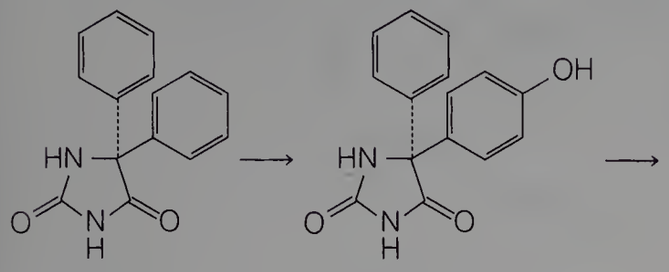
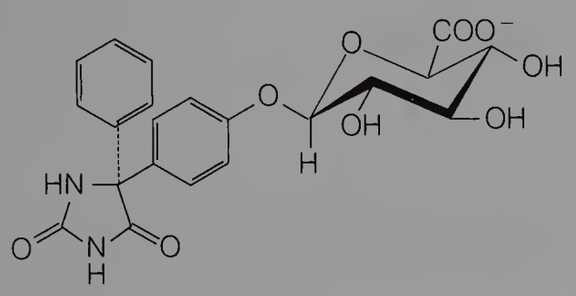


FIG. 3-4. *Examples of drugs and xenobiotics that undergo aromatic hydroxylation in humans. Arrow indicates site of aromatic hydroxylation.*

In contrast, the (*R*)(+)-enantiomer is metabolized by keto reduction[45] (see "Stereochemical Aspects of Drug Metabolism," below).

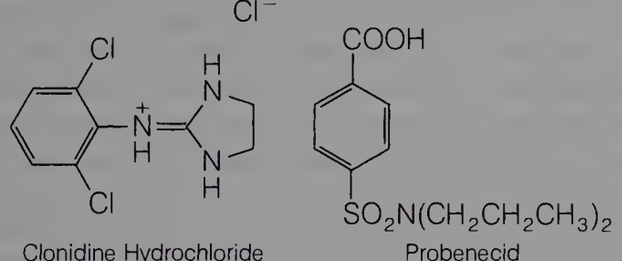


Phenytoin *p*-Hydroxyphenytoin



O-Glucuronide Conjugate

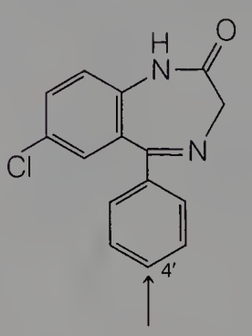
Often, the substituents attached to the aromatic ring may influence the ease of hydroxylation.[46] As a general rule, microsomal aromatic hydroxylation reactions appear to proceed most readily in activated (electron-rich) rings, whereas deactivated aromatic rings (e.g., those containing electron-withdrawing groups Cl, $—\overset{+}{N}R_3$, COOH, SO_2NHR) are generally slow or resistant to hydroxylation. The deactivating groups (Cl, $—\overset{+}{N}H{=}C$) present in the antihypertensive clonidine (Catapres) may explain why this drug undergoes little aromatic hydroxylation in humans.[48] The uricosuric agent probenecid (Benemid), with its electron-withdrawing carboxy and sulfamido groups, has not been reported to undergo any aromatic hydroxylation.[49]



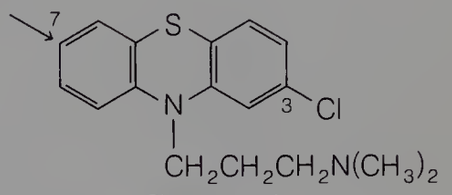
Clonidine Hydrochloride Probenecid

For compounds in which two aromatic rings are present, hydroxylation occurs preferentially in the more electron-rich ring. For example, aromatic hydroxylation of diazepam (Valium) occurs primarily in the more activated ring to yield 4′-hydroxydiazepam.[50] A similar situation is seen in the 7-hydroxylation of the antipsychotic agent chlorpromazine (Thorazine)[51] and in the *para*-hydroxylation of *p*-chlorobiphenyl to *p*′-chloro-*p*′-hydroxybiphenyl.[52]

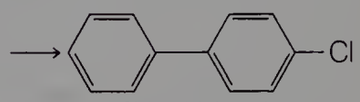
Recent environmental pollutants, such as polychlorinated biphenyls (PCBs) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), have attracted considerable public concern over their toxicity and health hazards. These compounds appear to be resistant to aromatic oxidation because of the numerous electronegative chlorine atoms present in the aromatic rings composing their structures. The metabolic stability coupled to the lipophilicity of these environmental contaminants probably explains their long persistence in the body once absorbed.[53,54]



Diazepam

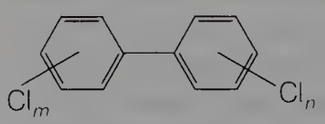


Chlorpromazine



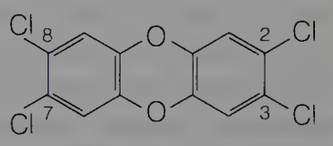
p-Chlorobiphenyl

Arene oxide intermediates are formed when a double bond in aromatic moieties is epoxidized. Arene oxides are of significant toxicologic concern because these intermediates are electrophilic and chemically reactive (owing to the strained three-membered epoxide ring). Detoxification of arene oxides occurs mainly by spontaneous rearrangement to arenols, but enzymatic hydration to *trans*-dihydrodiols and enzymatic conjugation with GSH also play very important roles (Fig. 3-5).[37,38] If not effectively detoxified by the first three pathways in Fig. 3-5, arene oxides will bind covalently with nucleophilic groups present on proteins, DNA, and RNA, thereby leading to serious cellular toxicity.[5,37]



Polychlorinated Biphenyl Mixtures

The number of chlorine atoms (*m*, *n*) present in two aromatic rings varies considerably



2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)

Quantitatively, the most important detoxification reaction for arene oxides is the spontaneous rearrangement to the corresponding arenols. Many times, this rearrangement is accompanied by a novel intramolecular hydride (deuteride) migration called the "NIH shift."[55] It was named after the National Institutes of Health (NIH) laboratory in Bethesda, Maryland, where this process was discovered. The general features of the NIH shift are illustrated with the mixed function aromatic oxidation of 4-deuterioanisole to 3-deuterio-4-hydroxyanisole in Fig. 3-6.[56]

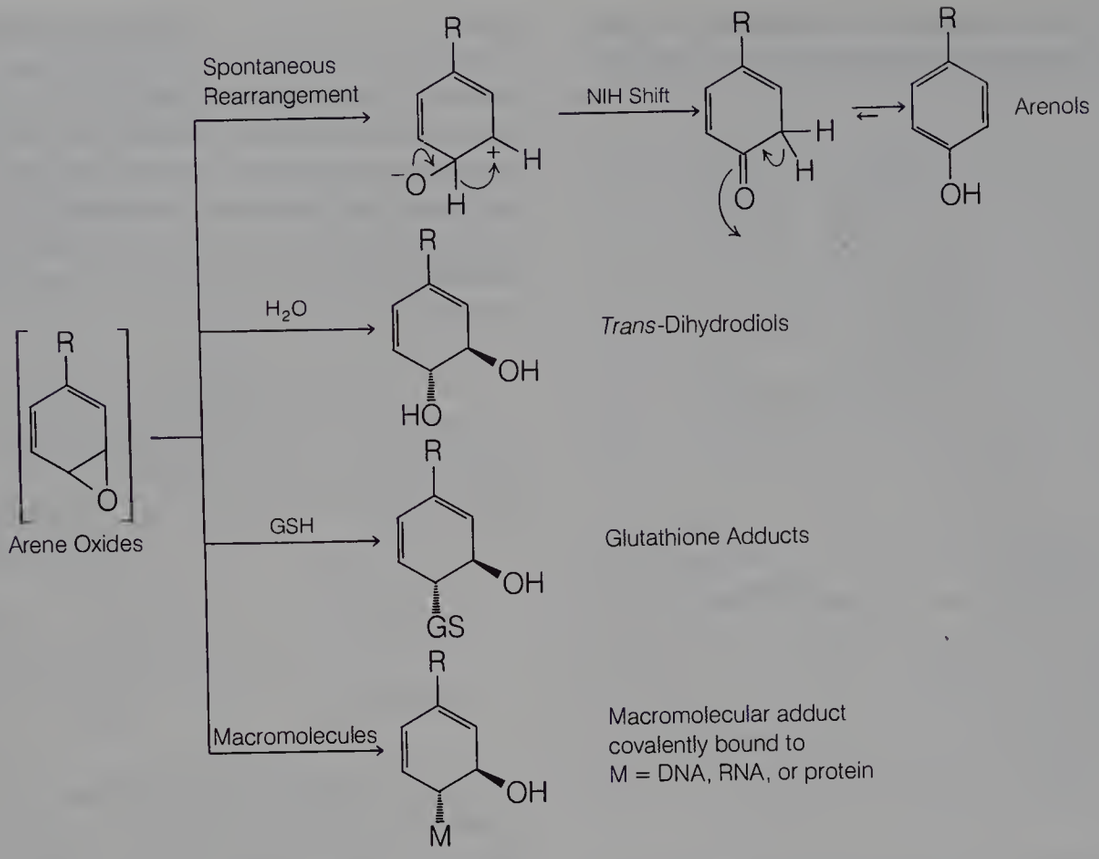


FIG. 3-5. *Possible reaction pathways for arene oxides.*[37,38]

After its metabolic formation, the arene oxide ring opens in the direction that generates the most resonance-stabilized carbocation (positive charge on C-3 carbon is resonance-stabilized by the OCH_3 group). The zwitterionic species (positive charge on the C-3 carbon atom and negative charge on the oxygen atom) then undergoes a 1,2-deuteride shift (NIH shift) to form the dienone. Final transformation of the dienone to 3-deuterio-4-hydroxyanisole occurs with the preferential loss of a proton because of the weaker bond energy of the C—H bond (as compared with the C—D bond). Thus, the deuterium is retained in the molecule by undergoing this intramolecular NIH shift. The experimental observation of an NIH shift for aromatic hydroxylation of a drug or xenobiotic is taken as indirect evidence for the involvement of an arene oxide.

In addition to the NIH shift, the zwitterionic species may undergo direct loss of D^+ to generate 4-hydroxyanisole, in which there is no retention of deuterium (Fig. 3-6). The alternative pathway (direct loss of D^+) may be more favorable than the NIH shift in some aromatic oxidation reactions. Therefore, depending on the substituent group on the arene, some aromatic hydroxylation reactions do not display any NIH shift.

Two extremely important enzymatic reactions also aid in neutralizing the reactivity of arene oxides. The first of these involves the hydration (i.e., nucleophilic attack of water on the epoxide) of arene oxides to yield inactive *trans*-dihydrodiol metabolites (Fig. 3-5). This reaction is catalyzed by microsomal enzymes called "epoxide hydrases."[57] Often, epoxide hydrase inhibitors, such as cyclohexene oxide and 1,1,1-trichloropropene-2,3-oxide, have been utilized to demonstrate the detoxification role of these enzymes. Addition of these inhibitors is accompanied frequently by an increase in toxicity of the arene oxide being tested because formation of nontoxic dihydrodiols is blocked. For example, the mutagenicity of benzo[*a*]pyrene-4,5-oxide, as measured by the Ames *Salmonella typhimurium* test system, is potentiated when cyclohexene oxide is added.[58] Dihydrodiol metabolites have been reported in the metabolism of several aromatic hydrocarbons (e.g., naphthalene, benzo[*a*]pyrene, and other related polycyclic aromatic hydrocarbons).[37] A few drugs (e.g., phenytoin,[59] phenobarbital,[60] glutethimide[61]) also have been observed to yield dihydrodiol products as minor metabolites in humans. Dihydrodiol products are susceptible to conjugation with glucuronic acid, as well as an enzymatic dehydrogenation

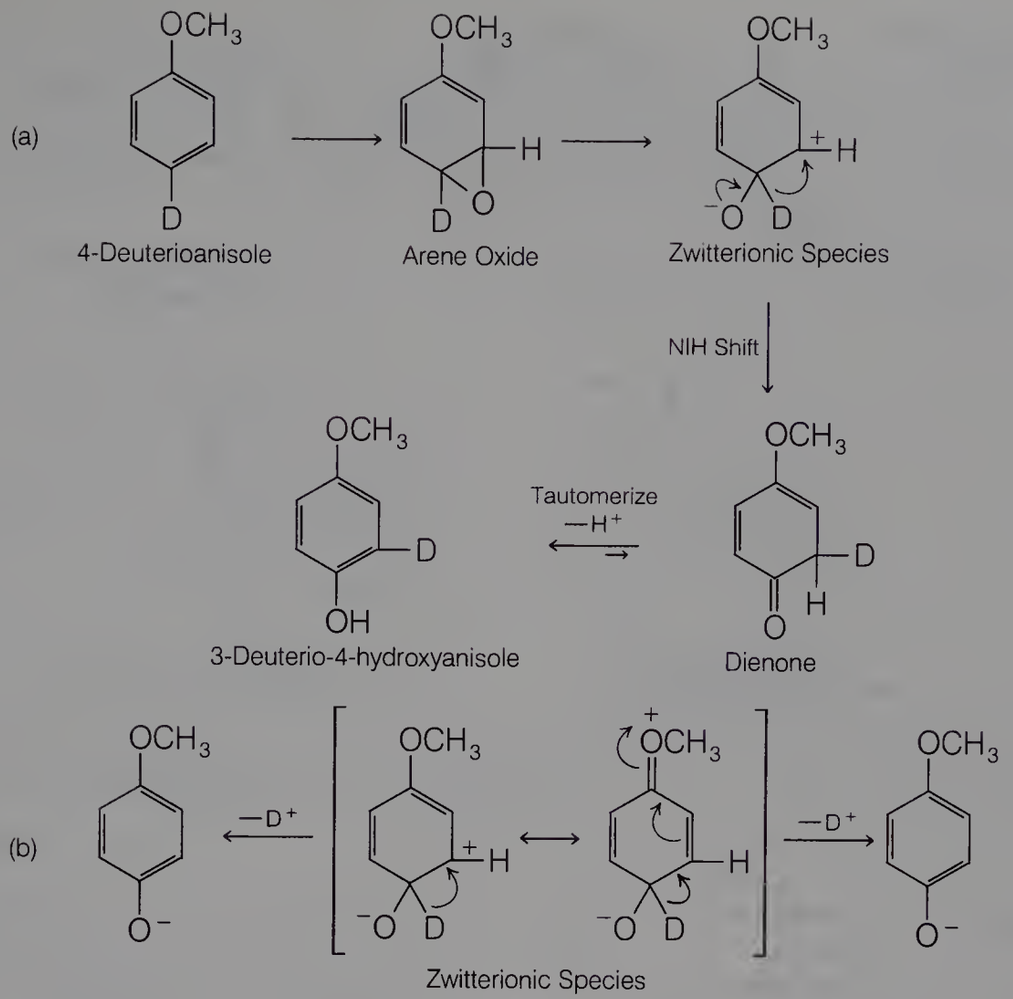
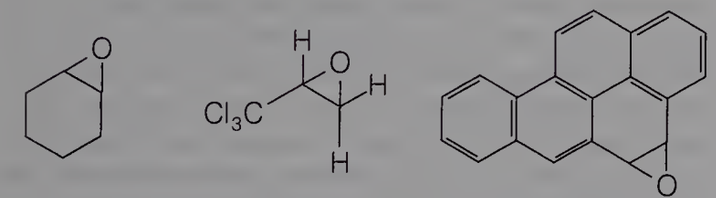


FIG. 3-6. **(a)** *General features of the NIH shift or 1,2-hydride (deuteride) shift in the mixed function oxidation of 4-deuterioanisole to 3-deuterio-4-hydroxyanisole.* **(b)** *Direct loss of D^+ from zwitterionic species, leading to no retention of deuterium in 4-hydroxyanisole.*

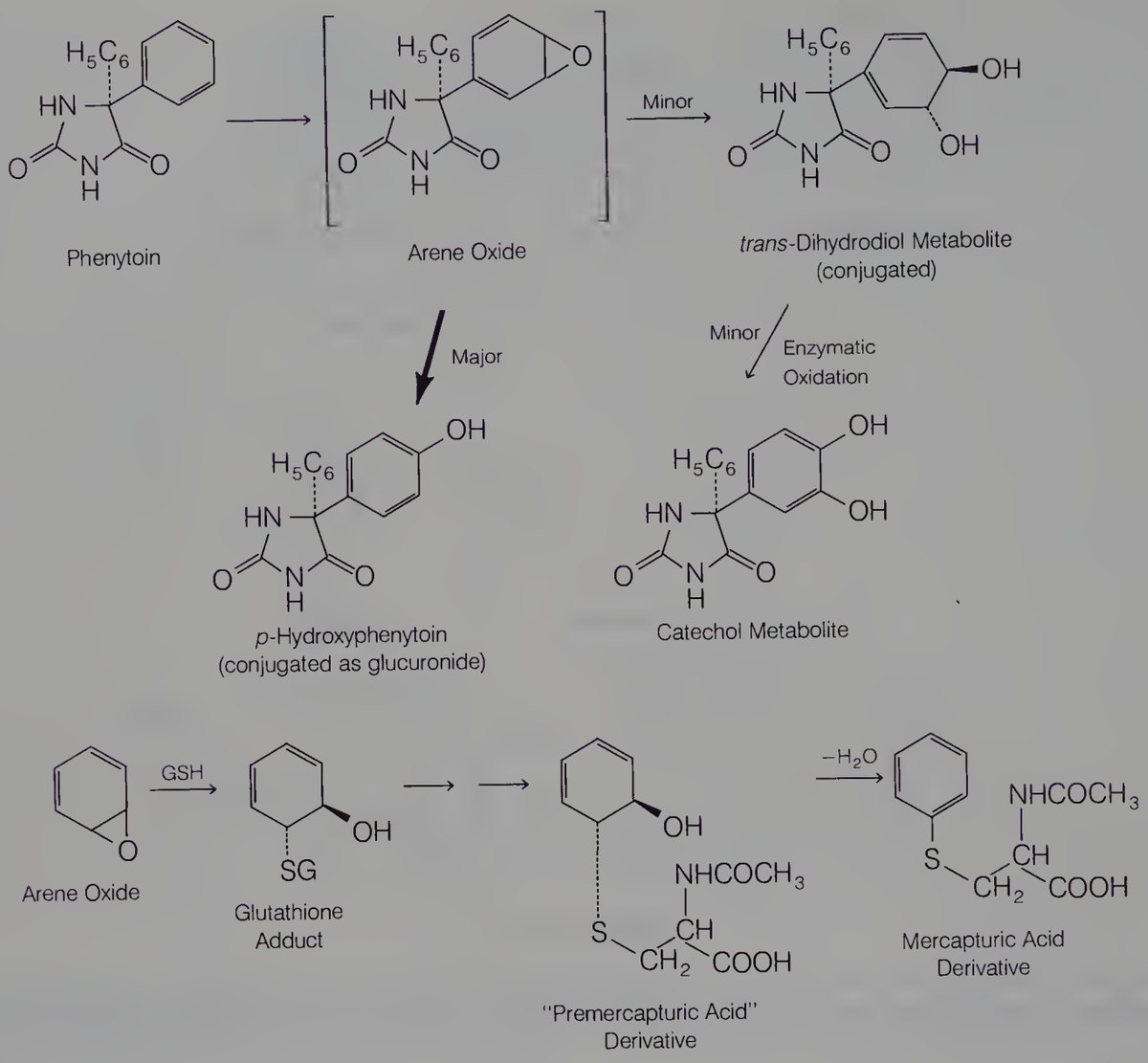
to the corresponding catechol metabolite, as exemplified by the metabolism of phenytoin.[59]



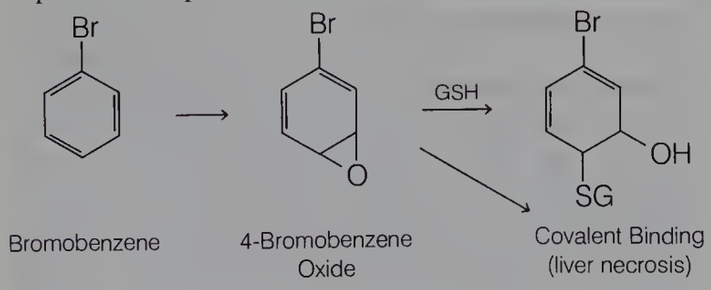
Cyclohexane oxide | 1,1,1-Trichloropropene 2,3-oxide | Benzo[*a*]pyrene 4,5-oxide

A second enzymatic reaction involves nucleophilic ring opening of the arene oxide by the sulfhydryl group present in GSH to yield the corresponding *trans*-1,2-dihydro-1-*S*-glutathionyl-2-hydroxy adduct, or GSH adduct (Fig. 3-5).[37] The reaction is catalyzed by various GSH *S*-transferases.[62] Because GSH is found in practically all mammalian tissues, it plays an important role in the detoxification not only of arene oxides but also of a variety of other chemically reactive and potentially toxic intermediates. Initially, GSH adducts formed from arene oxides are modified in a series of reactions to yield "premercapturic acid" or mercapturic acid metabolites.[63] Since it is classified as a phase II pathway, GSH conjugation will be covered in greater detail in a later section.

Because of their electrophilic and reactive nature, arene oxides also may undergo spontaneous reactions with nucleophilic functionalities present on biomacromolecules.[38] Such reactions lead to modified protein, DNA, and RNA structures and often cause dramatic alterations in how these macromolecules function. Much of the cytotoxicity and irreversible lesions caused by arene oxides are presumed to be the result of their covalent binding to cellular components. Several well-established examples of reactive arene oxides that cause serious toxicity are presented in the following.



Administration of bromobenzene to rats causes severe liver necrosis.[64] Extensive in vivo and in vitro studies indicate that the liver damage results from the interaction of a chemically reactive metabolite, 4-bromobenzene oxide, with hepatocytes.[65] Extensive covalent binding to hepatic tissue was confirmed using radiolabeled bromobenzene. The severity of necrosis correlated well with the amount of covalent binding to hepatic tissue. It was demonstrated, with diethyl maleate or large doses of bromobenzene in rats, that the depletion of hepatic GSH led to more severe liver necrosis.



Polycyclic aromatic hydrocarbons are ubiquitous environmental contaminants that are formed from auto emission, refuse burning, industrial processes, cigarette smoke, and other combustion processes. Benzo[*a*]pyrene, a potent carcinogenic agent, is perhaps the most extensively studied of the polycyclic aromatic hydrocarbons.[66] From inspection of its structure, aromatic hydroxylation of benzo[*a*]pyrene obviously can occur at a number of positions. The identification of several dihydrodiol metabolites is viewed as indirect evidence for the formation and involvement of arene oxides in the metabolism of benzo[*a*]pyrene. Although certain arene oxides of benzo[*a*]pyrene (e.g., 4,5-oxide, 7,8-oxide, 9,10-oxide) appear to display some mutagenic and tumorigenic activity, it does not appear that they represent the ultimate reactive species responsible for benzo[*a*]pyrene's carcinogenicity. In recent years, extensive studies have led to the characterization of a specific sequence of metabolic reactions (Fig. 3-7) that give rise to a highly reactive intermediate that covalently binds to DNA. Metabolic activation of benzo[*a*]-pyrene to the ultimate carcinogenic species involves an initial epoxidation reaction to form the 7,8-oxide, which is then converted by epoxide hydrase to (−)-7(*R*),8(*R*)-dihydroxy-7,8-dihydrobenzo[*a*]pyrene.[67] The two-step enzymatic formation of this *trans*-dihydrodiol is stereospecific. Subsequent epoxidation at the 9,10-double bond of the latter metabolite

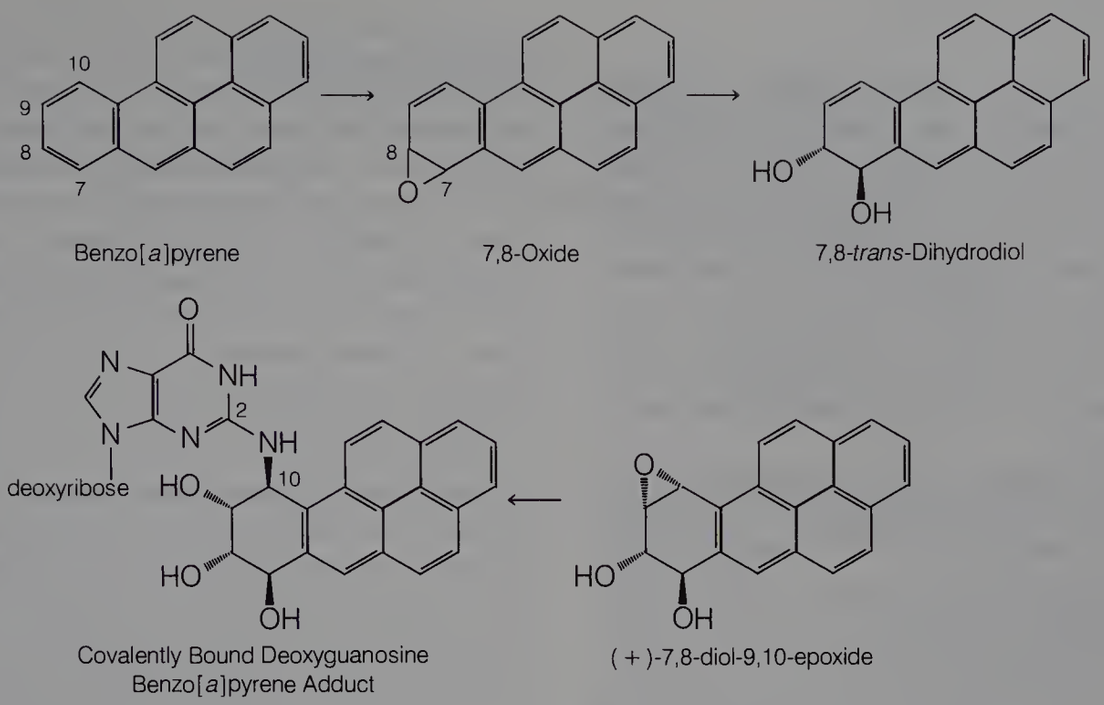
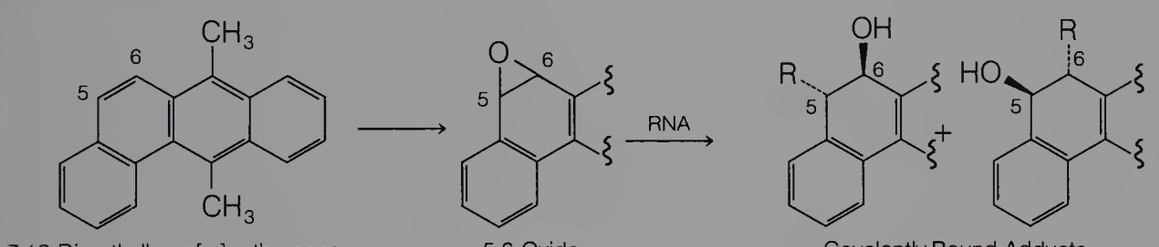
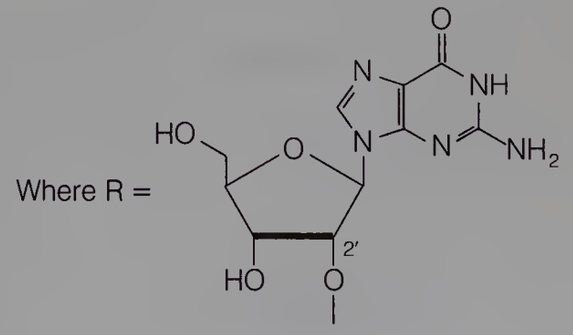


FIG. 3-7. *Metabolic sequence leading to the formation of the ultimate carcinogenic species of benzo[a]pyrene. (+)−7R,8S-dihydroxy-9R,10R-oxy- 7,8,9,10-tetrahydrobenzo[a]pyrene or (+)-7,8-diol-9,10-epoxide.*

generates predominantly (+)-7(*R*),8(*S*)-dihydroxy-9(*R*), 10(*R*)-oxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene or (+)7,8-diol-9,10-epoxide. It is this key electrophilic diol epoxide metabolite that readily reacts with DNA to form many covalently bound adducts.[68,69] Careful degradation studies have shown that the principal adduct involves attack of the C-2 amino group of deoxyguanosine at C-10 of the diol epoxide. Clearly, these reactions are responsible for genetic code alterations that ultimately lead to the malignant transformations. Covalent binding of the diol epoxide metabolite to deoxyadenosine and to deoxycytidine also has been established.[69]

Another carcinogenic polycyclic aromatic hydrocarbon, 7,12-dimethylbenz[*a*]anthracene, also forms covalent adducts to nucleic acids (RNA).[70] The ultimate carcinogenic reactive species apparently is the 5,6-oxide that results from epoxidation of the 5,6-double bond in this aromatic hydrocarbon. The arene oxide intermediate binds covalently to guanosine residues of RNA to yield the two adducts shown below.





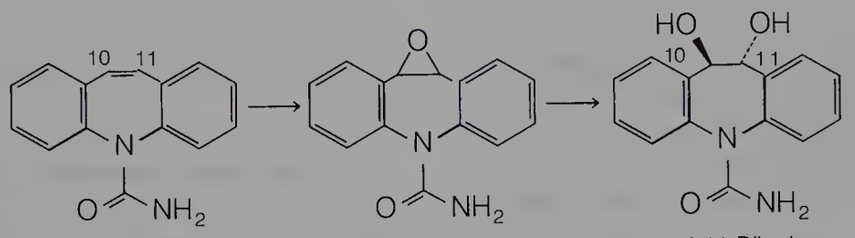
OXIDATION OF OLEFINS

The metabolic oxidation of olefinic carbon–carbon double bonds leads to the corresponding epoxide (or oxirane). Epoxides derived from olefins generally tend to be somewhat more stable than the arene oxides formed from aromatic compounds. A few epoxides are stable enough that they are directly measurable in biologic fluids (e.g., plasma, urine). As with their arene oxide counterparts, epoxides are susceptible to enzymatic hydration by epoxide hydrase to form *trans*-1,2-dihydrodiols (also called 1,2-diols or 1,2-dihydroxy compounds).[57] In addition, several epoxides undergo GSH conjugation.[71]

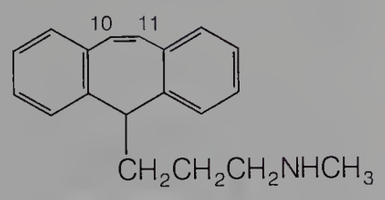
A well-known example of olefinic epoxidation is the metabolism, in humans, of the anticonvulsant drug carbamazepine (Tegretol) to carbamazepine-10,11-epoxide.[72] The epoxide is reasonably stable and can be measured quantitatively in the plasma of patients receiving the parent drug. The epoxide metabolite may have marked anticonvulsant activity and, therefore, may contribute substantially to the therapeutic effect of the parent drug.[73] Subsequent hydration of the epoxide produces 10,11-dihydroxycarbamazepine, an important urinary metabolite (10% to 30%) in humans.[72]

Epoxidation of the olefinic 10,11-double bond in the antipsychotic agent protriptyline (Vivactil)[74] and in the H_1-histamine antagonist cyproheptadine (Periactin)[75] also has been demonstrated. Frequently, the epoxides formed from the biotransformation of an olefinic compound are minor products, owing to their further conversion to the corresponding 1,2-diols. For example, dihydroxyalcofenac is a major human urinary metabolite of the anti-inflammatory agent alcofenac.[76] However, the epoxide metabolite from which it is derived is present in minute amounts. The presence of the dihydroxy metabolite (called secodiol) of secobarbital but not the epoxide product has been reported in humans.[77]

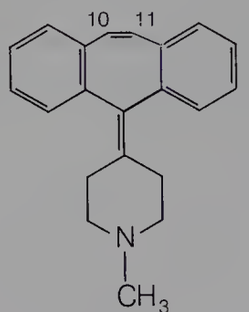
Indirect evidence for the formation of epoxides comes also from the isolation of GSH or mercapturic acid metabolites. After administration of styrene to rats, two urinary metabolites have been identified as the isomeric mercapturic acid derivatives resulting from nucleophilic attack of GSH on the intermediate epoxide.[78] In addition, styrene oxide covalently binds to rat liver microsomal proteins and nucleic acids.[79] These results indicate that styrene oxide is relatively reactive toward nucleophiles (e.g., GSH and nucleophilic groups on protein and nucleic acids).



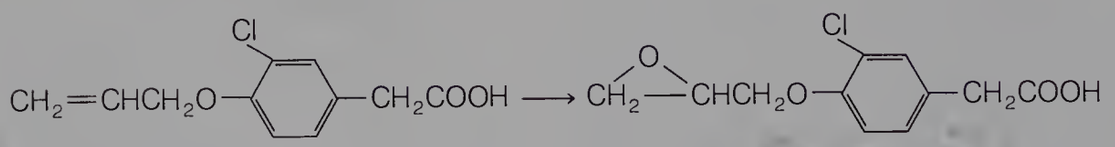
Carbamazepine — Carbamazepine-10,11-epoxide — *trans*-10,11-Dihydroxy-carbamazepine



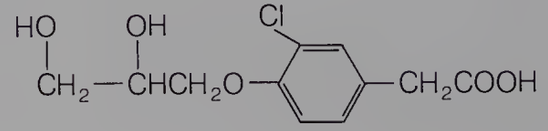
Protriptyline



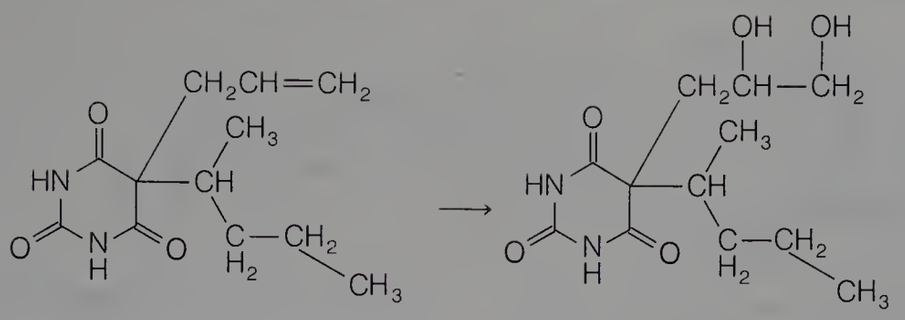
Cyproheptadine



Alcofenac — Alcofenac Epoxide



Dihydroxyalcofenac



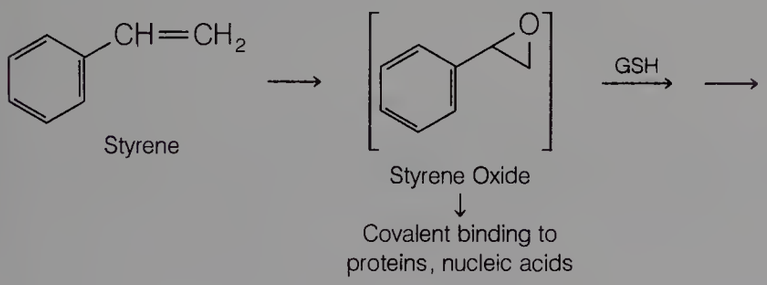
Secobarbital

Secodiol
5-(2,3-Dihydroxypropyl)-5-
(1-methylbutyl)-barbituric Acid

There apparently are divers metabolically generated epoxides that display similar chemical reactivity toward nucleophilic functionalities. Accordingly, the toxicity of some olefinic compounds may be a consequence of their metabolic conversion to chemically reactive epoxides.[80] One example that clearly links metabolic epoxidation as a biotoxification pathway involves aflatoxin B_1. This naturally occurring carcinogenic agent contains an olefinic (C2–C3) double bond adjacent to a cyclic ether oxygen. The hepatocarcinogenicity of aflatoxin B_1 has been clearly linked to its metabolic oxidation to the corresponding 2,3-oxide, which is extremely reactive.[81] Extensive in vitro and in vivo metabolic studies indicate that this 2,3-oxide binds covalently to DNA, RNA, and proteins. A major DNA adduct has been isolated and characterized as 2,3-dihydro-2-(N^7-guanyl)-3-hydroxyaflatoxin B_1.[82]

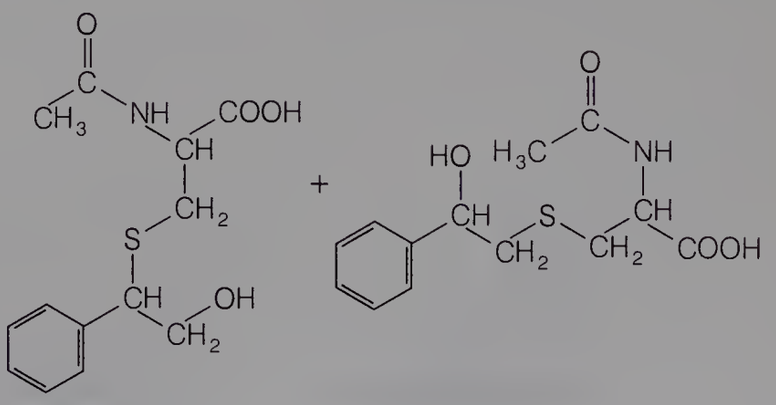
Other olefinic compounds, such as vinyl chloride,[83] stilbene,[84] and the carcinogenic estrogenic agent diethylstilbestrol (DES),[85] undergo metabolic epoxidation. The corresponding epoxide metabolites may be the reactive species responsible for mediating the cellular toxicity seen with these compounds.

An interesting group of olefin-containing compounds causes the destruction of cytochrome P-450.[86] Compounds belonging to this group include allylisopropylacetamide,[87] secobarbital,[88] and the volatile anesthetic agent fluroxene.[89] It is believed that the olefinic moiety present in these compounds is activated metabolically by cytochrome P-450 to form a very reactive intermediate that covalently binds to the heme portion of cytochrome P-450.[90] The abnormal heme derivatives, or "green pigments," that result from this covalent interaction have been characterized as *N*-alkylated protoporphyrins in which the *N*-alkyl moiety is derived directly from the olefin administered.[86,90] Chronic administration of the foregoing three agents is expected to lead to inhibition of oxidative drug metabolism, potential drug interactions, and prolonged pharmacologic effects.



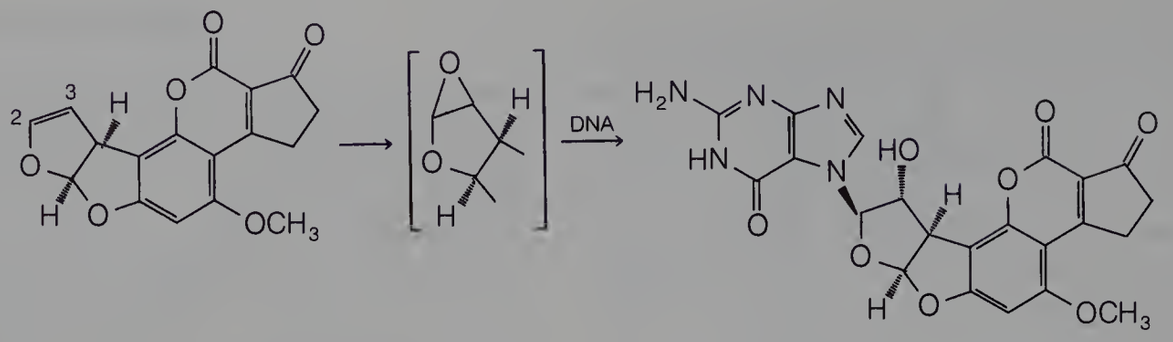
Styrene

Styrene Oxide
↓
Covalent binding to
proteins, nucleic acids

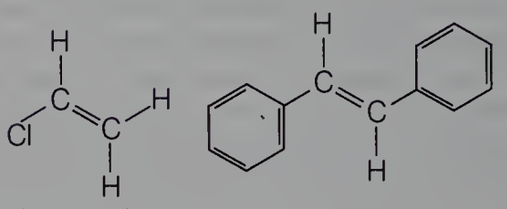


Mercapturic Acid
Derivative (major)

Mercapturic Acid
Derivative (minor)

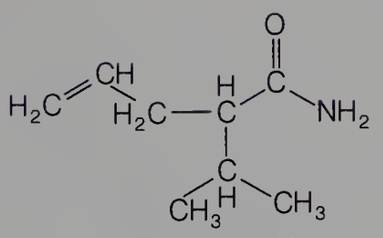


Aflatoxin B_1 | 2,3-Expoxide | 2,3-Dihydro-2-(N^7-guanyl)-3-hydroxyaflatoxin B_1

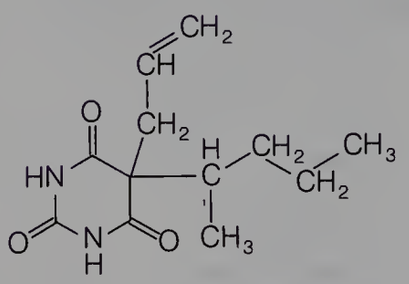


Vinyl chloride | Stilbene

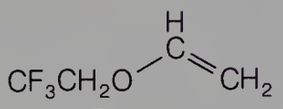
Diethylstilbestrol (DES)



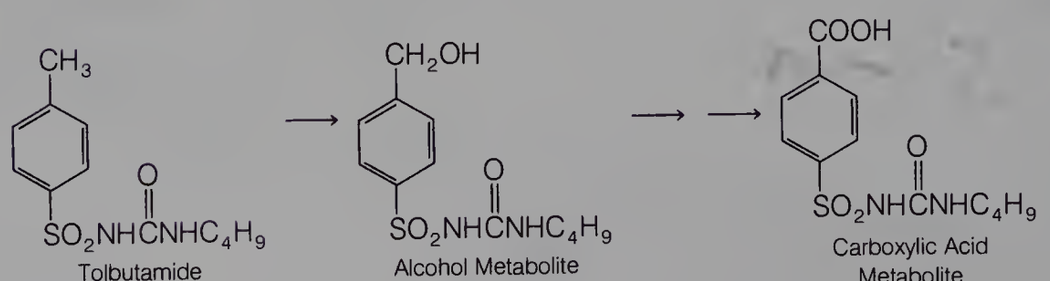
Allylisopropylacetamide



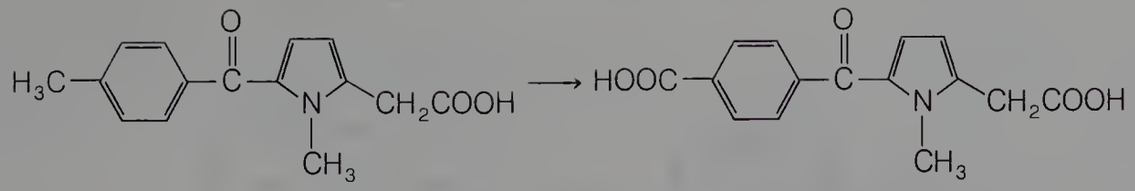
Secobarbital



Fluroxene



Tolbutamide | Alcohol Metabolite | Carboxylic Acid Metabolite



Tolmetin — Dicarboxylic Acid Metabolite

OXIDATION AT BENZYLIC CARBON ATOMS

Carbon atoms attached to aromatic rings (benzylic position) are susceptible to oxidation, thereby forming the corresponding alcohol (or carbinol) metabolite.[47] Primary alcohol metabolites often are oxidized further to aldehydes and carboxylic acids ($CH_2OH \rightarrow CHO \rightarrow COOH$), and secondary alcohols are converted to ketones by soluble alcohol and aldehyde dehydrogenases.[91] Alternatively, the alcohol may be conjugated directly with glucuronic acid.[92] The benzylic carbon atom present in the oral hypoglycemic agent tolbutamide (Orinase) is oxidized extensively to the corresponding alcohol and carboxylic acid. Both metabolites have been isolated in the urine of humans.[93] Similarly, the "benzylic" methyl group in the anti-inflammatory agent tolmetin (Tolectin) undergoes oxidation to yield the dicarboxylic acid product as the major metabolite in humans.[94] The sedative hypnotic agent methaqualone has been observed to undergo benzylic oxidation at its C-2′ methyl group to give 2′-hydroxymethylmethaqualone as a minor metabolite.[95] Benzylic hydroxylation occurs to a significant extent in the metabolism of the β-adrenergic blocker metoprolol (Lopressor) to yield α-hydroxymetoprolol.[96] Additional examples of drugs and xenobiotics undergoing benzylic oxidation are shown in Fig. 3-8.

OXIDATION AT ALLYLIC CARBON ATOMS

Microsomal hydroxylation at allylic carbon atoms is commonly observed in drug metabolism. An illustrative example of allylic oxidation is given by the psychoactive component of marijuana, Δ^1-THC. This molecule contains three allylic carbon centers (C-7, C-6, and C-3). Allylic hydroxylation occurs extensively at C-7 to yield 7-hydroxy-Δ^1-THC as the major plasma metabolite in humans.[10] Pharmacologic studies show that this 7-hydroxy metabolite is as active or even more active than Δ^1-THC itself and may contribute significantly to the overall central nervous system psychotomimetic effects of the parent compound.[103] Hydroxylation also occurs to a minor extent at the allylic C-6 position to give both the epimeric 6α- and 6β-hydroxy metabolites.[10] Metabolism does not occur at C-3, presumably because of steric hindrance.

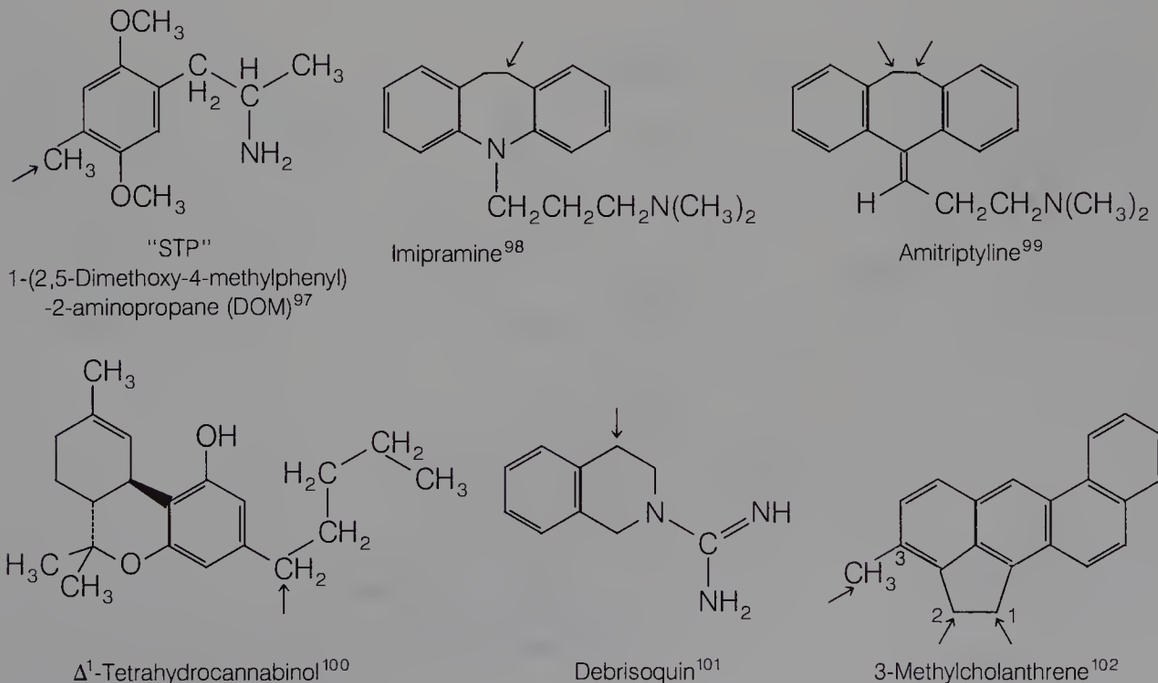
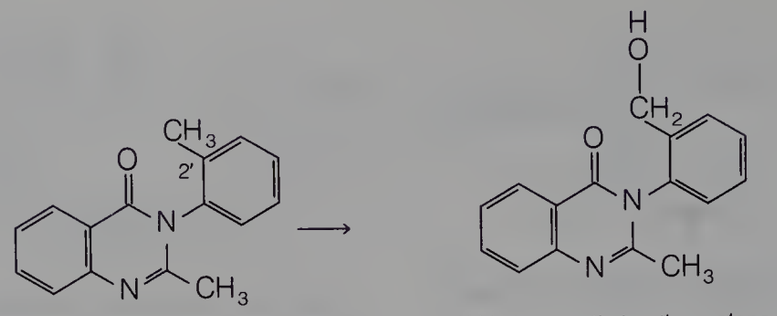
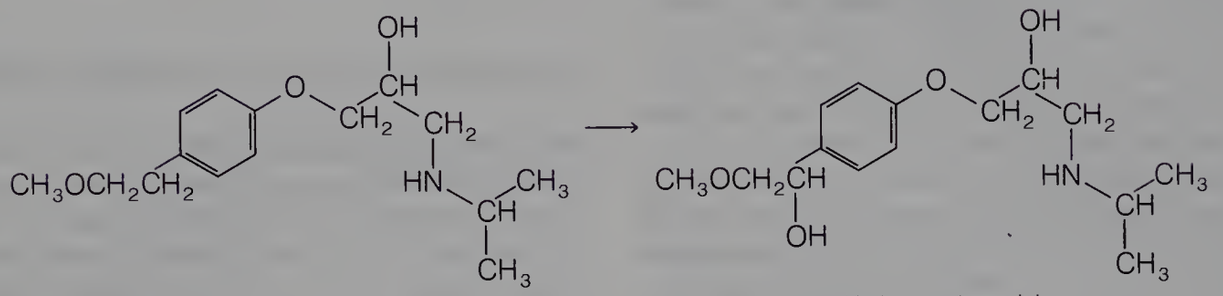


FIG. 3-8. *Examples of drugs and xenobiotics undergoing benzylic hydroxylation. Arrow indicates site of hydroxylation.*



Methaqualone

2'-Hydroxymethylmethaqualone



Metroprolol

α-Hydroxymetroprolol

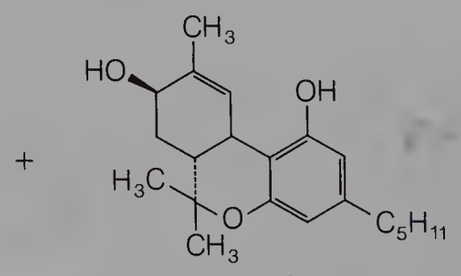
The antiarrhythmic agent quinidine is metabolized by allylic hydroxylation to 3-hydroxyquinidine, the principal plasma metabolite found in humans.[104] This metabolite shows significant antiarrhythmic activity in animals and possibly in humans.[105]

Other examples of allylic oxidation include the sedative hypnotic hexobarbital (Sombulex) and the analgesic pentazocine (Talwin). The 3′-hydroxylated metabolite formed from hexobarbital is susceptible to glucuronide conjugation as well as further oxidation to the 3′-oxo compound.[106] Hexobarbital is a chiral barbiturate derivative that exists in two enantiomeric forms. Studies in humans indicate that the pharmacologically less active (*R*)(−)-enantiomer is metabolized more rapidly than its (*S*)(+)-isomer.[107] Pentazocine undergoes allylic hydroxylation at the two terminal methyl groups of its *N*-butenyl side chain to yield either the *cis* or *trans* alcohol metabolites shown in the diagrams. In humans, greater amounts of the *trans* alcohol are formed.[108]

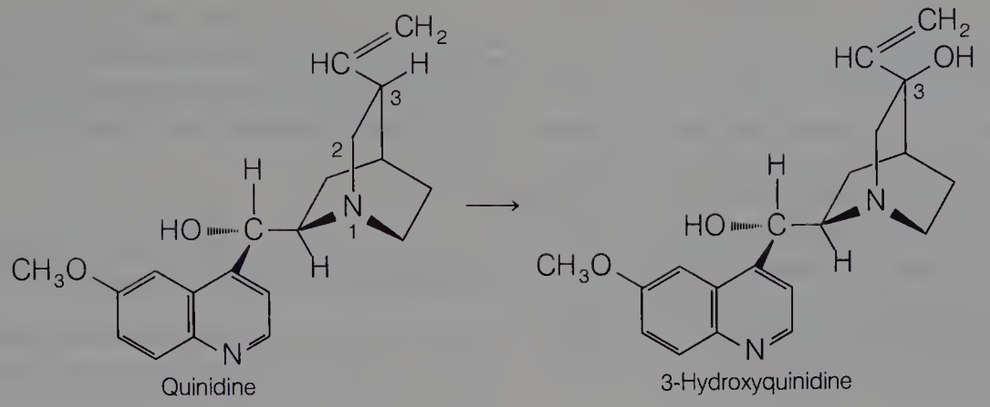
Δ¹-THC

7-Hydroxy-Δ¹-THC

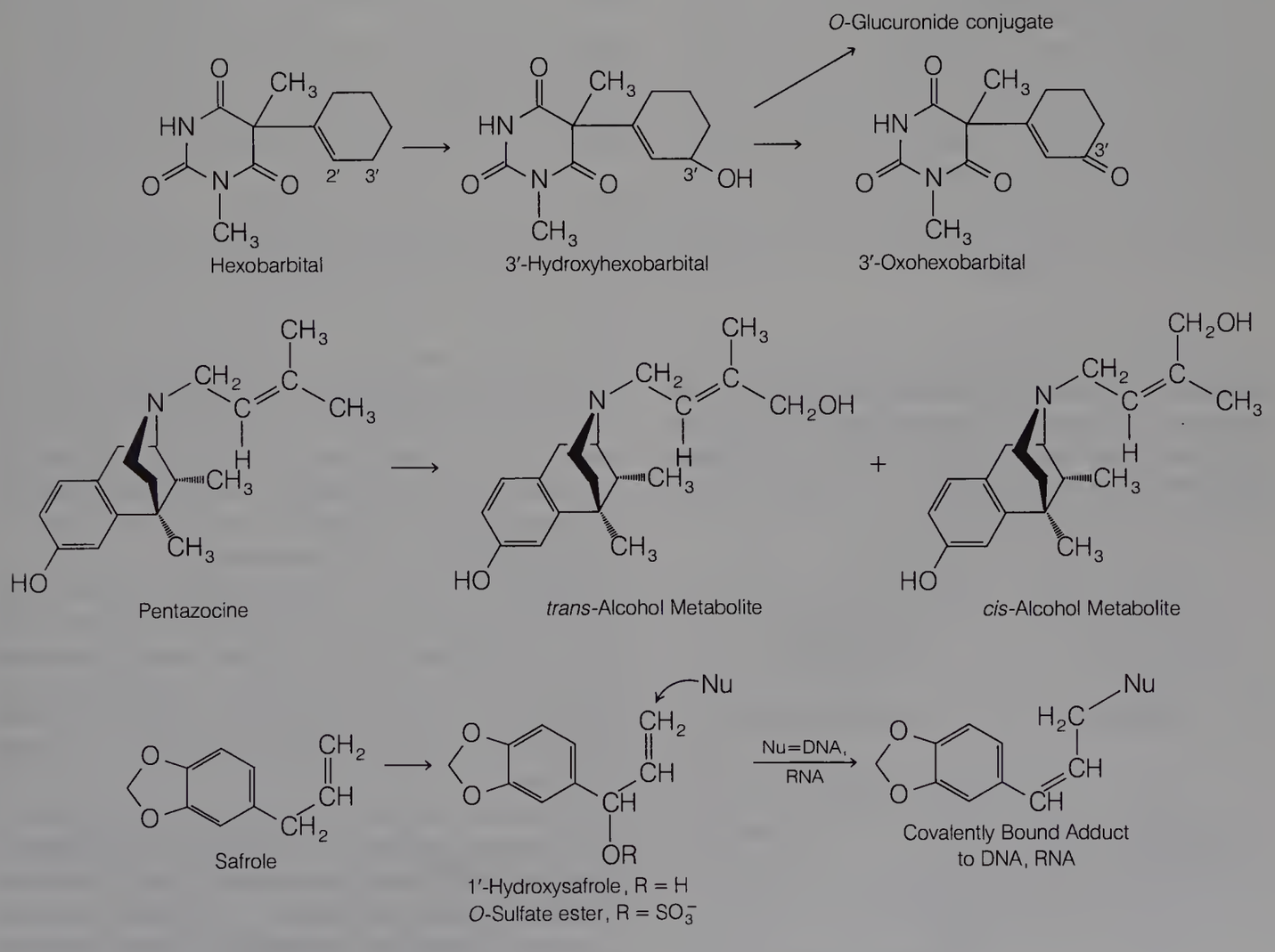
6α-Hydroxy-Δ¹-THC



6β-Hydroxy-Δ¹-THC



For the hepatocarcinogenic agent safrole, allylic hydroxylation is involved in a bioactivation pathway leading to the formation of chemically reactive metabolites.[109] This process involves initial hydroxylation at the C-1′ carbon of safrole. It should be noted that this center is both allylic and benzylic. The hydroxylated metabolite then undergoes further conjugation to form a sulfate ester. This chemically reactive ester intermediate presumably undergoes nucleophilic displacement reactions with DNA or RNA in vitro to form covalently bound adducts.[110] As shown in the scheme below, nucleophilic attack by DNA, RNA, or other nucleophiles is facilitated by a good leaving group (e.g., SO_4^{2-}) at the C-1′ position. The leaving group tendency of the alcohol OH group itself is not great enough to facilitate displacement reactions. Importantly, allylic hydroxylation generally is not a pathway that leads to the generation of reactive intermediates. Its involvement in the biotoxification of safrole appears to be an exception.



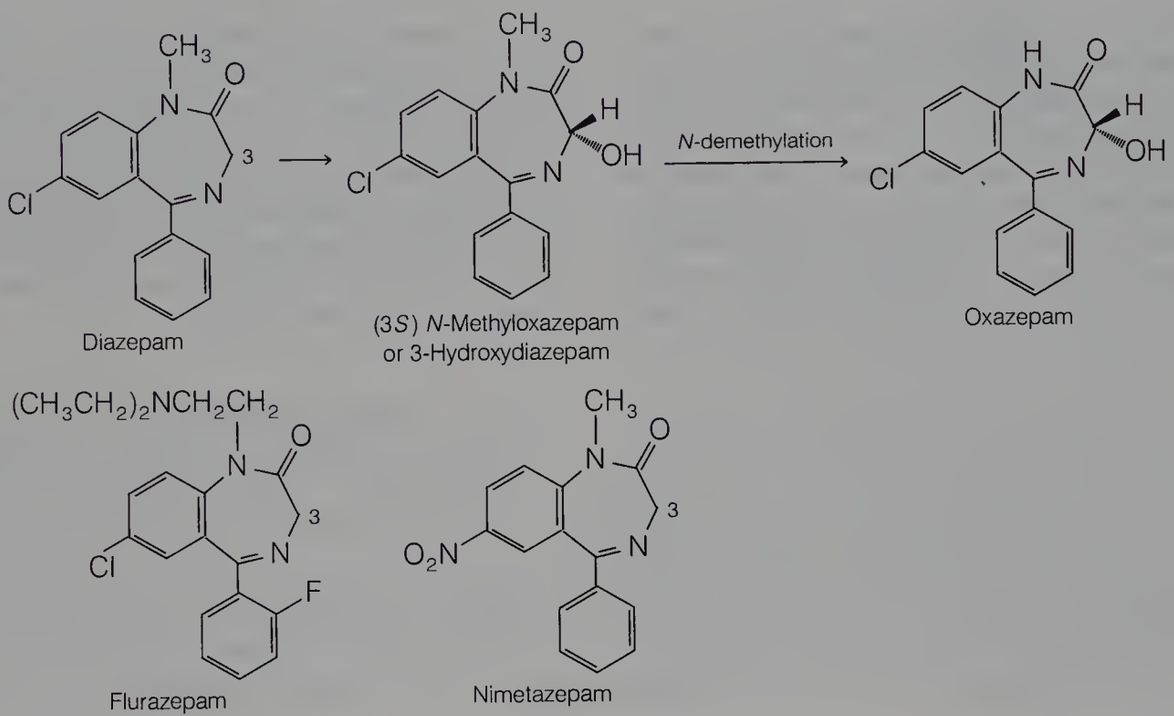
OXIDATION AT CARBON ATOMS α TO CARBONYLS AND IMINES

The mixed function oxidase system also oxidizes carbon atoms adjacent (i.e., α) to carbonyl and imino (C=N) functionalities. An important class of drugs undergoing this type of oxidation is the benzodiazepines. For example, diazepam (Valium), flurazepam (Dalmane), and nimetazepam are oxidized to their corresponding 3-hydroxy metabolites.[111] The C-3 carbon atom undergoing hydroxylation is α to both a lactam carbonyl and an imino functionality.

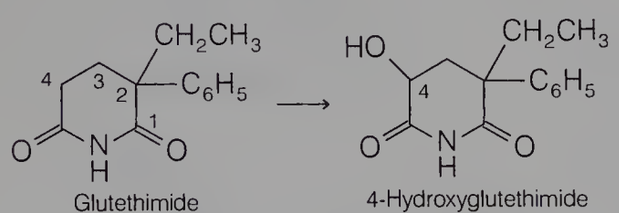
Hydroxylation of the carbon atom α to carbonyl functionalities generally occurs only to a limited extent in drug metabolism. An illustrative example involves the hydroxylation of the sedative hypnotic glutethimide (Doriden) to 4-hydroxyglutethimide.[113]

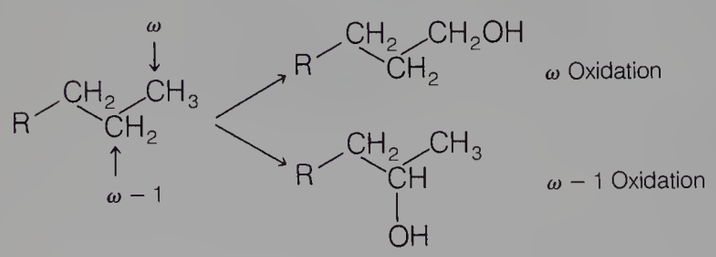
OXIDATION AT ALIPHATIC AND ALICYCLIC CARBON ATOMS

Alkyl or aliphatic carbon centers are subject to mixed function oxidation. Metabolic oxidation at the terminal methyl group often is referred to as *ω-oxidation*, and oxidation of the penultimate carbon atom (i.e., next-to-the-last carbon) is called *ω − 1 oxidation*.[47] The initial alcohol metabolites formed from these enzymatic ω- and ω − 1 oxidations are susceptible to further oxidation to yield aldehyde, ketones, or carboxylic acids. Alternatively, the alcohol metabolites may undergo glucuronide conjugation.



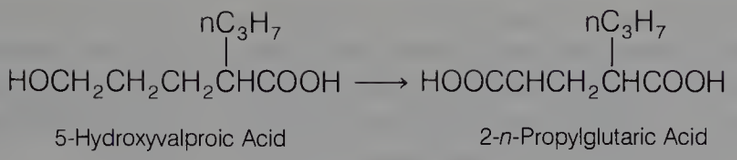
For diazepam, the hydroxylation reaction proceeds with remarkable stereoselectivity to form primarily (90%) 3-hydroxydiazepam (also called *N*-methyloxazepam) having the (*S*) absolute configuration at C-3.[112] Further *N*-demethylation of the latter metabolite gives rise to the pharmacologically active 3(*S*)(+)-oxazepam.

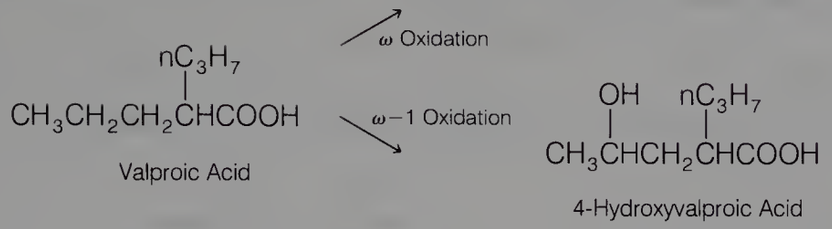


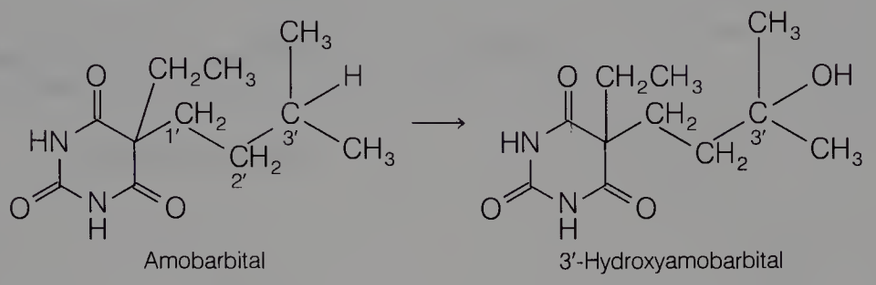


Aliphatic ω- and ω − 1 hydroxylations commonly take place in drug molecules having straight or branched alkyl chains. For example, the antiepileptic agent valproic acid (Depakene) undergoes both ω- and ω − 1 oxidation to the 5-hydroxy and 4-hydroxy metabolites, respectively.[114] Further oxidation of the 5-hydroxy metabolite yields 2-*n*-propylglutaric acid.

Numerous barbiturates and oral hypoglycemic sulfonylureas also have aliphatic side chains that are susceptible to oxidation. For example, the sedative hypnotic amobarbital (Amytal) undergoes extensive ω − 1 oxidation to the corresponding 3′-hydroxylated metabolite.[115] Other barbiturates, such as pentobarbital,[116] thiamylal,[117] and secobarbital,[77]





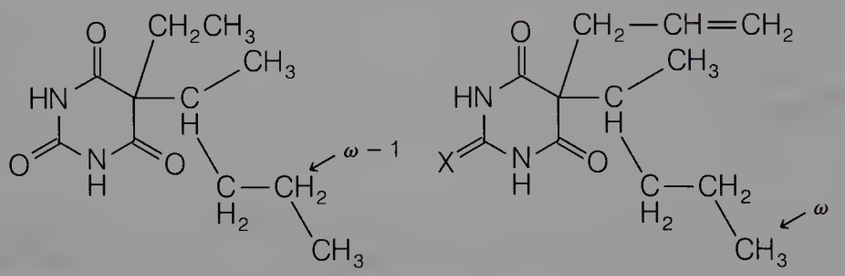


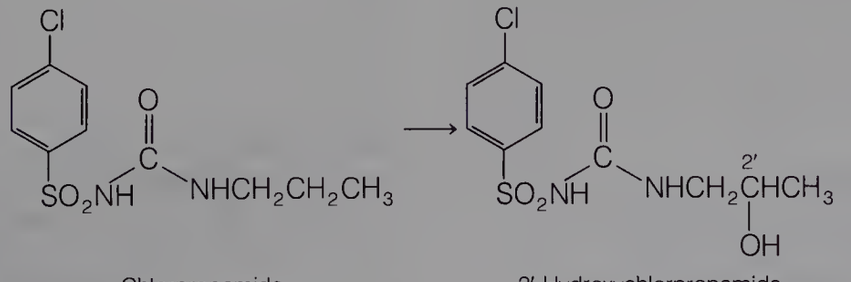
have been reported to be metabolized by way of ω and $\omega - 1$ oxidation.

The *n*-propyl side chain attached to the oral hypoglycemic agent chlorpropamide (Diabinese) undergoes extensive $\omega - 1$ hydroxylation to yield the secondary alcohol 2′-hydroxychlorpropamide as a major urinary metabolite in humans.[118]

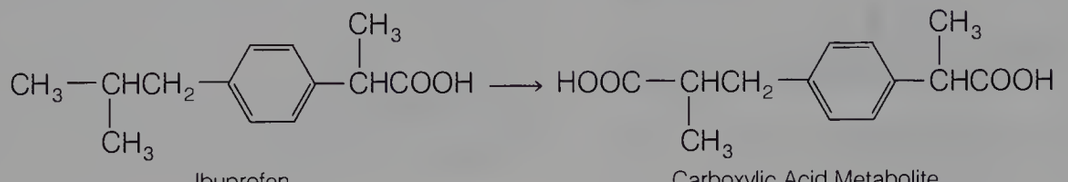
Omega and $\omega - 1$ oxidation of the isobutyl moiety present in the anti-inflammatory agent ibuprofen (Motrin) yield the corresponding carboxylic acid and tertiary alcohol metabolites.[119] Additional examples of drugs reported to undergo aliphatic hydroxylation include meprobamate,[120] glutethimide,[113] ethosuximide,[121] and phenylbutazone.[122]

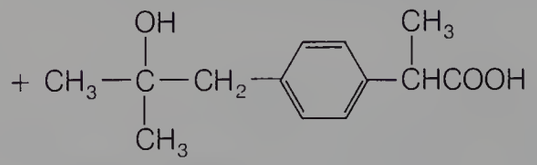
The cyclohexyl group is commonly found in many medicinal agents and is also susceptible to mixed function oxidation (alicyclic hydroxylation).[47] Enzymatic introduction of a hydroxyl group into a monosubstituted cyclohexane ring generally occurs at C-3 or C-4 and can lead to *cis* and *trans* conformational stereoisomers, as shown in the diagrammed scheme.

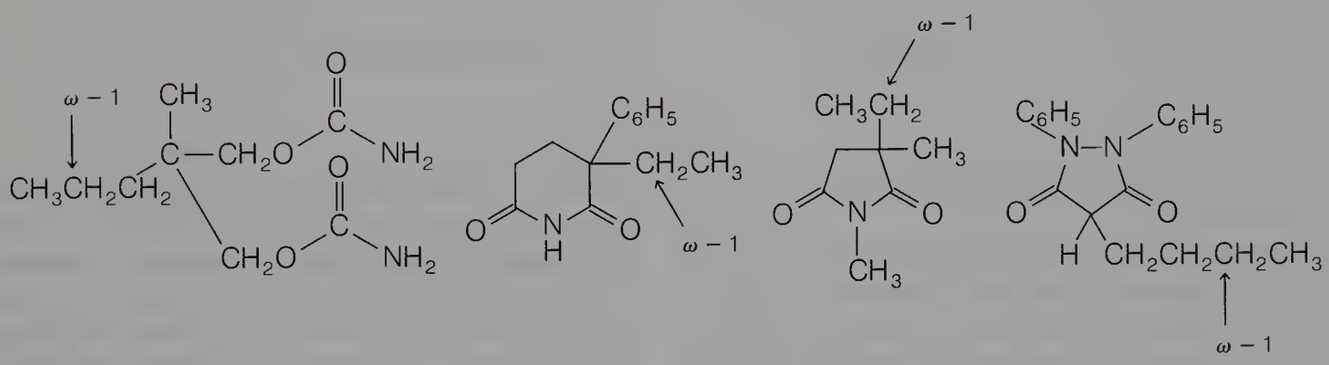


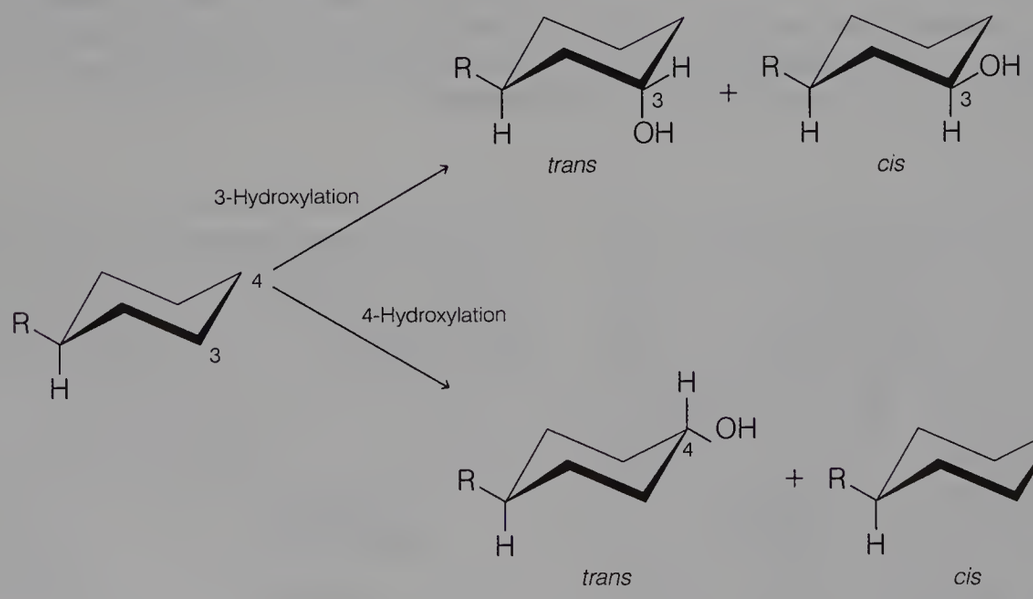


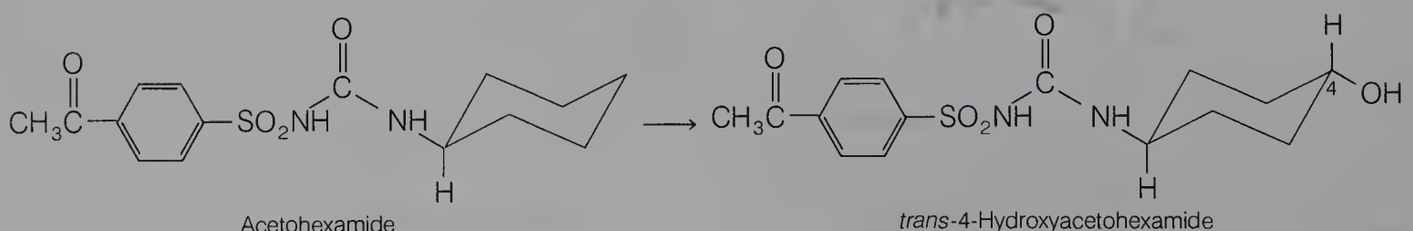
An example illustrating this hydroxylation pathway is seen in the metabolism of the oral hypoglycemic agent acetohexamide (Dymelor). In humans, the *trans*-4-hydroxycyclohexyl product has been reported as a major metabolite.[123] Small amounts of the other possible stereoisomers, namely, the *cis*-4-, *cis*-3-, and *trans*-3-hydroxycyclohexyl derivatives, also have been detected. Another related oral hypoglycemic agent, glipizide, is oxidized in humans to the *trans*-4- and *cis*-3-hydroxylcyclohexyl metabolites in about a 6:1 ratio.[124]

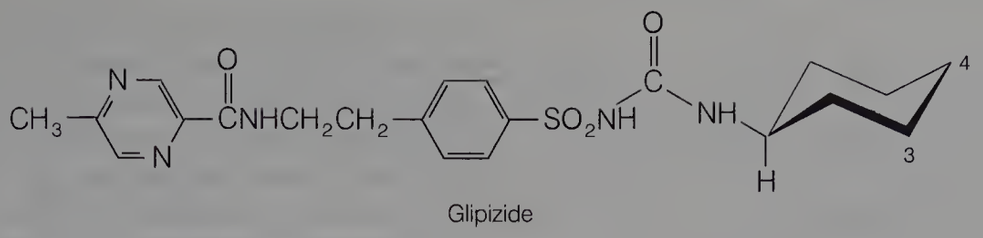




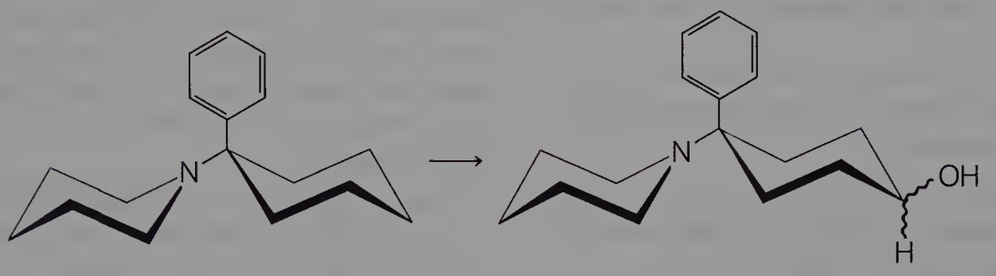






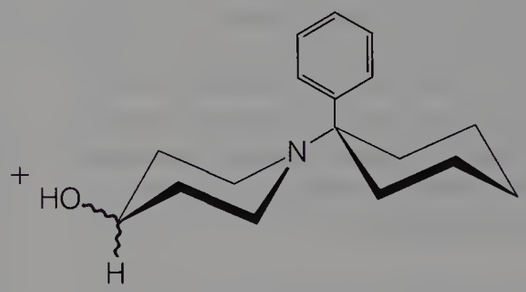


Glipizide

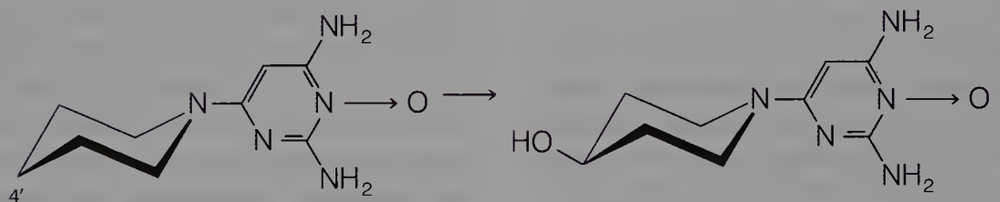


Phencyclidine

4-Hydroxycyclohexyl Metabolite



4-Hydroxypiperidyl Metabolite



Minoxidil

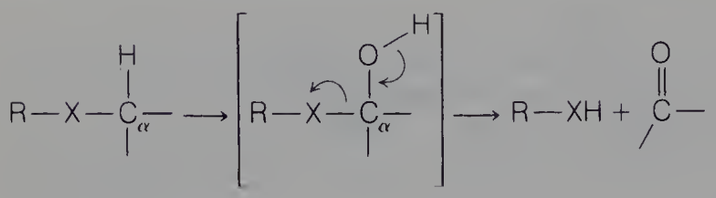
4′-Hydroxyminoxidil

Two human urinary metabolites of phencyclidine (PCP) have been identified as the 4-hydroxypiperidyl and 4-hydroxycyclohexyl derivatives of the parent compound.[125a] Thus, from these results, it appears that "alicyclic" hydroxylation of the six-membered piperidyl moiety may parallel closely the hydroxylation pattern of the cyclohexyl moiety. The stereo-chemistry of the hydroxylated centers in the two metabolites has not been established clearly. Biotransformation of the antihypertensive agent minoxidil (Loniten) yields the 4′-hydroxypiperidyl metabolite. In the dog, this product is a major urinary metabolite (29% to 47%), whereas in humans it is detected in small amounts (approximately 3%).[125b]

OXIDATION INVOLVING CARBON–HETEROATOM SYSTEMS

Nitrogen and oxygen functionalities are commonly found in most drugs and foreign compounds, whereas sulfur functionalities occur only occasionally. Metabolic oxidation of carbon–nitrogen, carbon–oxygen, and carbon–sulfur systems principally involves two basic types of biotransformation process:

1. Hydroxylation of the α-carbon atom attached directly to the heteroatom (*N*, *O*, *S*). The resulting intermediate is often unstable and decomposes with the cleavage of the carbon–heteroatom bond:



Where X = N,O,S Usually Unstable

Oxidative *N*-, *O*-, and *S*-dealkylation as well as oxidative deamination reactions fall under this mechanistic pathway.

2. Hydroxylation or oxidation of the heteroatom (*N*, *S* only, e.g., *N*-hydroxylation, *N*-oxide formation, sulfoxide, and sulfone formation).

Several structural features frequently determine which pathway will predominate, especially in carbon–nitrogen systems. Metabolism of some nitrogen-containing compounds is complicated by the fact that carbon- or nitrogen-hydroxylated products may undergo secondary reactions to form other, more complex metabolic products (e.g., oxime, nitrone, nitroso, imino). Other oxidative processes that do not fall under the foregoing two basic categories will be discussed individually in the appropriate carbon–heteroatom section. The metabolism of carbon–nitrogen systems will be discussed first, followed by the metabolism of carbon–oxygen and carbon–sulfur systems.

Oxidation Involving Carbon–Nitrogen Systems

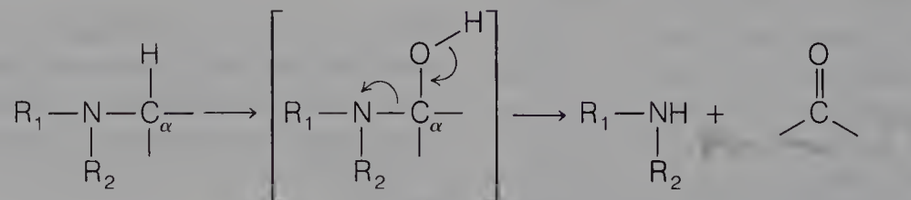
Metabolism of nitrogen functionalities (e.g., amines, amides) is of importance because such functional groups are found in many natural products (e.g., morphine, cocaine, nicotine) and in numerous important drugs (e.g., phenothiazines, antihistamines, tricyclic antidepressants, β-adrenergic agents, sympathomimetic phenylethylamines, barbiturates, benzodiazepines).[126] The following discussion divides nitrogen-containing compounds into three basic classes:

1. Aliphatic (tertiary, secondary, and primary) and alicyclic (tertiary, secondary) amines
2. Aromatic and heterocyclic nitrogen compounds
3. Amides

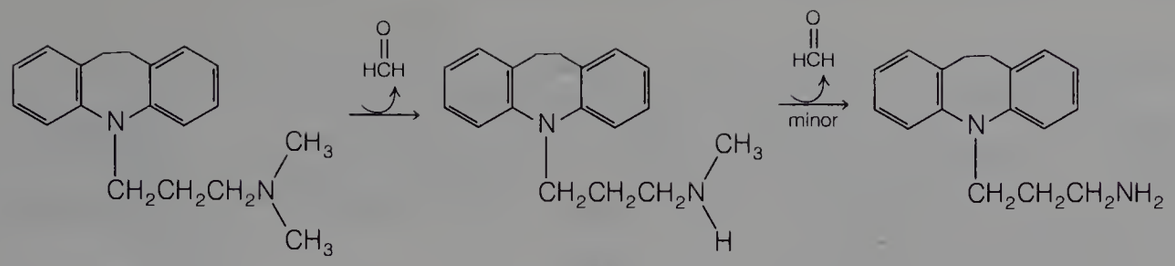
The susceptibility of each class of these nitrogen compounds to either α-carbon hydroxylation or *N*-oxidation and the metabolic products that are formed will be discussed.

The hepatic enzymes responsible for carrying out α-carbon hydroxylation reactions are the cytochrome P-450 mixed function oxidases. However, the *N*-hydroxylation or *N*-oxidation reactions appear to be catalyzed not only by cytochrome P-450 but also by a second class of hepatic mixed function oxidases called "amine oxidases" (sometimes called "*N*-oxidases").[127] These enzymes are NADPH-dependent flavoproteins and do not contain cytochrome P-450.[128] They require NADPH and molecular oxygen to carry out *N*-oxidation.

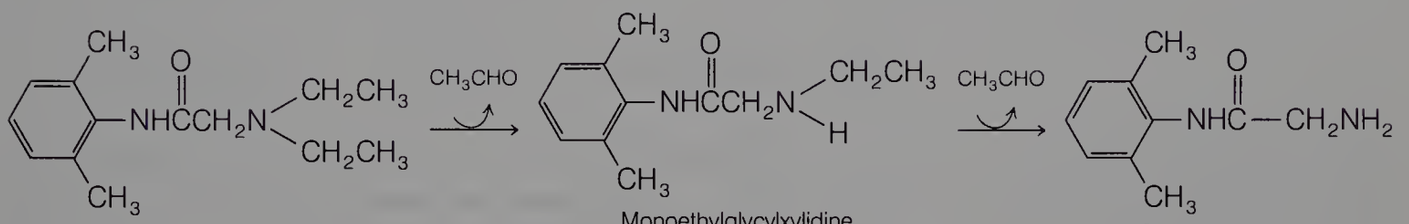
Tertiary Aliphatic and Alicyclic Amines. The oxidative removal of alkyl groups (particularly methyl groups) from tertiary aliphatic and alicyclic amines is carried out by hepatic cytochrome P-450 mixed function oxidase enzymes. This reaction is commonly referred to as "oxidative *N*-dealkylation."[129] The initial step involves α-carbon hydroxylation to form a carbinolamine intermediate, which is unstable and undergoes spontaneous heterolytic cleavage of the C—N bond to give a secondary amine and a carbonyl moiety (aldehyde or ketone).[130] In general, small alkyl groups, such as methyl, ethyl, and isopropyl, are removed rapidly.[129] *N*-Dealkylation of the *t*-butyl group is not possible by the carbinolamine pathway because α-carbon hydroxylation cannot occur. Removal of the first alkyl group from a tertiary amine occurs more rapidly than removal of the second alkyl group. In some instances, bisdealkylation of the tertiary aliphatic amine to the corresponding primary aliphatic amine occurs very slowly.[129] For example, the tertiary amine imipramine (Tofranil) is monodemethylated to desmethylimipramine (desipramine).[98,131] This major plasma metabolite is pharmacologically active in humans and contributes substantially to the antidepressant activity of the parent drug.[132] Very little of the bisdemethylated metabolite of imipramine is detected. In contrast, the local anesthetic and antiarrhythmic agent lidocaine is metabolized extensively by *N*-deethylation to both monoethylglycylxylidine and glycyl-2,6-xylidine in humans.[133]



Tertiary Amine Carbinolamine Secondary Amine Carbonyl Moiety (aldehyde or ketone)



Imipramine — Desmethylimipramine (desipramine) — Bisdesmethylimipramine

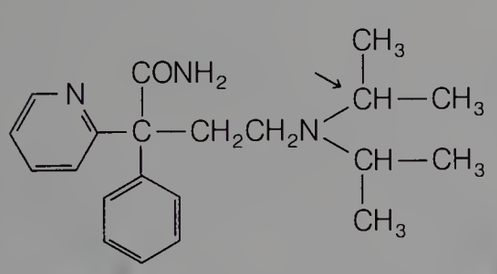


Lidocaine — Monoethylglycylxylidine (MEGX) — Glycyl-2,6-xylidine

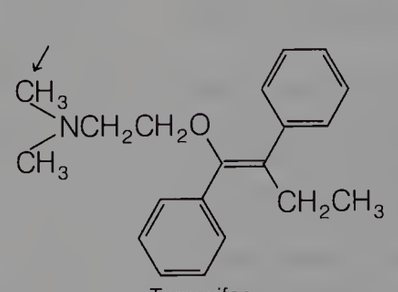
Numerous other tertiary aliphatic amine drugs are metabolized principally by oxidative *N*-dealkylation. Some of these include the antiarrhythmic disopyramide (Norpace),[134] the antiestrogenic agent tamoxifen (Nolvadex),[135] diphenhydramine (Benadryl),[136] chlorpromazine (Thorazine),[137] and (+)-α-propoxyphene (Darvon).[138] When the tertiary amine contains several different substituents capable of undergoing dealkylation, the smaller alkyl group is removed preferentially and more rapidly. For example, in benzphetamine (Didrex), the methyl group is removed much more rapidly than the benzyl moiety.[139]

An interesting cyclization reaction occurs with methadone upon *N*-demethylation. The demethylated metabolite normethadone undergoes a spontaneous cyclization reaction to form the enamine metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenyl-pyrrolidine (EDDP).[140] Subsequent *N*-demethylation of EDDP and isomerization of the double bond leads to 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline (EMDP).

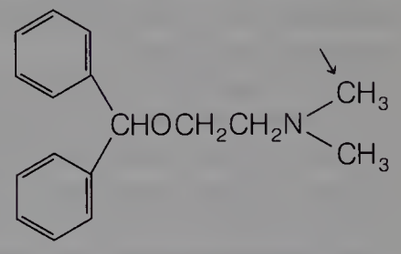
Many times, bisdealkylation of a tertiary amine leads to the corresponding primary aliphatic amine metabolite, which is susceptible to further oxidation. For example, the bisdesmethyl metabolite of the H_1-histamine antagonist brompheniramine (Dimetane) undergoes oxidative deamination and further oxidation to the corresponding propionic acid metabolite.[141] Oxidative deamination will be discussed in greater detail when we examine the metabolic reactions of secondary and primary amines.



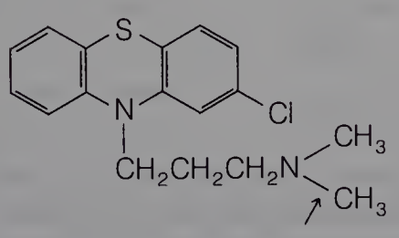
Disopyramide



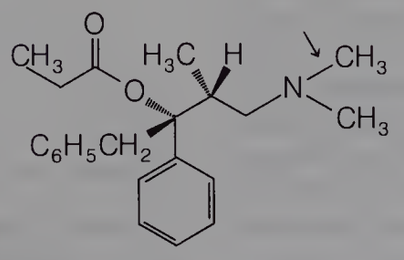
Tamoxifen



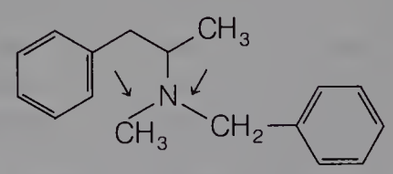
Diphenhydramine



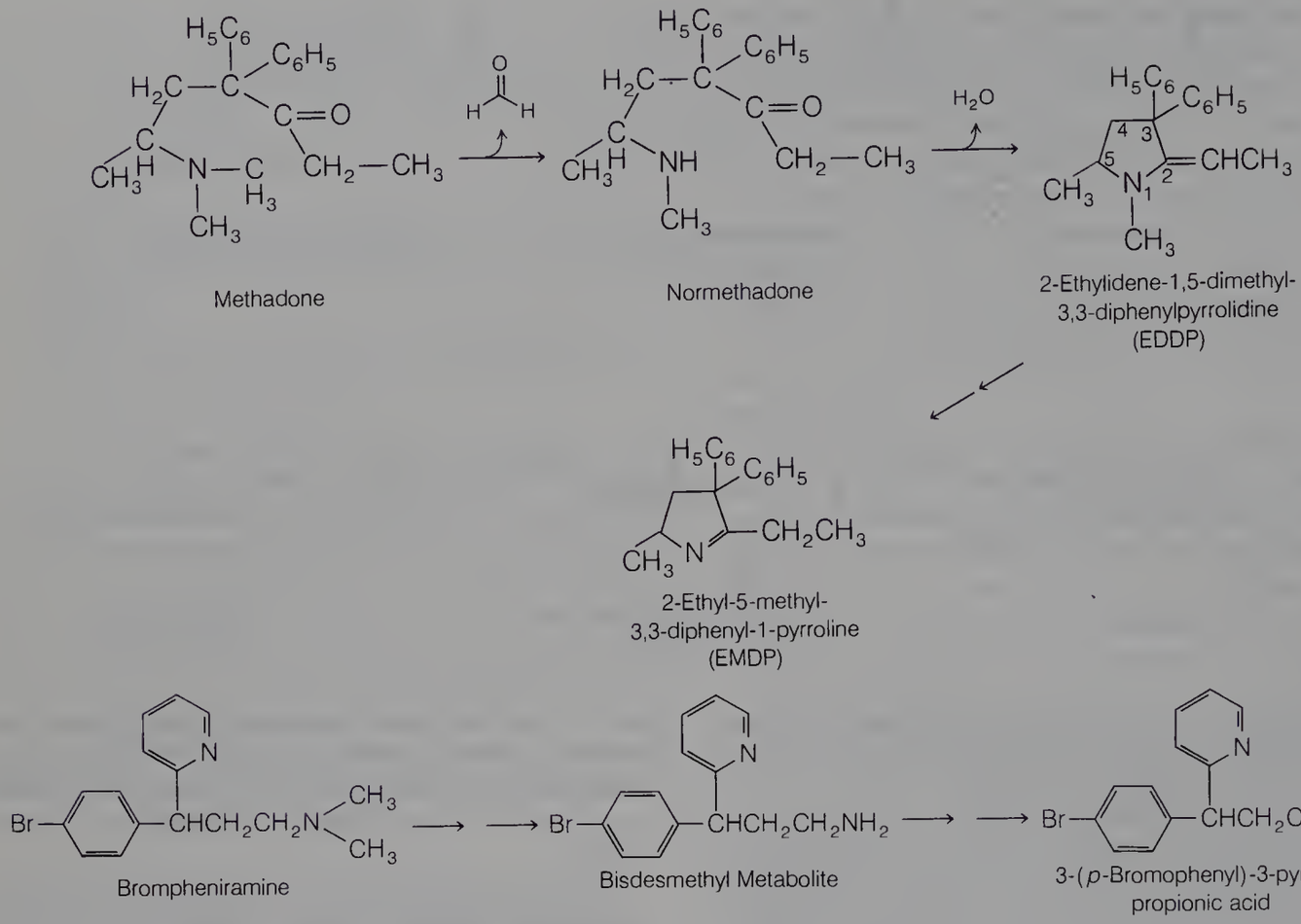
Chlorpromazine



(+)-α-Propoxyphene

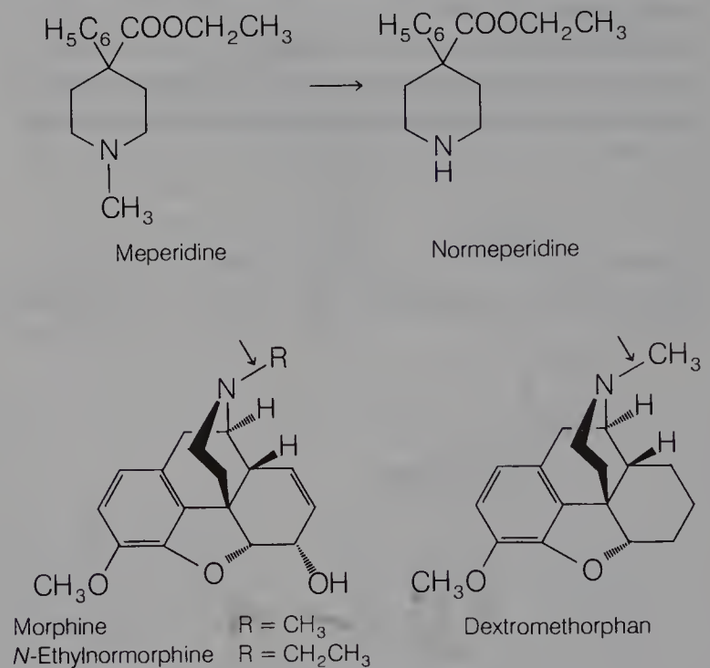


Benzphetamine (*N*-demethylation and *N*-debenzylation)

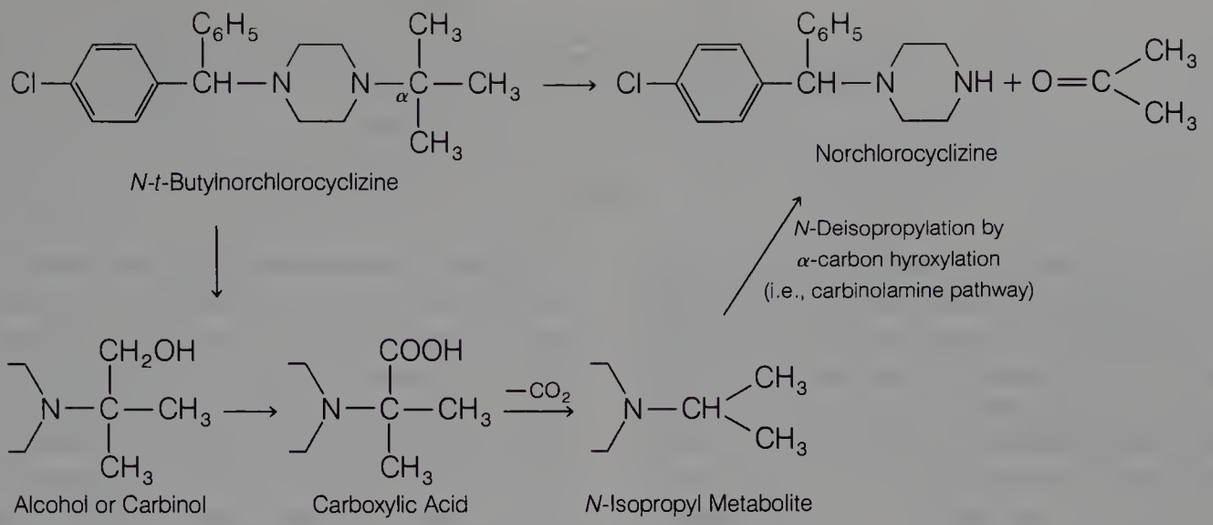


Similar to their aliphatic counterparts, alicyclic tertiary amines are susceptible to oxidative *N*-dealkylation reactions. For example, the analgesic meperidine (Demerol) is metabolized principally by this pathway to yield normeperidine as a major plasma metabolite in humans.[142] Morphine, *N*-ethylnormorphine, and dextromethorphan also undergo *N*-dealkylation to some extent.[143]

Direct *N*-dealkylation of *t*-butyl groups, as discussed earlier, is not possible by the α-carbon hydroxylation pathway. However, in vitro studies indicate that *N*-*t*-butylnorchlorocyclizine is indeed metabolized to significant amounts of norchlorocyclizine, whereby the *t*-butyl group is lost.[144] Careful studies showed that the *t*-butyl group is removed by initial hydroxylation of one of the methyl groups of the *t*-butyl moiety to the carbinol or alcohol product.[145] Further oxidation generates the corresponding carboxylic acid, which upon decarboxylation forms the *N*-isopropyl derivative. The *N*-isopropyl intermediate is dealkylated by the normal α-carbon hydroxylation (i.e., carbinolamine) pathway to give norchlorocyclizine and acetone. Whether this is a general method for the loss of *t*-butyl groups from amines is still unclear. Indirect *N*-dealkylation of *t*-butyl groups is not observed significantly. The *N*-*t*-butyl group present in many β-adrenergic antagonists, such as terbutaline and salbutamol, remains intact and does not appear to undergo any significant metabolism.[146]

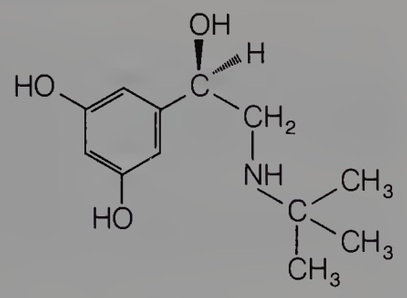


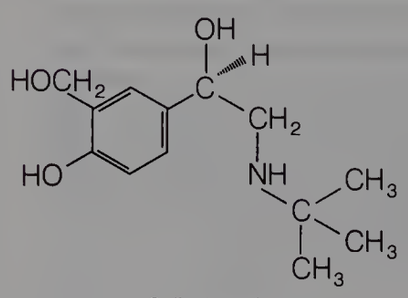
Alicyclic tertiary amines often generate lactam metabolites by α-carbon hydroxylation reactions. For example, the tobacco alkaloid nicotine is hydroxylated initially at the ring carbon atom α to the nitrogen to yield a carbinolamine intermediate. Furthermore, enzymatic oxidation of this cyclic carbinolamine generates the lactam metabolite cotinine.[147]

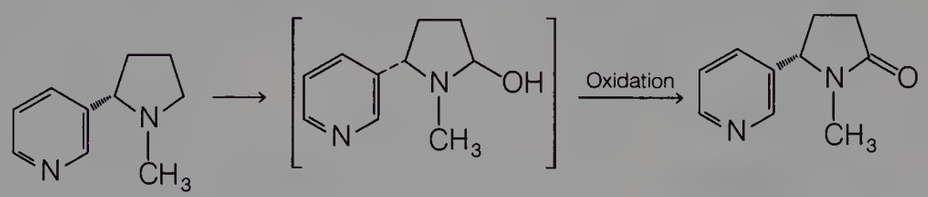


Formation of lactam metabolites also has been reported to occur to a minor extent for the antihistamine cyproheptadine (Periactin)[148] and the antiemetic diphenidol (Vontrol).[149]

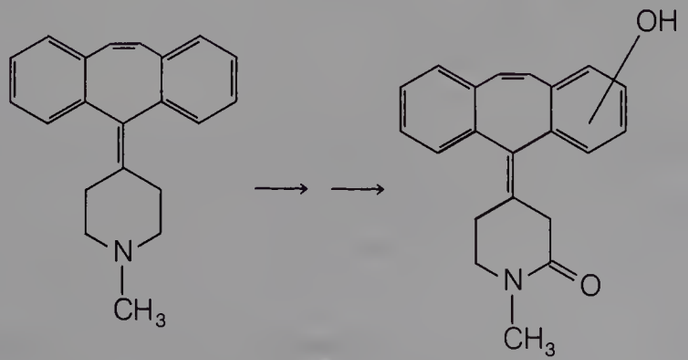
N-Oxidation of tertiary amines occurs with several drugs.[150] The true extent of *N*-oxide formation often is complicated by the susceptibility of *N*-oxides to undergo in vivo reduction back to the parent tertiary amine. Tertiary amines such as H_1-histamine antagonists (e.g., orphenadrine, tripelenamine), phenothiazines (e.g., chlorpromazine), tricyclic antidepressants (e.g., imipramine), and narcotic analgesics (e.g., morphine, codeine, and meperidine) have been reported to form *N*-oxide products. In some instances, *N*-oxides possess pharmacologic activity.[151] For example, comparison of imipramine *N*-oxide with imipramine indicates that the *N*-oxide itself possesses antidepressant and cardiovascular activity similar to the parent drug.[152]

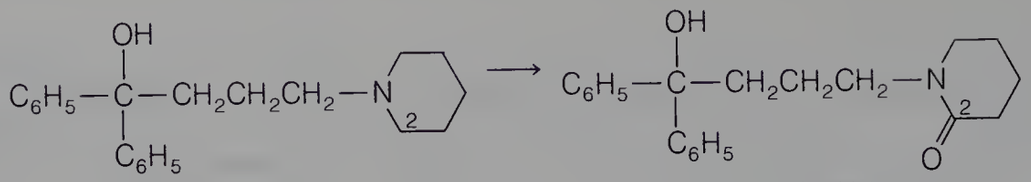

Terbutaline


Salbutamol



Nicotine Carbinolamine Cotinine

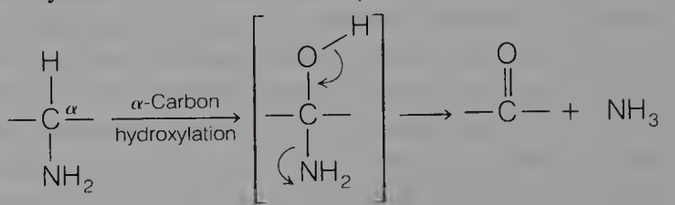

Cyproheptadine Lactam Metabolite



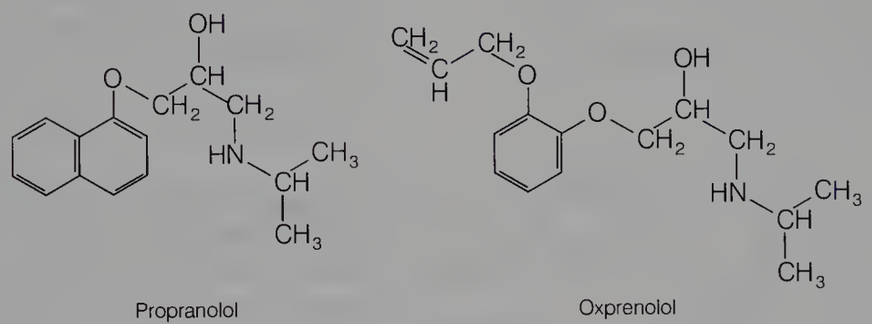
Secondary and Primary Amines. Secondary amines (either parent compounds or metabolites) are susceptible to oxidative *N*-dealkylation, oxidative deamination, and *N*-oxidation reactions.[129,153] As in tertiary amines, *N*-dealkylation of secondary amines proceeds by the carbinolamine pathway. Dealkylation of secondary amines gives rise to the corresponding primary amine metabolite. For example, the β-adrenergic blockers propranolol[39] and oxprenolol[154] undergo *N*-deisopropylation to the corresponding primary amines. *N*-Dealkylation appears to be a significant biotransformation pathway for the secondary amine drugs methamphetamine[155] and ketamine,[156] yielding amphetamine and norketamine, respectively.

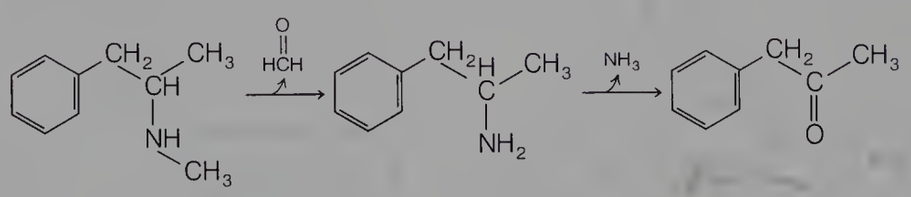
The primary amine metabolites formed from oxidative dealkylation are susceptible to *oxidative deamination*. This process is similar to *N*-dealkylation in that it involves an initial α-carbon hydroxylation reaction to form a carbinolamine intermediate, which then undergoes subsequent carbon–nitrogen cleavage to the carbonyl metabolite and ammonia. If α-carbon hydroxylation cannot occur, then oxidative deamination is not possible. For example, deamination does not occur for norketamine because α-carbon hydroxylation cannot take place.[156] For methamphetamine, oxidative deamination of primary amine metabolite amphetamine produces phenylacetone (see below).[155]

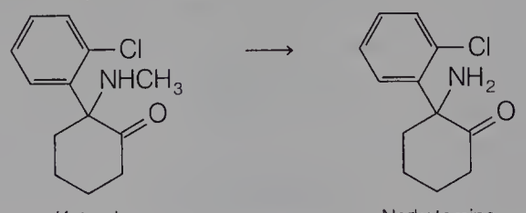
In general, dealkylation of secondary amines is believed to take place before oxidative deamination occurs. However, there is some evidence that this may not always be true. Direct deamination of the secondary amine also has occurred. For example, in addition to undergoing deamination through its desisopropyl primary amine metabolite, propranolol can undergo a direct oxidative deamination reaction (also by α-carbon hydroxylation) to yield the aldehyde metabolite and isopropylamine (Fig. 3-9).[157] How much direct oxidative deamination contributes to the metabolism of secondary amines remains unclear.



Some secondary alicyclic amines, similarly to their tertiary amine analogues, are metabolized to their corresponding lactam derivatives. For example, the anorectic agent phenmetrazine (Preludin) is metabolized principally to the lactam product 3-oxophenmetrazine.[158] In humans, this lactam metabolite is a major urinary product. Methylphenidate







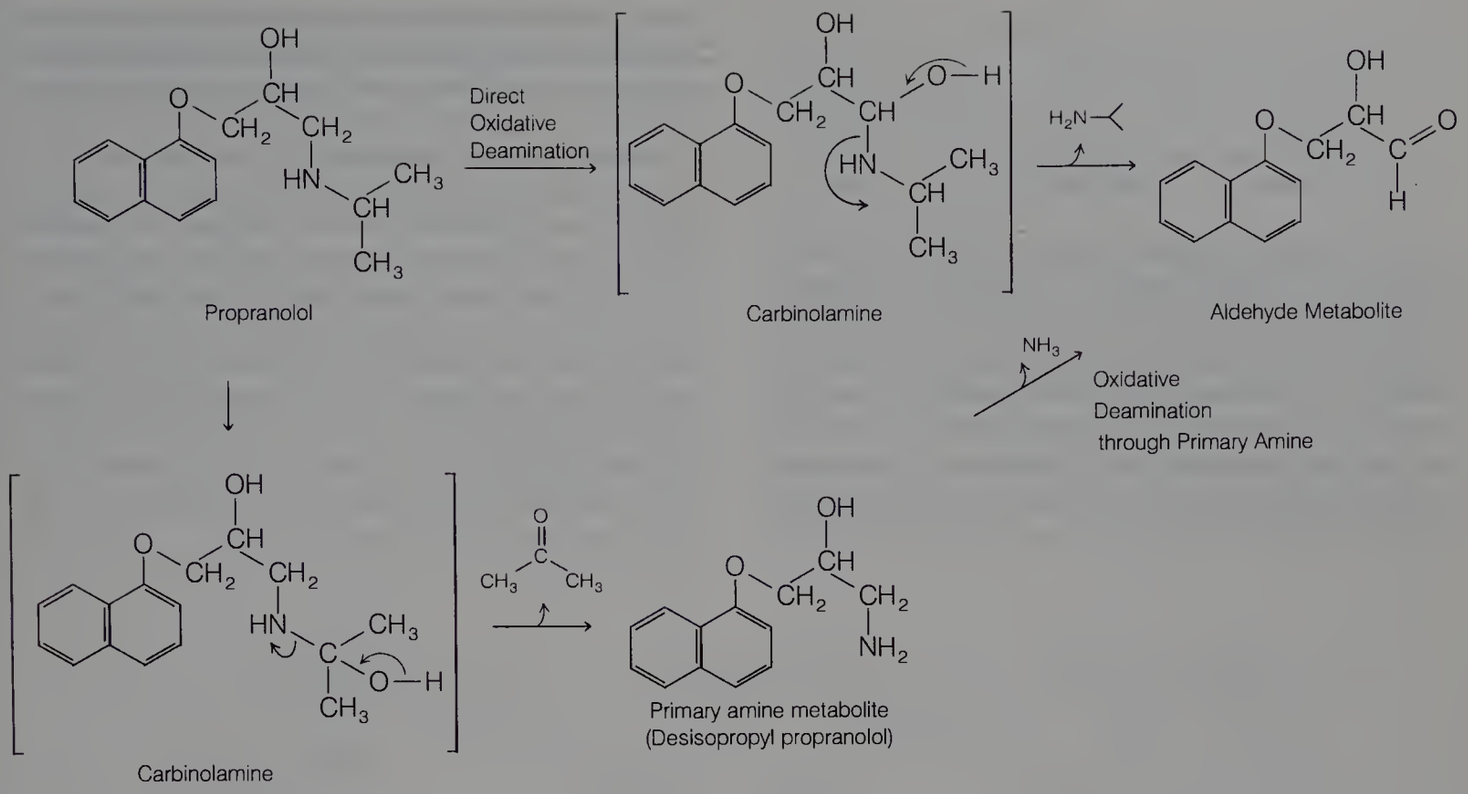
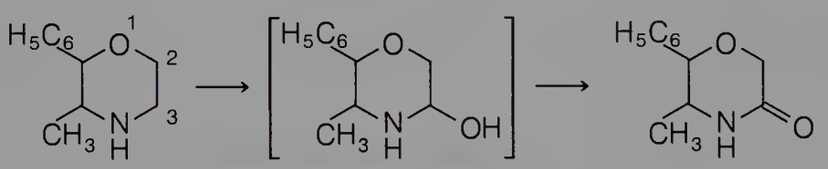
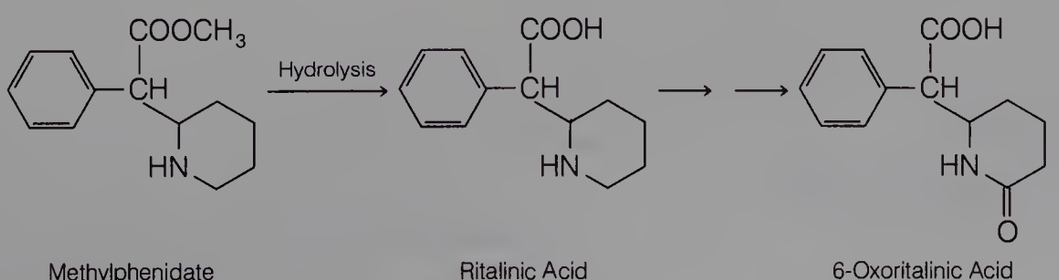


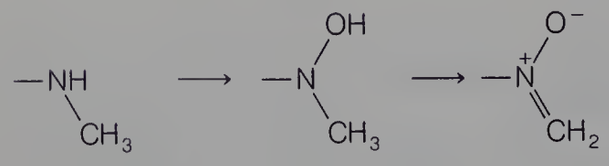
FIG. 3-9. *The metabolism of propranolol to its aldehyde metabolite by direct deamination of the parent compound and by deamination of its primary amine metabolite, desisopropyl propranolol.*

(Ritalin) also has been reported to yield a lactam metabolite, 6-oxoritalinic acid, by oxidation of its hydrolyzed metabolite, ritalinic acid, in humans.[159]

Metabolic *N*-oxidation of secondary aliphatic and alicyclic amines leads to several *N*-oxygenated products.[153] *N*-Hydroxylation of secondary amines generates the corresponding *N*-hydroxylamine metabolites. Often, these hydroxylamine products are susceptible to further oxidation (either spontaneous or enzymatic) to the corresponding nitrone derivatives. For example, *N*-benzylamphetamine has been observed to undergo metabolism to both the corresponding *N*-hydroxylamine and the nitrone metabolites.[160] In humans, the nitrone metabolite of phenmetrazine (Preludin) found in the urine is believed to be formed by further oxidation of the *N*-hydroxylamine intermediate *N*-hydroxyphenmetrazine.[158] Importantly, in comparison with oxidative dealkylation and deamination, *N*-oxidation occurs to a much lesser extent for secondary amines.



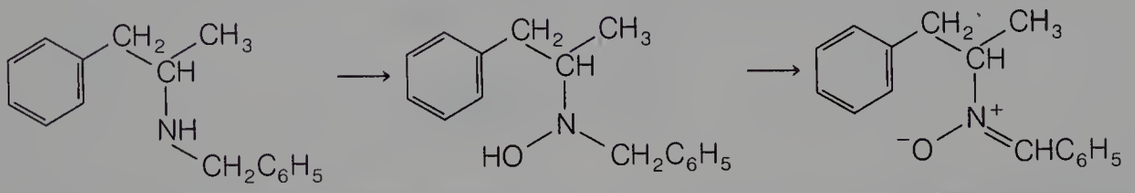




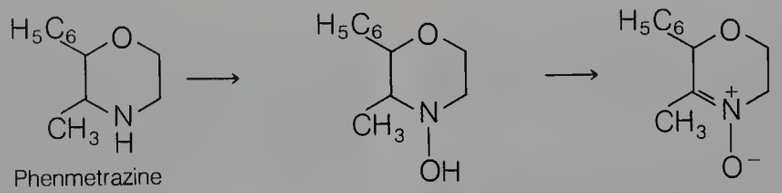
Secondary amine Hydroxylamine Nitrone

Primary aliphatic amines (whether parent drugs or metabolites) are biotransformed by oxidative deamination (through the carbinolamine pathway) or by *N*-oxidation. In general, oxidative deamination of most exogenous primary amines is carried out by the mixed function oxidases discussed earlier. However, endogenous primary amines, such as dopamine, norepinephrine, tryptamine, and serotonin, are metabolized through oxidative deamination by a specialized family of enzymes called monoamine oxidases (MAO).[161] These enzymes are involved primarily in inactivating the foregoing neurotransmitter amines. MAO apparently plays no significant role in the metabolism of xenobiotic primary amines.

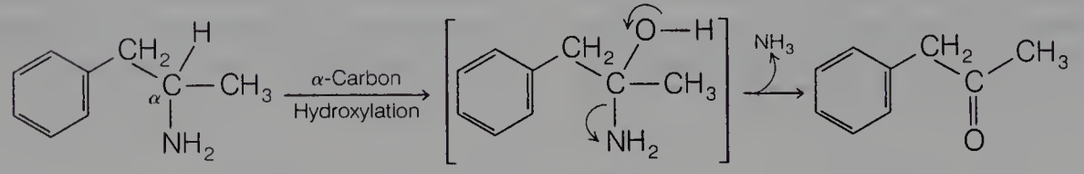
Structural features, especially the α-substituents of the primary amine, often determine whether carbon or nitrogen oxidation will occur. For example, compare amphetamine with its α-methyl homologue phentermine. In amphetamine, α-carbon hydroxylation can occur to form the carbinolamine intermediate, which is converted to the oxidatively deaminated product phenylacetone.[46] With phentermine, α-carbon hydroxylation is not possible and precludes oxidative deamination for this drug. Consequently, phentermine would be expected to undergo *N*-oxidation readily. In humans, *p*-hydroxylation and *N*-oxidation are the main pathways for biotransformation of phentermine.[162]



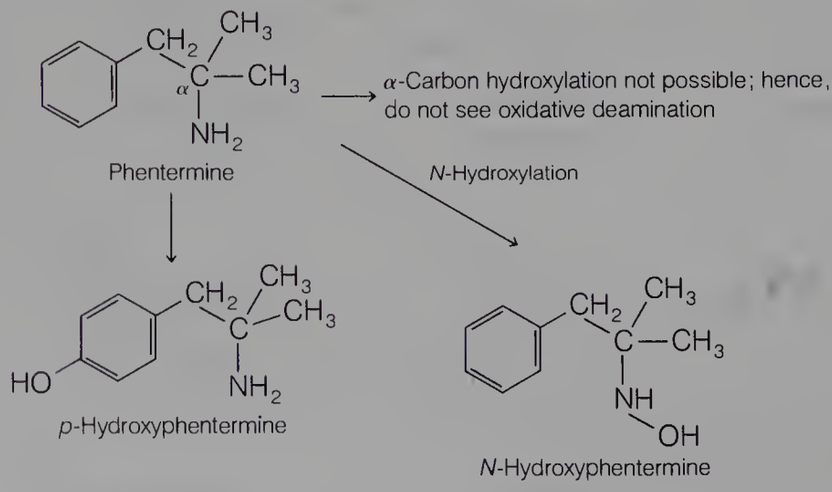
N-Benzylamphetamine Hydroxylamine Metabolite Nitrone Metabolite



Phenmetrazine *N*-Hydroxyphenmetrazine Nitrone Metabolite

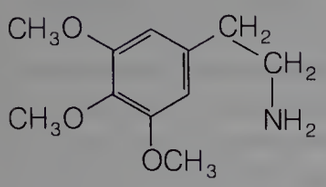


Amphetamine Carbinolamine Phenylacetone

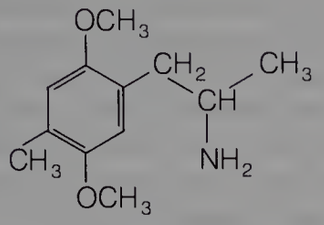


Phentermine

Indeed, *N*-hydroxyphentermine is an important (5%) urinary metabolite in humans.[162] As shall be discussed shortly, *N*-hydroxylamine metabolites are susceptible to further oxidation to yield other *N*-oxygenated products.



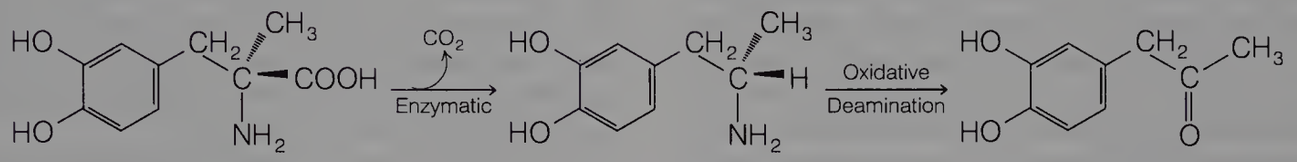
Mescaline



1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane
DOM or "STP"

kylated to their corresponding primary amine metabolites, which are amenable to oxidative deamination. (*S*)(+)-α-Methyldopamine resulting from decarboxylation of the antihypertensive agent (*S*)(−)-α-methyldopa (Aldomet) is deaminated to the corresponding ketone metabolite 3,4-dihydroxyphenylacetone.[164] In humans, this ketone is a major urinary metabolite.

The *N*-hydroxylation reaction is not restricted to α-substituted primary amines such as phentermine. Amphetamine has been observed to undergo some *N*-hydroxylation in vitro to *N*-hydroxyamphetamine.[165] However, *N*-hydroxyamphetamine is susceptible to further conversion to the imine or oxidation to the oxime intermediate. Note that the oxime intermediate arising from this *N*-oxidation pathway can undergo hydrolytic cleavage to yield phenylacetone, the same



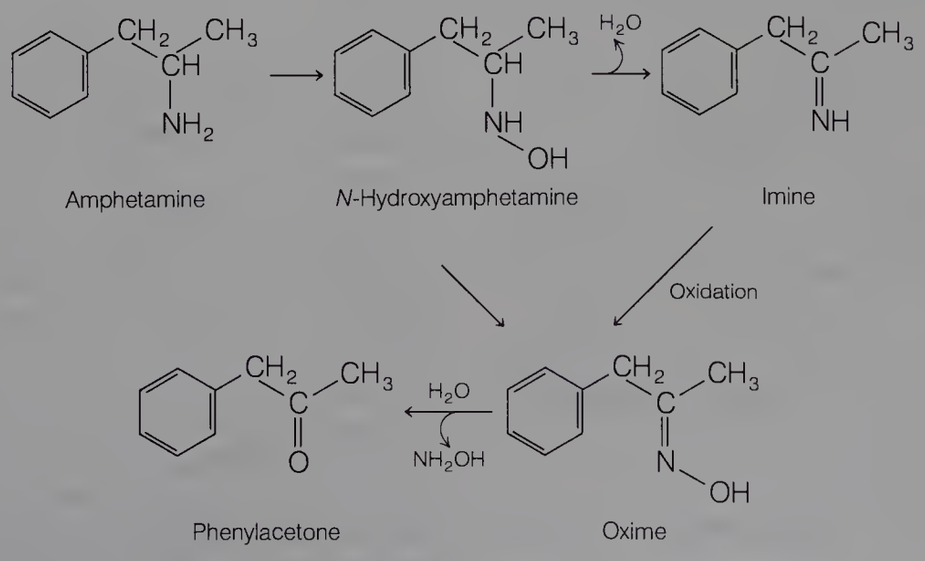
S(−)-α-Methyldopa

S(+)-α-Methyldopamine

3,4-Dihydroxyphenylacetone

Xenobiotics, such as the hallucinogenic agents mescaline[163] and 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM or "STP"),[97] are oxidatively deaminated. Primary amine metabolites arising from *N*-dealkylated or decarboxylation reactions also undergo deamination. The example of the bisdesmethyl primary amine metabolite derived from bromopheniramine was discussed earlier (see section on tertiary aliphatic and alicyclic amines).[141] In addition, many tertiary aliphatic amines (e.g., antihistamines) and secondary aliphatic amines (e.g., propranolol) are deal-

product obtained by the α-carbon hydroxylation (carbinolamine) pathway.[166] Thus, conversion of amphetamine to phenylacetone may arise through either the α-carbon hydroxylation or the *N*-oxidation pathway. The debate concerning the relative importance of the two pathways is ongoing.[167,168] The general consensus, however, is that both metabolic pathways (carbon and nitrogen oxidation) are probably operative. Whether α-carbon or nitrogen oxidation predominates in the metabolism of amphetamine appears to be species-dependent.



Amphetamine

N-Hydroxyamphetamine

Imine

Phenylacetone

Oxime

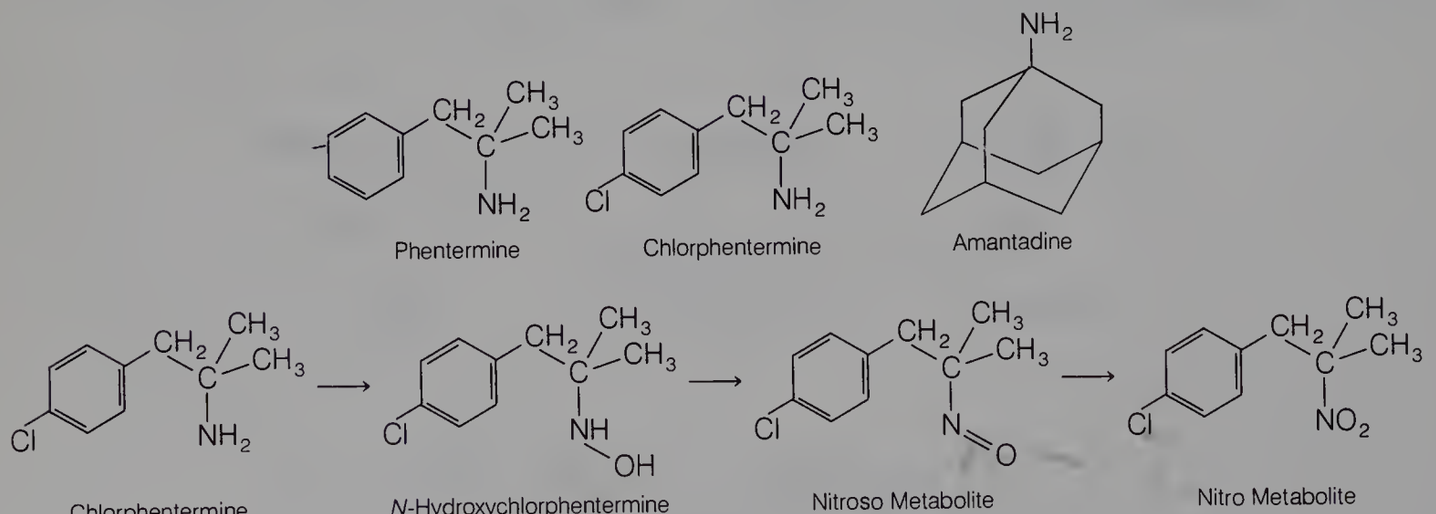
In primary aliphatic amines, such as phentermine,[162] chlorphentermine (*p*-chlorphentermine),[168] and amantadine,[169] *N*-oxidation appears to be the major biotransformation pathway because α-carbon hydroxylation cannot occur. In humans, chlorphentermine is *N*-hydroxylated extensively. About 30% of a dose of chlorphentermine is found in the urine (48 hours) as *N*-hydroxychlorphentermine (free and conjugated) and an additional 18% as other products of *N*-oxidation (presumably the nitroso and nitro metabolites).[168] In general, *N*-hydroxylamines are chemically unstable and susceptible to spontaneous or enzymatic oxidation to the nitroso and nitro derivatives. For example, the *N*-hydroxylamine metabolite of phentermine undergoes further oxidation to the nitroso and nitro products.[162] The antiviral and antiparkinsonian agent amantadine (Symmetrel) has been reported to undergo *N*-oxidation to yield the corresponding *N*-hydroxy and nitroso metabolites in vitro.[169]

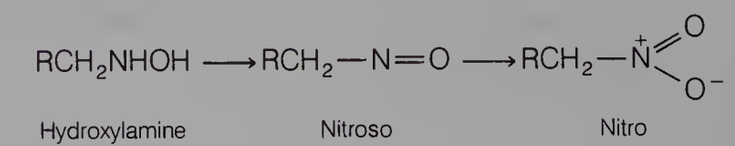
Aromatic Amines and Heterocyclic Nitrogen Compounds. The biotransformation of aromatic amines parallels the carbon and nitrogen oxidation reactions seen for aliphatic amines.[170,171] For tertiary aromatic amines, such as *N,N*-dimethylaniline, oxidative *N*-dealkylation as well as *N*-oxide formation take place.[172] Secondary aromatic amines may undergo *N*-dealkylation or *N*-hydroxylation to give the corresponding *N*-hydroxylamines. Further oxidation of the *N*-hydroxylamine leads to nitrone products, which in turn may be hydrolyzed to primary hydroxylamines.[173] Tertiary and secondary aromatic amines are encountered rarely in medicinal agents. In contrast, primary aromatic amines are found in many drugs and often are generated from enzymatic reduction of aromatic nitro compounds, reductive cleavage of azo compounds, and hydrolysis of aromatic amides.

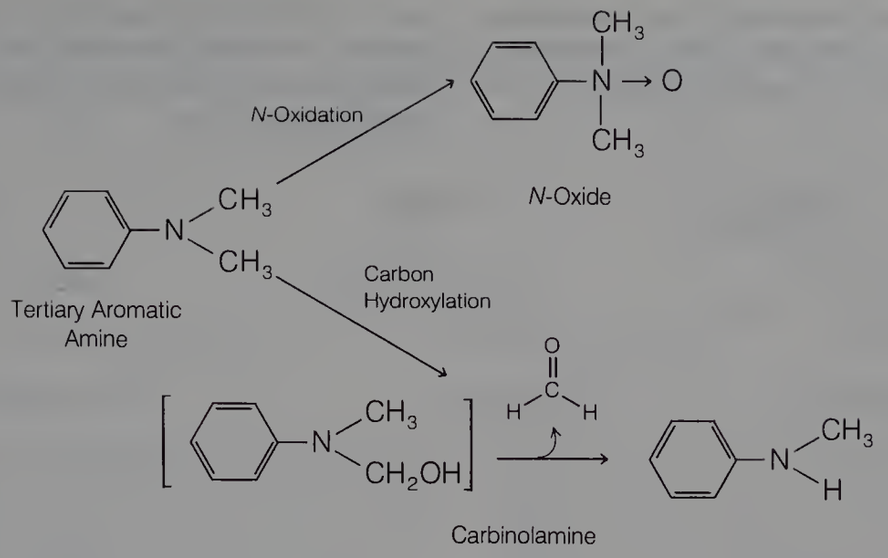
N-Oxidation of primary aromatic amines generates the *N*-hydroxylamine metabolite. For example, aniline is metabolized to the corresponding *N*-hydroxy product.[171] Oxidation of the hydroxylamine derivative to the nitroso derivative also can occur. When considering primary aromatic amine drugs or metabolites, *N*-oxidation constitutes only a minor pathway in comparison with other biotransformation pathways, such as *N*-acetylation and aromatic hydroxylation, in humans. However, some *N*-oxygenated metabolites have been reported. For example, the antileprotic agent dapsone and its *N*-acetylated metabolite are metabolized significantly to their corresponding *N*-hydroxylamine derivatives.[174] The *N*-hydroxy metabolites are further conjugated with glucuronic acid.

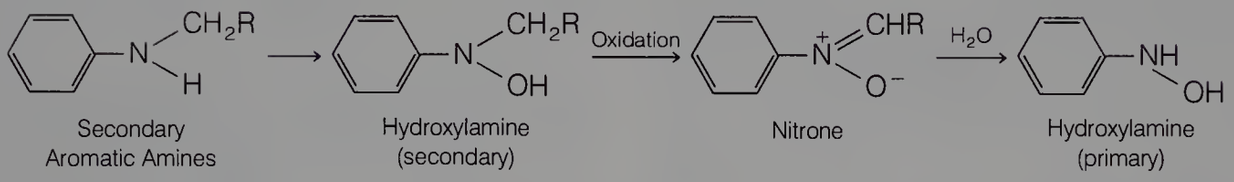
Methemoglobinemia toxicity caused by several aromatic amines, including aniline and dapsone, is a result of the bioconversion of the aromatic amine to its *N*-hydroxy derivative. Apparently, the *N*-hydroxylamine oxidizes the Fe^{2+} form of hemoglobin to its Fe^{3+} form. This oxidized (Fe^{3+}) state of hemoglobin (called "methemoglobin" or "ferrihemoglobin") is no longer capable of transporting oxygen and leads to serious hypoxia or anemia.[175]

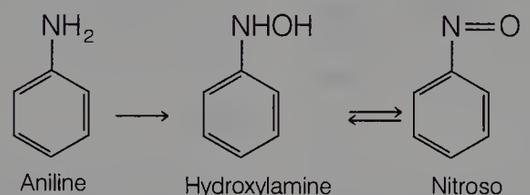
Divers aromatic amines (especially azoamino dyes) are known to be carcinogenic. *N*-Oxidation plays an important role in bioactivating these aromatic amines to potentially reactive electrophilic species that covalently bind to cellular protein, DNA, or RNA. A well-studied example is the carcinogenic agent *N*-methyl-4-aminoazobenzene.[176] *N*-Oxidation of this compound leads to the corresponding hydroxylamine, which undergoes sulfate conjugation. Owing to the good leaving group ability of the sulfate (SO_4^{2-}) anion, this sulfate conjugate can ionize spontaneously to form a highly reactive, resonance-stabilized nitrenium species. Covalent

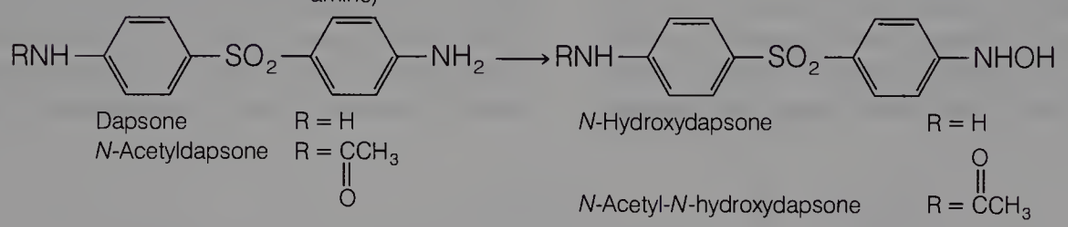






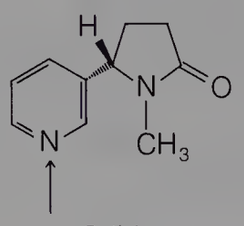


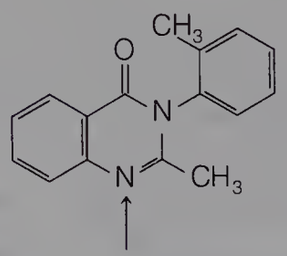




adducts between this species and DNA, RNA, and protein have been characterized.[177] The sulfate ester is believed to be the ultimate carcinogenic species. Thus, the example indicates that certain aromatic amines can be bioactivated to reactive intermediates by *N*-hydroxylation and *O*-sulfate conjugation. Whether primary hydroxylamines can be bioactivated similarly is unclear. In addition, it is not known if this biotoxification pathway plays any substantial role in the toxicity of aromatic amine drugs.

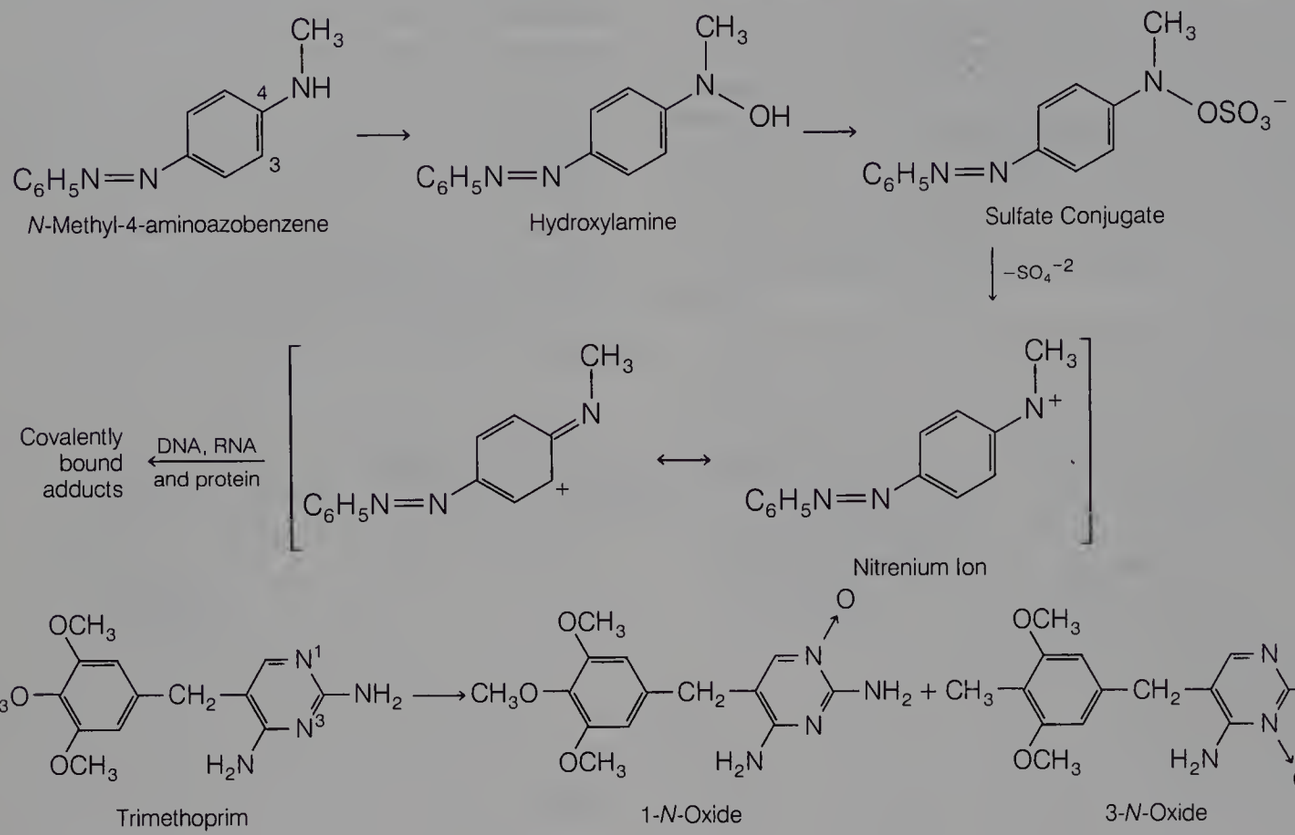
N-Oxidation of the nitrogen atoms present in aromatic heterocyclic moieties of many drugs occurs to a minor extent. For example, in humans, *N*-oxidation of the folic acid antagonist trimethoprim (Proloprim, Trimpex) has yielded approximately equal amounts of the isomeric 1-*N*-oxide and 3-*N*-oxide as minor metabolites.[178] The pyridinyl nitrogen atom present in cotinine (the major metabolite of nicotine)





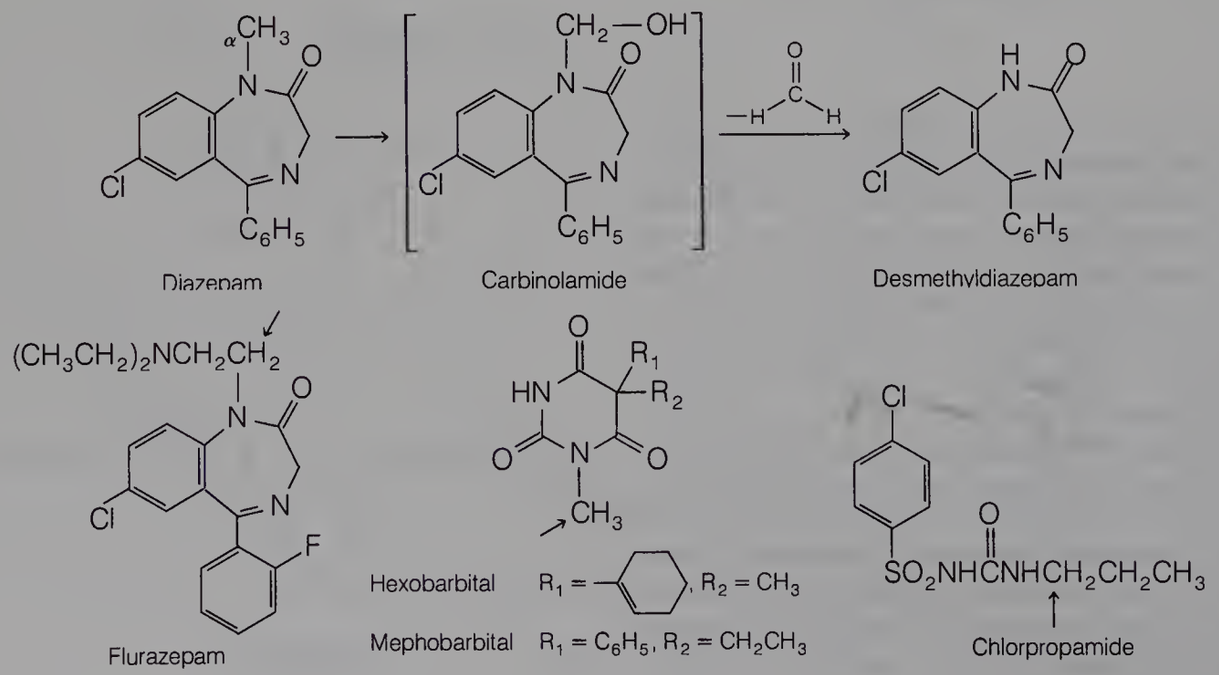
undergoes oxidation to yield the corresponding *N*-oxide metabolite.[179] Formation of an *N*-oxide metabolite of methaqualone (Quaalude, Parest) also has been observed in humans.[180]

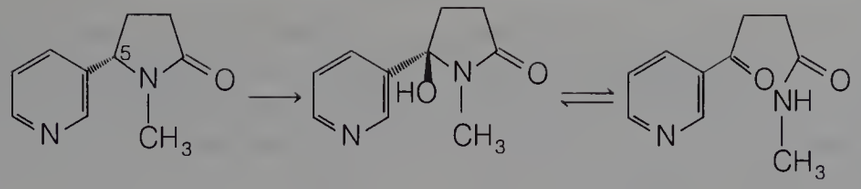
which is unstable and fragments to form the *N*-dealkylated product. For example, diazepam undergoes extensive *N*-demethylation to the pharmacologically active metabolite desmethyldiazepam.[181]



Amides. Amide functionalities are susceptible to oxidative carbon–nitrogen bond cleavage (by way of α-carbon hydroxylation) and *N*-hydroxylation reactions. Oxidative dealkylation of many *N*-substituted amide drugs and xenobiotics has been reported. Mechanistically, oxidative dealkylation proceeds by way of an initially formed carbinolamide,

Various other *N*-alkyl substituents present in benzodiazepines (e.g., flurazepam)[111] and in barbiturates (e.g., hexobarbital and mephobarbital)[105] are similarly oxidatively *N*-dealkylated. Alkyl groups attached to the amide moiety of some sulfonylureas, such as the oral hypoglycemic chlorpropamide,[182] also are subject to dealkylation to a minor extent.



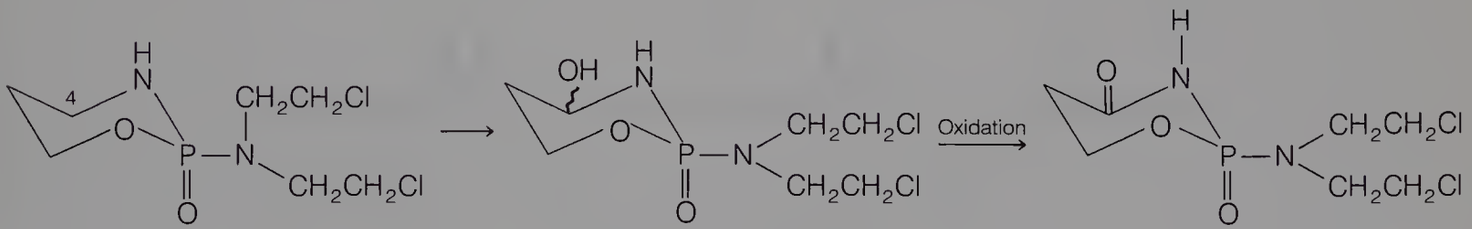


Cotinine 5-Hydroxycotinine γ-(3-Pyridyl)-γ-oxo-*N*-methylbutyramide

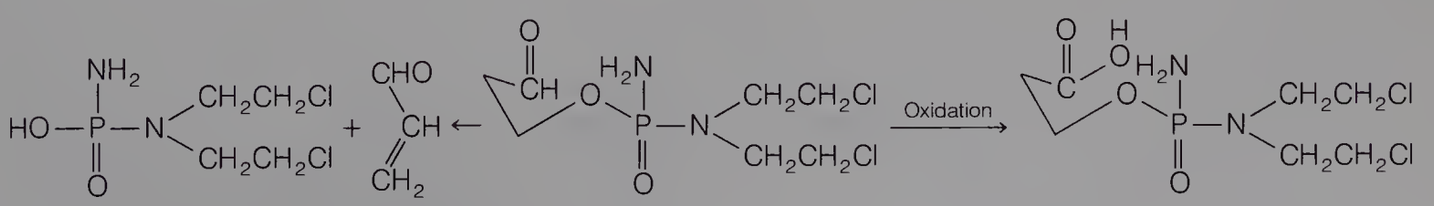
In the cyclic amides or lactams, hydroxylation of the alicyclic carbon α to the nitrogen atom also leads to carbinolamides. An example of this pathway is the conversion of cotinine to 5-hydroxycotinine. Interestingly, the latter carbinolamide intermediate is in tautomeric equilibrium with the ring-opened metabolite γ-(3-pyridyl)-γ-oxo-*N*-methylbutyramide.[183]

Metabolism of the important cancer chemotherapeutic agent cyclophosphamide (Cytoxan) follows a hydroxylation pathway similar to that just described for cyclic amides. This drug is a cyclic phosphoramide derivative and, for the most part, is the phosphorous counterpart of a cyclic amide. Because cyclophosphamide itself is pharmacologically inactive,[184] metabolic bioactivation is required for the drug to mediate its antitumorigenic or cytotoxic effects. The key biotransformation pathway leading to the active metabolite involves an initial carbon hydroxylation reaction at C-4 to form the carbinolamide 4-hydroxycyclophosphamide.[185] 4-Hydroxycyclophosphamide is in equilibrium with the ring-opened dealkylated metabolite aldophosphamide. Although it has potent cytotoxic properties, aldophosphamide undergoes a further elimination reaction (reverse Michael reaction) to generate acrolein and the phosphoramide mustard *N,N*-bis(2-chloro-ethyl)phosphorodiamidic acid. The latter metabolite is the principal species responsible for cyclophosphamide's antitumorigenic properties and chemotherapeutic effect. Enzymatic oxidation of 4-hydroxycyclophosphamide and aldophosphamide leads to the relatively nontoxic metabolites 4-ketocyclophosphamide and carboxycyclophosphamide, respectively.

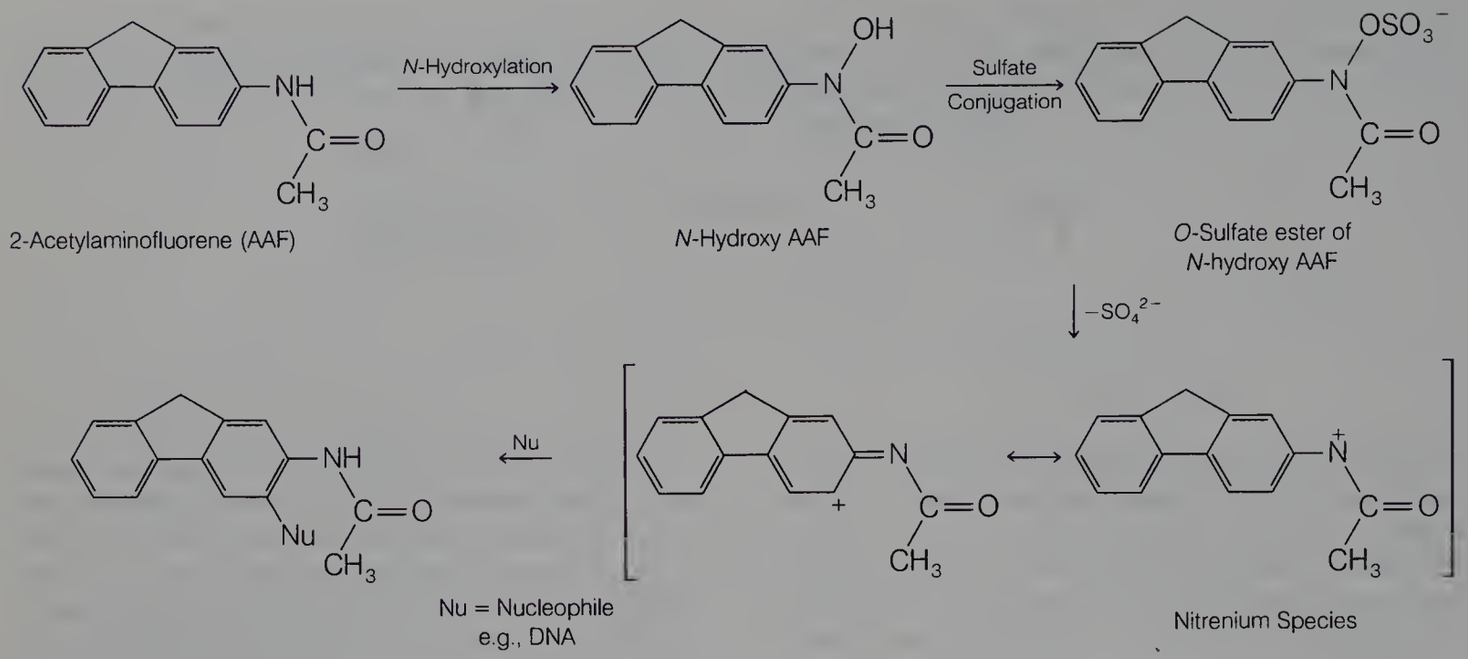
N-Hydroxylation of aromatic amides, which occurs to a minor extent, is of some toxicologic interest since this biotransformation pathway may lead to the formation of chemically reactive intermediates. Several examples of cytotoxicity or carcinogenicity associated with metabolic *N*-hydroxylation of the parent aromatic amide have been reported. For example, the well-known hepatocarcinogenic 2-acetylaminofluorene (AAF) undergoes an *N*-hydroxylation reaction catalyzed by cytochrome P-450 to form the corresponding *N*-hydroxy metabolite (also called a hydroxamic



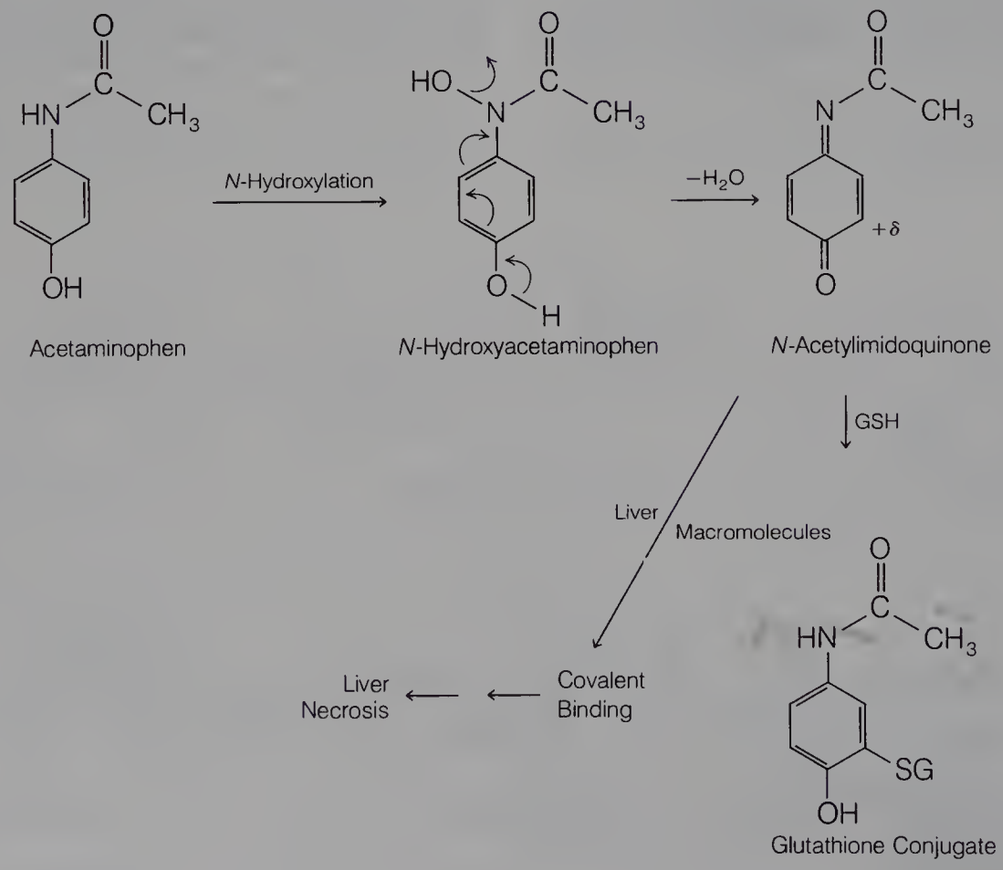
Cyclophosphamide 4-Hydroxycyclophosphamide 4-Ketocyclophosphamide



Phosphoramide Mustard *N,N*-bis(2-Chloroethyl)-phosphorodiamidic Acid Acrolein Aldophosphamide Carboxyphosphamide



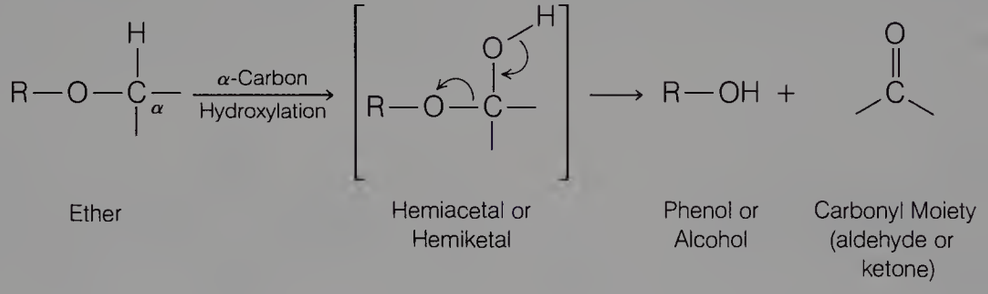
acid).[186] Further conjugation of this hydroxamic acid produces the corresponding *O*-sulfate ester, which ionizes to generate the electrophilic nitrenium species. Covalent binding of this reactive intermediate to DNA is known to occur and is likely to be the initial event that ultimately leads to malignant tumor formation.[187] Sulfate conjugation plays an important role in this biotoxification pathway (see "Sulfate Conjugation," below, for further discussion).

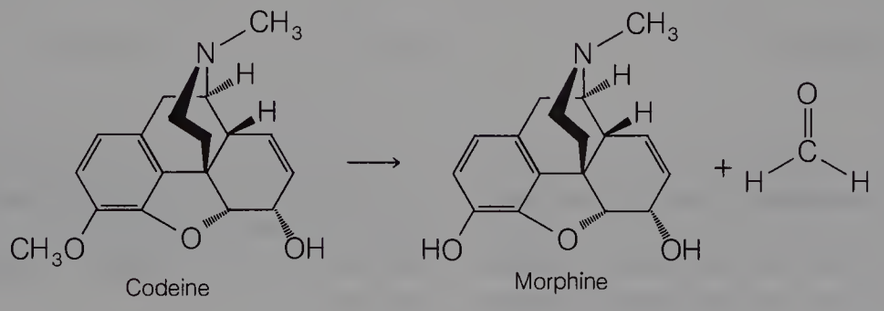


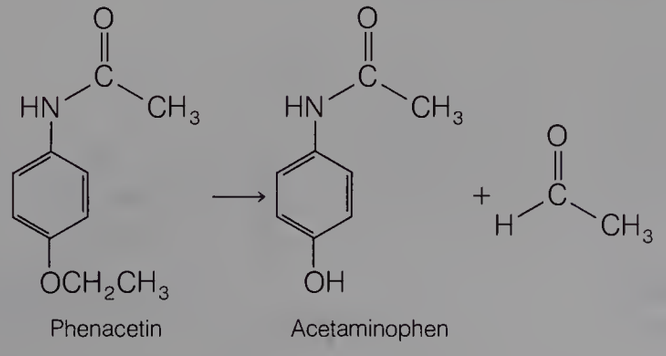
Acetaminophen is a relatively safe and nontoxic analgesic agent if used at therapeutic doses. When large doses of this drug are ingested, extensive liver necrosis is produced in humans and animals.[188] Considerable evidence argues that this hepatotoxicity is dependent upon the formation of a metabolically generated reactive intermediate.[189] Until recently,[190] the accepted bioactivation pathway was believed to involve an initial *N*-hydroxylation reaction to form *N*-hydroxyacetaminophen.[191] Spontaneous dehydration of this *N*-hydroxyamide produces *N*-acetylimidoquinone, the proposed reactive metabolite. Usually, the GSH present in the liver combines with this reactive metabolite to form the corresponding GSH conjugate. If GSH levels are sufficiently depleted by large doses of acetaminophen, covalent binding of the reactive intermediate occurs with macromolecules present in the liver, thereby leading to cellular necrosis. However, studies indicate that the reactive *N*-acetylimidoquinone intermediate is not formed from *N*-hydroxyacetaminophen.[189,190] It probably arises through some other oxidative process. Therefore, the mechanistic formation of the reactive metabolite of acetaminophen remains unclear.

Oxidation Involving Carbon–Oxygen Systems

Oxidative *O*-dealkylation of carbon–oxygen systems (principally ethers) is catalyzed by microsomal mixed function oxidases.[129] Mechanistically, the biotransformation involves an initial α-carbon hydroxylation to form either a hemiacetal or a hemiketal, which undergoes spontaneous carbon–oxygen bond cleavage to yield the dealkylated oxygen species (phenol or alcohol) and a carbon moiety (aldehyde or ketone). Small alkyl groups (e.g., methyl or ethyl) attached to oxygen are *O*-dealkylated rapidly. For example, morphine is the metabolic product resulting from *O*-demethylation of codeine.[192] The antipyretic and analgesic activities of phenacetin in humans appear to be a consequence of *O*-deethylation to the active metabolite acetaminophen.[193] Several other drugs containing ether groups, such as indomethacin (Indocin),[194] prazosin (Minipress),[195] and metoprolol (Lopressor),[96] have been reported to undergo significant *O*-demethylation to their corresponding phenolic or alcoholic metabolites, which are further conjugated. In many drugs that have several nonequivalent methoxy groups, one particular methoxy group often appears to be *O*-demethylated selectively or preferentially. For example, the 3,4,5-trimethoxyphenyl moiety in both mescaline[196] and trimethoprim[178] undergoes *O*-demethylation to yield predominantly the corresponding 3-*O*-demethylated metabolites. 4-*O*-Demethylation also occurs to a minor extent for both drugs. The phenolic and alcoholic metabolites formed from oxidative *O*-demethylation are susceptible to conjugation, particularly glucuronidation.

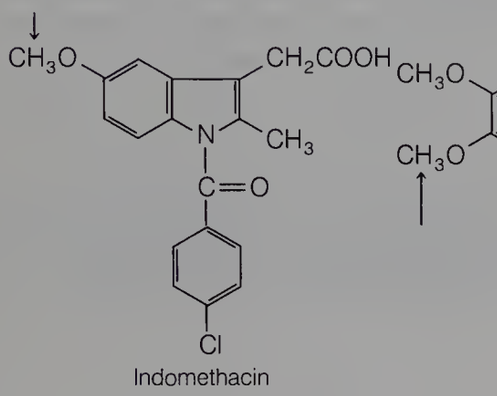




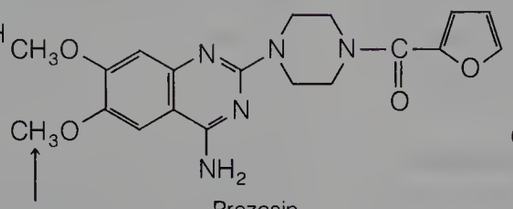


Oxidation Involving Carbon–Sulfur Systems

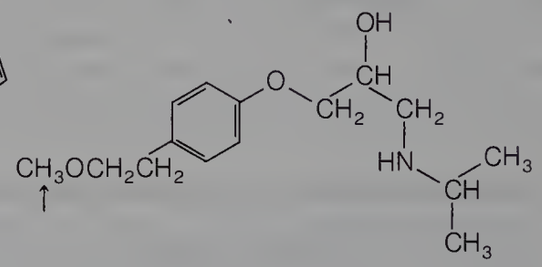
Carbon–sulfur functional groups are susceptible to metabolic *S*-dealkylation, desulfuration, and *S*-oxidation reactions. The first two processes involve oxidative carbon–sulfur bond cleavage. *S*-Dealkylation is analogous to *O*- and *N*-dealkylation mechanistically (i.e., involves α-carbon hydroxylation) and has been observed for various sulfur xenobiotics.[129,197] For example, 6-(methylthio)purine is demethylated oxidatively in rats to 6-mercaptopurine.[198] *S*-Demethylation of methitural[199] and *S*-debenzylation of 2-benzylthio-5-trifluoromethylbenzoic acid also have been reported. In contrast to *O*- and *N*-dealkylation, examples of drugs undergoing *S*-dealkylation in humans are limited, owing to the small number of sulfur-containing medicinals and to competing metabolic *S*-oxidation processes (see diagram).



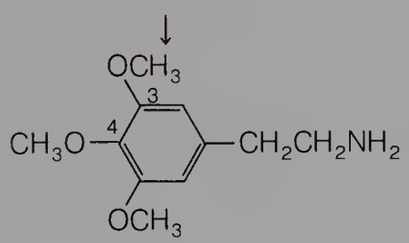
Indomethacin



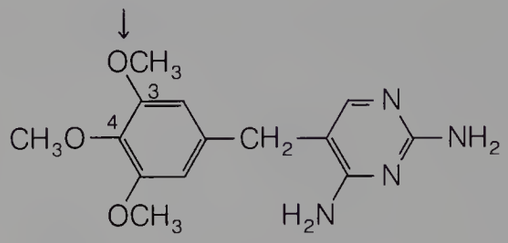
Prazosin



Metoprolol



Mescaline

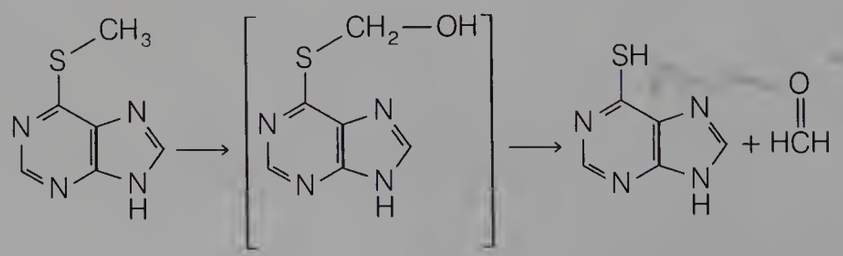


Trimethoprim

Oxidative conversion of carbon–sulfur double bonds (C=S) (thiono) to the corresponding carbon–oxygen double bond (C=O) is called "desulfuration." A well-known drug example of this metabolic process is the biotransformation of thiopental to its corresponding oxygen analogue pentobarbital.[200] An analogous desulfuration reaction also occurs with the P=S moiety present in a number of organophosphate insecticides, such as parathion.[201] Desulfuration of parathion leads to the formation of paraoxon, which is the active metabolite responsible for the anticholinesterase activity of the parent drug. The mechanistic details of desulfuration are poorly understood, but it appears to involve microsomal oxidation of the C=S or P=S double bond.[202]

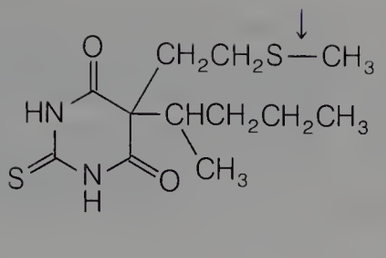
Organosulfur xenobiotics commonly undergo *S*-oxidation to yield sulfoxide derivatives. Several phenothiazine derivatives are metabolized by this pathway. For example, both sulfur atoms present in thioridazine (Mellaril)[203] are susceptible to *S*-oxidation. Oxidation of the 2-methylthio group yields the active sulfoxide metabolite mesoridazine. Interestingly, mesoridazine is twice as potent an antipsychotic agent as thioridazine in humans and has been introduced into clinical use as Serentil.[204]

S-Oxidation constitutes an important pathway in the metabolism of the H_2-histamine antagonists cimetidine (Tagamet)[205] and metiamide.[206] The corresponding sulfoxide derivatives are the major urinary metabolites found in humans.

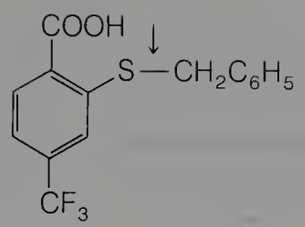


6-(Methylthio)-purine

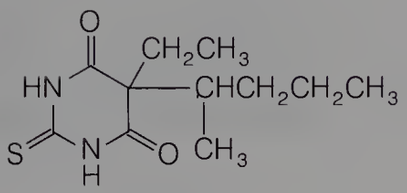
6-Mercaptopurine



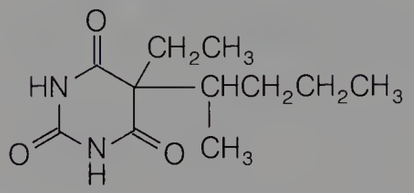
Methitural



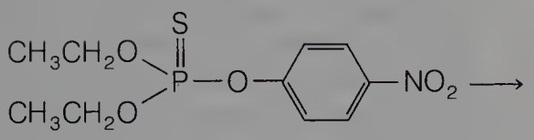
2-Benzylthio-5-trifluoromethylbenzoic Acid



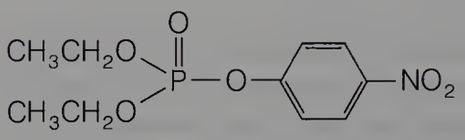
Thiopental



Pentobarbital

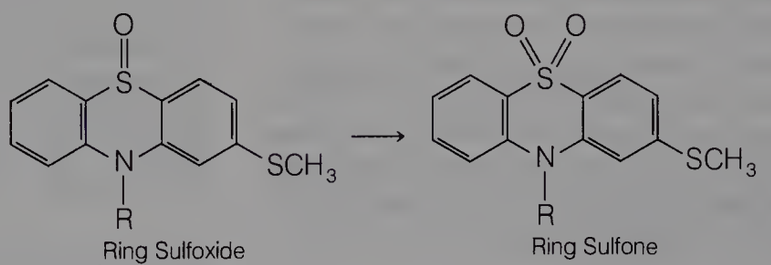


Parathion



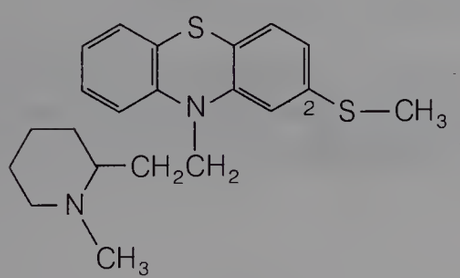
Paraoxon

Sulfoxide drugs and metabolites may be further oxidized to sulfones (—SO_2—). The sulfoxide group present in the immunosuppressive agent oxisuran is metabolized to a sulfone moiety.[207] In humans, dimethylsulfoxide (DMSO) is found primarily in the urine as the oxidized product dimethylsulfone. Sulfoxide metabolites, such as those of thioridazine, have been reported to undergo further oxidation to their sulfone —SO_2— derivatives.[203]

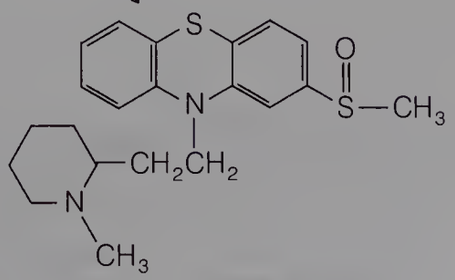


Ring Sulfoxide

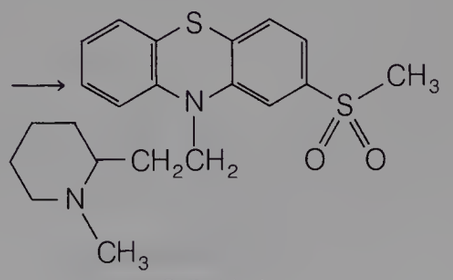
Ring Sulfone



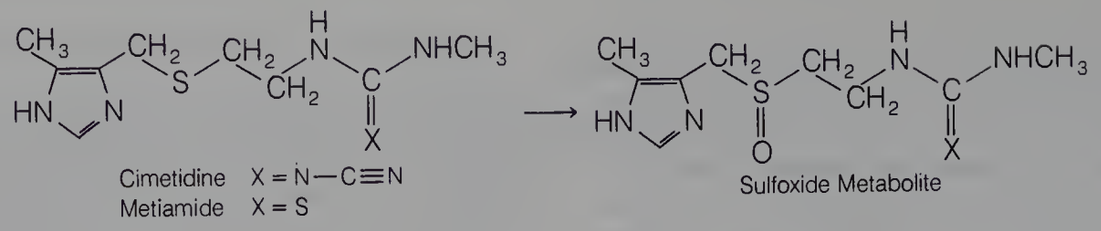
Thioridazine

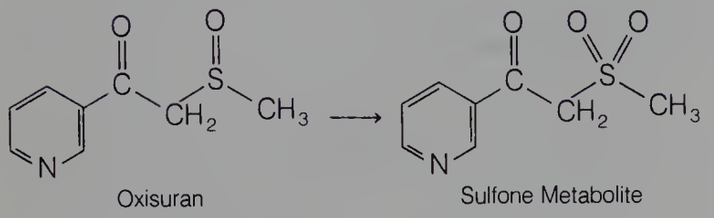


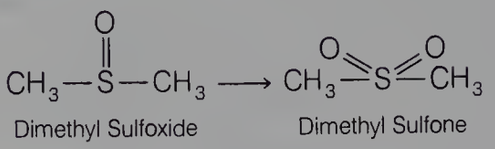
Mesoridazine



Sulforidazine



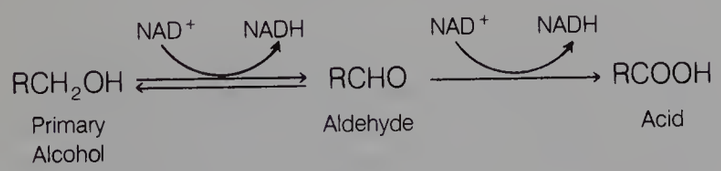




OXIDATION OF ALCOHOLS AND ALDEHYDES

Many oxidative processes (e.g., benzylic, allylic, alicyclic, or aliphatic hydroxylation) generate alcohol or carbinol metabolites as intermediate products. If not conjugated, these alcohol products are further oxidized to aldehydes (if primary alcohols) or to ketones (if secondary alcohols). Aldehyde metabolites resulting from oxidation of primary alcohols or from oxidative deamination of primary aliphatic amines often undergo facile oxidation to generate polar carboxylic acid derivatives.[91] As a general rule, primary alcoholic groups and aldehyde functionalities are quite vulnerable to oxidation. Several drug examples in which primary alcohol metabolites and aldehyde metabolites are oxidized to carboxylic acid products were cited in earlier sections.

Although secondary alcohols are susceptible to oxidation, this reaction is not often important because the reverse reaction, namely, reduction of the ketone back to the secondary alcohol, occurs quite readily. In addition, the secondary alcohol group, being polar and functionalized, is more likely to be conjugated than the ketone moiety.

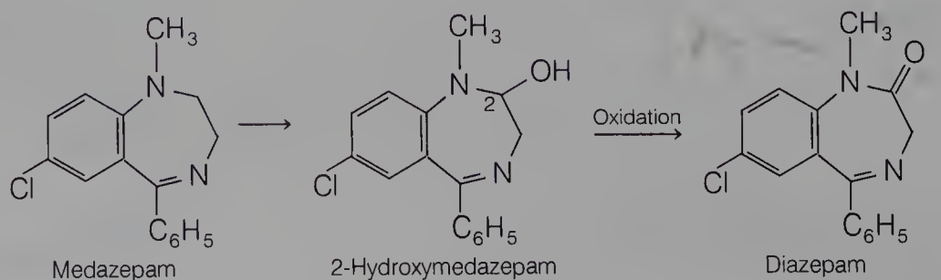


The bioconversion of alcohols to aldehydes and ketones is catalyzed by soluble alcohol dehydrogenases present in the liver and other tissues. NAD^+ is required as a coenzyme, although $NADP^+$ also may serve as a coenzyme. The reaction catalyzed by alcohol dehydrogenase is reversible but often proceeds to the right because the aldehyde formed is further oxidized to the acid. Several aldehyde dehydrogenases, including aldehyde oxidase and xanthine oxidase, carry out the oxidation of aldehydes to their corresponding acids.[91,208,209]

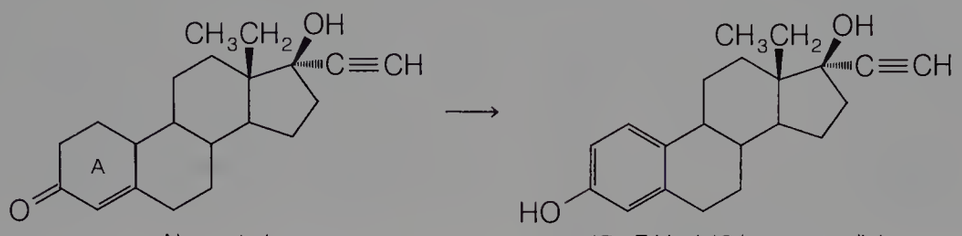
Metabolism of cyclic amines to their lactam metabolites has been observed for various drugs (e.g., nicotine, phenmetrazine, and methylphenidate). It appears that soluble or microsomal dehydrogenase and oxidases are involved in oxidizing the carbinol group of the intermediate carbinolamine to a carbonyl moiety.[209] For example, in the metabolism of medazepam to diazepam, the intermediate carbinolamine (2-hydroxymedazepam) undergoes oxidation of its 2-hydroxy group to a carbonyl moiety. A microsomal dehydrogenase carries out this oxidation.[210]

OTHER OXIDATIVE BIOTRANSFORMATION PATHWAYS

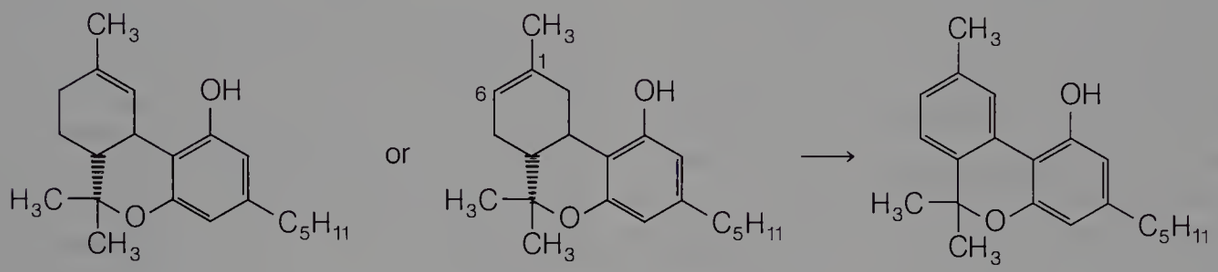
In addition to the many oxidative biotransformations discussed already, oxidative aromatization or dehydrogenation and oxidative dehalogenation reactions also occur. Meta-



bolic aromatization has been reported for norgestrel. Aromatization or dehydrogenation of the A ring present in this steroid leads to the corresponding phenolic product 17α-ethinyl-18-homoestradiol as a minor metabolite in women.[211] In mice, the terpene ring of Δ^1-THC or $\Delta^{1,6}$-THC undergoes aromatization to give cannabinol.[212]



Norgestrel 17α-Ethinyl-18-homoestradiol



Δ^1-THC $\Delta^{1,6}$-THC Cannabinol

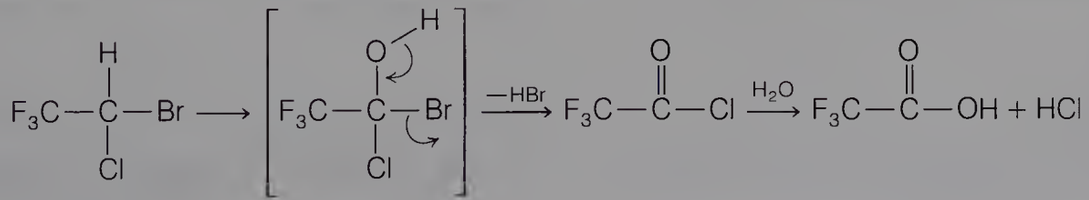
Many halogen-containing drugs and xenobiotics are metabolized by oxidative dehalogenation. For example, the volatile anesthetic agent halothane is metabolized principally to trifluoroacetic acid in humans.[213] It has been postulated that this metabolite arises from cytochrome P-450-mediated hydroxylation of halothane to form an initial carbinol intermediate that spontaneously eliminates hydrogen bromide (dehalogenation) to yield trifluoroacetyl chloride. The latter acyl chloride is chemically reactive and reacts rapidly with water to form trifluoroacetic acid. Alternatively, it can acylate tissue nucleophiles. Indeed, in vitro studies indicate that halothane is metabolized to a reactive intermediate (presumably trifluoroacetyl chloride), which covalently binds to liver microsomal proteins.[214] Chloroform also appears to be metabolized oxidatively by a similar dehalogenation pathway to yield the chemically reactive species phosgene. Phosgene may be responsible for the hepato- and nephrotoxicity associated with chloroform.[215]

A final example of oxidative dehalogenation concerns the antibiotic chloramphenicol. In vitro studies have demonstrated that the dichloroacetamide portion of the molecule undergoes oxidative dechlorination to yield a chemically reactive oxamyl chloride intermediate that is capable of reacting with water to form the corresponding oxamic acid metabolite or of acylating microsomal proteins.[216] Thus, it appears that in several instances oxidative dehalogenation can lead to the formation of toxic and reactive acyl halide intermediates.

REDUCTIVE REACTIONS

Reductive processes play an important role in the metabolism of many compounds containing carbonyl, nitro, and azo groups. Bioreduction of carbonyl compounds generates alcohol derivatives,[91,217] whereas nitro and azo reductions lead to amino derivatives.[218] The hydroxyl and amino moieties of the metabolites are much more susceptible to conjugation than the functional groups of the parent compounds. Hence, reductive processes, as such, facilitate drug elimination.

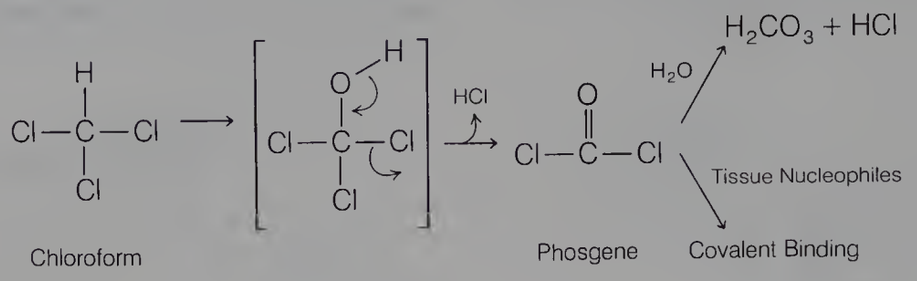
Reductive pathways that are encountered less frequently in drug metabolism include reduction of *N*-oxides to their

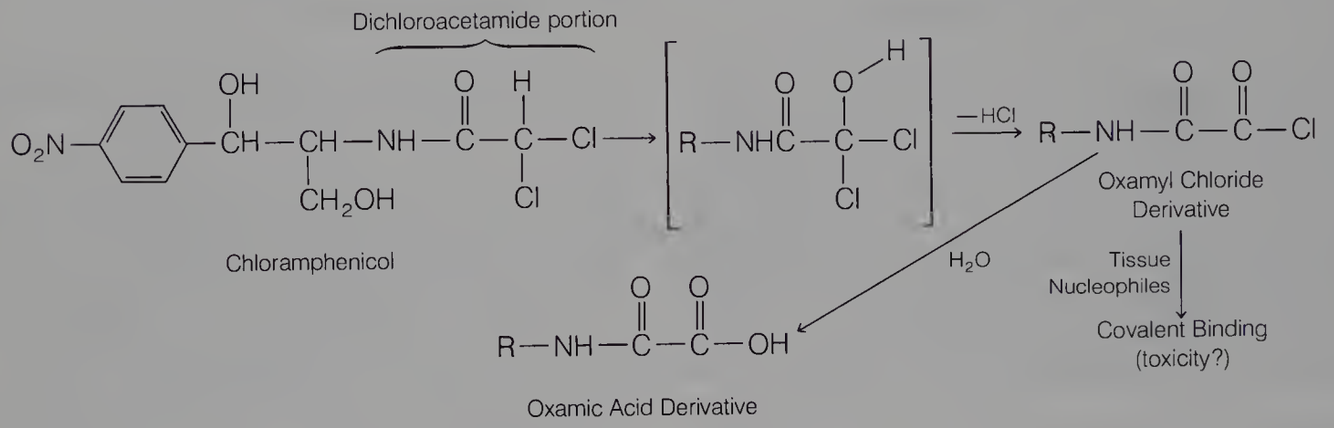


Halothane Carbinol Intermediate Trifluoroacetyl Chloride Trifluoroacetic Acid

corresponding tertiary amines and reduction of sulfoxides to sulfides. Reductive cleavage of disulfide linkages and reduction of carbon–carbon double bonds also occur but constitute only minor pathways in drug metabolism.

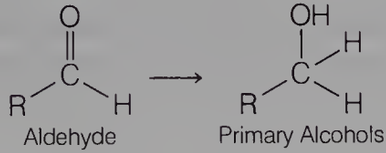
Divers soluble enzymes, called "aldo–keto reductases," carry out bioreduction of aldehydes and ketones.[91,219] They are found in the liver and other tissues (e.g., kidney). As a general class, these soluble enzymes have similar physi-

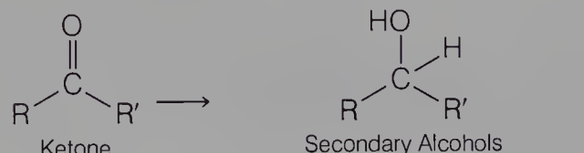




REDUCTION OF ALDEHYDE AND KETONE CARBONYLS

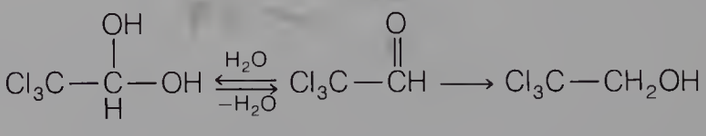
The carbonyl moiety, particularly the ketone group, is encountered frequently in many drugs. In addition, metabolites containing ketone and aldehyde functionalities often arise from oxidative deamination of xenobiotics (e.g., propranolol, chlorpheniramine, amphetamine). Owing to their ease of oxidation, aldehydes are metabolized mainly to carboxylic acids. Occasionally, aldehydes are reduced to primary alcohols. Ketones, however, are generally resistant to oxidation and are reduced mainly to secondary alcohols. Alcohol metabolites arising from reduction of carbonyl compounds generally undergo further conjugation (e.g., glucuronidation).





ochemical properties and broad substrate specificities and require NADPH as a cofactor. Oxidoreductase enzymes that carry out both oxidation and reduction reactions also are capable of reducing aldehydes and ketones.[219] For example, the important liver alcohol dehydrogenase is an NAD^+-dependent oxidoreductase that oxidizes ethanol and other aliphatic alcohols to aldehydes and ketones. However, in the presence of NADH or NADPH, the same enzyme system is capable of reducing carbonyl derivatives to their corresponding alcohols.[91]

Few aldehydes undergo bioreduction because of the relative ease of oxidation of aldehydes to carboxylic acids. However, one frequently cited example of a parent aldehyde drug undergoing extensive enzymatic reduction is the sedative–hypnotic chloral hydrate. Bioreduction of this hydrated aldehyde yields trichloroethanol as the major metabolite in humans.[220] Interestingly, this alcohol metabolite is pharmacologically active. Further glucuronidation of the alcohol leads to an inactive conjugated product that is readily excreted in the urine.



Aldehyde metabolites resulting from oxidative deamination of drugs also have been observed to undergo reduction to a minor extent. For example, in humans the β-adrenergic blocker propranolol is converted to an intermediate aldehyde by *N*-dealkylation and oxidative deamination. Although the aldehyde is oxidized primarily to the corresponding carboxylic acid (naphthoxylactic acid), a small fraction also is reduced to the alcohol derivative (propranolol glycol).[221]

Two major polar urinary metabolites of the histamine H_1 antagonist chlorpheniramine have been identified in dogs as the alcohol and carboxylic acid products (conjugated), derived respectively from reduction and oxidation of an aldehyde metabolite. The aldehyde precursor arises from bis-*N*-demethylation and oxidative deamination of chlorpheniramine.[222]

Bioreduction of ketones often leads to the creation of an asymmetric center and, thereby, two possible stereoisomeric alcohols.[91,223] For example, reduction of acetophenone by a soluble rabbit kidney reductase leads to the enantiomeric alcohols (*S*)(−)- and (*R*)(+)-methylphenylcarbinol, with the (*S*)(−)-isomer predominating (3 : 1 ratio).[224] The preferential formation of one stereoisomer over the other is termed *product stereoselectivity* in drug metabolism.[223] Mechanistically, ketone reduction involves a "hydride" transfer from the reduced nicotinamide moiety of the cofactor NADPH or NADH to the carbonyl carbon atom of the ketone. It is generally agreed that this step proceeds with considerable *stereoselectivity*.[91,223] Consequently, it is not surprising to find many reports of xenobiotic ketones that are reduced preferentially to a predominant stereoisomer. Often, ketone reduction yields alcohol metabolites that are pharmacologically active.

Although many ketone-containing drugs undergo significant reduction, only a few selected examples will be presented in detail here. Those xenobiotics that are not discussed in the text have been structurally tabulated in Fig. 3-10. The keto group undergoing reduction has been designated with an arrow.

Ketones lacking asymmetric centers in their molecules, such as acetophenone or the oral hypoglycemic acetohexamide, usually give rise to predominantly one enantiomer upon reduction. In humans, acetohexamide is metabolized rapidly in the liver to give principally (*S*)(−)-hydroxyhexamide.[225] This metabolite is as active a hypoglycemic agent as its parent compound and is eliminated through the kidneys.[226] Acetohexamide usually is not recommended in diabetic patients with renal failure, owing to the possible accumulation of its active metabolite, hydroxyhexamide.

When chiral ketones are reduced, they yield two possible diastereomeric or epimeric alcohols. For example, the (*R*)(+)-enantiomer of the oral anticoagulant warfarin undergoes extensive reduction of its side chain keto group to generate the (*R,S*)(+)-alcohol as the major plasma metabolite in humans.[45,234] Small amounts of the (*R,R*)(+)-diastereomer also are formed. In contrast, the (*S*)(−)-enantiomer undergoes little ketone reduction and is primarily 7-hydroxylated (i.e., aromatic hydroxylation) in humans.

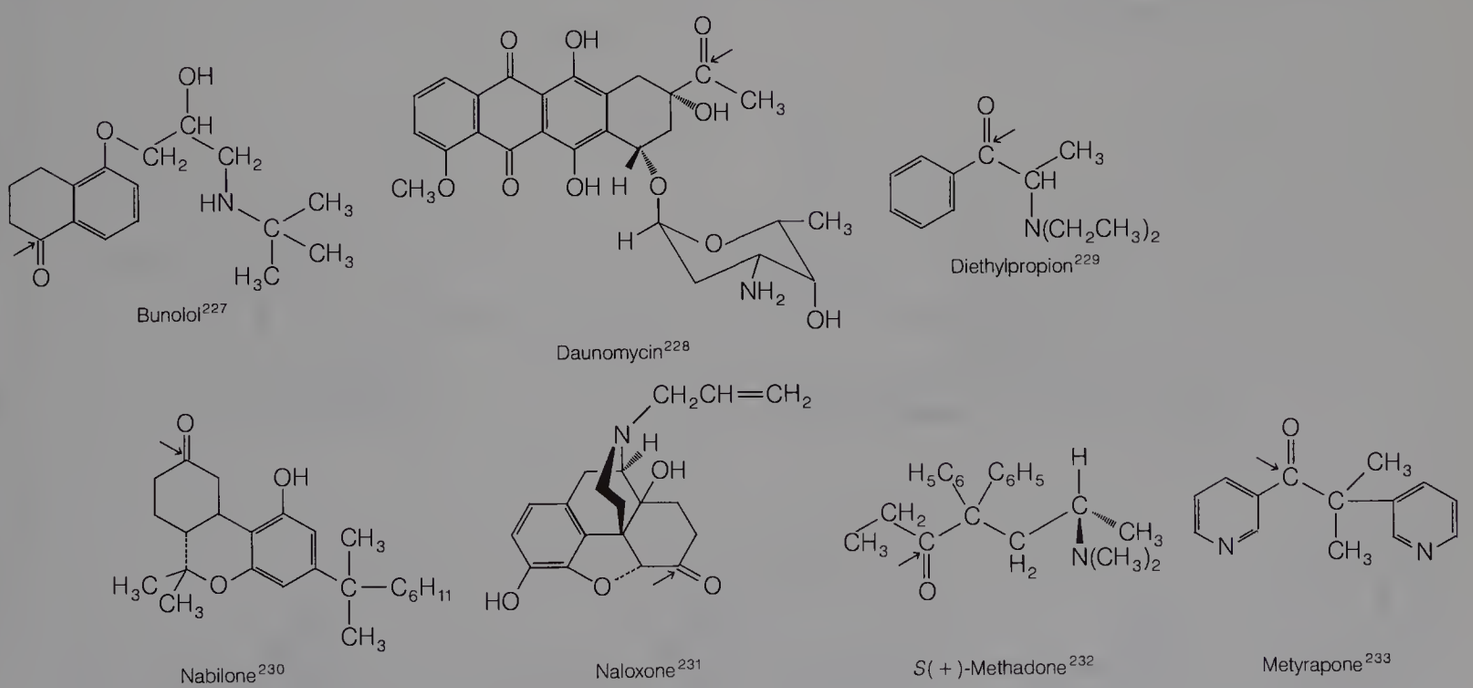
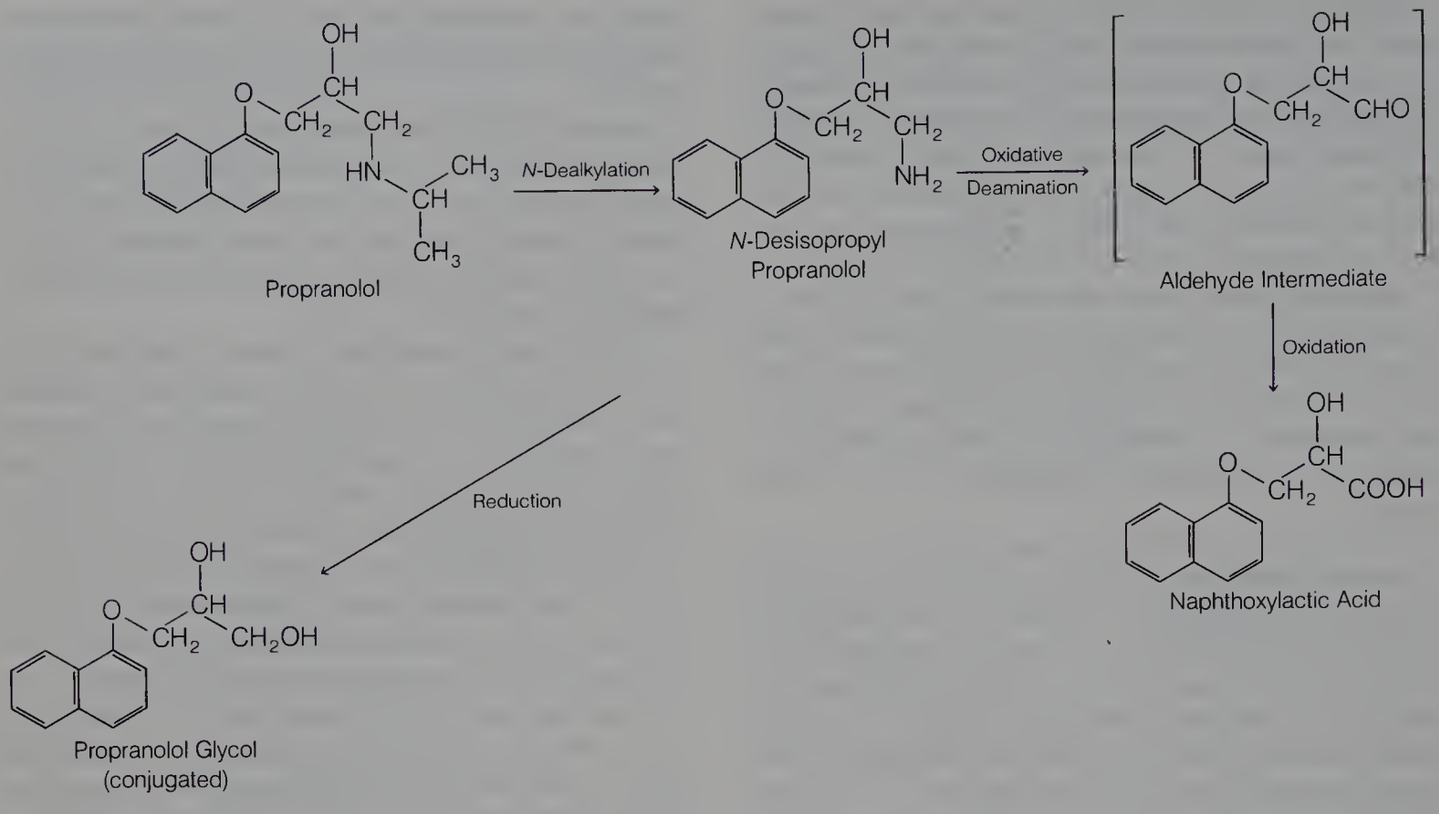
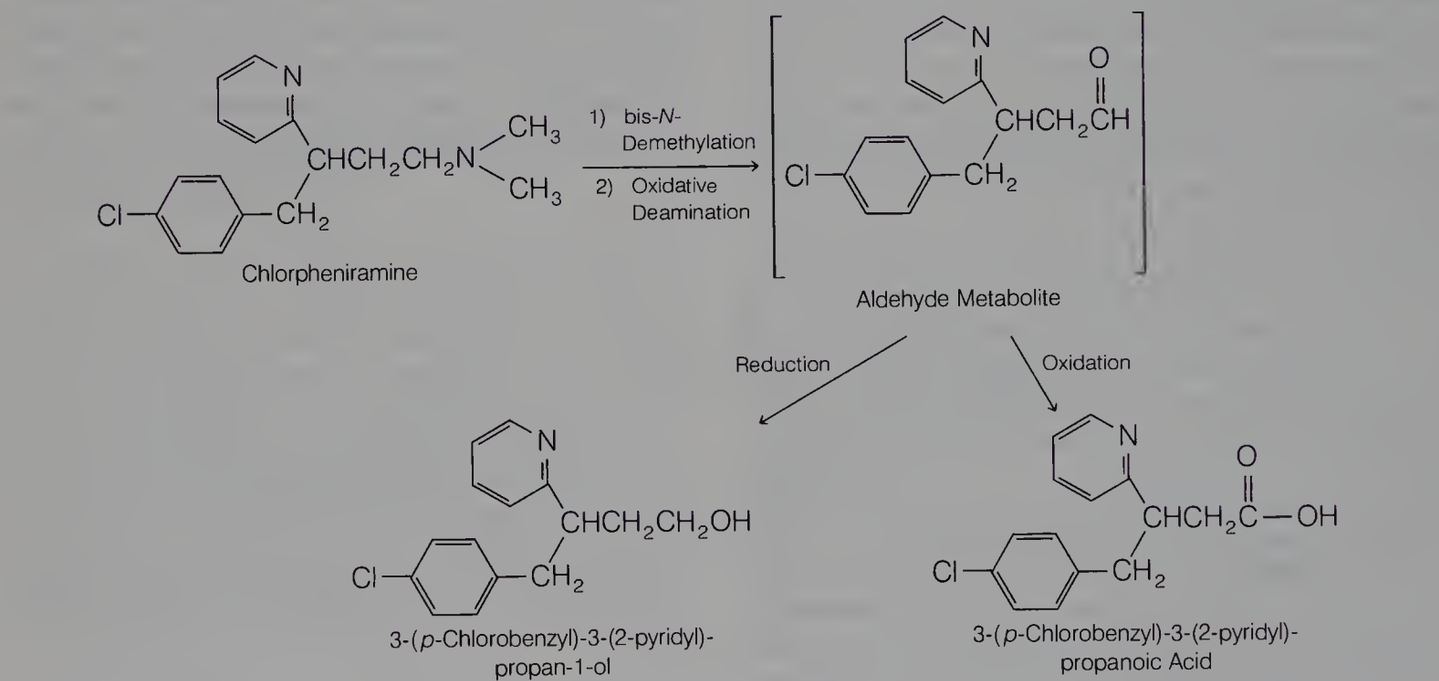
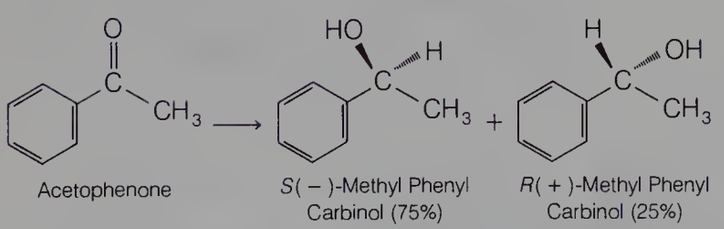
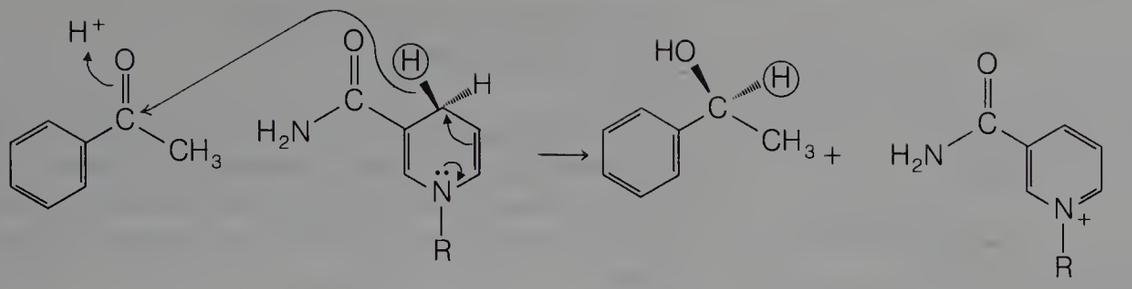


FIG. 3-10. *Additional examples of xenobiotics that undergo extensive ketone reduction, which were not covered in the text. Arrow indicates the keto group undergoing reduction.*

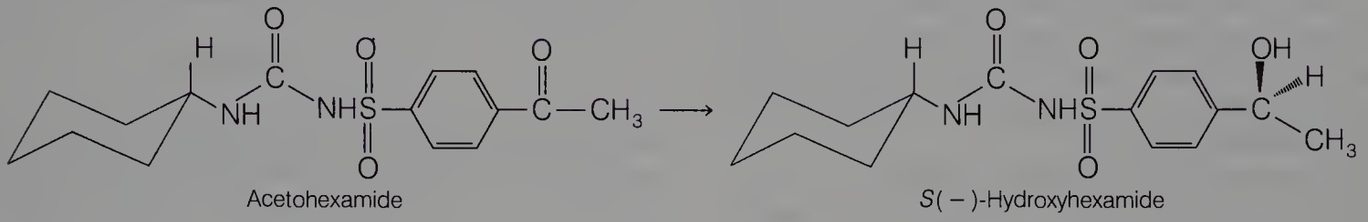

Propranolol
N-Dealkylation
N-Desisopropyl Propranolol
Oxidative Deamination
Aldehyde Intermediate
Oxidation
Naphthoxylactic Acid
Reduction
Propranolol Glycol (conjugated)


Chlorpheniramine
1) bis-N-Demethylation
2) Oxidative Deamination
Aldehyde Metabolite
Reduction
Oxidation
3-(p-Chlorobenzyl)-3-(2-pyridyl)-propan-1-ol
3-(p-Chlorobenzyl)-3-(2-pyridyl)-propanoic Acid


Acetophenone
S(−)-Methyl Phenyl Carbinol (75%)
R(+)-Methyl Phenyl Carbinol (25%)



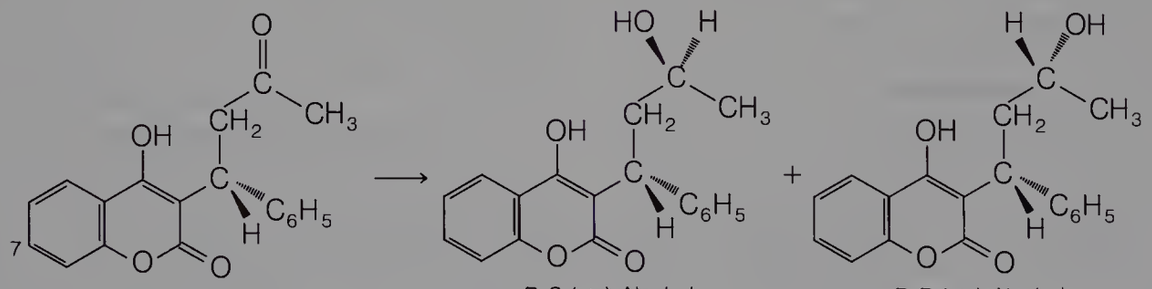
Acetophenone | Reduced Nicotamide Moiety of NADPH or NADH | *S*(−)-Methyl Phenyl Carbinol | Oxidized Nicotamide Moiety of $NADP^+$ or NAD^+



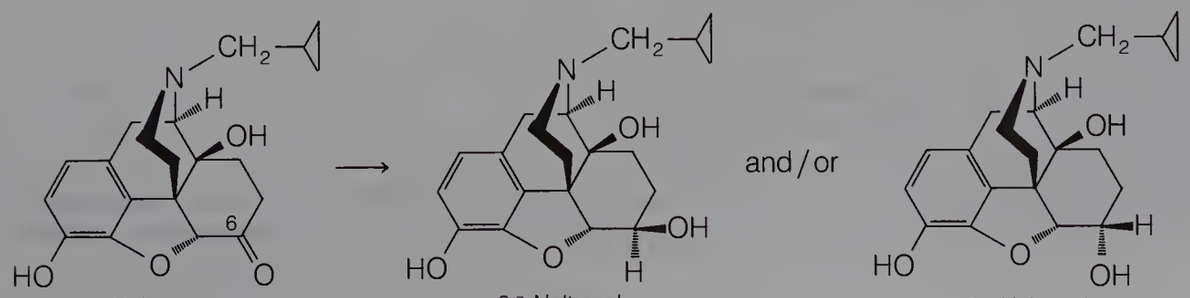
Acetohexamide | *S*(−)-Hydroxyhexamide

Reduction of the 6-keto functionality in the narcotic antagonist naltrexone can lead to either the epimeric 6α- or 6β-hydroxy metabolites depending on the animal species.[231] In human and rabbit, bioreduction of naltrexone is highly stereoselective and generates only 6β-naltrexol, whereas in chicken, reduction yields only 6α-naltrexol.[231,235] However, in monkey and guinea pig, both epimeric alcohols are formed (predominantly 6β-naltrexol).[236] It appears that in the latter two species, reduction of naltrexone to the epimeric 6α- and 6β-alcohols is carried out by two distinctly different reductases found in the liver.[235,236]

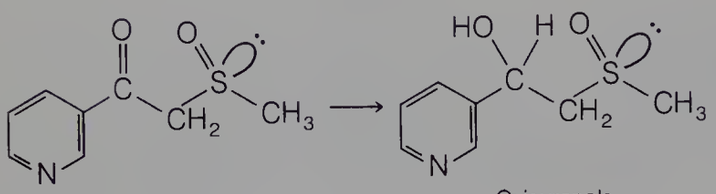
Reduction of oxisuran appears not be an important pathway by which the parent drug mediates its immunosuppressive effects. Studies indicate that oxisuran has its greatest immunosuppressive effects in those species that form alcohols as their major metabolic products (e.g., human, rat).[237,238] In species in which reduction is a minor pathway (e.g., dog), oxisuran shows little immunosuppressive activity.[238] These findings indicate that the oxisuran alcohols (oxisuranols) are pharmacologically active and contribute substantially to the overall immunosuppressive effect of the parent drug. The sulfoxide group in oxisuran is chiral, by virtue of the lone pair of electrons on sulfur. Therefore, reduction of oxisuran leads to diastereomeric alcohols.



R(+)-Warfarin | *R*,*S*-(+)-Alcohol Major Diastereomer | *R*,*R*-(+)-Alcohol Minor Diastereomer



Naltrexone | 6β-Naltrexol | and/or | 6α-Naltrexol



Oxisuran

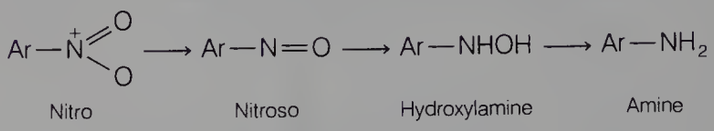
Oxisuranols
(diastereomeric mixture)

Reduction of α,β-unsaturated ketones results in reduction not only of the ketone group but of the carbon–carbon double bond as well. Steroidal drugs often fall into this class, including norethindrone, a synthetic progestin found in many oral contraceptive drug combinations. In women, the major plasma and urinary metabolite of norethindrone is the 3β, 5β-tetrahydro derivative.[239]

Ketones resulting from metabolic oxidative deamination processes are also susceptible to reduction. For example, rabbit liver microsomal preparations metabolize amphetamine to phenylacetone, which is reduced subsequently to 1-phenyl-2-propanol.[240] In humans, a minor urinary metabolite of (−)-ephedrine has been identified as the diol derivative formed from keto reduction of the oxidatively deaminated product 1-hydroxy-1-phenylpropan-2-one.[241]

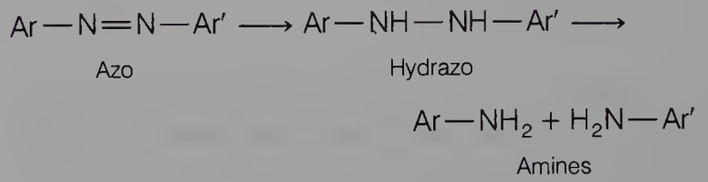
REDUCTION OF NITRO AND AZO COMPOUNDS

The reduction of aromatic nitro and azo xenobiotics leads to aromatic primary amine metabolites.[218] Aromatic nitro compounds are reduced initially to the nitroso and hydroxylamine intermediates, as shown in the metabolic sequence below:

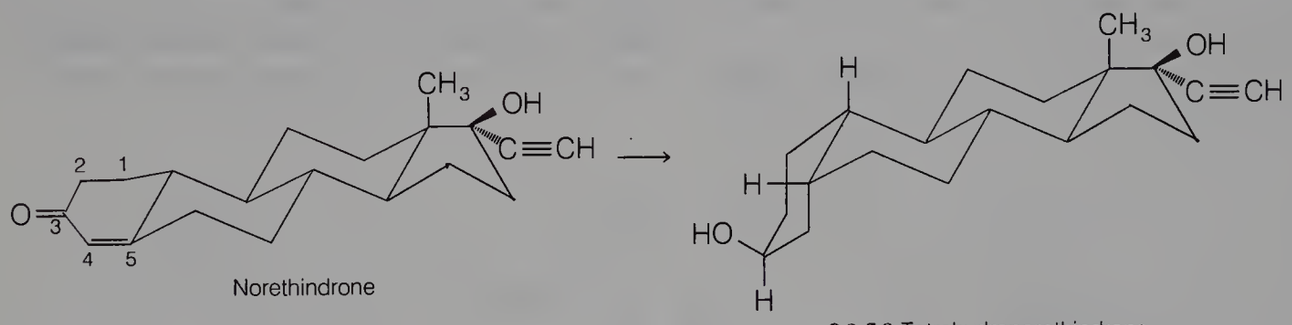


Nitro — Nitroso — Hydroxylamine — Amine

Azo reduction, however, is believed to proceed via a hydrazo intermediate (—NH—NH—) that subsequently is cleaved reductively to yield the corresponding aromatic amines:

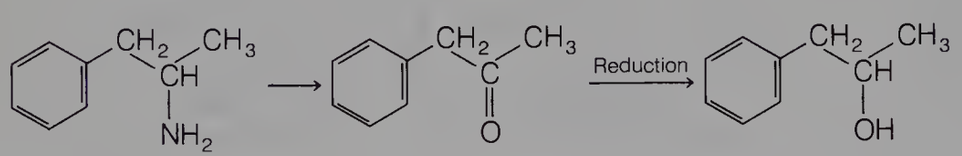


Azo — Hydrazo — Amines

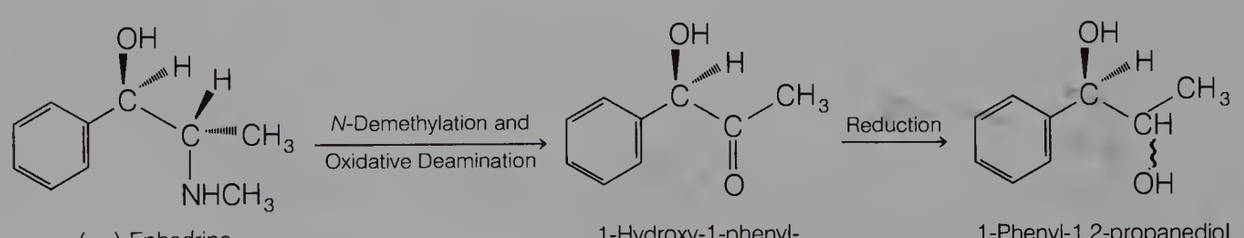


Norethindrone

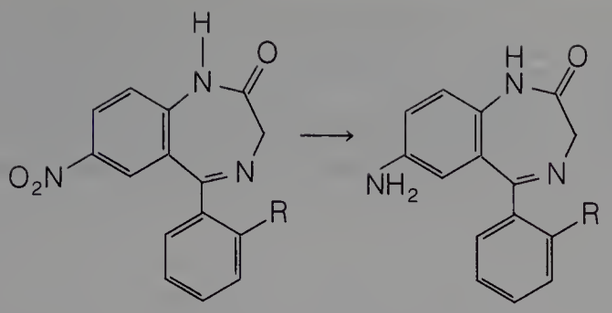
$3\beta,5\beta$-Tetrahydronorethindrone



Amphetamine — Phenylacetone — 1-Phenyl-2-propanol

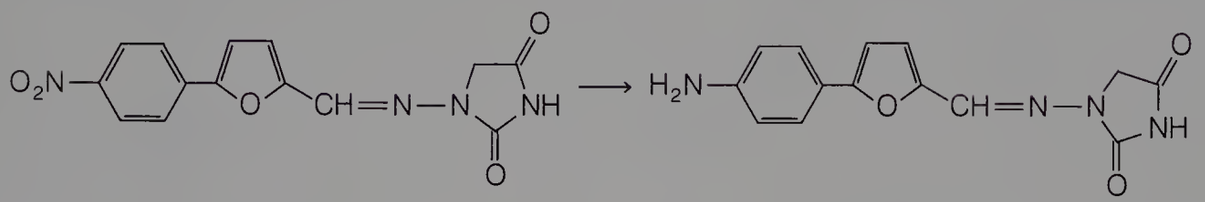


(−)-Ephedrine — 1-Hydroxy-1-phenylpropan-2-one — 1-Phenyl-1,2-propanediol (as glucuronide conjugate)



Clonazepam, R = Cl
Nitrazepam, R = H

7-Amino Metabolite



Dantrolene

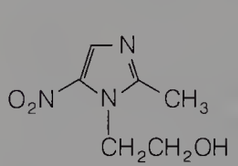
Aminodantrolene

Bioreduction of nitro compounds is carried out by NADPH-dependent microsomal and soluble nitro reductases present in the liver. A multicomponent hepatic microsomal reductase system requiring NADPH appears to be responsible for azo reduction.[242,243] In addition, bacterial reductases present in the intestine are capable of reducing nitro and azo compounds, especially those that are absorbed poorly or excreted mainly in the bile.[244]

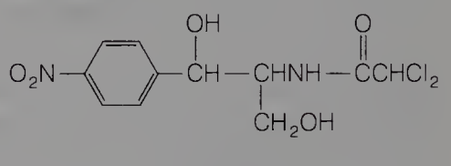
Various aromatic nitro drugs undergo enzymatic reduction to the corresponding aromatic amines. For example, the 7-nitro benzodiazepine derivatives clonazepam and nitrazepam are metabolized extensively to their respective 7-amino metabolites in humans.[245,246] The skeletal muscle relaxant dantrolene (Dantrium) also has been reported to undergo reduction to aminodantrolene in humans.[247]

For some nitro xenobiotics, bioreduction appears to be a minor metabolic pathway in vivo, owing to competing oxidative and conjugative reactions. However, under artificial anaerobic in vitro incubation conditions, these same nitro xenobiotics are enzymatically reduced rapidly. For example, most of the urinary metabolites of metronidazole found in humans are either oxidation or conjugation products. Reduced metabolites of metronidazole have not been detected.[248] However, when incubated anaerobically with guinea pig liver preparations, metronidazole undergoes considerable nitro reduction.[249]

Bacterial reductase present in the intestine also tends to complicate in vivo interpretations of nitro reduction. For example, in rats the antibiotic chloramphenicol is not reduced in vivo by the liver but is excreted in the bile and, subsequently, reduced by intestinal flora to form the amino metabolite.[250]

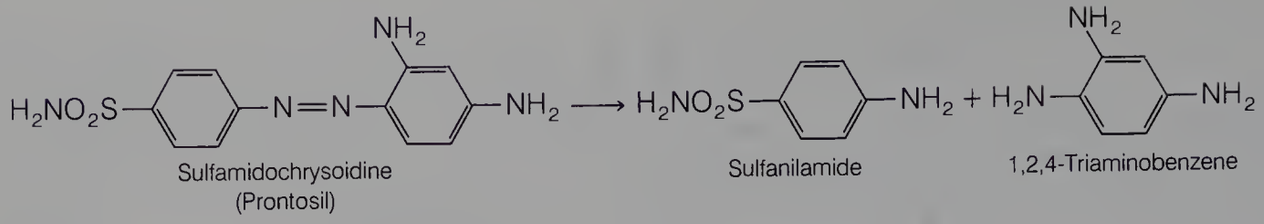


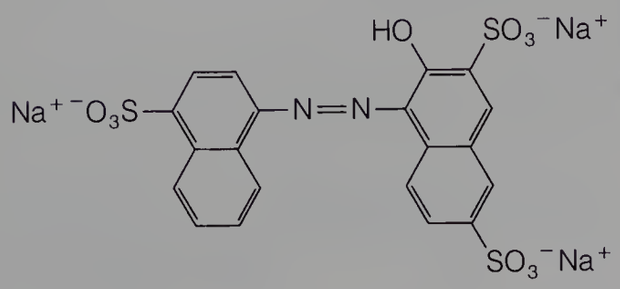
Metronidazole

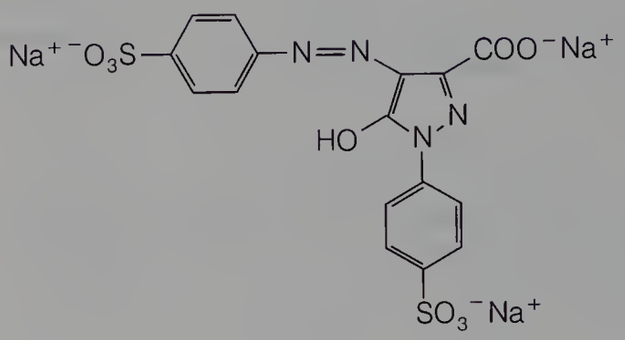


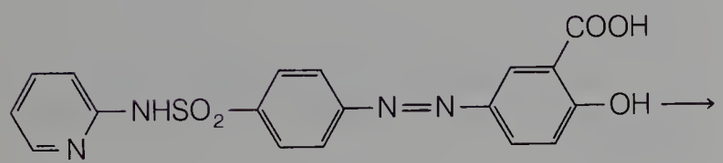
Chloramphenicol

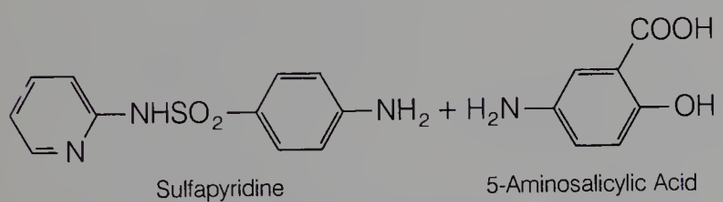
The enzymatic reduction of azo compounds is best exemplified by the conversion of sulfamidochrysoidine (Prontosil) to the active sulfanilamide metabolite in the liver.[251] This reaction has historical significance, for it led to the discovery of sulfanilamide as an antibiotic and eventually to the development of many of the therapeutic sulfonamide drugs. Bacterial reductases present in the intestine play a significant role in reducing azo xenobiotics, particularly those that are absorbed poorly.[244] For example, the two azo dyes tartrazine[252] and amaranth[253] have poor oral absorption as a result of the many polar and ionized sulfonic acid groups present in their structures. Therefore, these two azo compounds are metabolized primarily by bacterial reductases present in the intestine. The importance of intestinal reduction is further revealed in the metabolism of sulfasalazine (formerly salicylazosulfapyridine, Azulfidine), a drug used in the treatment of ulcerative colitis. The drug is absorbed poorly and undergoes reductive cleavage of the azo linkage to yield sulfapyridine and 5-aminosalicylic acid.[254] The reaction occurs primarily in the colon and is carried out principally by intestinal bacteria. Studies in germ-free rats, lacking intestinal flora, have demonstrated that sulfasalazine is not reduced to any appreciable extent.[255]

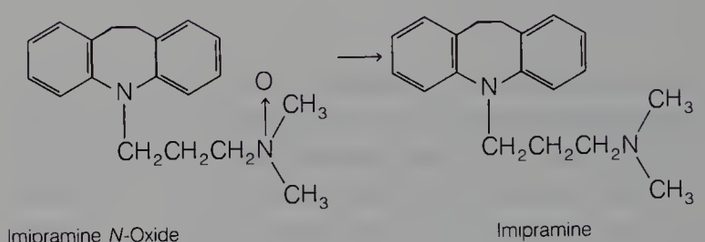








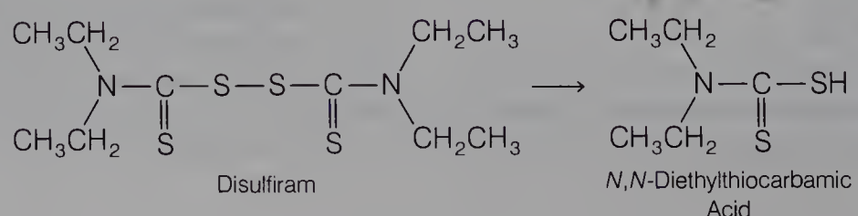




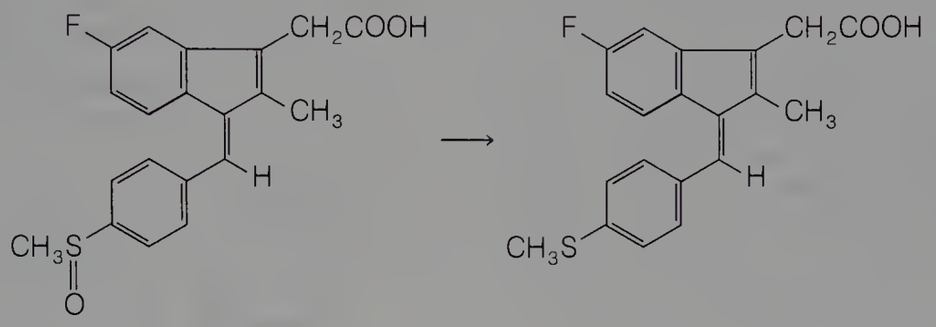
MISCELLANEOUS REDUCTIONS

Several minor reductive reactions also occur. Reduction of *N*-oxides to the corresponding tertiary amine occurs to some extent. This reductive pathway is of interest because several tertiary amines are oxidized to form polar and water-soluble *N*-oxide metabolites. If reduction of *N*-oxide metabolites occurs to a significant extent, drug elimination of the parent tertiary amine would be impeded. *N*-Oxide reduction often is assessed by administering the pure synthetic *N*-oxide in vitro or in vivo and then attempting to detect the formation of the tertiary amine. For example, imipramine *N*-oxide undergoes reduction in rat liver preparations.[256]

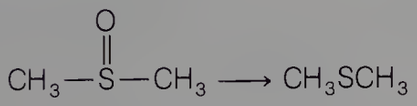
Reduction of sulfur-containing functional groups, such as the disulfide and sulfoxide moieties, also constitutes a minor reductive pathway. Reductive cleavage of the disulfide bond in disulfiram (Antabuse) yields *N,N*-diethyldithiocarbamic acid (free or glucuronidated) as a major metabolite in humans.[257] Although sulfoxide functionalities are oxidized mainly to sulfones (—SO_2—), they sometimes undergo reduction to sulfides. The importance of this reductive pathway is seen in the metabolism of the anti-inflammatory agent sulindac (Clinoril). Studies in humans show that sulindac undergoes reduction to an active sulfide that is responsible for the overall anti-inflammatory effect of the parent drug.[258] Sulindac or its sulfone metabolite exhibits little anti-inflam-



matory activity. Another example of sulfide formation involves the reduction of DMSO to dimethyl sulfide. In humans, DMSO is metabolized to a minor extent by this pathway. The characteristic unpleasant odor of dimethyl sulfide is evident on the breath of patients who use this agent.[259]



Sulindac — Sulindac Sulfide Metabolite



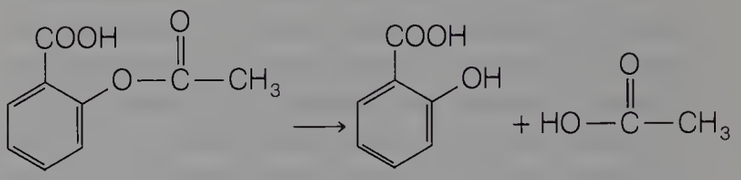
Dimethyl Sulfoxide — Dimethyl Sulfide

HYDROLYTIC REACTIONS

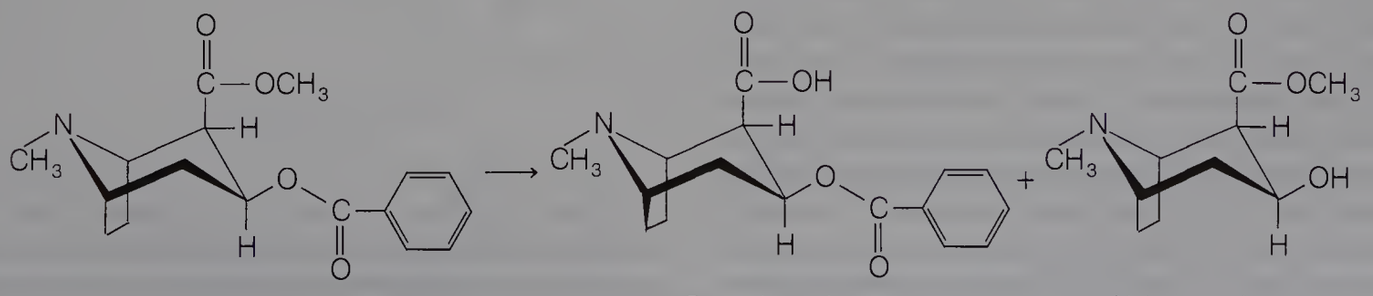
HYDROLYSIS OF ESTERS AND AMIDES

The metabolism of ester and amide linkages in many drugs is catalyzed by hydrolytic enzymes present in various tissues and in plasma. The metabolic products formed (carboxylic acids, alcohols, phenols, and amines) generally are polar and functionally more susceptible to conjugation and excretion than the parent ester or amide drugs. The enzymes carrying out ester hydrolysis include several nonspecific esterases found in the liver, kidney, and intestine as well as the pseudocholinesterases present in plasma.[260,261] Amide hydrolysis appears to be mediated by liver microsomal amidases, esterases, and deacylases.[261]

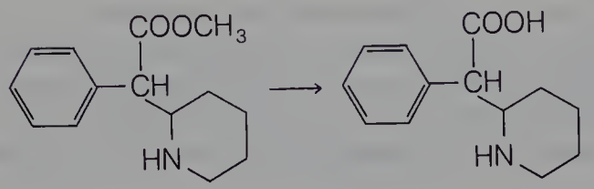
Hydrolysis is a major biotransformation pathway for drugs containing the ester functionality. This is because of the relative ease of hydrolyzing the ester linkage. A classic example of ester hydrolysis is the metabolic conversion of aspirin (acetylsalicylic acid) to salicylic acid.[262] Of the two ester moieties present in cocaine, it appears that, in general, the methyl group is hydrolyzed preferentially to yield benzoylecgonine as the major urinary metabolite in humans.[263] However, the hydrolysis of cocaine to methylecgonine also has been demonstrated to occur in plasma and blood to a minor extent.[264] Methylphenidate (Ritalin) is biotransformed rapidly by hydrolysis to yield ritalinic acid as the major urinary metabolite in humans.[265] Often, ester hydrolysis of the parent drug leads to pharmacologically active metabolites. For example, hydrolysis of diphenoxylate in humans leads to diphenoxylic acid (difenoxin), which is apparently five times more potent an antidiarrheal agent than the parent ester.[266] The rapid metabolism of clofibrate (Atromid-S) yields *p*-chlorophenoxyisobutyric acid (CPIB) as the major plasma metabolite in humans.[267] Studies in rats indicate that the free acid CPIB is responsible for clofibrate's hypolipidemic effect.[268]



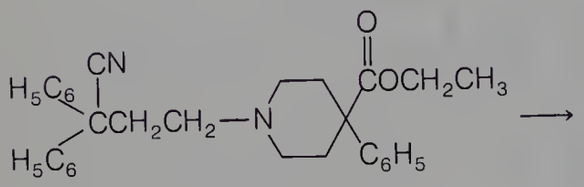
Aspirin (Acetylsalicylic acid) — Salicylic Acid — Acetic Acid



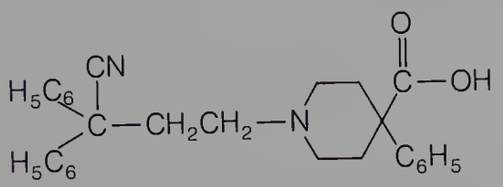
Cocaine — Benzoylecgonine — Methylecgonine



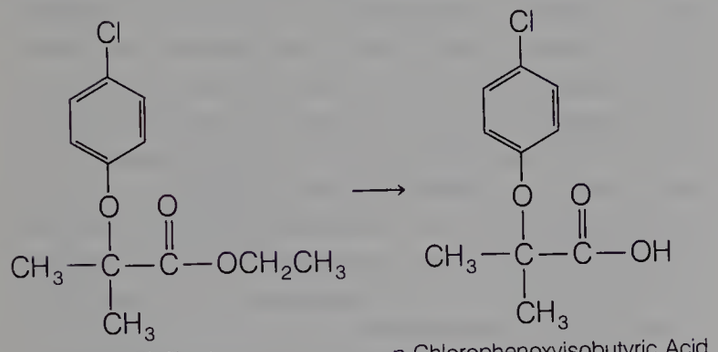
Methylphenidate Ritalinic Acid



Diphenoxylate

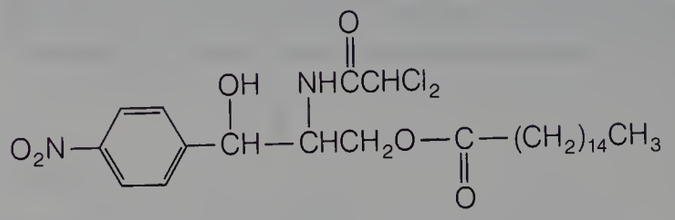


Diphenoxylic Acid
(Difenoxin)

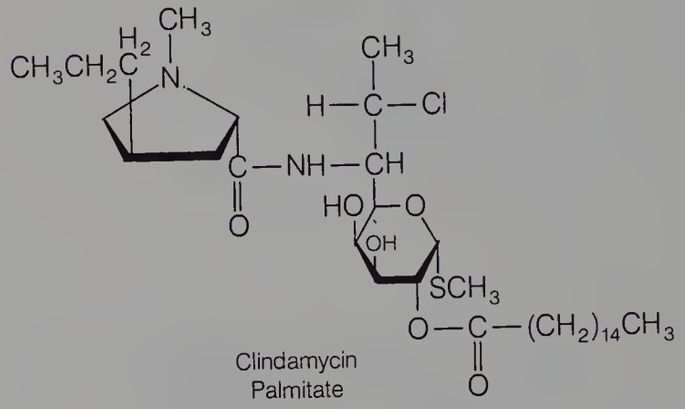


Clofibrate *p*-Chlorophenoxyisobutyric Acid

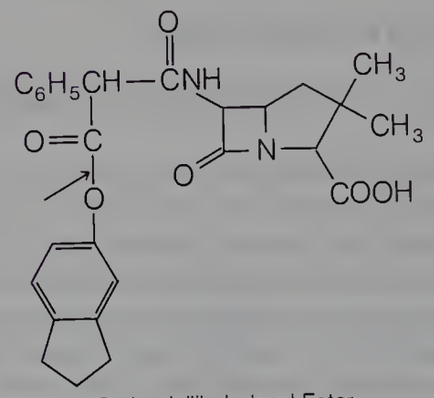
Many parent drugs have been chemically modified or derivatized to generate so-called *prodrugs* to overcome some undesirable property (e.g., bitter taste, poor absorption, poor solubility, irritation at site of injection). The rationale behind the prodrug concept was to develop an agent that, once inside the biologic system, would be biotransformed to the active parent drug.[17] The presence of esterases in many tissues and plasma makes ester derivatives logical prodrug candidates because hydrolysis would cause the ester prodrug to revert to the parent compound. For example, antibiotics, such as chloramphenicol and clindamycin, have been derivatized as their palmitate esters to minimize their bitter taste and to improve their palatability in pediatric liquid suspensions.[269] After oral administration, intestinal esterases and lipases hydrolyze the palmitate esters to the free antibiotics. To improve the poor oral absorption of carbenicillin, a lipophilic indanyl ester has been formulated (Geocillin).[270] Once orally absorbed, the ester is hydrolyzed rapidly to the parent drug. A final example involves derivatization of prednisolone to its C-21 hemisuccinate sodium salt. This water-soluble derivative is extremely useful for parenteral administration and is metabolized to the parent steroid drug by plasma and tissue esterases.[271]



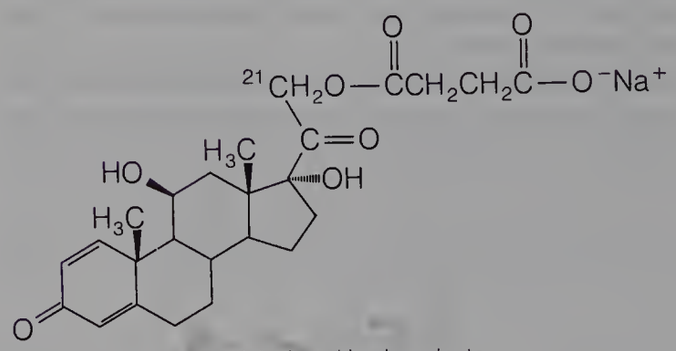
Chloramphenicol
Palmitate



Clindamycin
Palmitate



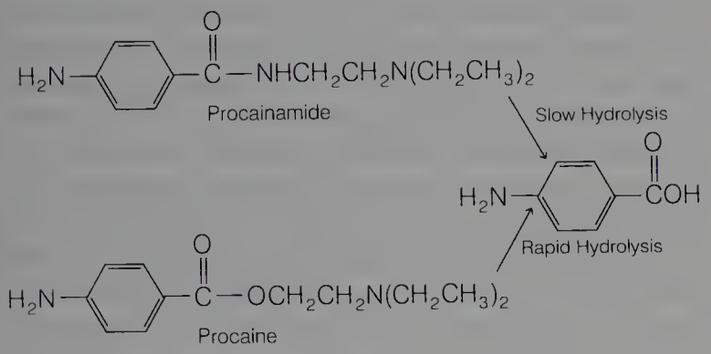
Carbenicillin Indanyl Ester



Prednisolone Hemisuccinate
Sodium Salt

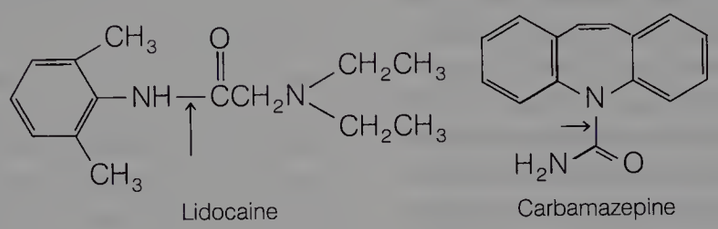
Amides are hydrolyzed slowly in comparison to esters.[261] For example, hydrolysis of the amide bond of procainamide is relatively slow compared with hydrolysis of the ester linkage in procaine.[260,272] Drugs in which amide cleavage has

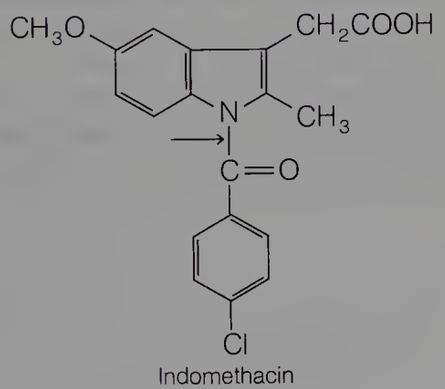
been reported to occur to some extent include lidocaine,[273] carbamazepine,[72] indomethacin,[194] and prazosin (Minipress).[195] Amide linkages present in barbiturates (e.g., hexobarbital)[274] as well as in hydantoins (e.g., 5-phenylhydantoin)[275a] and succinimides (phensuximide)[275a] are also susceptible to hydrolysis.

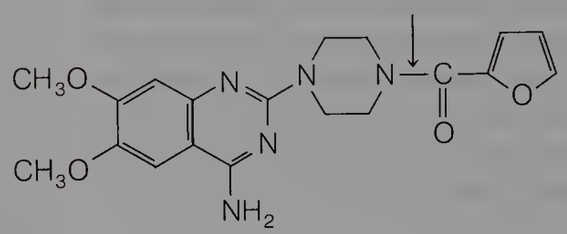


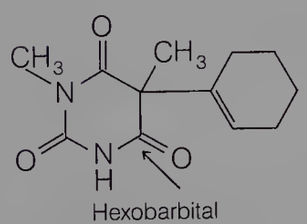
is a well-recognized hydrolytic reaction.[275b] Examples of peptides or protein hormones undergoing hydrolysis include human insulin, growth hormone (GH), prolactin, parathyroid hormone (PTH), and atrial natriuretic factor (ANF), to name a few.[275c]

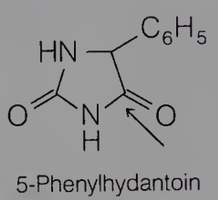
In addition to hydrolysis of amides and esters, hydrolytic cleavage of other moieties occurs to a minor extent in drug metabolism,[8] including the hydrolysis of phosphate esters (e.g., diethylstilbestrol diphosphate), sulfonylureas, cardiac glycosides, carbamate esters, and organophosphate compounds. Glucuronide and sulfate conjugates also are capable of undergoing hydrolytic cleavage by β-glucuronidase and sulfatase enzymes. These hydrolytic reactions are discussed in the following section. Finally, the hydration or hydrolytic cleavage of epoxides and arene oxides by epoxide hydrase is considered a hydrolytic reaction.

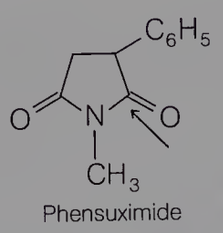












MISCELLANEOUS HYDROLYTIC REACTIONS

Hydrolysis of recombinant human peptide drugs and hormones at the N- or C-terminal amino acids by carboxy- and aminopeptidases and proteases in blood and other tissues

PHASE II, OR CONJUGATION, REACTIONS

Phase I, or functionalization, reactions do not always produce hydrophilic or pharmacologically inactive metabolites. However, various phase II, or conjugation, reactions are ca-

pable of converting these metabolites to more polar and water-soluble products. Many conjugative enzymes accomplish this objective by attaching small, polar, and ionizable endogenous molecules, such as glucuronic acid, sulfate, glycine, and glutamine, to the phase I metabolite or parent xenobiotic. The resulting conjugated products are relatively water-soluble and readily excretable. In addition, they generally are biologically inactive and nontoxic. Other phase II reactions, such as methylation and acetylation, do not generally increase water solubility but mainly serve to terminate or attenuate pharmacologic activity. The role of GSH is to combine with chemically reactive compounds to prevent damage to important biomacromolecules, such as DNA, RNA, and proteins. Thus, phase II reactions can be regarded as truly detoxifying pathways in drug metabolism, with a few exceptions.

A distinguishing feature of most phase II reactions is that the conjugating group (glucuronic acid, sulfate, methyl, and acetyl) is activated initially in the form of a coenzyme before transfer or attachment of the group is made to the accepting substrate by the appropriate transferase enzyme. In other cases, such as glycine and glutamine conjugation, the substrate is activated initially. Many endogenous compounds, such as bilirubin, steroids, catecholamines, and histamine, also undergo conjugation reactions and utilize the same coenzymes, although they appear to be mediated by more specific transferase enzymes. The phase II conjugative pathways to be discussed include those listed earlier in this chapter. Although other conjugative pathways (e.g., conjugation with glycosides, phosphate, other amino acids, conversion of cyanide to thiocyanate) exist, they are of only minor importance in drug metabolism and will not be covered in this chapter.

GLUCURONIC ACID CONJUGATION

Glucuronidation is the most common conjugative pathway in drug metabolism for several reasons: (1) a readily available supply of D-glucuronic acid (derived from D-glucose); (2) numerous functional groups that can combine enzymatically with glucuronic acid; and (3) the glucuronyl moiety (with its ionized carboxylate [pK_a 3.2] and polar hydroxyl groups), when attached to xenobiotic substrates, greatly increases the water solubility of the conjugated product.[92,276,277] Formation of β-glucuronides involves two steps, synthesis of an activated coenzyme, uridine-5′-diphospho-α-D-glucuronic acid (UDPGA), and subsequent transfer of the glucuronyl group from UDPGA to an appropriate substrate.[92,277] The transfer step is catalyzed by microsomal enzymes called UDP-glucuronyltransferases. They are found primarily in the liver but also occur in many other tissues, including kidney, intestine, skin, lung, and brain.[277]

The sequence of events involved in glucuronidation is summarized in Fig. 3-11.[92,277] The synthesis of the coenzyme UDPGA utilizes α-D-glucose-1-phosphate as its initial precursor. Note that all glucuronide conjugates have the β-configuration or β-linkage at C-1 (hence, the term "*β-glucuronides*"). In contrast, the coenzyme UDPGA has an α-linkage. In the enzymatic transfer step, it appears that nucleophilic displacement of the α-linked UDP moiety from UDPGA by the substrate RXH proceeds with complete inversion of configuration at C-1 to give the β-glucuronide. Glucuronidation of one functional group is usually sufficient to effect excretion of the conjugated metabolite; diglucuronide conjugates usually do not occur.

The diversity of functional groups undergoing glucuronidation is illustrated in Box 3-2 and Figure 3-12. Metabolic products are classified as oxygen-, nitrogen-, sulfur-, or car-

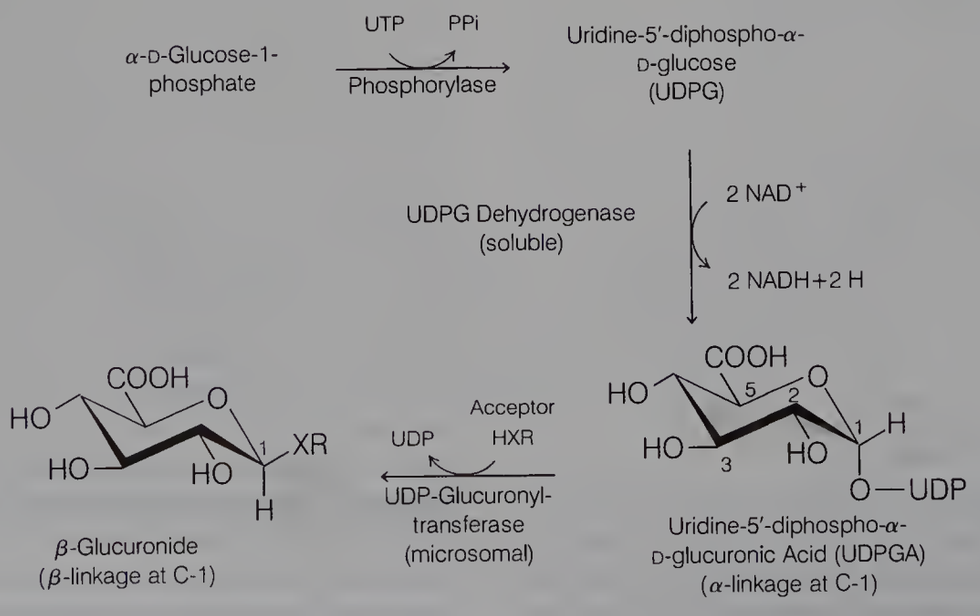


FIG. 3-11. *Formation of UDPGA and β-glucuronide conjugates.*

BOX 3-2. TYPES OF COMPOUNDS FORMING OXYGEN, NITROGEN, SULFUR, AND CARBON GLUCURONIDES

(For structures and site of β-glucuronide attachment, see Fig. 3-12).

OXYGEN GLUCURONIDES

Hydroxyl compounds

Phenols: morphine, acetaminophen, *p*-hydroxyphenytoin
Alcohols: tricholoroethanol, chloramphenicol, propranolol
Enols: 4-hydroxycoumarin
N-Hydroxyamines: *N*-hydroxydapsone
N-Hydroxyamides: *N*-hydroxy-2-acetylaminofluorene

Carboxyl compounds

Aryl acids: benzoic acid, salicylic acid
Arylalkyl acids: naproxen, fenoprofen

NITROGEN GLUCURONIDES

Arylamines: 7-amino-5-nitroindazole
Alkylamines: desipramine
Amides: meprobamate
Sulfonamides: sulfisoxazole
Tertiary amines: cyproheptadine, tripelennamine

SULFUR GLUCURONIDES

Sulfhydryl groups: methimazole, propylthiouracil, diethylthiocarbamic acid

CARBON GLUCURONIDES

3,5-Pyrazolidinedione: phenylbutazone, sulfinpyrazone

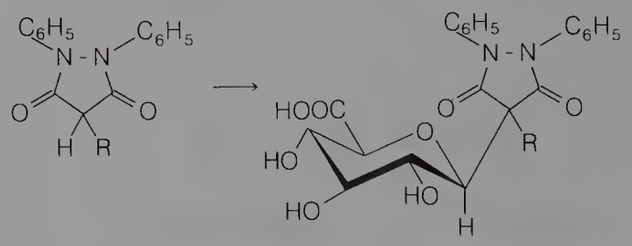
bon-glucuronide, according to the heteroatom attached to the C-1 atom of the glucuronyl group. Two important functionalities, the hydroxy and carboxy, form *O*-glucuronides. Phenolic and alcoholic hydroxyls are the most common functional groups undergoing glucuronidation in drug metabolism. As we have seen, phenolic and alcoholic hydroxyl groups are present in many parent compounds and arise through various phase I metabolic pathways. Morphine,[278] acetaminophen,[279] and *p*-hydroxyphenytoin (the major metabolite of phenytoin)[41] represent a few examples of phenolic compounds that undergo considerable glucuronidation. Alcoholic hydroxyls, such as those present in trichloroethanol (major metabolite of chloral hydrate),[220] chloramphenicol,[280] and propranolol,[281] are also commonly glucuronidated. Occurring less frequently is glucuronidation of other hydroxyl groups, such as enols (—C=C—OH),[282] *N*-hydroxylamines (RNHOH),[174] and *N*-hydroxylamides (RCNHOH).[186] For examples, refer to the list of glucuronides in Box 3-2.

The carboxy group is also subject to conjugation with glucuronic acid. For example, arylaliphatic acids, such as the anti-inflammatory agents naproxen[283] and fenoprofen,[284] are excreted primarily as their *O*-glucuronide derivatives in humans. Carboxylic acid metabolites such as those arising from chlorpheniramine[222] and propranolol[221] (see "Reduction of Aldehyde and Ketone Carbonyls," above) have been observed to form *O*-glucuronide conjugates. Aryl acids (e.g., benzoic acid,[285] salicylic acid[286]) also undergo conjugation with glucuronic acid, but a more important pathway for these compounds appears to be conjugation with glycine.

The formation of *N*-glucuronides with aromatic amines, aliphatic amines, amides, and sulfonamides occurs occasionally. Representative examples are found in the list of glucuronides in Box 3-2. Glucuronidation of aromatic and aliphatic amines is generally a minor pathway in comparison with *N*-acetylation or oxidative processes (e.g., oxidative deamination). Tertiary amines, such as the antihistaminic agents cyproheptadine (Periactin)[291] and tripelennamine,[292] have been observed to form interesting quaternary ammonium glucuronide metabolites.

Because the thiol group (SH) does not commonly occur in xenobiotics, *S*-glucuronide products have been reported for only a few drugs. For instance, the thiol groups present in methimazole (Tapazole),[293] propylthiouracil,[294] and *N,N*-diethyldithiocarbamic acid (major reduced metabolite of disulfiram, Antabuse)[295] have been demonstrated to undergo conjugation with glucuronic acid.

The formation of glucuronides attached directly to a carbon atom is relatively novel in drug metabolism. Studies in humans have shown that conjugation of phenylbutazone (Butazolidin)[296] and sulfinpyrazone (Anturane)[297] yield the corresponding *C*-glucuronide metabolites:

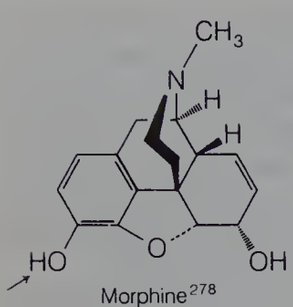


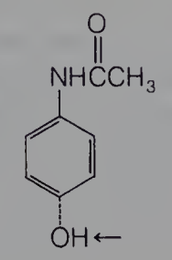
C-Glucuronide Metabolite

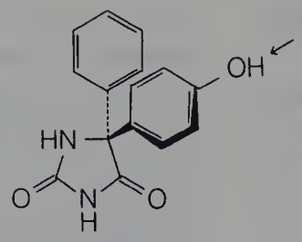
Phenylbutazone, R = $CH_2CH_2CH_2CH_3$

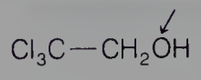
Sulfinpyrazone, R = $CH_2CH_2S(=O)C_6H_5$

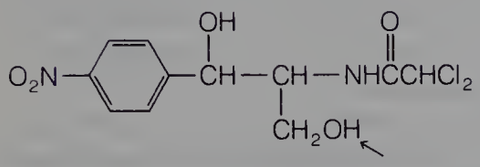
Besides xenobiotics, a number of endogenous substrates, notably bilirubin[298] and steroids,[299] are eliminated as glucuronide conjugates. Glucuronide conjugates are excreted primarily in the urine. However, as the relative molecular mass of the conjugate exceeds 300 Da, biliary excretion may become an important route of elimination.[300] Glucuronides that are excreted in the bile are susceptible to hydrolysis by β-glucuronidase enzymes present in the intestine. The hydrolyzed product may be reabsorbed in the intestine, thus leading to enterohepatic recycling.[19] β-Glucuronidases are also present in many other tissues, including the liver, the endocrine system, and the reproductive organs. Although the function of these hydrolytic enzymes in drug metabolism is unclear, it appears that, in terms of hormonal and endocrine

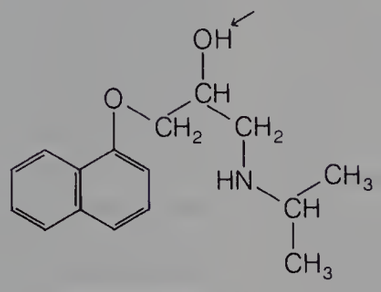

Morphine[278]

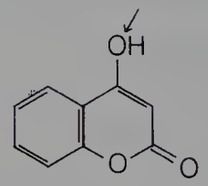

Acetaminophen[279]

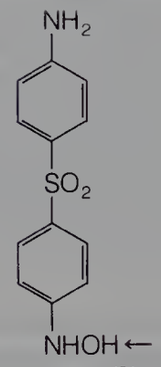

p-Hydroxyphenytoin[41]

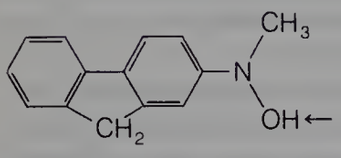

Trichloroethanol[220]

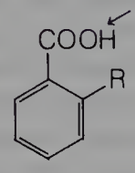

Chloramphenicol[280]

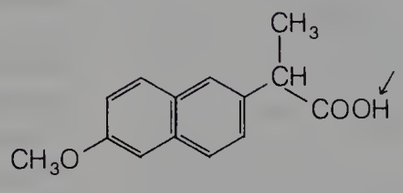

Propranolol[281]

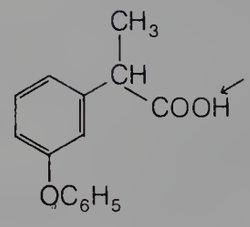

4-Hydroxycoumarin[282]

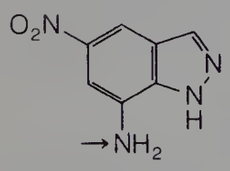

N-Hydroxydapsone[174]

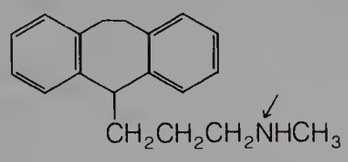

N-Hydroxy-2-acetyl-aminofluorene[186]

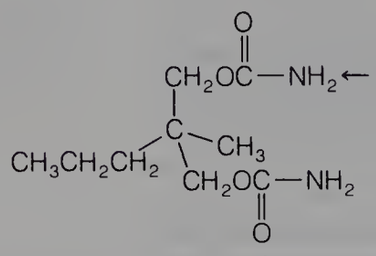

Benzoic acid[285], R = H
Salicylic acid[286], R = OH

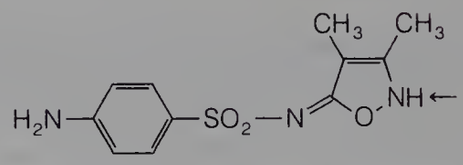

Naproxen[283]

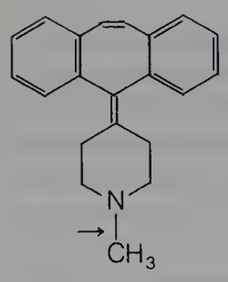

Fenoprofen[284]

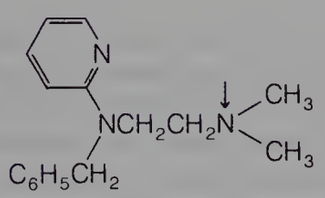

7-Amino-5-nitroindazole[287]

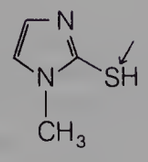

Desipramine[288]

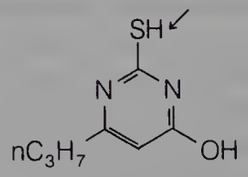

Meprobamate[289]


Sulfisoxazole[290]


Cyproheptadine[291]


Tripelennamine[292]


Methimazole[293]


Propylthiouracil[294]

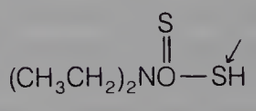

Diethylthio-carbamic Acid[295]

FIG. 3-12. *Structure of compounds that undergo glucuronidation (arrows indicate sites of β-glucuronide attachment).*

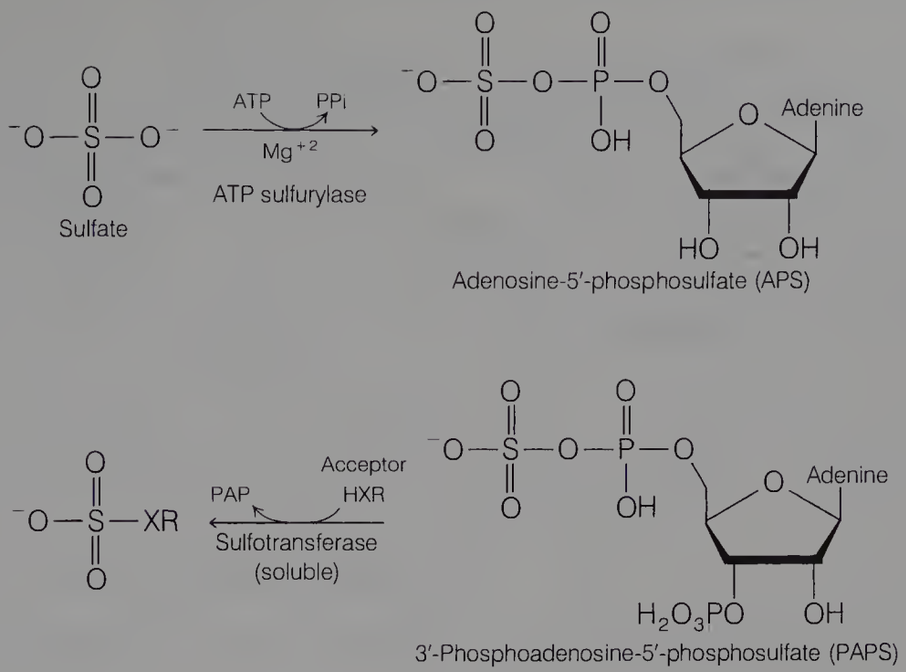


FIG. 3-13. *Formation of PAPS and sulfate conjugates.*

regulation, β-glucuronidases may liberate active hormones (e.g., steroids) from their inactive glucuronide conjugates.[19]

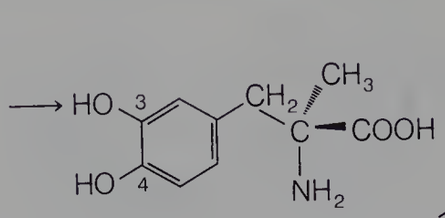
In neonates and children, glucuronidating processes often are not developed fully. In such subjects, drugs and endogenous compounds (e.g., bilirubin) that are metabolized normally by glucuronidation may accumulate and cause serious toxicity. For example, neonatal hyperbilirubinemia may be attributable to the inability of newborns to conjugate bilirubin with glucuronic acid.[301] Similarly, the inability of infants to glucuronidate chloramphenicol has been suggested to be responsible for the "gray baby syndrome," which results from accumulation of toxic levels of the free antibiotic.[302]

SULFATE CONJUGATION

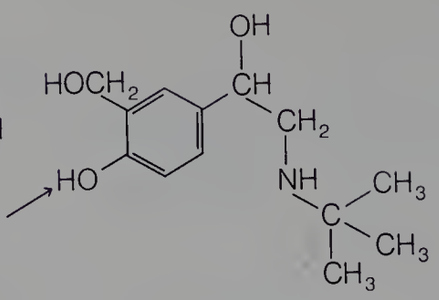
Conjugation of xenobiotics with sulfate occurs primarily with phenols and, occasionally, with alcohols, aromatic amines, and *N*-hydroxy compounds.[303–305] In contrast to glucuronic acid, the amount of available sulfate is rather limited. A significant portion of the sulfate pool is utilized by the body to conjugate numerous endogenous compounds, such as steroids, heparin, chondroitin, catecholamines, and thyroxine. The sulfate conjugation process involves activation of inorganic sulfate to the coenzyme 3′-phosphoadenosine-5′-phosphosulfate (PAPS). Subsequent transfer of the sulfate group from PAPS to the accepting substrate is catalyzed by various soluble sulfotransferases present in the liver and other tissues (e.g., kidney, intestine).[305] The sequence of events involved in sulfoconjugation is depicted in Fig. 3-13. Sulfate conjugation generally leads to water-soluble and inactive metabolites. However, it appears that the *O*-sulfate conjugates of some *N*-hydroxy compounds give rise to chemically reactive intermediates that are toxic.[186]

Phenols compose the main group of substrates undergoing sulfate conjugation. Thus, drugs containing phenolic moieties are often susceptible to sulfate formation. For example, the antihypertensive agent α-methyldopa (Aldomet) is metabolized extensively to its 3-*O*-sulfate ester in humans.[306] The β-adrenergic bronchodilators salbutamol (albuterol)[307] and terbutaline (Brethine, Bricanyl)[308] also undergo sulfate conjugation as their principal route of metabolism in humans. However, for many phenols, sulfoconjugation may represent only a minor pathway. Glucuronidation of phenols is frequently a competing reaction and may predominate as the conjugative route for some phenolic drugs. In adults, the major urinary metabolite of the analgesic acetaminophen is the *O*-glucuronide conjugate, with the *O*-sulfate conjugate being formed in small amounts.[279] Interestingly, in infants and young children (ages 3 to 9 years) a different urinary excretion pattern is observed: the *O*-sulfate conjugate is the main urinary product.[309] The explanation for this reversal stems from the fact that neonates and young children have a decreased glucuronidating capacity owing to undeveloped glucuronyltransferases or low levels of these enzymes. Sulfate conjugation, however, is well developed and becomes the main route by which acetaminophen is conjugated in this pediatric-aged group.

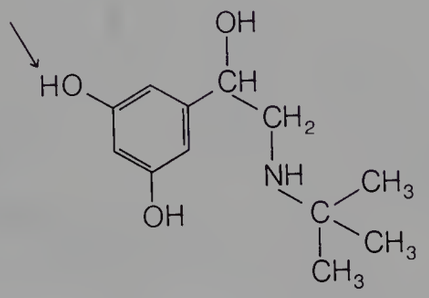
Other functionalities, such as alcohols (e.g., aliphatic C_1 to C_5 alcohols, diethylene glycol)[310] and aromatic amines (e.g., aniline, 2-naphthylamine),[311] are also capable of forming sulfate conjugates. These reactions, however, have only minor importance in drug metabolism. The sulfate conjugation of *N*-hydroxylamines and *N*-hydroxylamides takes place occasionally as well. *O*-Sulfate ester conjugates of *N*-hydroxy compounds are of considerable toxicologic concern because they can lead to reactive intermediates that are responsible for cellular toxicity. The carcinogenic agents *N*-methyl-4-aminoazobenzene and 2-acetylaminofluorene are believed to mediate their toxicity through *N*-hydroxylation to the corresponding *N*-hydroxy compounds (see earlier section on *N*-hydroxylation of amines and amides). Sulfoconju-



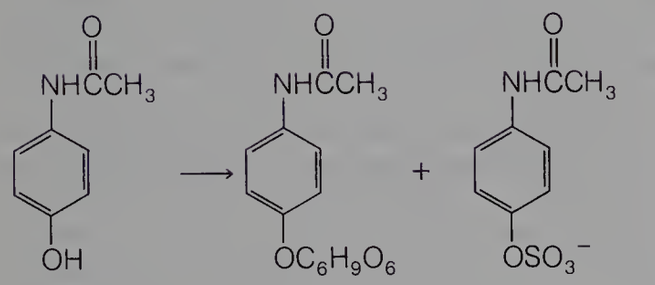
α-Methyldopa



Salbutamol
(Albuterol)



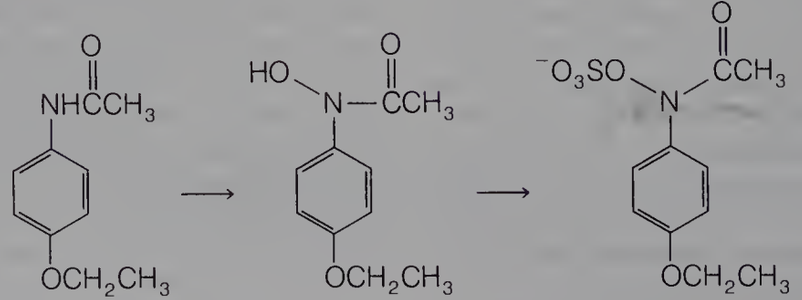
Terbutaline



Acetaminophen — *O*-Glucuronide Conjugate — *O*-Sulfate Conjugate

gation of the *N*-hydroxy metabolites yields *O*-sulfate esters, which presumably are the ultimate carcinogenic species. Loss of SO_4^{2-} from the foregoing sulfate conjugates generates electrophilic nitrenium species, which may react with nucleophilic groups (e.g., NH_2, OH, SH), present in proteins, DNA, and RNA, to form covalent linkages that lead to structural and functional alteration of these crucial biomacromolecules.[312] The consequences of this are cellular toxicity (tissue necrosis) or alteration of genetic code material, leading eventually to cancer. Some evidence supporting the role of sulfate conjugation in the metabolic activation of *N*-hydroxy compounds to reactive intermediates comes from the observation that the degree of hepatotoxicity and hepatocarcinogenicity of *N*-hydroxy-2-acetylaminofluorene is markedly dependent on the level of sulfotransferase activity in the liver.[313]

The analgesic phenacetin is metabolized to *N*-hydroxyphenacetin and subsequently conjugated with sulfate.[314] The *O*-sulfate conjugate of *N*-hydroxyphenacetin has been demonstrated to bind covalently to microsomal proteins.[315] This pathway may represent one route leading to reactive intermediates, which are responsible for the hepatotoxicity and nephrotoxicity associated with phenacetin. Other pathways (e.g., arene oxides) leading to reactive electrophilic intermediates are also possible.[6]



Phenacetin — *N*-Hydroxyphenacetin — *O*-Sulfate conjugate of *N*-Hydroxyphenacetin

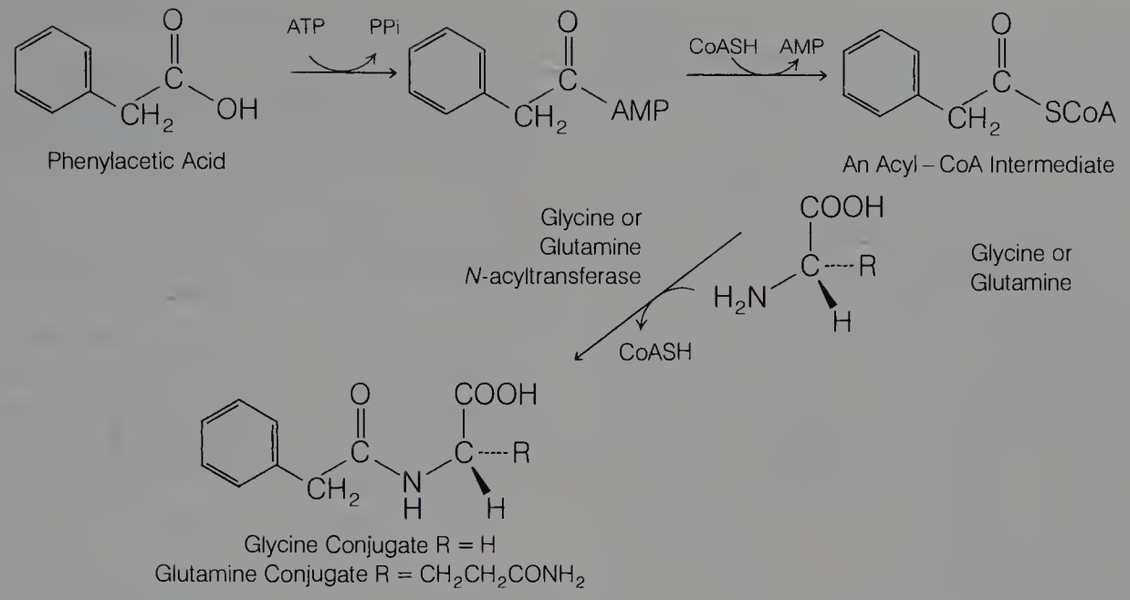
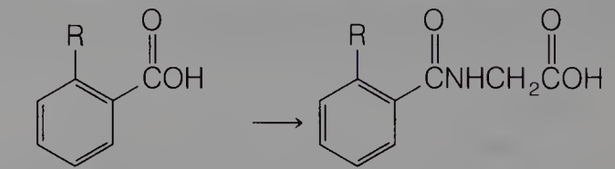


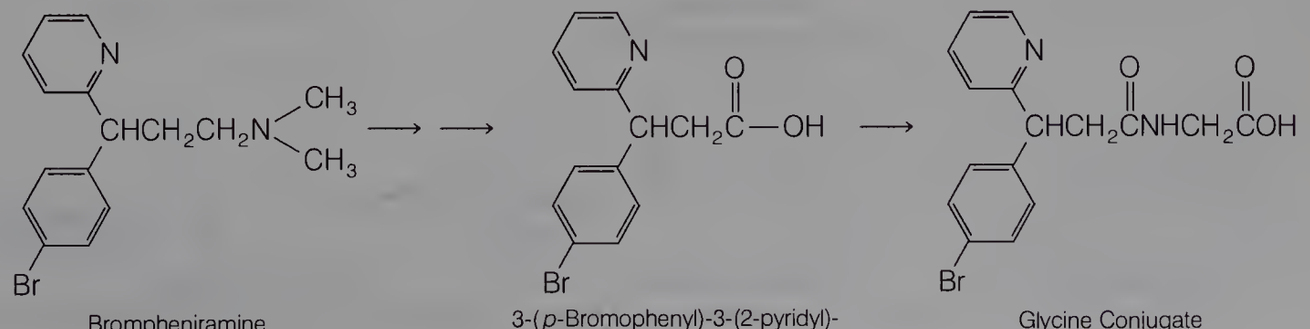
FIG. 3-14. *Formation of glycine and glutamine conjugates of phenylacetic acid.*

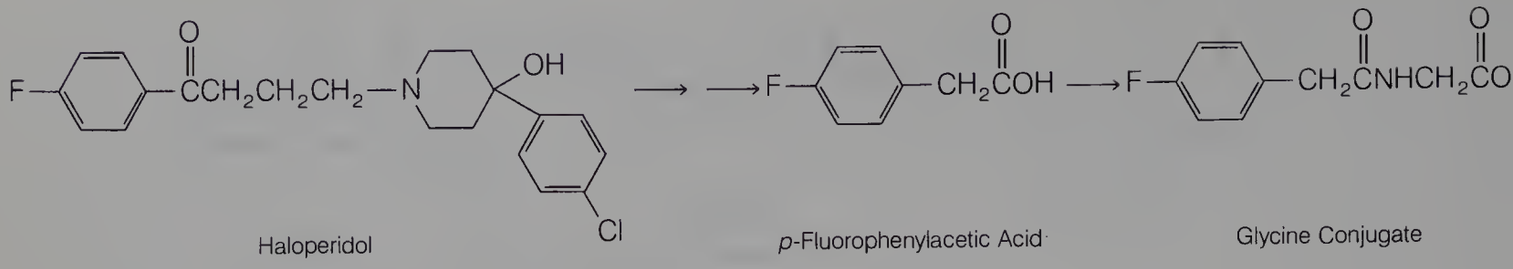
CONJUGATION WITH GLYCINE, GLUTAMINE, AND OTHER AMINO ACIDS

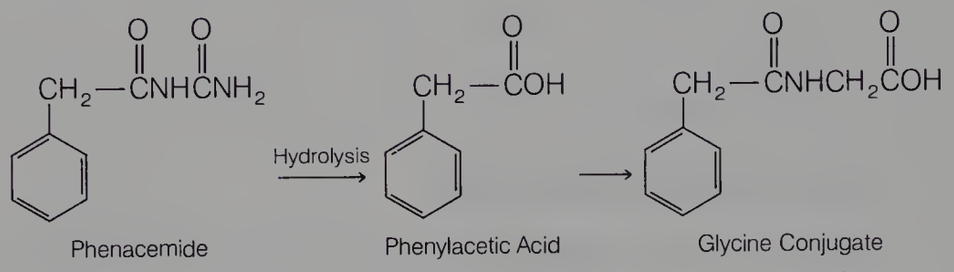
The amino acids glycine and glutamine are utilized by mammalian systems to conjugate carboxylic acids, particularly aromatic acids and arylalkyl acids.[316,317] Glycine conjugation is common to most mammals, whereas glutamine conjugation appears mainly confined to humans and other primates. The quantity of amino acid conjugates formed from xenobiotics is minute because of the limited availability of amino acids in the body and competition with glucuronidation for carboxylic acid substrates. In contrast with glucuronic acid and sulfate, glycine and glutamine are not converted to activated coenzymes. Instead, the carboxylic acid substrate is activated with adenosine triphosphate (ATP) and coenzyme A (CoA) to form an acyl–CoA complex. The latter intermediate, in turn, acylates glycine or glutamine under the influence of specific glycine or glutamine *N*-acyltransferase enzymes. The activation and acylation steps take place in the mitochondria of liver and kidney cells. The sequence of metabolic events associated with glycine and glutamine conjugation of phenylacetic acid is summarized in Fig. 3-14. Amino acid conjugates, being polar and water-soluble, mainly are excreted renally and sometimes in the bile.

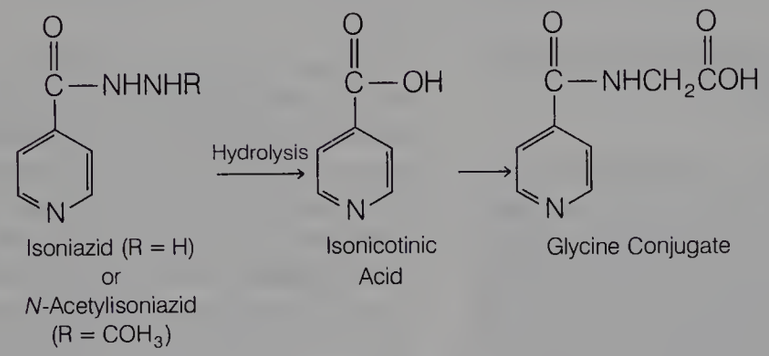
Aromatic acids and arylalkyl acids are the major substrates undergoing glycine conjugation. The conversion of benzoic acid to its glycine conjugate, hippuric acid, is a well-known metabolic reaction in many mammalian systems.[318] The extensive metabolism of salicylic acid (75% of dose) to salicyluric acid in humans is another illustrative example.[319] Carboxylic acid metabolites resulting from oxidation or hydrolysis of many drugs are also susceptible to glycine conjugation. For example, the H_1-histamine antagonist brompheniramine is oxidized to a propionic acid metabolite that is conjugated with glycine in both human and dog.[141] Similarly, *p*-fluorophenylacetic acid, derived from the metabolism of the antipsychotic agent haloperidol (Haldol), is found as the glycine conjugate in the urine of rats.[320] Phenylacetic acid and isonicotinic acid, resulting from the hydrolysis of, respectively, the anticonvulsant phenacemide (Phenurone)[321] and the antituberculosis agent isoniazid,[322] also are conjugated with glycine to some extent.





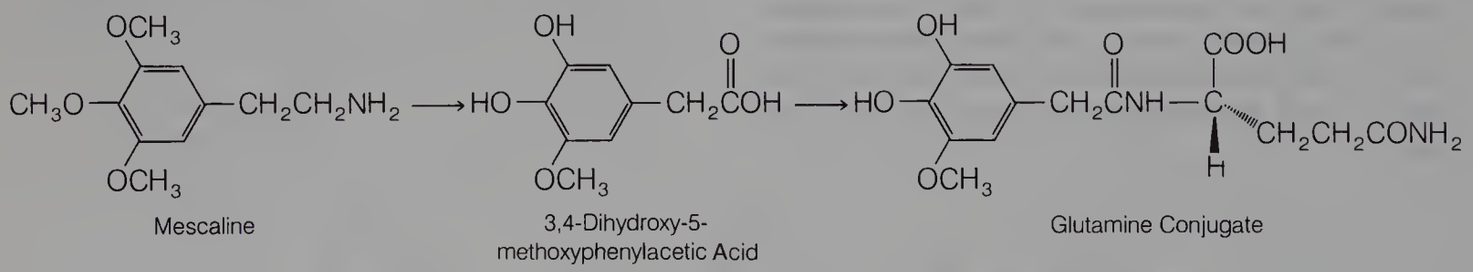


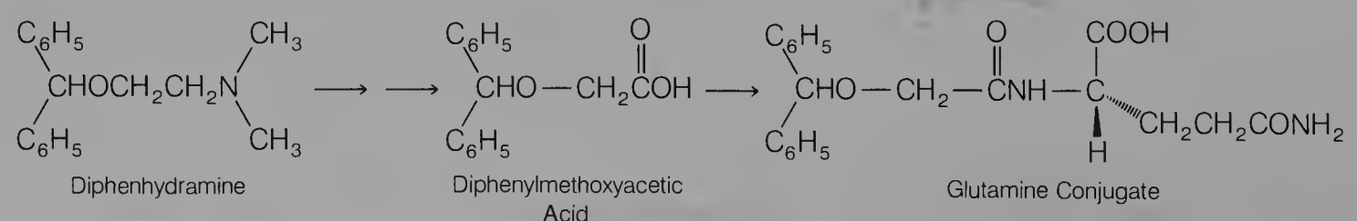




Glutamine conjugation occurs mainly with arylacetic acids, including endogenous phenylacetic[323] and indolylacetic acid.[324] A few glutamine conjugates of drug metabolites have been reported. For example, in humans the 3,4-dihydroxy-5-methoxyphenylacetic acid metabolite of mescaline is found as a conjugate of glutamine.[325] Diphenylmethoxyacetic acid, a metabolite of the antihistamine diphenhydramine (Benadryl), is biotransformed further to the corresponding glutamine derivative in the rhesus monkey.[326]

Several other amino acids are involved in the conjugation of carboxylic acids, but these reactions occur only occasionally and appear to be highly substrate- and species-dependent.[317,327] Ornithine (in birds), aspartic acid and serine (in rats), alanine (in mouse and hamster), taurine ($H_2NCH_2CH_2SO_3H$) (in mammals and pigeons), and histidine (in African bats) are among these amino acids.[327]





GSH OR MERCAPTURIC ACID CONJUGATES

GSH conjugation is an important pathway by which chemically reactive electrophilic compounds are detoxified.[328,329] It is now generally accepted that reactive electrophilic species manifest their toxicity (e.g., tissue necrosis, carcinogenicity, mutagenicity, teratogenicity) by combining covalently with nucleophilic groups present in vital cellular proteins and nucleic acids.[4,330] Many serious drug toxicities may be explainable also in terms of covalent interaction of metabolically generated electrophilic intermediates with cellular nucleophiles.[5,6] GSH protects vital cellular constituents against chemically reactive species by virtue of its nucleophilic sulfhydryl (SH) group. It is the SH group that reacts with electron-deficient compounds to form *S*-substituted GSH adducts (Fig. 3-15).[328,329]

GSH is a tripeptide (γ-glutamyl-cysteinylglycine) found in most tissues. Xenobiotics conjugated with GSH usually are not excreted as such but undergo further biotransformation to give *S*-substituted *N*-acetylcysteine products called mercapturic acids.[63,71,329] This process involves enzymatic cleavage of two amino acids (namely, glutamic acid and glycine) from the initially formed GSH adduct and subsequent *N*-acetylation of the remaining *S*-substituted cysteine residue. The formation of GSH conjugates and their conversion to mercapturic acid derivatives are outlined in Fig. 3-15.

Conjugation of a wide spectrum of substrates with GSH is catalyzed by a family of cytoplasmic enzymes known as glutathione *S*-transferases.[62] These enzymes are found in most tissues, particularly the liver and kidney. Degradation of GSH conjugates to mercapturic acids is carried out principally by renal and hepatic microsomal enzymes (Fig. 3-15).[63] Unlike other conjugative phase II reactions, GSH conjugation does not require the initial formation of an activated coenzyme or substrate. The inherent reactivity of the nucleophilic GSH toward an electrophilic substrate usually provides sufficient driving force. The substrates susceptible to GSH conjugation are quite varied and encompass many chemically different classes of compounds. A major prerequisite is that the substrate be sufficiently electrophilic. Compounds that react with GSH do so by two general mechanisms: (1) nucleophilic displacement at an electron-deficient carbon or heteroatom or (2) nucleophilic addition to an electron-deficient double bond.[328]

Many aliphatic and arylalkyl halides (Cl, Br, I), sulfates

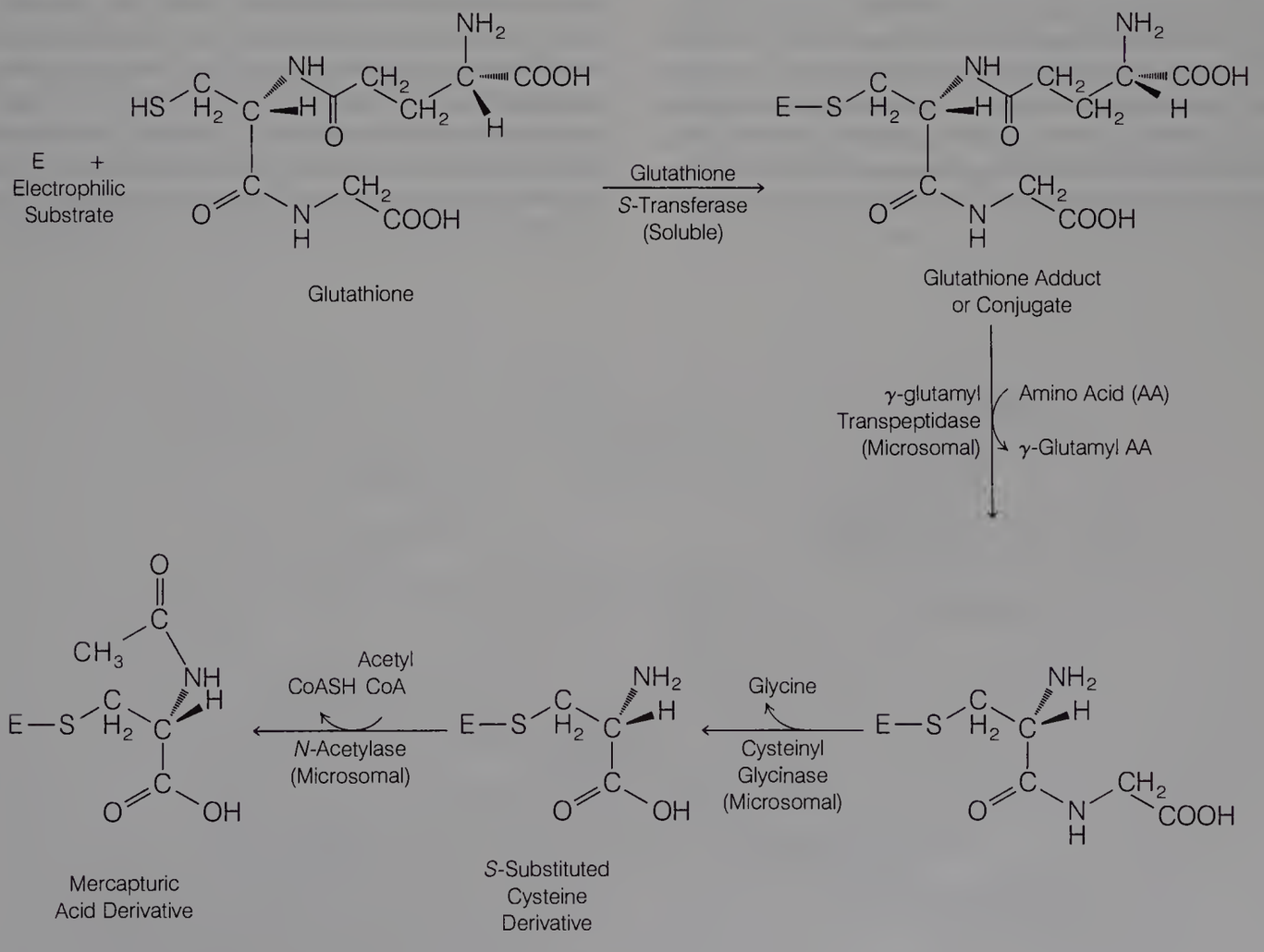
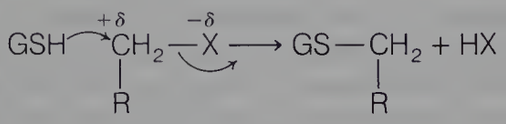


FIG. 3-15. *Formation of GSH conjugates of electrophilic xenobiotics or metabolites (E) and their conversion to mercapturic acids.*

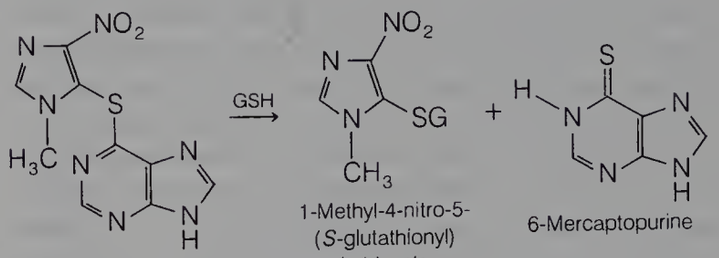
(OSO_3^-), sulfonates (OSO_2R), nitrates (NO_2), and organophosphates (O-P[OR]$_2$) possess electron-deficient carbon atoms that react with GSH (by aliphatic nucleophilic displacement) to form GSH conjugates, as shown:



R = Alkyl, Aryl, Benzylic, Allylic
X = Br, Cl, I, OSO_3^-, OSO_2R, $OPO(OR)_2$

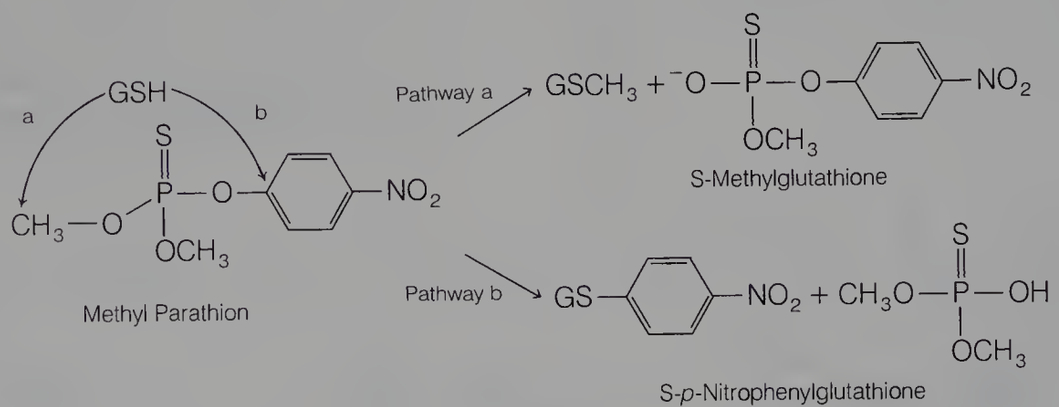
The carbon center is rendered electrophilic as a result of the electron-withdrawing group (e.g., halide, sulfate, phosphate) attached to it. Nucleophilic displacement often is facilitated when the carbon atom is benzylic or allylic or when X is a good leaving group (e.g., halide, sulfate). Many industrial chemicals, such as benzyl chloride ($C_6H_5CH_2Cl$), allyl chloride (CH_2=$CHCH_2Cl$), and methyl iodide, are known to be toxic and carcinogenic. The reactivity of these three halides toward GSH conjugation in mammalian systems is demonstrated by the formation of the corresponding mercapturic acid derivatives.[329] Organophosphate insecticides, such as methyl parathion, are detoxified by two different GSH pathways.[331] Pathway *a* involves aliphatic nucleophilic substitution and yields *S*-methylglutathione. Pathway *b* involves aromatic nucleophilic substitution and produces *S*-*p*-nitrophenylglutathione. Aromatic or heteroaromatic nucleophilic substitution reactions with GSH occur only when the ring is rendered sufficiently electron-deficient by the presence of one or more strongly electron-withdrawing substituents (e.g., NO_2, Cl). For example, 2,4-dichloronitrobenzene is susceptible to nucleophilic substitution by GSH, whereas chlorobenzene is not.[332]

The metabolism of the immunosuppressive drug azathioprine (Imuran) to 1-methyl-4-nitro-5-(*S*-glutathionyl)imidazole and 6-mercaptopurine is an example of heteroaromatic nucleophilic substitution involving GSH.[333] Interestingly, 6-mercaptopurine formed in this reaction appears to be responsible for azathioprine's immunosuppressive activity.[334]

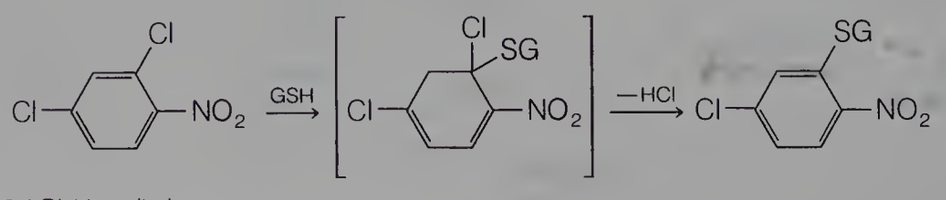


Azathioprine — 1-Methyl-4-nitro-5-(*S*-glutathionyl) imidazole — 6-Mercaptopurine

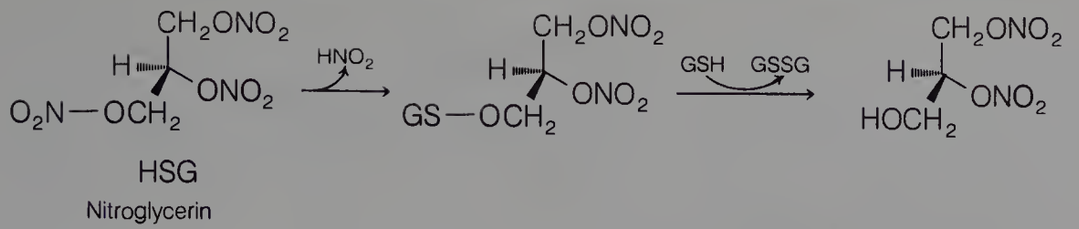
Arene oxides and aliphatic epoxides (or oxiranes) represent a very important class of substrates that are conjugated and detoxified by GSH.[335] The three-membered oxygen-containing ring in these compounds is highly strained and, therefore, reactive toward ring cleavage by nucleophiles (e.g., GSH, H_2O, or nucleophilic groups present on cellular macromolecules). As discussed previously, arene oxides and epoxides are intermediary products formed from cytochrome P-450 oxidation of aromatic compounds (arenes) and olefins, respectively. If reactive arene oxides (e.g., benzo[*a*]pyrene-4,5-oxide, 4-bromobenzene oxide) and aliphatic epoxides (e.g., styrene oxide) are not "neutralized" or detoxified by glutathione *S*-transferase, epoxide hydrase, or other pathways, they ultimately covalently bind to cellular macromolecules to cause serious cytotoxicity and carcinogenicity. The isolation of GSH or mercapturic acid adducts from benzo[*a*]pyrene, bromobenzene, and styrene clearly

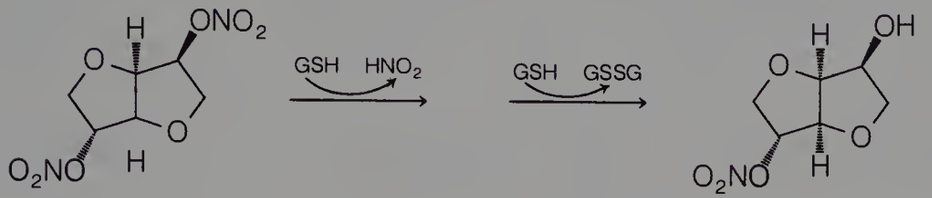


Methyl Parathion



2,4-Dichloronitrobenzene

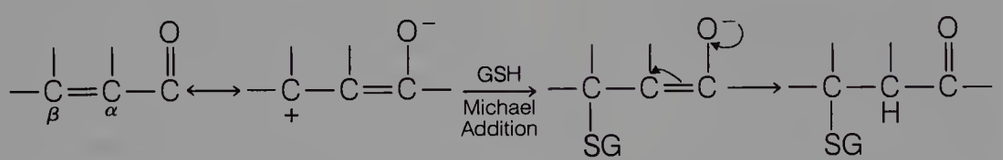


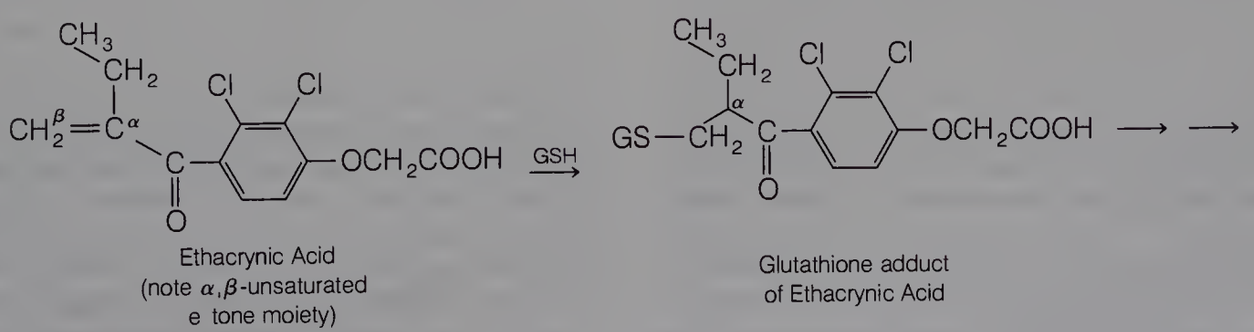


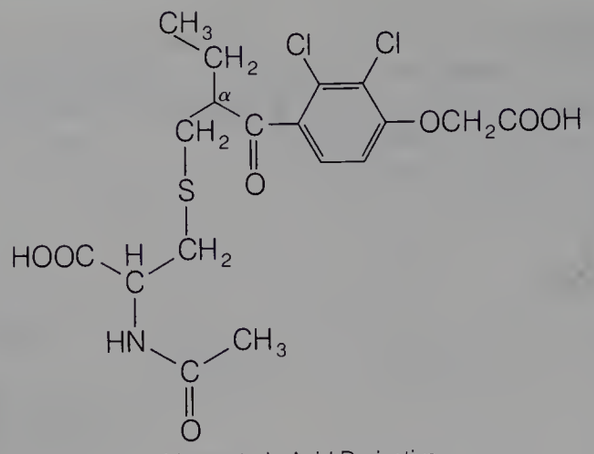
demonstrates the importance of GSH in reacting with the reactive epoxide metabolites generated from these compounds.

GSH conjugation involving substitution at heteroatoms, such as oxygen, is seen often with organic nitrates. For example, nitroglycerin (Nitrostat) and isosorbide dinitrate (Isordil) are metabolized by a pathway involving an initial GSH conjugation reaction. However, the GSH conjugate products are not metabolized to mercapturic acids but, instead, are converted enzymatically to the corresponding alcohol derivatives and glutathione disulfide (GSSG).[336]

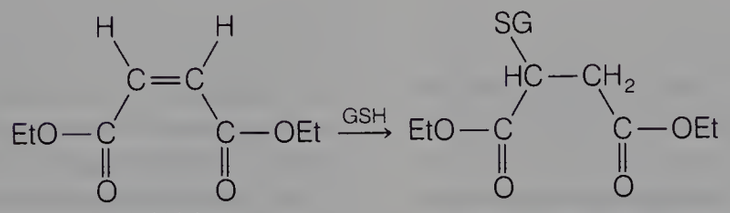
The nucleophilic addition of GSH to electron-deficient carbon–carbon double bonds occurs mainly in compounds with α,β-unsaturated double bonds. In most instances, the double bond is rendered electron-deficient by resonance or conjugation with a carbonyl group (ketone or aldehyde), ester, nitrile, or other. Such α,β-unsaturated systems undergo so-called Michael addition reactions with GSH to yield the corresponding GSH adduct.[328,329] For example, in rats and dogs the diuretic agent ethacrynic acid (Edecrin) reacts with GSH to form the corresponding GSH or mercapturic acid derivatives.[337] The compound diethyl maleate is readily conjugated with GSH and has been used experimentally to deplete hepatic GSH stores in laboratory animals.[338] Several other α,β-unsaturated compounds, such as acrolein, crotonaldehyde, *o*-chlorobenzylidenemalononitrile, and arecoline, form mercapturic acid or GSH derivatives.[329] It should be emphasized that not all α,β-unsaturated compounds are conjugated with GSH. Many steroidal agents possessing α,β-unsaturated carbonyl moieties, such as prednisone and digitoxigenin, have not been observed to undergo any significant conjugation with GSH. Steric factors, de-



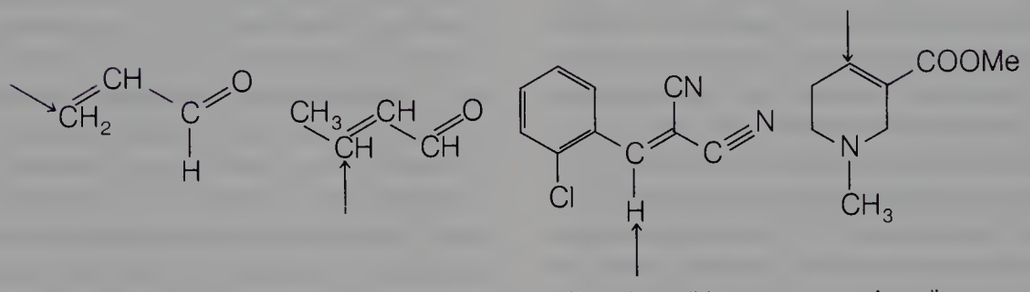




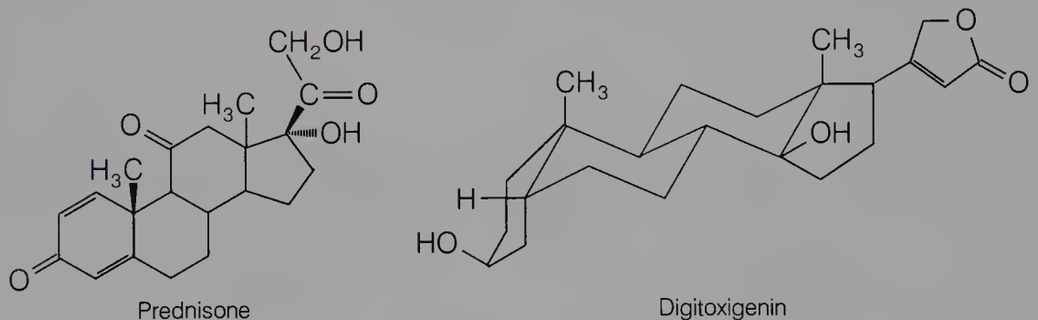
Mercapturic Acid Derivative



Diethyl Maleate



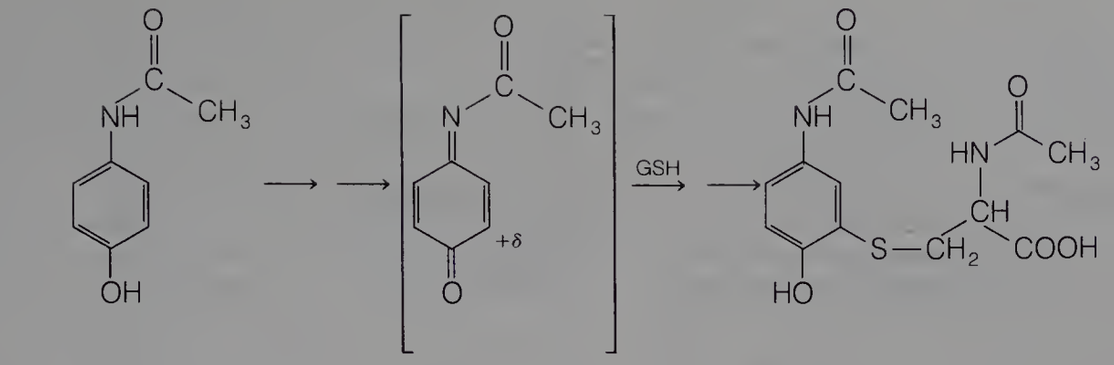
Acrolein | Crotonaldehyde | *o*-Chlorobenzylidene-malononitrile | Arecoline

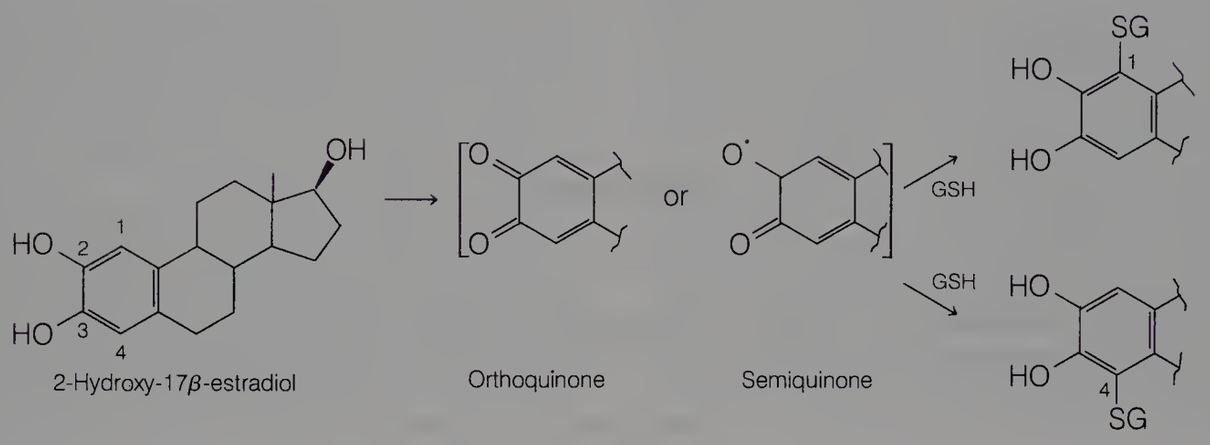


Prednisone | Digitoxigenin

creased reactivity of the double bond, as well as other factors (e.g., susceptibility to metabolic reduction of the ketone or the C=C double bond) may account for these observations.

Occasionally, metabolic oxidative biotransformation reactions may generate chemically reactive α,β-unsaturated systems that react with GSH. For example, metabolic oxidation of acetaminophen presumably generates the chemically reactive intermediate *N*-acetylimidoquinone. Michael addition of GSH to the imidoquinone leads to the corresponding mercapturic acid derivative in both animals and humans.[189,191] 2-Hydroxyestrogens, such as 2-hydroxy-17β-estradiol, undergo conjugation with GSH to yield the two isomeric mercapturic acid or GSH derivatives. Although the exact mechanism is unclear, it appears that the 2-hydroxyestrogen is oxidized to a chemically reactive orthoquinone or semiquinone intermediate that reacts with GSH at either the electrophilic C-1 or C-4 position.[339]





In most instances, GSH conjugation is regarded as a detoxifying pathway that protects cellular macromolecules, such as protein and DNA, against harmful electrophiles. In a few cases, GSH conjugation has been implicated in causing toxicity. Often, this is because the GSH conjugates are themselves electrophilic (e.g., vicinal dihaloethanes) or give rise to metabolic intermediates (e.g., cysteine metabolites of haloalkenes) that are electrophilic.[329] 1,2-Dichloroethane, for example, reacts with GSH to produce *S*-(2-chloroethyl)glutathione; the nucleophilic sulfur group in this conjugate can internally displace the chlorine group to give rise to an electrophilic three-membered ring episulfonium ion. The covalent interaction of the episulfonium intermediate with the guanosine moiety of DNA may contribute to the mutagenic and carcinogenic effects observed for 1,2-dichloroethane.[329b] The metabolic conversion of GSH conjugates to reactive cysteine metabolites has been shown to be responsible for the nephrotoxicity associated with some halogenated alkanes and alkenes.[329c] The activation pathway appears to involve γ-glutamyl transpeptidase and cysteine conjugate β-lyase, two enzymes that apparently target the conjugates to the kidney.

ACETYLATION

Acetylation constitutes an important metabolic route for drugs containing primary amino groups.[316,340] This encompasses primary aromatic amines ($ArNH_2$), sulfonamides ($H_2NC_6H_4SO_2NHR$), hydrazines (—$NHNH_2$), hydrazides (—$CONHNH_2$), and primary aliphatic amines. The amide derivatives formed from acetylation of these amino functionalities are generally inactive and nontoxic. Because water solubility is not enhanced greatly by *N*-acetylation, it appears that the primary function of acetylation is to terminate pharmacologic activity and detoxification. However, a few reports indicate that acetylated metabolites may be as active as (e.g., *N*-acetylprocainamide)[341] or more toxic than (e.g., *N*-acetylisoniazid)[342] their corresponding parent compounds.

The acetyl group utilized in *N*-acetylation of xenobiotics is supplied by acetyl-CoA.[316] Transfer of the acetyl group from this cofactor to the accepting amino substrate is carried out by soluble *N*-acetyltransferases present in hepatic reticuloendothelial cells. Other extrahepatic tissues, such as the lung, spleen, gastric mucosa, red blood cells, and lymphocytes, also show acetylation capability. *N*-Acetyltransferase enzymes display broad substrate specificity and catalyze the acetylation of several drugs and xenobiotics (Fig. 3-16).[340] Aromatic compounds possessing a primary amino group, such as aniline,[316] *p*-aminobenzoic acid,[343] *p*-aminosalicylic acid,[325] procainamide (Pronestyl),[341,343] and dapsone (Avlosulfon),[344] are especially susceptible to *N*-acetylation. Aromatic amine metabolites resulting from the reduction of aryl nitro compounds also are *N*-acetylated. For example, the anticonvulsant clonazepam (Clonopin) undergoes nitro re-

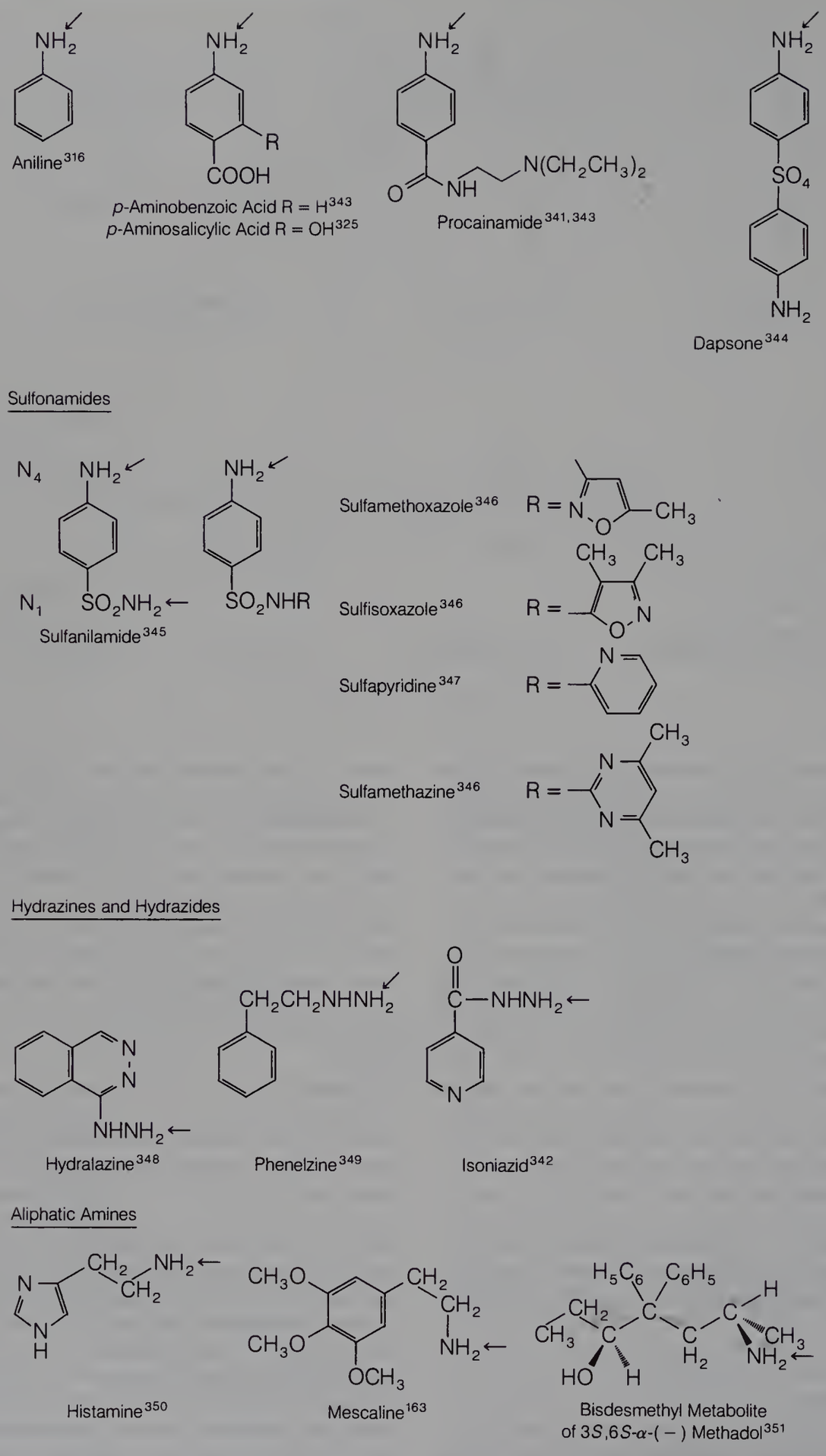


FIG. 3-16. *Examples of different types of compound undergoing* N*-acetylation (arrows indicate sites of* N*-acetylation).*

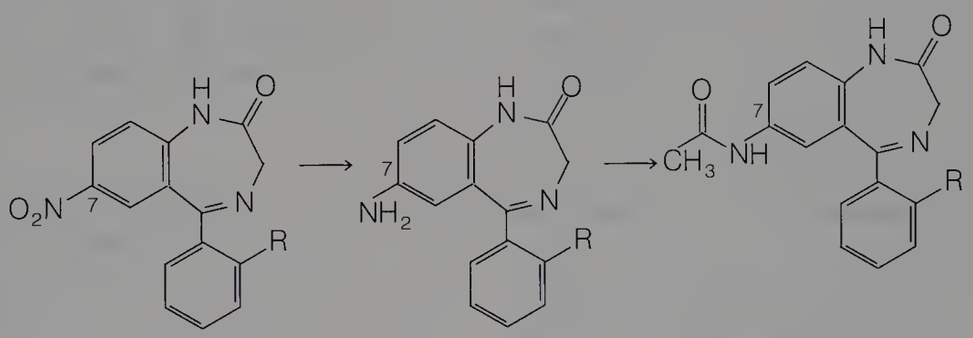
duction to its 7-amino metabolite, which in turn is *N*-acetylated.[245] Another related benzodiazepam analogue, nitrazepam, follows a similar pathway.[246]

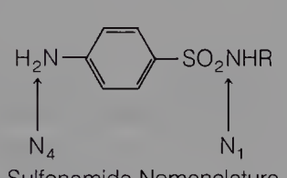
The metabolism of a number of sulfonamides, such as sulfanilamide,[345] sulfamethoxazole (Gantanol),[346] sulfisoxazole (Gantrisin),[346] sulfapyridine[347] (major metabolite from azo reduction of sulfasalazine, Azulfidine), and sulfamethazine,[316] occurs mainly by acetylation at the N-4 position. With sulfanilamide, acetylation also takes place at the sulfamido N-1 position.[345] *N*-Acetylated metabolites of sulfonamides tend to be less water-soluble than their parent compounds and have the potential of crystallizing out in renal tubules (*crystalluria*), thereby causing kidney damage. The frequency of crystalluria and renal toxicity is especially prominent with older sulfonamide derivatives, such as sulfathiazole.[1,327] However, newer sulfonamides, such as sulfisoxazole and sulfamethoxazole, are metabolized to relatively water-soluble acetylated derivatives, which are less likely to precipitate out.

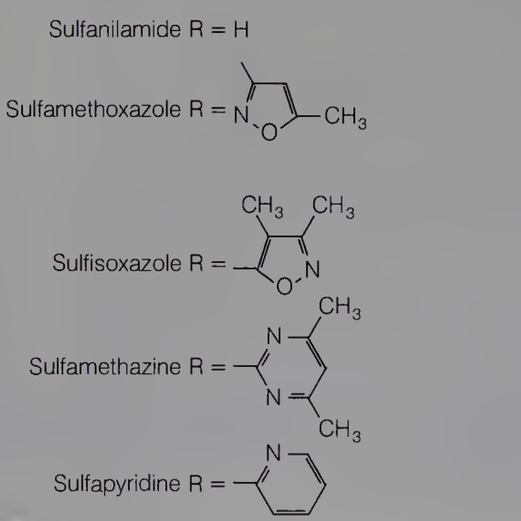
The biotransformation of hydrazine and hydrazide derivatives also proceeds by acetylation. The antihypertensive hydralazine (Apresoline)[348] and the MAO inhibitor phenelzine (Nardil)[349] are two representative hydrazine compounds that are metabolized by this pathway. It should be noted that the initially formed *N*-acetyl derivative of hydralazine is unstable and cyclizes intramolecularly to form 3-methyl-*s*-triazolo[3,4-α]phthalazine as the major isolable hydralazine metabolite in humans.[348] The antituberculosis drug isoniazid or isonicotinic acid hydrazide (INH) is metabolized extensively to *N*-acetylisoniazid.[342]

The acetylation of some primary aliphatic amines such as histamine,[350] mescaline,[163] and the bis-*N*-demethylated metabolite of $\alpha(-)$-methadol[351] also has been reported. In comparison with oxidative deamination processes, *N*-acetylation is only a minor pathway in the metabolism of this class of compounds.

The acetylation pattern of several drugs (e.g., isoniazid, hydralazine, procainamide) in the human population displays a bimodal character in which the drug is conjugated either rapidly or slowly with acetyl-CoA.[352,353] This phenomenon is termed *acetylation polymorphism*. Individuals are classified as being either slow or rapid acetylator phenotypes. This variation in acetylating ability is genetic and is caused mainly by differences in *N*-acetyltransferase activity. The proportion of rapid and slow acetylators varies widely among different ethnic groups throughout the world. For example, a high proportion of Eskimos and Asians are rapid acetylators, whereas Egyptians and some Western European groups are mainly slow acetylators.[353] Other populations are intermediate between these two extremes. Because of the



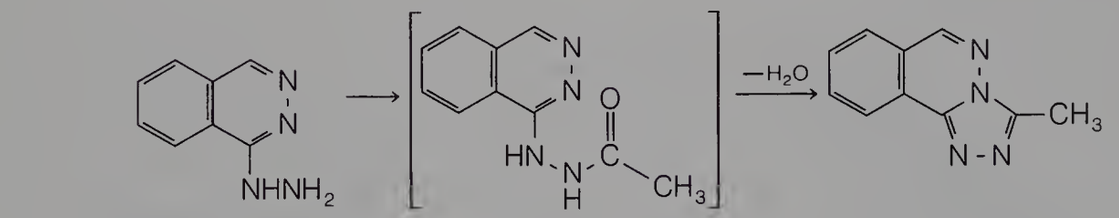


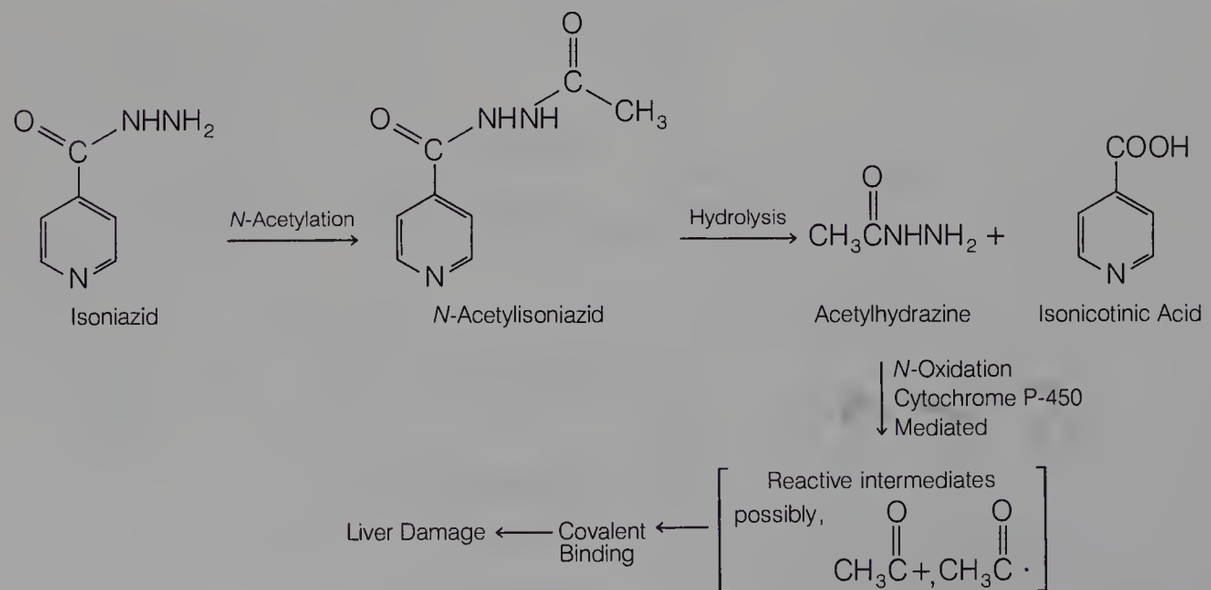


bimodal distribution of the human population into rapid and slow acetylators, there appears to be significant individual variation in therapeutic and toxicologic responses to drugs displaying acetylation polymorphism.[316,352,353] It seems that slow acetylators are more likely to develop adverse reactions, whereas rapid acetylators are more likely to show an inadequate therapeutic response to standard doses of the drug. The antituberculosis drug isoniazid illustrates many of these points. The plasma half-life of isoniazid in rapid acetylators ranges from 45 to 80 minutes, whereas in slow acetylators the half-life is about 140 to 200 minutes.[354] Thus, for a given fixed-dosing regimen, slow acetylators tend to accumulate higher plasma concentrations of isoniazid than do rapid acetylators. Higher concentrations of isoniazid may explain the greater therapeutic response (i.e., higher cure rate) among slow acetylators, but they probably also account for the greater incidence of adverse effects (e.g., peripheral neuritis and drug-induced systemic lupus erythematosus syndrome) observed among slow acetylators.[353] Slow acetylators of isoniazid apparently are also more susceptible to certain drug interactions involving drug metabolism. For example, phenytoin toxicity associated with concomitant use with isoniazid appears to be more prevalent in slow acetylators than in rapid acetylators.[355] Isoniazid inhibits the metabolism of phenytoin, thereby leading to an accumulation of high and toxic plasma levels of phenytoin.

Interestingly, patients who are rapid acetylators appear to be more likely to develop isoniazid-associated hepatitis.[342] This liver toxicity presumably arises from initial hydrolysis of the *N*-acetylated metabolite *N*-acetylisoniazid to acetylhydrazine. The latter metabolite is further converted (by cytochrome P-450 enzyme systems) to chemically reactive acylating intermediates that covalently bind to hepatic tissue, causing necrosis. Pathologic and biochemical studies in experimental animals appear to support this hypothesis. Therefore, rapid acetylators run a greater risk of incurring liver injury by virtue of producing more acetylhydrazine.

It appears that the tendency of drugs such as hydralazine and procainamide to cause lupus erythematosus syndrome and to elicit formation of antinuclear antibodies (ANA) is related to acetylator phenotype, with greater prevalence in slow acetylators.[356] Rapid acetylation may prevent the immunologic triggering of ANA formation and the lupus syndrome. Interestingly, the *N*-acetylated metabolite of procainamide has been shown to be as active an antiarrhythmic agent as the parent drug[341] and to have a half-life twice as long in humans.[357] These findings indicate that *N*-acetylprocainamide may be a promising alternative to procainamide as an antiarrhythmic agent with decreased lupus-inducing potential.





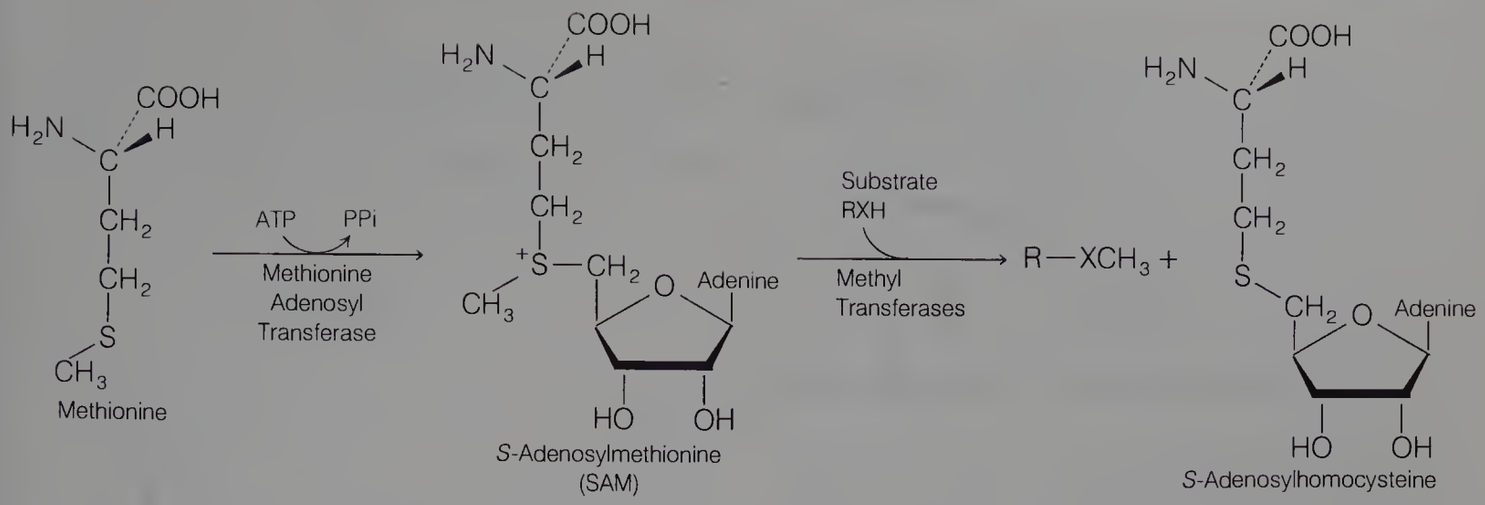
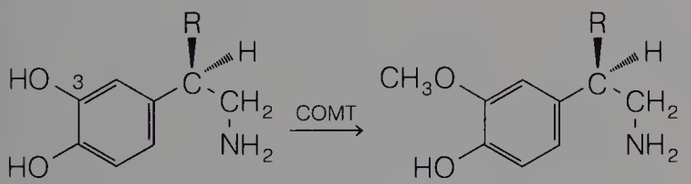


FIG. 3-17. *Conjugation of exogenous and endogenous substrates (RXH) by methylation.*

METHYLATION

Methylation reactions play an important role in the biosynthesis of many endogenous compounds (e.g., epinephrine and melatonin) and in the inactivation of numerous physiologically active biogenic amines (e.g., norepinephrine, dopamine, serotonin, and histamine).[358] However, methylation constitutes only a minor pathway for conjugating drugs and xenobiotics. Methylation generally does not lead to polar or water-soluble metabolites, except when it creates a quaternary ammonium derivative. Most methylated products tend to be pharmacologically inactive, although there are a few exceptions.



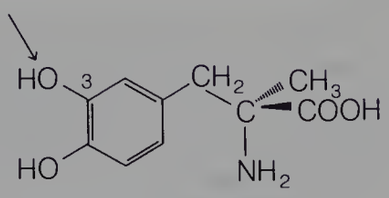
Norepinephrine, R = OH
Dopamine, R = H

Normetanephrine, R = OH
3-Methoxytyramine, R = H

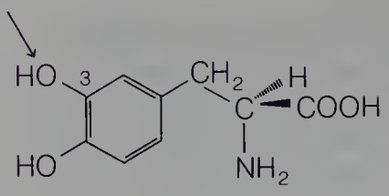
The coenzyme involved in methylation reactions is *S*-adenosylmethionine (SAM). The transfer of the activated methyl group from this coenzyme to the acceptor substrate is catalyzed by various cytoplasmic and microsomal methyltransferases (Fig. 3-17).[358,359] Methyltransferases having particular importance in the metabolism of foreign compounds include catechol-*O*-methyltransferase (COMT), phenol-*O*-methyltransferase, and nonspecific *N*-methyltransferases and *S*-methyltransferases.[358] One of these enzymes, COMT, should be familiar because it carries out *O*-methylation of such important neurotransmitters as norepinephrine and dopamine, the consequence of which is termination of their activity. Besides being present in the central and peripheral nerves, COMT is distributed widely in other mammalian tissues, particularly the liver and kidney. The other methyltransferases mentioned are located primarily in the liver, kidney, or lungs. Transferases that specifically methylate histamine, serotonin, and epinephrine usually are not involved in the metabolism of xenobiotics.[358]

Foreign compounds undergoing methylation include catechols, phenols, amines, and *N*-heterocyclic and thiol compounds. Catechol and catecholamine-like drugs are metabolized by COMT to inactive monomethylated catechol products. Examples of drugs undergoing significant *O*-methylation by COMT in humans include the antihypertensive (*S*)(−)α-methyldopa (Aldomet),[360] the antiparkinsonism agent (*S*)(−)-dopa (levodopa),[361] isoproterenol (Isuprel),[362] and dobutamine (Dobutrex).[363] The student should note the marked structural similarities between these drugs and the endogenous catecholamines such as norepinephrine and dopamine. In the foregoing four drugs, COMT selectively *O*-methylates only the phenolic OH at C-3. Bismethylation does not occur. Catechol metabolites arising from aromatic hydroxylation of phenols (e.g., 2-hydroxylation of 17α-ethinylestradiol)[44] and from the arene oxide dihydrodiol–catechol pathway (see earlier section on oxidation of aromatic moieties, e.g., the catechol metabolite of phenytoin)[364] also undergo *O*-methylation. Substrates undergoing *O*-methylation by COMT are required to contain an aromatic 1,2-dihydroxy group (i.e., catechol group). Resorcinol (1,3-dihydroxybenzene) or *p*-hydroquinone (1,4-dihydroxybenzene) derivatives are not substrates for COMT. This would explain why isoproterenol undergoes extensive *O*-methylation[362] but terbutaline (which contains a resorcinol moiety) does not.[308]

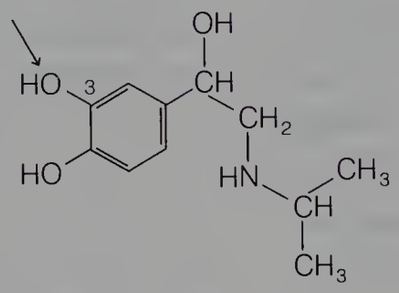
Occasionally, phenols have been reported to undergo *O*-methylation but only to a minor extent.[358] One interesting example involves the conversion of morphine to its *O*-methylated derivative, codeine, in humans. This metabolite is formed in significant amounts in tolerant subjects and may account for up to 10% of the morphine dose.[365]



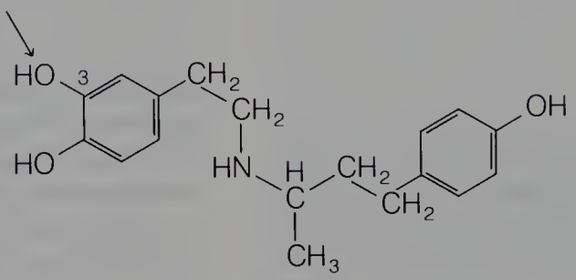
S(−)-α-Methyldopa



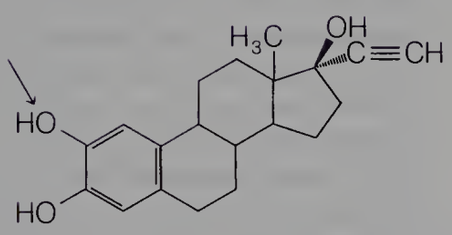
S(−)-Dopa



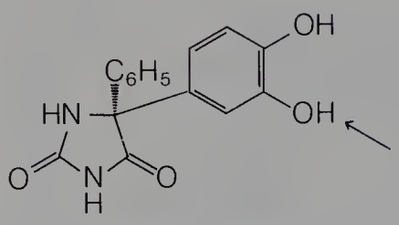
Isoproterenol



Dobutamine



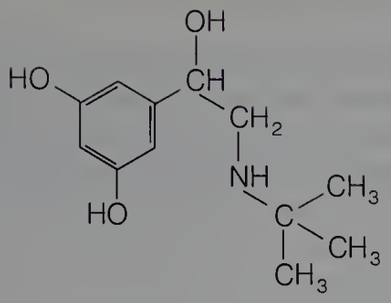
2-Hydroxy-17α-ethinylestradiol



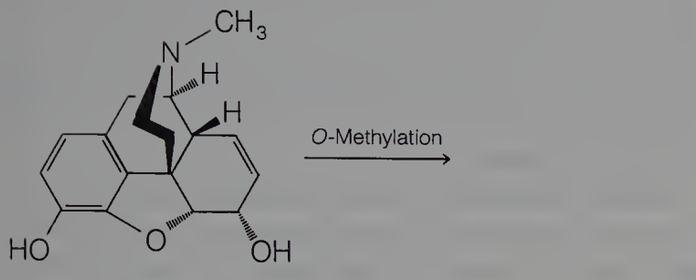
Catechol Metabolite of Phenytoin

Although *N*-methylation of endogenous amines (e.g., histamine, norepinephrine) occurs commonly, biotransformation of nitrogen-containing xenobiotics to *N*-methylated metabolites occurs to only a limited extent. Some examples reported include the *N*-methylation of the antiviral and antiparkinsonism agent amantadine (Symmetrel) in dogs[366] and the in vitro *N*-methylation of norephedrine in rabbit lung preparations.[358] *N*-Methylation of nitrogen atoms present in heterocyclic compounds (e.g., pyridine derivatives) also takes place. For example, the pyridinyl nitrogens of nicotine[147] and nicotinic acid[367] are *N*-methylated to yield quaternary ammonium products.

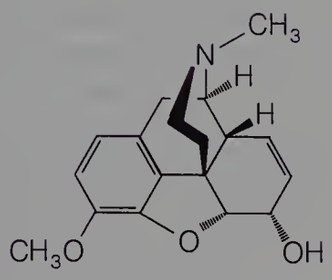
Thiol-containing drugs, such as propylthiouracil,[368] 2,3-dimercapto-1-propanol (BAL),[369] and 6-mercaptopurine,[370] also have been reported to undergo *S*-methylation.



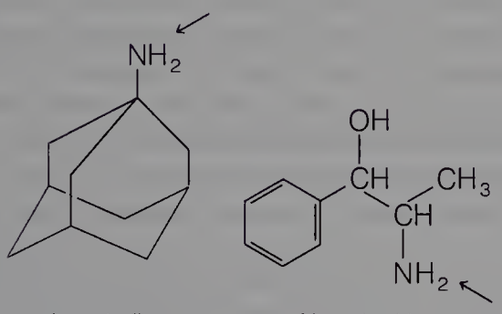
Terbutaline
(not a substrate for COMT)



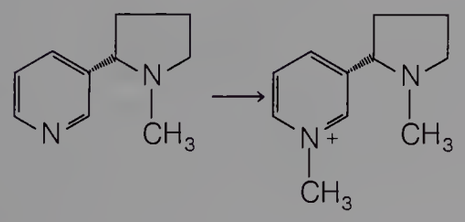
Morphine



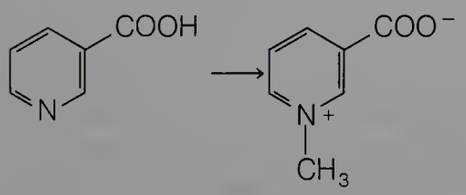
Codeine



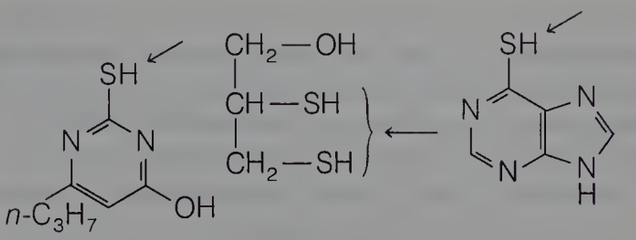
Amantadine Norephedrine



Nicotine



Nicotinic Acid Trigonelline



Propylthiouracil 2,3-Dimercapto-1-propanol (BAL) 6-Mercaptopurine

FACTORS AFFECTING DRUG METABOLISM

Drugs and xenobiotics often are metabolized by several different phase I and phase II pathways to give a number of metabolites. The relative amount of any particular metabolite is determined by the concentration and activity of the enzyme(s) responsible for the biotransformation. The rate of metabolism of a drug is particularly important for its pharmacologic action as well as its toxicity. For example, if the rate of metabolism of a drug is decreased, this generally leads to an increase in the intensity and duration of the drug. In addition, decreased metabolic elimination may lead to accumulation of toxic levels of the drug. Conversely, an increase in the rate of metabolism leads to decreases in intensity and duration of action, as well as to decreased efficacy. Many factors may affect drug metabolism, and they will be discussed in the following sections. These include age, species and strain, genetic or hereditary factors, sex, enzyme induction, and enzyme inhibition.[29,371]

AGE DIFFERENCES

Age-related differences in drug metabolism are generally quite apparent in the newborn.[372,373] In most fetal and newborn animals, undeveloped or deficient oxidative and conjugative enzymes are chiefly responsible for the reduced metabolic capability seen. In general, the ability to carry out metabolic reactions increases rapidly after birth and approaches adult levels in about 1 to 2 months. An illustration of the influence of age on drug metabolism is seen in the duration of action (sleep time) of hexobarbital in newborn and adult mice.[374] When given a dose of 10 mg/kg body weight, the newborn mouse sleeps more than 6 hours. In contrast, the adult mouse sleeps for fewer than 5 minutes when given the same dose.

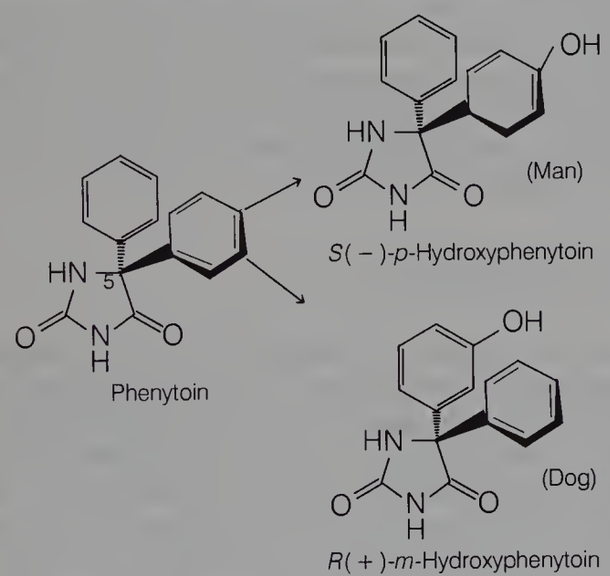
In humans, oxidative and conjugative (e.g., glucuronidation) capabilities of newborns are also low compared with adults. For example, the oxidative (cytochrome P-450) metabolism of tolbutamide appears to be decreased markedly in newborns.[375] In comparison with the half-life of 8 hours in adults, the plasma half-life of tolbutamide in infants is greater than 40 hours. As discussed earlier, infants possess poor glucuronidating ability owing to a deficiency in glucuronyltransferase activity. The inability of infants to conjugate chloramphenicol with glucuronic acid appears to be respon-

sible for the accumulation of toxic levels of this antibiotic, resulting in the so-called gray baby syndrome.[302] Similarly, neonatal hyperbilirubinemia (or kernicterus) results from the inability of newborn babies to glucuronidate bilirubin.[301]

The effect of old age on drug metabolism has not been as well studied. There is some evidence in animals and humans that drug metabolism diminishes with old age.[376] However, much of the evidence is based on prolonged plasma half-lives of drugs that are metabolized totally or mainly by hepatic microsomal enzymes (e.g., antipyrine, phenobarbital, acetaminophen). The quantitative importance of old age on drug metabolism is not now known.

SPECIES AND STRAIN DIFFERENCES

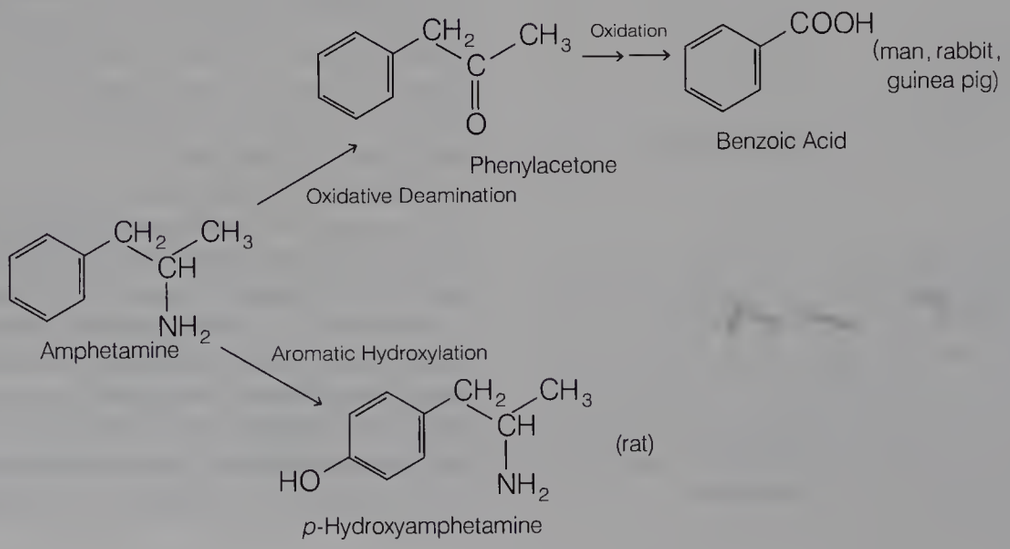
The metabolism of many drugs and foreign compounds is often species-dependent. Different animal species may biotransform a particular xenobiotic by similar or markedly different metabolic pathways. Even within the same species, there may be individual variations (strain differences) that may result in significant differences in a specific metabolic pathway.[377,378]



Species variation has been observed in many oxidative biotransformation reactions. For example, metabolism of amphetamine occurs by two main pathways: oxidative deamination or aromatic hydroxylation. In human, rabbit, and guinea pig, oxidative deamination appears to be the predominant pathway, whereas in rat aromatic hydroxylation appears to be the more important route.[379] Phenytoin is another drug showing marked species difference in metabolism. In human, phenytoin undergoes aromatic oxidation to yield primarily (*S*)(−)-*p*-hydroxyphenytoin, whereas in dog oxidation occurs mainly to give (*R*)(+)-*m*-hydroxyphenytoin.[380] There is a dramatic difference not only in the position (i.e., *meta* or *para*) of aromatic hydroxylation but also in which of the two phenyl rings (at C-5 of phenytoin) undergoes aromatic oxidation.

Species differences in many conjugation reactions also have been observed. Often, these differences are caused by the presence or absence of transferase enzymes involved in the conjugative process. For example, cats lack glucuronyltransferase enzymes and, therefore, tend to conjugate phenolic xenobiotics by sulfation instead.[381] In pigs, the situation is reversed: pigs are not able to conjugate phenols with sulfate (owing to lack of sulfotransferase enzymes) but appear to have good glucuronidation capability.[381] The conjugation of aromatic acids with amino acids (e.g., glycine, glutamine) has been noted to be dependent on the animal species, as well as on the substrate. For example, glycine conjugation is a common conjugation pathway for benzoic acid in many animals. However, in certain birds (e.g., duck, goose, turkey), glycine is replaced by the amino acid ornithine.[382] Phenylacetic acid is a substrate for both glycine and glutamine conjugation in humans and other primates. However, in nonprimates, such as the rabbit and rat, phenylacetic acid is excreted only as the glycine conjugate.[383]

Strain differences in drug metabolism, particularly in inbred mice and rabbits, have been noted. These differences apparently are caused by genetic variations in the amount of metabolizing enzyme present among the different strains.



For example, in vitro studies indicate that cottontail rabbit liver microsomes metabolize hexobarbital about ten times more rapidly than New Zealand rabbit liver microsomes.[384] In humans, interindividual differences in drug metabolism will be considered under hereditary or genetic factors.

HEREDITARY OR GENETIC FACTORS

Marked individual differences in the metabolism of several drugs exist in humans.[354] Apparently, genetic or hereditary factors are mainly responsible for the large differences seen in the rate of metabolism of these drugs. One frequently cited example that dramatically illustrates the influence of genetic factors on drug metabolism concerns the biotransformation of the antituberculosis agent isoniazid, or INH. Metabolism of isoniazid occurs primarily by *N*-acetylation.[342] Studies indicate that individuals differ markedly in their ability to acetylate the drug, either slowly or rapidly. Rapid acetylators appear to have more hepatic *N*-acetyltransferase enzymes than do slow acetylators. In addition, the level of *N*-acetyltransferase is genetically determined and is transmitted as an autosomal recessive trait in humans. As discussed earlier in the section on acetylation, the proportion of rapid and slow acetylators varies widely among different ethnic groups. For instance, a high proportion (90%) of Eskimos and Asians are rapid acetylators, whereas Egyptians and Mediterranean Jews are mainly slow acetylators.[353]

The rate at which isoniazid is acetylated is clinically important in terms of therapeutic response and toxicity. In general, it seems that rapid acetylators are more likely to show an inadequate therapeutic response (lower cure rate against tuberculosis), whereas slow acetylators are more likely to develop a greater incidence of adverse effects (e.g., peripheral neuritis, lupus erythematosus syndrome).[353] Other drugs, such as hydralazine, procainamide, and dapsone, show a similar bimodal distribution in the rate at which they are acetylated.[353]

Genetic factors also appear to influence the rate of oxidation of drugs like phenytoin, phenylbutazone, dicumarol, and nortriptyline.[385,386] The rate of oxidation of these drugs varies widely among different individuals; however, these differences do not appear to be distributed bimodally, as in acetylation. In general, individuals who tend to oxidize one drug rapidly are also likely to oxidize other drugs rapidly. Numerous studies in twins (identical and fraternal) and in families indicate that oxidation of these drugs is under genetic control.[386]

SEX DIFFERENCES

The rate of metabolism of xenobiotics also varies according to sex in some animal species. For example, a marked difference is observed between female and male rats. Adult male rats metabolize several foreign compounds at a much faster rate than female rats (e.g., *N*-demethylation of aminopyrine, hexobarbital oxidation, glucuronidation of *o*-aminophenol). Apparently, this sex difference is also dependent on the substrate because some xenobiotics are metabolized at the same rate in both female and male rats. Differences in microsomal oxidation have been shown to be under the control of sex hormones, particularly androgens. The anabolic action of androgens seems to increase metabolism.[387]

Sex differences in drug metabolism appear to be species-dependent. Rabbits and mice, for example, do not show a significant sex difference in drug metabolism.[387] In humans, there have been a few reports of sex differences in metabolism. For instance, nicotine and aspirin seem to be metabolized differently in women and men.[388]

ENZYME INDUCTION

The activity of hepatic microsomal enzymes, such as the cytochrome P-450 mixed function oxidase system, can be increased markedly upon exposure to divers drugs, pesticides, polycyclic aromatic hydrocarbons, and environmental xenobiotics. The process by which the activity of these drug-metabolizing enzymes is increased is referred to as *enzyme induction*.[389–391] The increase in activity apparently is caused by an increase in the amount of newly synthesized enzyme. Enzyme induction often leads to an increase in the rate of drug metabolism and to a decrease in the duration of drug action.

Inducing agents may increase the rate of their own metabolism as well as those of other unrelated drugs or foreign compounds (Table 3-1).[29] Concomitant administration of two or more drugs often may lead to serious drug interactions as a result of enzyme induction. For instance, a clinically critical drug interaction occurs with phenobarbital and warfarin.[392] Induction of microsomal enzymes by phenobarbital causes an increase in the metabolism of warfarin and, consequently, a marked decrease in the anticoagulant effect. Therefore, if a patient is receiving warfarin anticoagulant

TABLE 3-1

DRUGS THAT INDUCE METABOLISM IN HUMANS

Inducing Agent	*Enhances Metabolism of*
Phenobarbital and other barbiturates	Coumarin anticoagulants, phenytoin, cortisol, testosterone, bilirubin, vitamin D, acetaminophen, oral contraceptives
Glutethimide	Glutethimide, warfarin
Phenylbutazone	Aminopyrine, cortisol
Meprobamate	Meprobamate
Ethanol	Pentobarbital, tolbutamide
Phenytoin	Cortisol, nortriptyline, oral contraceptives
Rifampin	Rifampin, hexobarbital, tolbutamide, coumarin anticoagulants, oral contraceptives, methadone, digitoxin, cortisol
Griseofulvin	Warfarin
Carbamazepine	Carbamazepine, warfarin, phenytoin

(From Nelson, S. D.: In Wolff, M. E. (ed.). Burger's Medicinal Chemistry, 4th ed., Part 1, p. 227. New York, Wiley-Interscience, 1980. Reprinted with permission.)

therapy and begins taking phenobarbital, careful attention must be paid to readjustment of the warfarin dose. Dosage readjustment also must be made if a patient receiving both warfarin and phenobarbital therapy suddenly stops taking the barbiturate. The ineffectiveness of oral contraceptives in women on concurrent phenobarbital or rifampin therapy has been attributed to the enhanced metabolism of estrogens (e.g., 17α-ethinylestradiol) caused by phenobarbital[393] and rifampin[394] induction.

Inducers of microsomal enzymes also may enhance the metabolism of endogenous compounds, such as steroidal hormones and bilirubin. For example, phenobarbital has been observed to increase the metabolism of cortisol, testosterone, vitamin D, and bilirubin in humans.[389] The enhanced metabolism of vitamin D_3 induced by phenobarbital and phenytoin appears to be responsible for the osteomalacia seen in patients on long-term use of these two anticonvulsant drugs.[395] Interestingly, phenobarbital causes the induction of glucuronyltransferase enzymes, thereby enhancing the conjugation of bilirubin with glucuronic acid. Phenobarbital has been used occasionally to treat hyperbilirubinemia in neonates.[396]

In addition to drugs, other chemicals, such as polycyclic aromatic hydrocarbons (e.g., benzo[*a*]pyrene, 3-methylcholanthrene) and environmental pollutants (e.g., pesticides, polychlorinated biphenyls, TCDD), may induce certain oxidative pathways and, thereby, alter drug response.[389,391] Cigarette smoke contains minute amounts of polycyclic aromatic hydrocarbons, such as benzo[*a*]pyrene, which are potent inducers of microsomal cytochrome P-450 enzymes. This induction has been noted to increase the oxidation of some drugs in smokers. For example, theophylline is metabolized more rapidly in smokers than in nonsmokers. This difference is reflected in the marked difference in the plasma half-life of theophylline between smokers ($T_{1/2}$ 4.1 hours) and nonsmokers ($T_{1/2}$ 7.2 hours).[397] Other drugs, such as phenacetin, pentazocine, and propoxyphene, also have been reported to undergo more rapid metabolism in smokers than in nonsmokers.[398]

Occupational and accidental exposures to chlorinated pesticides and insecticides also have stimulated drug metabolism. For instance, the half-life of antipyrine in workers occupationally exposed to the insecticides lindane and DDT has been reported to be significantly shorter (7.7 hours versus 11.7 hours) in contrast with control subjects.[399] A case has been reported in which a worker exposed to chlorinated insecticides showed a lack of response (i.e., decreased anticoagulant effect) to a therapeutic dose of warfarin.[400]

Multiple forms of cytochrome P-450 have been demonstrated.[28,36] Many chemicals selectively induce one or more distinct forms of cytochrome P-450.[28] These inducters fall into two categories: ''phenobarbital-like'' inducers or ''polycyclic aromatic hydrocarbon-like'' inducers (e.g., benzo[*a*]pyrene, 3-methylcholanthrene). Phenobarbital-like compounds generally induce one or more forms of cytochrome P-450, in which the spectral maximum of the reduced cytochrome P-450 carbon monoxide complex occurs at 450 nm. In contrast, polycyclic aromatic hydrocarbon-like chemicals induce a different form of cytochrome P-450, in which the reduced cytochrome P-450–carbon monoxide complex occurs at 448 nm.[25,28] This distinct enzyme form often is referred to as cytochrome P-448. Xenobiotics, such as benzo[*a*]pyrene, 3-methylcholanthrene, and TCDD, induce cytochrome P-448.[389] Cytochrome P-448 is particularly interesting in that it shows a greater selectivity for the oxidation of polycyclic aromatic hydrocarbons.

Enzyme induction also may affect toxicity of some drugs by enhancing the metabolic formation of chemically reactive metabolites. Particularly important is the induction of cytochrome P-450 enzymes involved in the oxidation of drugs to reactive intermediates. For example, the oxidation of acetaminophen to a reactive imidoquinone metabolite appears to be carried out by a phenobarbital-inducible form of cytochrome P-450 in rats and mice. Numerous studies in these two animals indicate that phenobarbital pretreatment leads to an increase in vivo hepatotoxicity and covalent binding, as well as to an increase in the formation of reactive metabolite in microsomal incubation mixtures.[188,189,191] Induction of cytochrome P-448 is of toxicologic concern because it is now well established that this particular enzyme is involved in the metabolism of polycyclic aromatic hydrocarbons to reactive and carcinogenic intermediates.[67,401] For example, the metabolic bioactivation of benzo[*a*]pyrene to its ultimate carcinogenic diol epoxide intermediate is carried out by cytochrome P-448 (see earlier section on aromatic oxidation for the bioactivation pathway of benzo[*a*]pyrene to its diol epoxide).[401] Thus, it is becoming increasingly apparent that enzyme induction may enhance the toxicity of some xenobiotics by increasing the rate of formation of reactive metabolites.

ENZYME INHIBITION

Several drugs and xenobiotics are capable of inhibiting drug metabolism.[29,371] With metabolism decreased, drug accumulation often occurs, leading to prolonged drug action and serious adverse effects. Enzyme inhibition can occur by divers mechanisms, including substrate competition, interference with protein synthesis, inactivation of drug-metabolizing enzymes, hepatotoxicity leading to impairment of enzyme activity, and others. Some drug interactions resulting from enzyme inhibition have been reported in humans.[402] For example, phenylbutazone has been noted to inhibit stereoselectively the metabolism of the more potent (*S*)(−)-enantiomer of warfarin. This inhibition may explain the excessive hypoprothrombinemia (increased anticoagulant effect) and many instances of hemorrhaging seen in patients on both warfarin and phenylbutazone therapy.[45] The metabolism of phenytoin is inhibited by drugs such as chloramphenicol, disulfiram, and isoniazid.[392] Interestingly, phenytoin toxicity as a result of enzyme inhibition by isoniazid appears to occur primarily in slow acetylators.[355] Several drugs, such as dicumarol, chloramphenicol, and phenylbutazone,[392]

have been observed to inhibit the biotransformation of tolbutamide, which may lead to a hypoglycemic response.

Other compounds, such as SK & F-525A (proadifen hydrochloride), metyrapone, piperonyl butoxide, and cobaltous chloride, have been used experimentally in animals as general inhibitors of microsomal enzymes.[29,403]

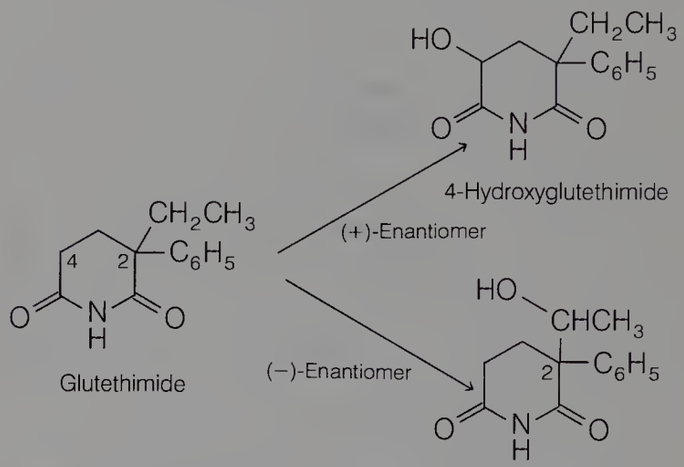
MISCELLANEOUS FACTORS AFFECTING DRUG METABOLISM[29,371]

Other factors also may influence drug metabolism. Dietary factors, such as the protein/carbohydrate ratio, affect the metabolism of a few drugs. Indoles present in vegetables, such as brussels sprouts, cabbage, and cauliflower, and polycyclic aromatic hydrocarbons present in charcoal-broiled beef cause enzyme induction and stimulate the metabolism of some drugs. Vitamins, minerals, starvation, and malnutrition also apparently have an influence on drug metabolism. Finally, physiologic factors, such as the pathologic state of the liver (e.g., hepatic cancer, cirrhosis, hepatitis), pregnancy, hormonal disturbances (e.g., thyroxine, steroids), and circadian rhythm, may markedly affect drug metabolism.

STEREOCHEMICAL ASPECTS OF DRUG METABOLISM

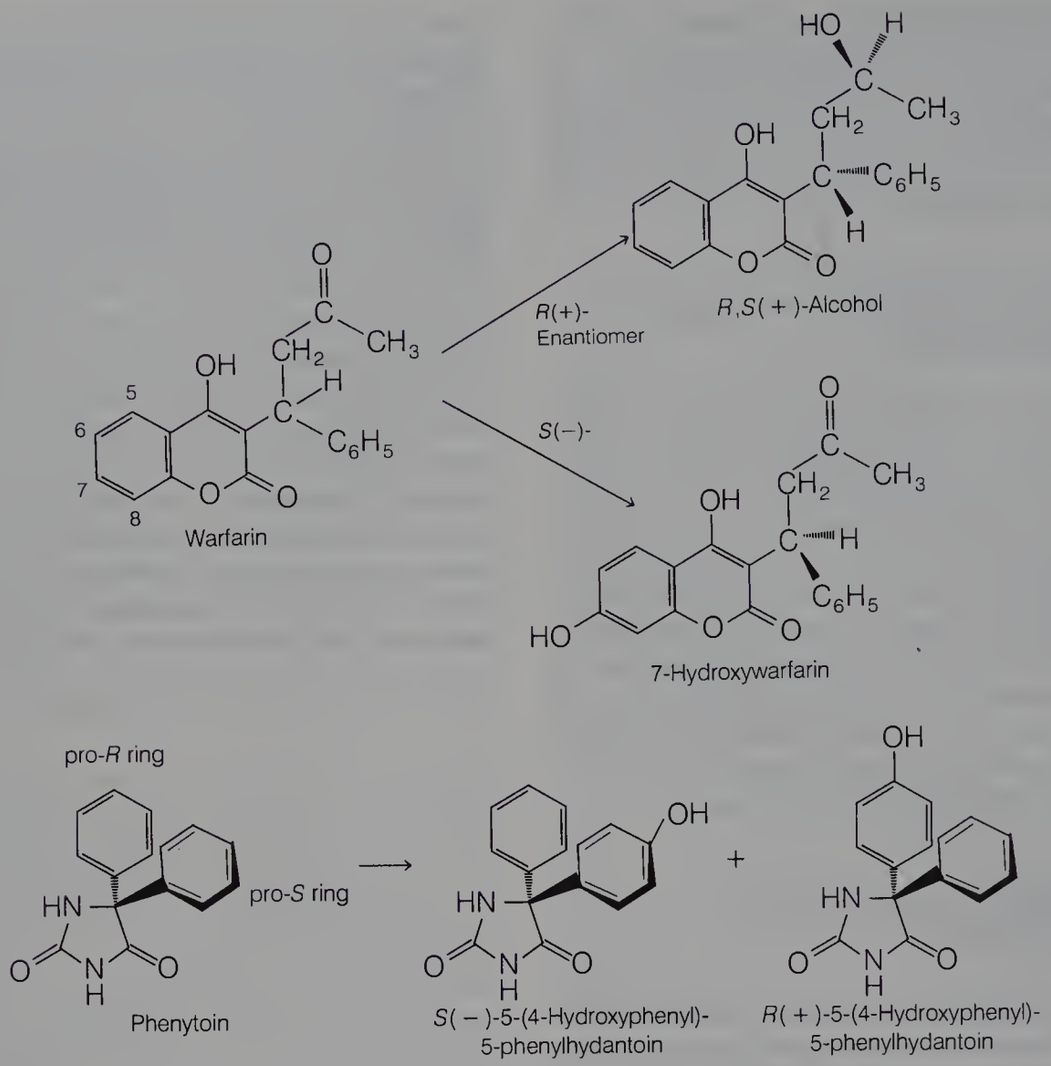
Many drugs (e.g., warfarin, propranolol, hexobarbital, glutethimide, cyclophosphamide, ketamine, and ibuprofen) often are administered as racemic mixtures in humans. The two enantiomers present in a racemic mixture may differ from one another in pharmacologic activity. Usually, one enantiomer tends to be much more active than the other. For example, the (*S*)(−)-enantiomer of warfarin is five times more potent as an oral anticoagulant than is the (*R*)(+)-enantiomer.[404] In some instances, the two enantiomers may have totally different pharmacologic activities. For example, (+)-α-propoxyphene (Darvon) is an analgesic, whereas (−)-α-propoxyphene (Novrad) is an antitussive.[405] Such differences in activity between stereoisomers should not be surprising since in Chapter 2 we learned that stereochemical factors generally have a dramatic influence on how the drug molecule interacts with the target receptors to elicit its pharmacologic response. By the same token, the preferential interaction of one stereoisomer with drug-metabolizing enzymes may lead one to anticipate differences in metabolism for the two enantiomers of a racemic mixture. Indeed, individual enantiomers of a racemic drug often are metabolized at different rates. For example, studies in humans indicate that the less active (+)-enantiomer of propranolol undergoes more rapid metabolism than the corresponding (−)-enantiomer.[406] Allylic hydroxylation of hexobarbital has been observed to occur more rapidly with the *R*(−)-enantiomer in humans.[407] The term "*substrate stereoselectivity*" is used frequently to denote the preference of one stereoisomer as a substrate for a metabolizing enzyme or metabolic process.[223]

Individual enantiomers of a racemic mixture also may be metabolized by different pathways. For instance, in dogs the (+)-enantiomer of the sedative hypnotic glutethimide (Doriden) is hydroxylated primarily α to the carbonyl to yield 4-hydroxyglutethimide, whereas the (−)-enantiomer undergoes aliphatic $\omega - 1$ hydroxylation of its C-2 ethyl group.[113] Dramatic differences in the metabolic profile of two enantiomers of warfarin also have been noted. In humans, the more active (*S*)(−)-isomer is 7-hydroxylated (aromatic hydroxylation), whereas the (*R*)(+)-isomer undergoes keto reduction to yield primarily the (*RS*) warfarin alcohol as the major plasma metabolite.[45,234] Although numerous other examples of substrate stereoselectivity or enantioselectivity in drug metabolism exist, the examples presented will suffice to emphasize the point.[223,408]



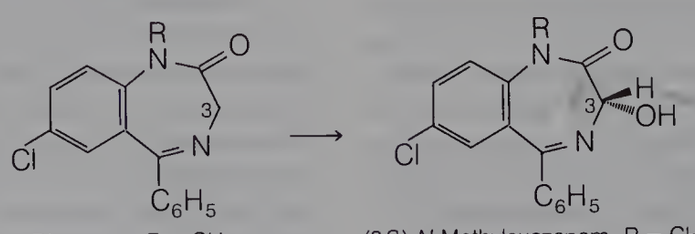
Drug biotransformation processes often lead to the creation of a new asymmetric center in the metabolite (i.e., stereoisomeric or enantiomeric products). The preferential metabolic formation of a stereoisomeric product is called *product stereoselectivity*.[223] For example, bioreduction of ketone xenobiotics, as a general rule, produces predominantly one stereoisomeric alcohol (see "Reduction of Ketone Carbonyls," above).[91,223] The preferential formation of (*S*)(−)-hydroxyhexamide from the hypoglycemic agent acetohexamide[225] and the exclusive generation of 6β-naltrexol from naltrexone[231] (see "Reduction of Ketone Carbonyls" for structure) are two examples of highly stereoselective bioreduction processes in humans.

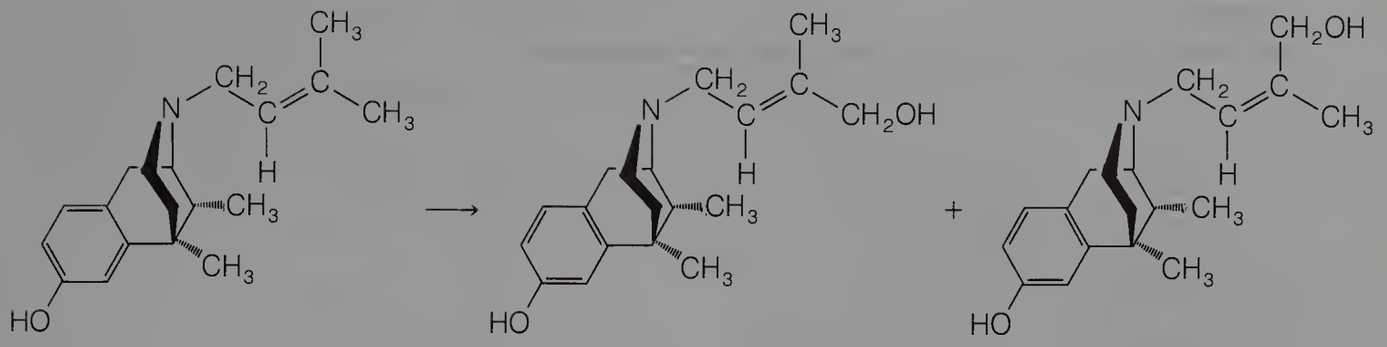
Oxidative biotransformations display product stereoselectivity, too. For example, phenytoin contains two phenyl rings in its structure, both of which a priori should be susceptible to aromatic hydroxylation. However, in humans, *p*-hydroxylation occurs preferentially (approximately 90%) at the pro-(*S*) phenyl ring to give primarily (*S*)(−)-5-(4-hydroxyphenyl)-5-phenylhydantoin. Although the other phenyl ring also is *p*-hydroxylated, it occurs only to a minor extent (10%).[380] Microsomal hydroxylation of the C-3 carbon of diazepam and desmethyldiazepam (using mouse liver prepa-



rations) has been reported to proceed with remarkable stereoselectivity to yield optically active metabolites having the 3(*S*) absolute configuration.[112] Interestingly, these two metabolites are pharmacologically active and one of them, oxazepam, is marketed as a drug (Serax). The allylic hydroxylation of the *N*-butenyl side group of the analgesic pentazocine (Talwin) leads to two possible alcohols (*cis* and *trans* alcohols). In human, mouse, and monkey, pentazocine is metabolized predominantly to the *trans* alcohol metabolite, whereas the rat primarily tends to form the *cis* alcohol.[106] The product stereoselectivity observed in this biotransformation involves *cis* and *trans* geometric stereoisomers.

The term "*regioselectivity*"[409] has been introduced in drug metabolism to denote the selective metabolism of two or more similar functional groups (e.g., OCH_3, OH, NO_2) or two or more similar atoms that are positioned in different regions of a molecule. For example, of the four methoxy groups present in papaverine, the 4′-OCH_3 group is regioselectively *O*-demethylated in several species (e.g., rat, guinea pig, rabbit, and dog).[410] Trimethoprim (Trimpex, Proloprim) has two heterocyclic sp^2 nitrogen atoms (N^1 and N^3) in its structure. In dogs, it appears that oxidation occurs regioselectively at N^3 to give the corresponding 3-*N*-oxide.[178] Nitroreduction of the 7-nitro group in 5,7-dinitroindazole to yield

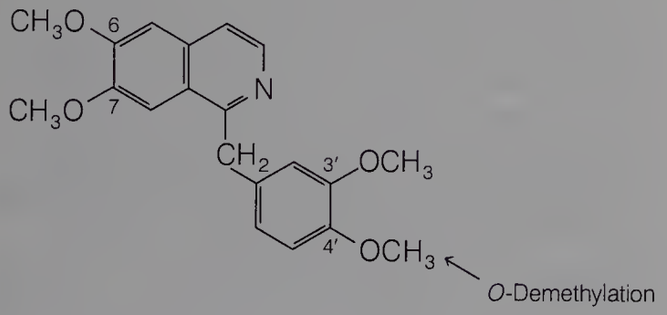


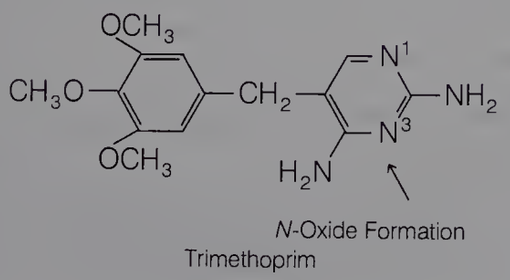


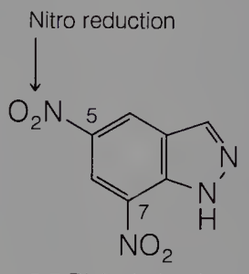
the 7-amino derivative in the mouse and rat occurs with a high degree of regioselectivity.[287] Substrates amenable to *O*-methylation by COMT appear to proceed with remarkable regioselectivity, as typified by the cardiotonic agent dobutamine (Dobutrex). *O*-Methylation occurs exclusively with the phenolic hydroxy group at C-3.[363]

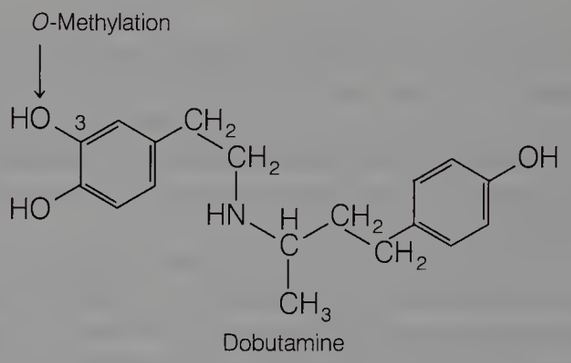
PHARMACOLOGICALLY ACTIVE METABOLITES

The traditional notion that drug metabolites are inactive and insignificant in drug therapy has changed dramatically in recent years. There is now increasing evidence to indicate that many drugs are biotransformed to pharmacologically active metabolites that contribute to the therapeutic as well as toxic effects of the parent compound. Metabolites that have been shown to have significant therapeutic activity in humans are listed in Table 3-2.[2,411] The parent drug from which the metabolite is derived and the biotransformation process involved also are given.









How significantly an active metabolite contributes to the therapeutic or toxic effects ascribed to the parent drug depends on its relative activity and quantitative importance (e.g., plasma concentration). In addition, whether the metabolite accumulates after repeated administration (e.g., desmethyldiazepam in geriatric patients) or in patients with renal failure are determinants.

From a clinical standpoint, active metabolites are especially important in patients with decreased renal function. If renal excretion is the major pathway for elimination of the active metabolite, then accumulation is likely to occur in patients with renal failure. Especially with drugs such as procainamide, clofibrate, and digitoxin caution should be exercised in treating patients with renal failure.[2,411] Many of the toxic effects seen for these drugs have been attributed to high plasma levels of their active metabolites. For example, the severe muscle weakness and tenderness (myopathy) seen with clofibrate in renal failure patients is believed to be caused by high levels of the active metabolite chlorophenoxyisobutyric acid.[412] Cardiovascular toxicity owing to

TABLE 3-2

PHARMACOLOGICALLY ACTIVE METABOLITES IN HUMANS

Parent Drug	*Metabolite*	*Biotransformation Process*
Acetohexamide	Hydroxyhexamide	Ketone reduction
Acetylmethadol	Noracetylmethadol	*N*-Demethylation
Amitriptyline	Nortriptyline	*N*-Demethylation
Azathioprine	6-Mercaptopurine	Glutathione conjugation
Carbamazepine	Carbamazepine-9, 10-epoxide	Epoxidation
Chloral hydrate	Trichloroethanol	Aldehyde reduction
Clofibrate	Chlorophenoxyisobutyric acid	Ester hydrolysis
Chlorpromazine	7-Hydroxychlorpromazine	Aromatic hydroxylation
Cortisone	Hydrocortisone	Ketone reduction
Diazepam	Desmethyldiazepam and oxazepam	*N*-Demethylation and 3-hydroxylation
Digitoxin	Digoxin	Alicyclic hydroxylation
Diphenoxylate	Diphenoxylic acid	Ester hydrolysis
Imipramine	Desipramine	*N*-Demethylation
Mephobarbital	Phenobarbital	*N*-Demethylation
Metoprolol	α-Hydroxymethylmetoprolol	Benzylic hydroxylation
Phenacetin	Acetaminophen	*O*-Deethylation
Phenylbutazone	Oxybutazone	Aromatic hydroxylation
Prednisone	Prednisolone	Ketone reduction
Primidone	Phenobarbital	Hydroxylation and oxidation to ketone
Procainamide	*N*-Acetylprocainamide	*N*-Acetylation
Propranolol	4-Hydroxypropranolol	Aromatic hydroxylation
Quinidine	3-Hydroxyquinidine	Allylic hydroxylation
Sulindac	Sulfide metabolite of sulindac	Sulfoxide reduction
Thioridazine	Mesoridazine	*S*-oxidation
Warfarin	Warfarin alcohols	Ketone reduction

digitoxin and procainamide in anephric subjects has been attributed to high plasma levels of digoxin and *N*-acetylprocainamide, respectively. In such situations, appropriate reduction in dosage and careful monitoring of plasma levels of the parent drug and its active metabolite often are recommended.

The pharmacologic activity of some metabolites has led many manufacturers to synthesize these metabolites and to market them as separate drug entities. For example, oxyphenbutazone (Tandearil, Oxalid) is the *p*-hydroxylated metabolite of the anti-inflammatory agent phenylbutazone (Butazolidin, Azolid), nortriptyline (Aventyl) is the *N*-demethylated metabolite of the tricyclic antidepressant amitriptyline (Elavil), oxazepam (Serax) is the *N*-demethylated and 3-hydroxylated metabolite of diazepam (Valium), and mesoridazine (Serentil) is the sulfoxide metabolite of the antipsychotic agent thioridazine (Mellaril).

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CHAPTER 4

Drug Latentiation and Prodrugs

Forrest T. Smith
C. Randall Clark

HISTORY

In 1958, Albert initially coined the term ''prodrug'' and used it to refer to a pharmacologically inactive compound that is transformed by the mammalian system into an active substance by either chemical or metabolic means.[1] This included both compounds that are designed to undergo a transformation in order to yield an active substance and those that were discovered by serendipity to do so. These two situations were distinguished by Harper, who in 1959 introduced the term ''drug latentiation'' to refer to drugs specifically designed to require bioactivation.[2]

These ideas have led to the development of a number of currently used drugs that have advantages over their nonprodrug counterparts. The type of prodrug that is to be produced depends upon the specific aspect of the drug's action that requires improvement and the type of functionality that is present in the active drug. Generally, prodrug approaches are undertaken to improve patient acceptability of the agent (i.e., reduce pain associated with administration), alter absorption, alter distribution, alter metabolism, or alter elimination. The chemical nature of the prodrugs that can be prepared is somewhat limited, however, by the chemical nature of the active species.

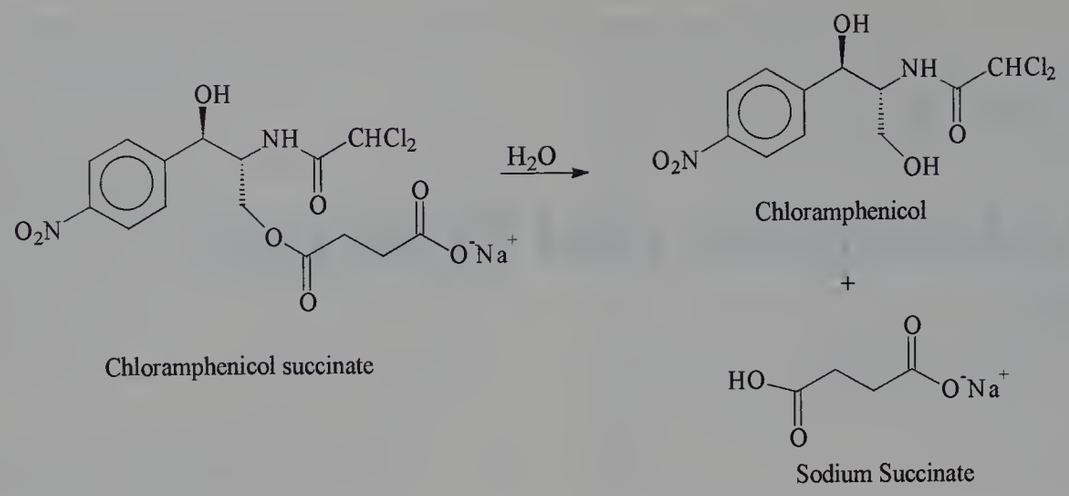
Recently, the terms ''hard drugs'' and ''soft drugs'' have been introduced.[3,4] Hard drugs are compounds designed to contain those structural characteristics necessary for pharmacological activity but in a form not susceptible to metabolic or chemical transformation. In this way, the production of any toxic metabolite is avoided, and there is an increased efficiency of action. Since the drug is not inactivated by metabolism, it may be less readily eliminated. On the other hand, soft drugs are active compounds that, after exerting their desired pharmacological effect, are designed to undergo metabolic inactivation to give a nontoxic product. Therefore, soft drugs are considered the opposite of prodrugs.

BASIC CONCEPTS

A prodrug by definition is inactive and must be converted into an active species within the biological system. There is a variety of mechanisms by which this conversion may be accomplished. Generally speaking, the conversion to an active form is most often carried out by metabolizing enzymes within the body. It is possible, however, for conversion to an active form to be accomplished by chemical means, e.g., hydrolysis or decarboxylation, although this is less commonly seen. Chemical transformation is not dependent upon the presence or relative amounts of metabolizing enzymes, and therefore less variability in activation is seen among patients; however, since such compounds are chemically unstable, storage of these compounds may present a problem.

Prodrugs can be conveniently grouped into carrier-linked prodrugs and bioprecursor prodrugs.[5] Carrier-linked prodrugs are drugs that have been attached through a metabolically labile linkage to another molecule, the so-called promoiety, which is not necessary for activity but may impart some desirable property to the drug, such as increased lipid or water solubility or site-directed delivery. There are several advantages that may be gained by generating a prodrug, such as increased absorption, alleviation of pain at the site of injection if the agent is given parenterally, elimination of an unpleasant taste associated with the drug, decreased toxicity, decreased metabolic inactivation, increased chemical stability, and a prolonged or shortened action, whichever is desired in a particular agent. An example of such a prodrug form of chloramphenicol is provided below (Scheme 1).[6]

Parenteral administration of a drug may cause pain at the site of injection, especially if the drug begins to precipitate out of solution and damage the surrounding tissue. This situation can be remedied by preparing a drug with increased solubility in the administered solvent. Since chloramphenicol has low water solubility, the succinate ester was prepared

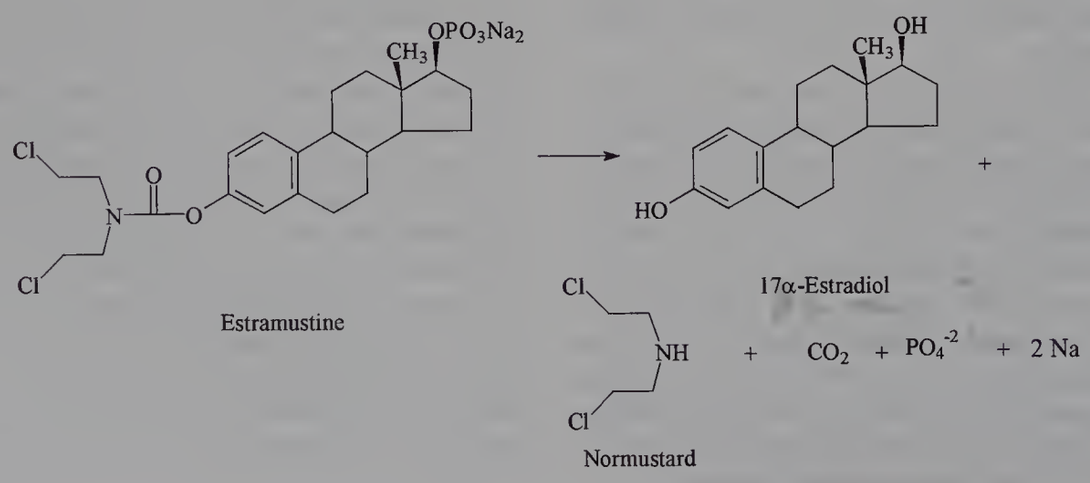


Scheme 1.

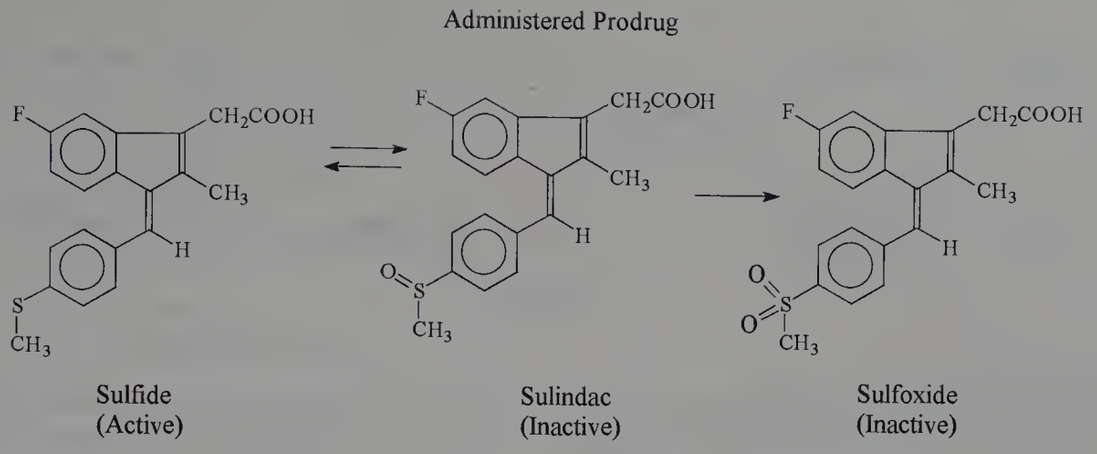
in order to increase the water solubility of the agent and facilitate parenteral administration. The succinate ester itself is inactive as an antibacterial agent, so it must be converted to chloramphenicol for this agent to be effective. This occurs in the plasma to give the active drug and succinate. The ester hydrolysis reaction can be catalyzed by esterases present in large amounts in the plasma. The ability to prepare ester-type prodrugs depends, of course, on the presence of either a hydroxyl group or a carboxyl moiety in the drug molecule. The promoiety should be easily and completely removed after it has served its function and should also be nontoxic, as is indeed the case with succinate. The selection of the appropriate promoiety is dependent upon which properties are sought for the agent. If it is desirable to increase water solubility, then a promoiety containing an ionizable function or numerous polar functional groups is used. If, on the other hand, the goal is to increase lipid solubility or decrease water solubility, then a nonpolar promoiety is appropriate.

A slight variation on the carrier-linked prodrug approach is seen with mutual prodrugs in which the carrier also has activity. An example of such an approach is seen in the antineoplastic agent estramustine, which is used in the treatment of prostatic cancer (Scheme 2).[7] Estramustine is composed of a phosphorylated steroid (17α-estradiol) linked to a normustard [$HN(CH_2CH_2Cl)_2$] through a carbamate linkage. The steroid portion of the molecule helps to concentrate the drug in the prostate, where hydrolysis occurs to give the normustard and CO_2. The normustard then acts as an alkylating agent and exerts a cytotoxic effect. The 17α-estradiol also has an anti-androgenic effect on the prostate and thereby slows the growth of the cancer cells. Since both the steroid and the normustard possess activity, estramustine is termed a mutual prodrug. Note that phosphorylation of the estradiol can be utilized to increase the water solubility, which also constitutes a prodrug modification. Both types of esters (carbamates and phosphates) are hydrolyzed by chemical or enzymatic means.

In contrast to carrier-linked prodrugs, bioprecursor prodrugs contain no promoiety but rather rely upon metabolism to introduce the necessary functionality to create an active species. For example, the nonsteroidal anti-inflammatory agent sulindac is inactive as the sulfoxide and must be reduced metabolically to the active sulfide (Scheme 3).[8] Sulindac is administered orally and absorbed in the small intes-



Scheme 2.



Scheme 3.

tines, and subsequently reduced to the active species. Administration of the inactive form has the benefit of reducing the gastrointestinal irritation associated with the sulfide. This example also points to one of the problems associated with this approach, namely, participation of alternate metabolic paths, which may inactivate the compound. In this case, after absorption of sulindac, irreversible metabolic oxidation of the sulfoxide to the sulfone can also occur to give an inactive compound.

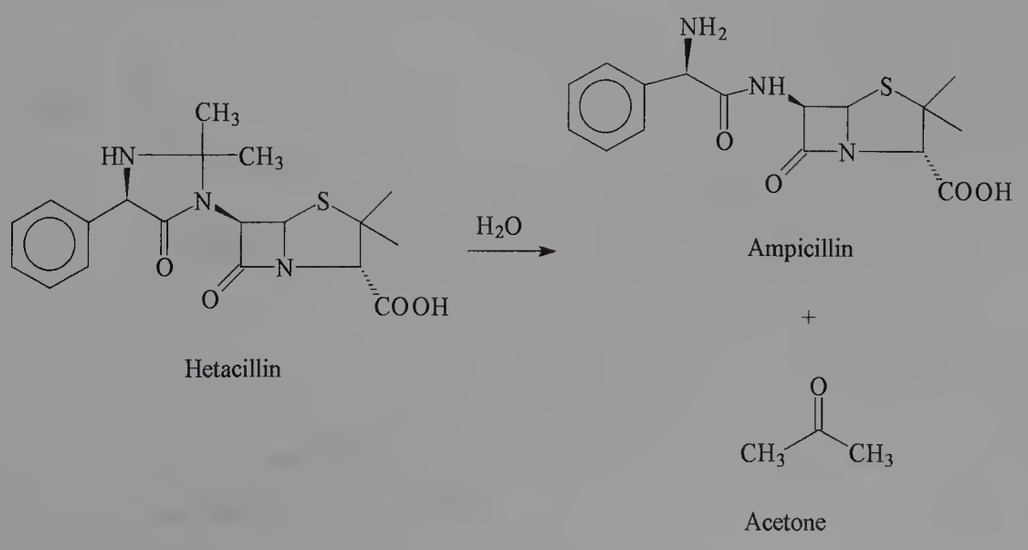
Although seen less frequently, some prodrugs rely upon chemical mechanisms for conversion of the prodrug to its active form. For example, hetacillin is a prodrug form of ampicillin in which the amide nitrogen and α-amino functionalities have been allowed to react with acetone to give an imidazolidinone ring system (Scheme 4).[9–14] This has the effect of decreasing the basicity of the α-amino group and reducing protonation in the small intestines so that the agent is more lipophilic. In this manner, the absorption of the drug from the small intestines is increased after oral dosing, and chemical hydrolysis after absorption regenerates ampicillin. In such an approach, it is necessary that the added moiety or promoiety, in this case acetone, be nontoxic and easily removed after it has performed its function.

PRODRUGS OF FUNCTIONAL GROUPS

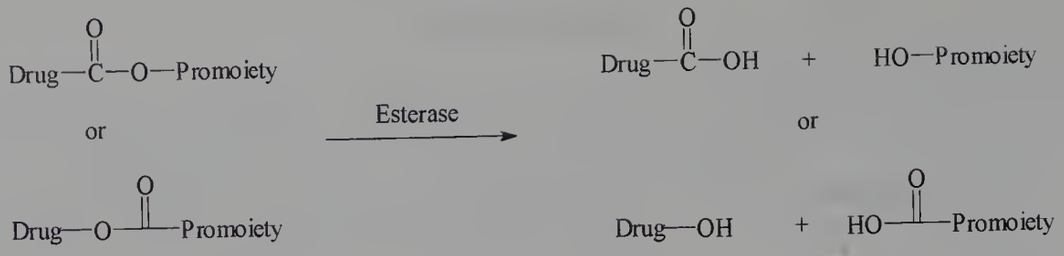
As mentioned above, there is a variety of different types of prodrugs, and a comprehensive discussion of each individual agent is beyond the scope of this chapter. However, the major types of prodrugs grouped according to functional group and, in the case of the bioprecursor drugs, grouped according to type of metabolic activation are discussed briefly here.

CARBOXYLIC ACIDS AND ALCOHOLS

Prodrugs of agents that contain carboxylic acid or alcohol functionalities can often be prepared by conversion to an ester. This is the most commonly seen type of prodrug, due to the ease with which the ester can be hydrolyzed to give



Scheme 4.



Scheme 5.

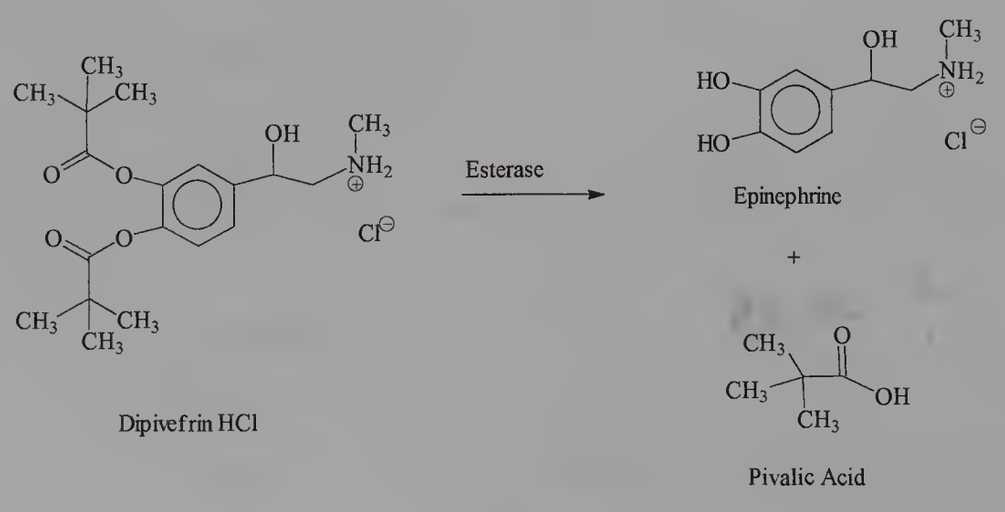
the active drug. Hydrolysis is normally accomplished by esterase enzymes present in plasma and other tissues capable of hydrolyzing a wide variety of ester linkages (Scheme 5).[15] Included below is a number of the different types of esterase enzymes that prodrugs may utilize:

Ester hydrolase
Lipase
Cholesterol esterase
Acetylcholinesterase
Carboxypeptidase
Cholinesterase

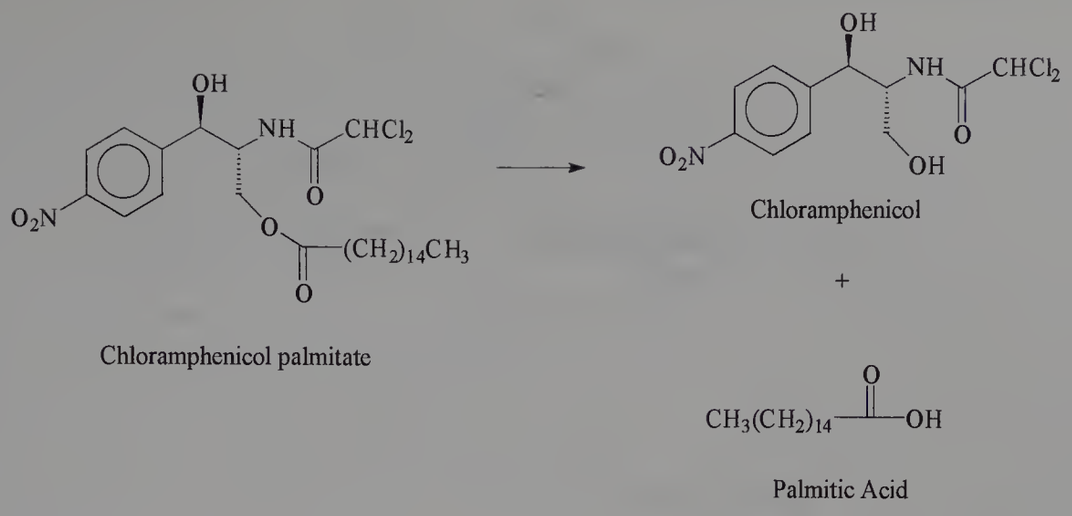
In addition to these agents, microflora present within the gut produce a wide variety of enzymes capable of hydrolyzing esters. It is also possible that chemical hydrolysis of the ester function may occur to some extent. An additional factor that has contributed to the popularity of esters as prodrugs is the ease with which they can be formed. If the drug molecule contains either an alcohol or carboxylic acid functionality, an ester prodrug may be easily synthesized. The carboxylic or alcohol promoiety can be chosen to provide a wide range of lipophilic or hydrophilic properties to the drug depending upon what is desired. Manipulation of the steric and electronic properties of the promoiety allows the rate and extent of hydrolysis to be controlled. This can be an important consideration when it is crucial that the active drug be revealed at the correct point in its movement through the biological system.

When it is desirable to decrease water solubility, a nonpolar alcohol or carboxylic acid is chosen as the prodrug moiety. By decreasing the hydrophilicity of the compound, a number of benefits may be achieved, including increased absorption, decreased dissolution in the aqueous environment of the stomach, and prolongation of the duration of action. An example of increased absorption by the addition of a nonpolar carboxylic acid is seen with dipivefrin HCl (Scheme 6). This is a prodrug form of epinephrine in which the catechol hydroxyl groups have been utilized in the formation of an ester linkage with pivalic acid.[16] The agent is used in the treatment of open-angle glaucoma. The increased lipophilicity relative to epinephrine allows the agent to move across the membrane of the eye easily when applied, achieving higher intraocular concentrations. Hydrolysis of the ester functions then occurs in the cornea, conjunctiva, and aqueous humor to generate the active form, epinephrine. By utilizing pivalic acid as the promoiety, the steric bulk around the scissile ester bond is increased, which slows the ester hydrolysis relative to less bulky groups, yet still allows this reaction to proceed after the drug has crossed the membrane barriers of the eye. In addition to this benefit, the catechol system is somewhat susceptible to oxidation, and protection of the catechol as the diester prevents this oxidation and the resulting drug inactivation from occurring.

Decreasing the water solubility of a drug by the formation of a prodrug may have additional benefits beyond simply increasing absorption. A number of agents have an unpleas-



Scheme 6.



Scheme 7.

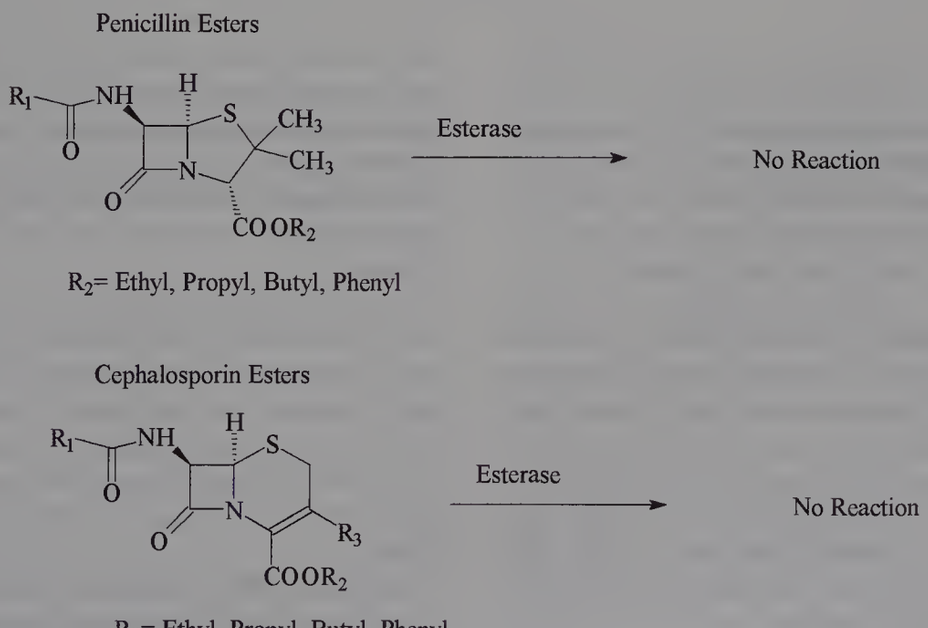
ant taste when given orally. This results when the drug begins to dissolve in the mouth and then is capable of interacting with taste receptors. This can present a significant problem, especially in pediatric patients, and may lead to low compliance. A prodrug with reduced water solubility does not dissolve to any appreciable extent in the mouth and therefore does not interact with taste receptors. This approach has been utilized in the case of the antibacterial chloramphenicol, which produces a bitter taste when given as the parent drug (Scheme 7). The hydrophobic palmitate ester does not dissolve to any appreciable extent in the mouth, so there is little chance for interaction with taste receptors.[17] The ester moiety is subsequently hydrolyzed in the gastrointestinal tract, and the agent is absorbed as chloramphenicol.

Listed below is a number of other agents that have been converted into ester prodrugs and other types of prodrugs in order to overcome an unpleasant taste:

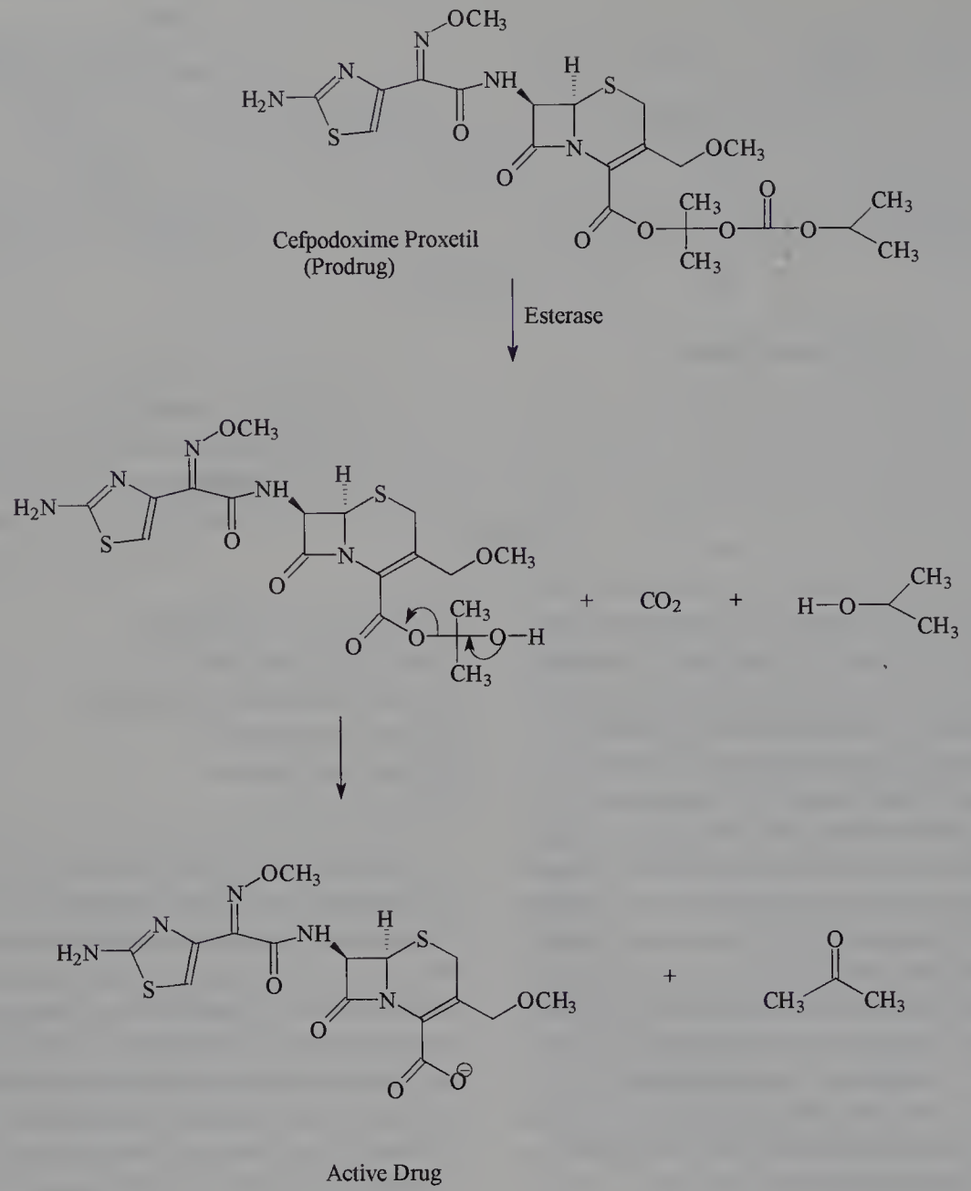
Chloramphenicol palmitate
N-Acetyl sulfisoxazole
N-Acetyl sulfamethoxypyridazine
Erythromycin estolate
Clindamycin palmitate
Troleandomycin

It should be mentioned that not all carboxylic esters are easily hydrolyzed in vivo. Steric inhibition around the ester, in some cases, prevents the prodrug from being hydrolyzed. This is seen in the β-lactams, in which it is often desirable to increase the hydrophobicity of the agent to improve absorption or prevent dissolution in the stomach, where acid-catalyzed decomposition may occur. Simple esters of the carboxylic acid moiety, however, are not hydrolyzed in vivo to the active carboxylate (Scheme 8).

A solution to this problem was to utilize the so-called



Scheme 8.



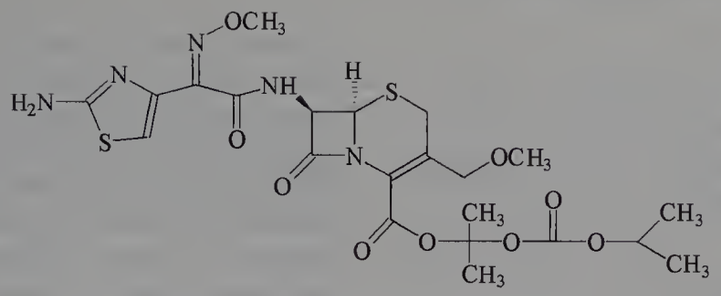
Scheme 9.

double ester approach, in which an additional ester or carbonate function was incorporated into the R_2 substituent further removed from the heterocyclic nucleus.[18,19] Hydrolysis of such a function occurred readily, and the moiety was selected so that chemical hydrolysis of the second ester occurred quickly. This is seen in the cephalosporin cefpodoxime proxetil, where a carbonate function was utilized (Scheme 9).[20] The carbonate is also susceptible to the action of esterase enzymes, and the unstable product undergoes further reaction to give the active carboxylate. This approach is frequently used to improve the absorption or prevent dissolution in the stomach, and the subsequent acid-catalyzed decomposition of amino penicillins and second- and third-generation cephalosporins (cefpodoxime proxetil has been classified as both a second- and third-generation agent) so that these agents can be administered orally (for several examples, see Scheme 10).

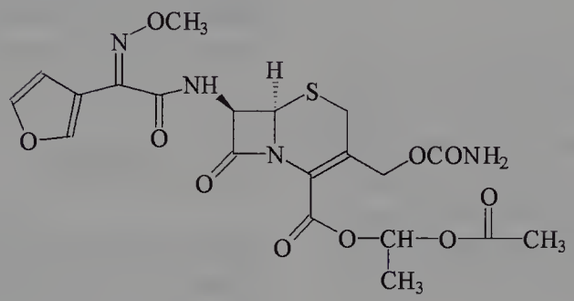
In order to increase the hydrophilicity of an agent, several different types of ester prodrugs have been utilized, including succinates, phosphates, and sulfonates. All are ionized at physiological pH and therefore increase the water solubility of the agents, making them more suitable for parenteral or oral administration where high water solubility is desirable (Scheme 11).

Succinate esters containing an ionizable carboxylate are useful when rapid in vivo hydrolysis of the ester functionality is required. The rapid hydrolysis is related to the intramolecular attack of the carboxylate on the ester linkage, which does not require the participation of enzymes (Scheme 12). As a result, these agents may be somewhat unstable in solution and should be dissolved immediately prior to administration.

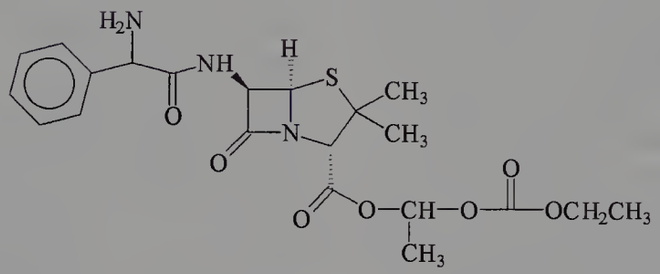
Phosphate esters of alcohols offer another method of increasing the water solubility of an agent. The phosphates are completely ionized at physiological pH and generally hydrolyzed rapidly in vivo by phosphatase enzymes. Ioniza-



Cefpodoxime Proxetil



Cefuroxime Axetil



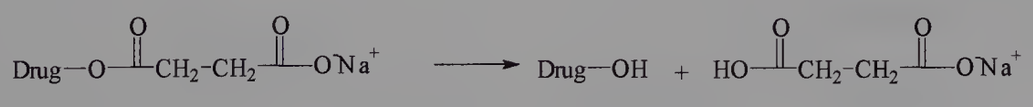
Bacampicillin

Scheme 10.

tion of the phosphate function imparts high stability to these derivatives in solution, and solutions for administration can be stored for long periods of time without hydrolysis of the phosphate. Such an approach has been utilized to produce clindamycin phosphate, which produces less pain at the injection site compared with clindamycin itself (Scheme 13). Pain after parenteral administration is associated with local irritation due to low aqueous solubility or highly acidic or basic solutions. In clindamycin phosphate, the reduction in pain is thought to be related to the increased water solubility of the agent.

AMINES

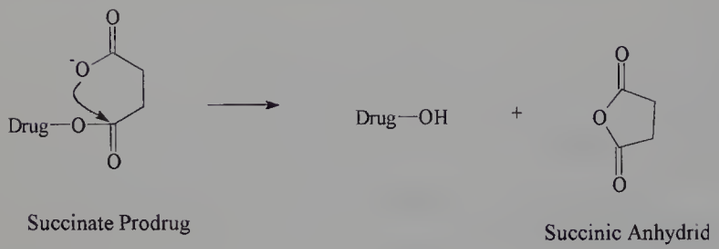
Derivatization of amines to give amides has not been widely used as a prodrug strategy due to high chemical stability of the amide linkage and the lack of amidase enzymes neces-



Succinates

Phosphates

Scheme 11.



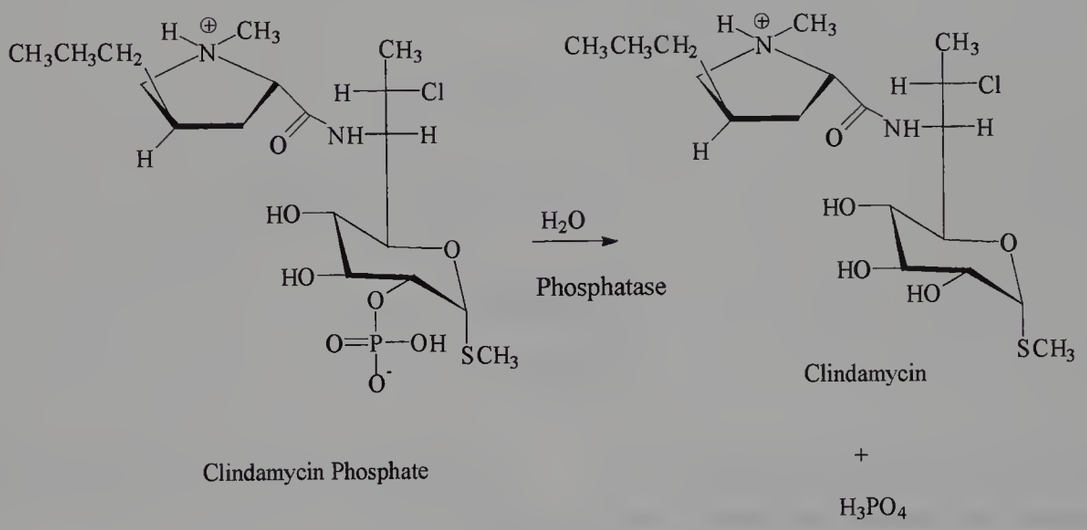
Scheme 12.

sary for hydrolysis. A more common approach has been to utilize Mannich bases as a prodrug form of the amines. Mannich bases result from the reaction of two amines with an aldehyde or ketone. As seen for hetacillin (Scheme 4), the effect of forming the Mannich base is to lower the basicity of the amine and thereby increase lipophilicity and absorption.

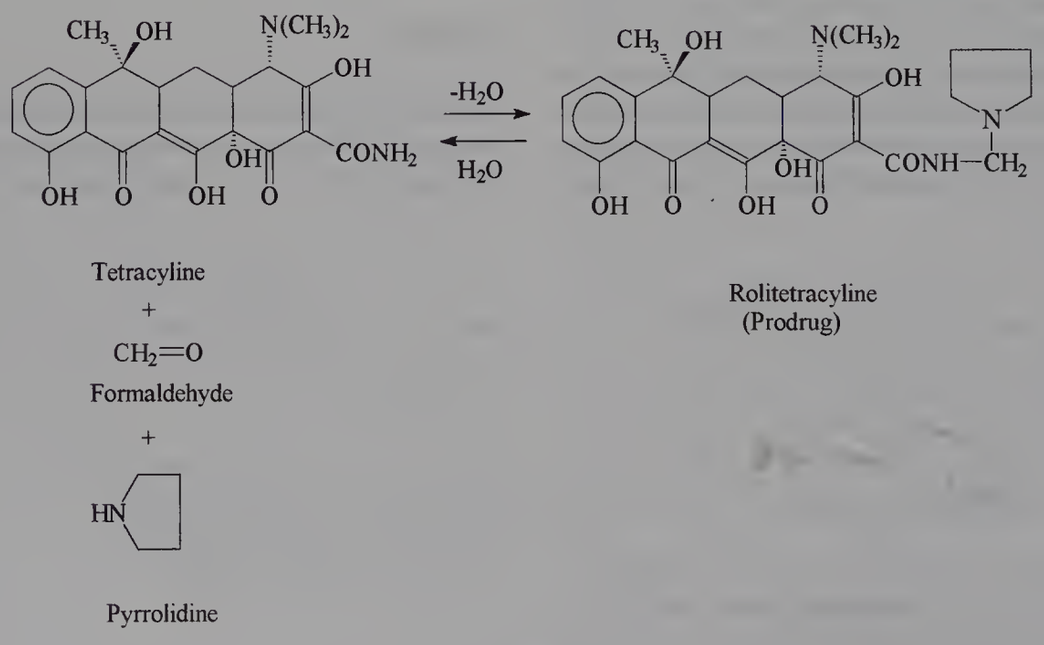
When nitrogen is present in an amide linkage, it is sometimes desirable to utilize the amide nitrogen as one of the amines necessary to form a Mannich base. This approach has been utilized in the antibiotic tetracycline, in which the amide nitrogen was allowed to react with formaldehyde and pyrrolidine to give the Mannich base rolitetracycline (Scheme 14).[21] In this case, the addition of the basic pyrrolidine nitrogen introduces an additional ionizable functionality and increases the water solubility of the parent drug. Hydrolysis of the Mannich base occurs completely and rapidly in aqueous media to give the active tetracycline.

AZO LINKAGE

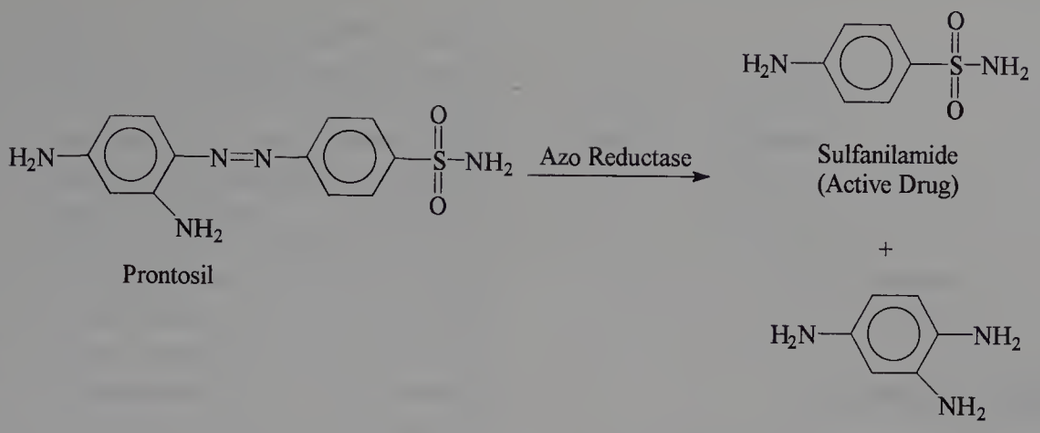
Amines have occasionally been incorporated into an azo linkage for the purpose of producing a prodrug. In fact, it



Scheme 13.



Scheme 14.



Scheme 15.

was an azo dye, prontosil, that led to the discovery of the sulfonamides as the first antibacterials to be used to treat systemic infections.[22] While prontosil itself was inactive in vitro, it was active in vivo, where it was converted by azo reductase enzymes in the gut to sulfanilamide, the active species (Scheme 15).

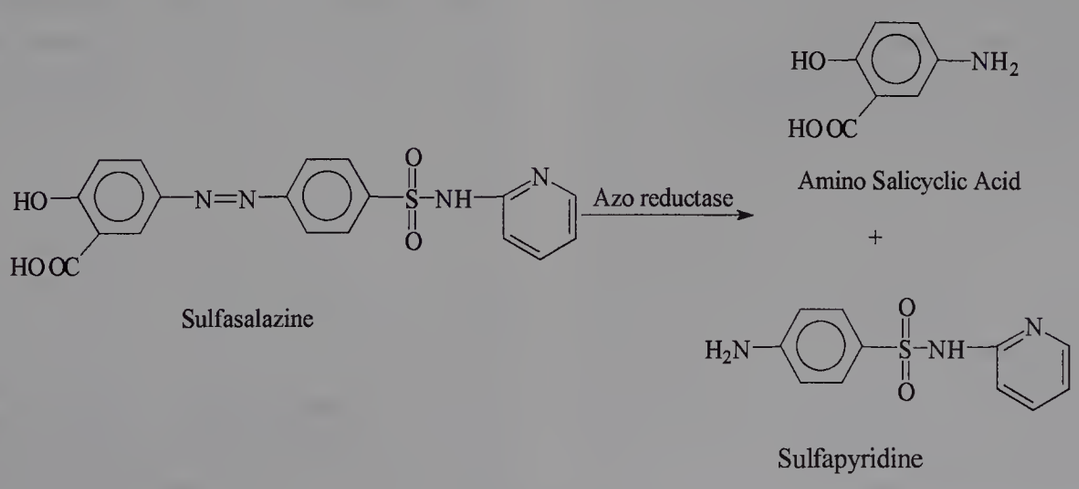
Although prontosil is no longer used as an antibacterial, this type of linkage appears in sulfasalazine, which is used in the treatment of ulcerative colitis. The azo linkage is broken in the gut by the action of azo reductases produced by microflora. This releases the active agent, amino salicylic acid, which has an anti-inflammatory effect on the colon, and sulfapyridine (Scheme 16). The advantage of this prodrug approach is that cleavage of the azo linkage and generation of amino salicylic acid prior to absorption prevents the systemic absorption of the agent and helps concentrate the active agent at the site of action.

CARBONYL COMPOUNDS

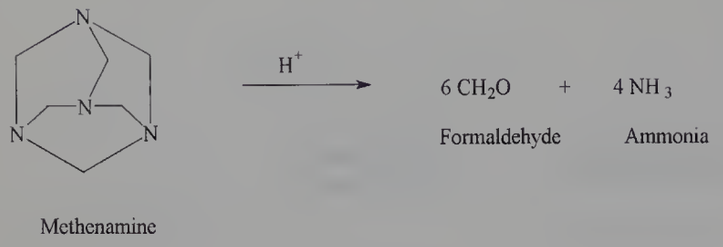
A number of different functionalities have been evaluated as prodrug derivatives of carbonyls, e.g., aldehydes and ketones, although this approach has not found wide clinical utility. These have generally involved derivatives in which the sp^2 hybridized carbonyl carbon is converted to an sp^3 hybridized carbon attached to two heteroatoms such as oxygen, nitrogen, or sulfur. Under hydrolysis conditions, these functionalities are reconverted to the carbonyl compounds. An example of this approach is methenamine, which is shown below (Scheme 17).[23]

Methenamine releases formaldehyde in the urine, which acts as an antibacterial agent by reacting with nucleophiles present in bacteria. The agent is administered in enteric-coated capsules to protect the agent from premature hydrolysis in the acidic environment of the stomach. After dissolution of the enteric-coated capsules occurs in the intestines, the agent is absorbed and moves into the bloodstream, eventually ending up in the urine, where the acidic pH catalyzes the chemical hydrolysis to give formaldehyde. By utilizing a prodrug approach, the systemic release of formaldehyde is prevented and toxicity is reduced.

Other prodrug approaches have involved the use of oximes, imines, and enol esters, although these types of compounds have not been used clinically. There are a number of agents that contain imine and oxime linkages such as many of the third-generation cephalosporins, e.g., cefotaxime and ceftizoxime, but these are not prodrugs.



Scheme 16.



Scheme 17.

BIOPRECURSOR PRODRUGS

As indicated previously, bioprecursor prodrugs do not contain a carrier or promoiety but rather contain latent functionality, which is metabolically or chemically transformed to the active drug molecule. The types of activation often involve oxidative activation, reductive activation, or phosphorylation. Of these, oxidation is commonly seen, because there are a number of endogenous enzymes that can carry out these transformations. Phosphorylation has been widely exploited in the development of antiviral agents, and many of the currently available agents depend upon this type of activation.

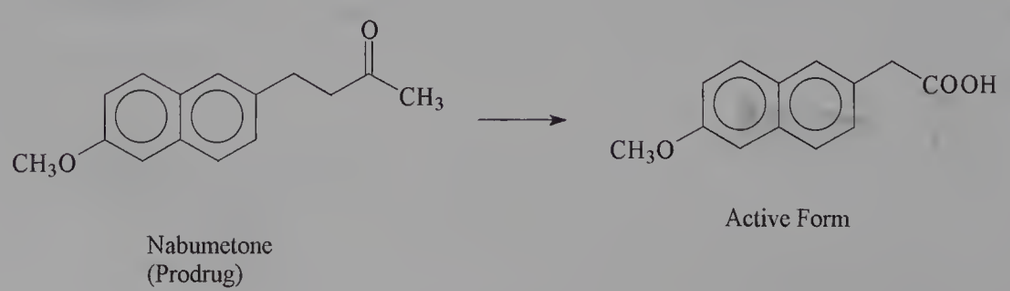
The abundance of oxidizing enzymes in the body has made this type of bioactivation a popular route. Isozymes of cytochrome P-450 are capable of oxidizing a wide variety of functionalities, generally to produce more polar compounds, which can be excreted directly or undergo Phase 2 conjugation reactions and subsequently undergo elimination. This occurs in a fairly predictable manner and therefore has been successfully exploited in prodrug approaches.

A good example of a prodrug that requires oxidative activation is the nonsteroidal anti-inflammatory drug (NSAID) nabumetone (Relafen) (Scheme 18).[24] The use of NSAIDs produces irritation of the stomach, which in patients with preexisting conditions or in patients taking large amounts of NSAIDs for extended periods of time may be severe. This irritation is associated in part with the presence of an acidic functionality in these agents. The carboxylic acid functionality commonly found in these agents is un-ionized in the highly acidic environment of the stomach. As a result, these agents are more lipophilic in nature and may pass into the cells of the stomach's mucosa. The intracellular pH of these cells is more basic than that of the stomach lumen, and the NSAID becomes ionized. This results in the back flow of H^+ from the lumen into these cells, with concomitant cellular damage. This type of damage could be prevented if the carboxylic acid function could be eliminated from these agents; however, this functional group is required for activity.

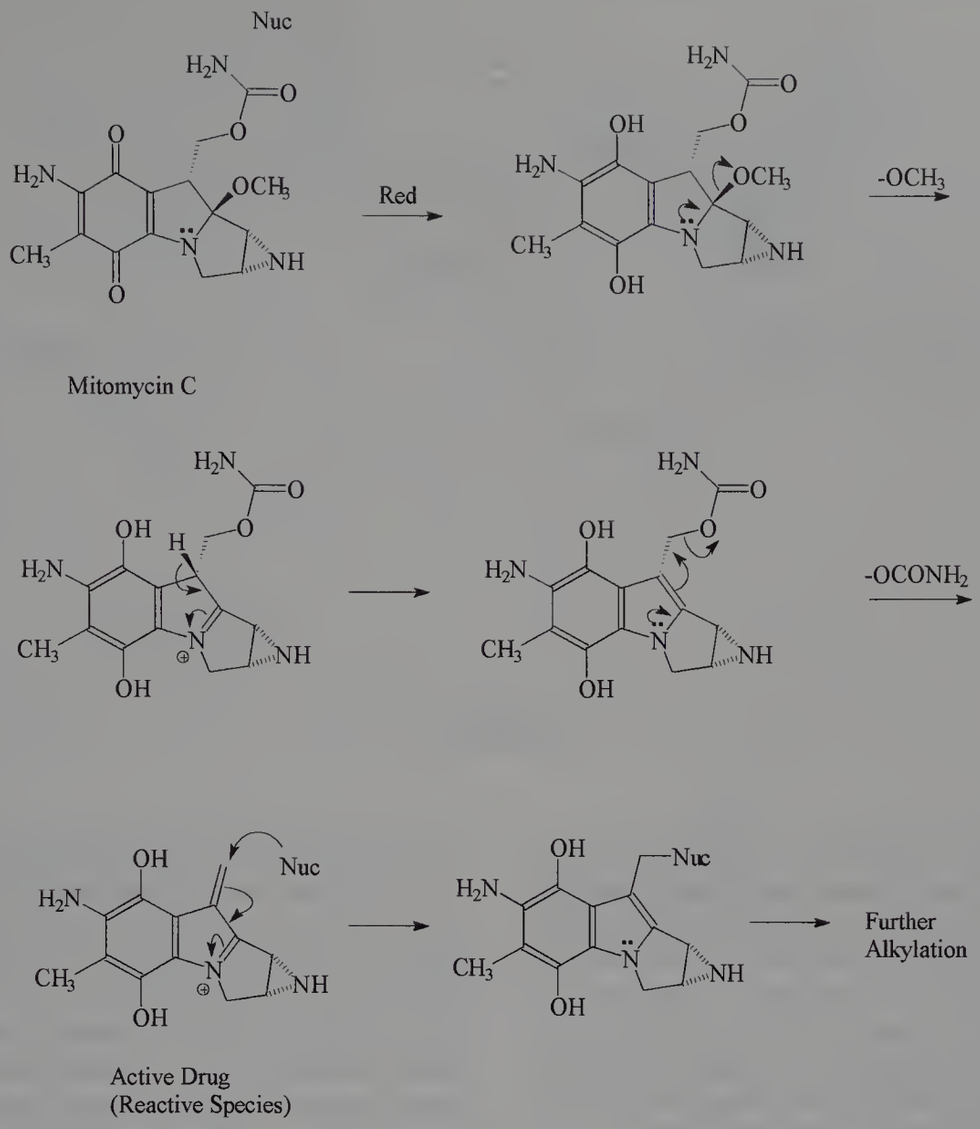
Nabumetone contains no acidic functionality and passes through the stomach without producing the irritation normally associated with this class of agents. Subsequent absorption occurs in the intestines, and metabolism in the liver produces the active compound as shown in Scheme 18. This approach, however, did not completely eliminate the gastric irritation associated with this agent since this is due only in part to a direct effect on the stomach. Inhibition of the target enzyme, cyclooxygenase, while having an anti-inflammatory effect, also results in the increased release of gastric acid, which irritates the stomach. So, while nabumetone exhibits reduced gastric irritation compared with other NSAIDs, this undesirable effect was not completely eliminated by a prodrug approach. Such an effect was also seen previously with the NSAID sulindac (Scheme 3), which exhibited reduced gastrointestinal irritation, but this side effect was not completely eliminated.

Reductive activation is occasionally seen as a method of prodrug activation but generally is less common than oxidative activation due to a lower number of reducing enzymes. One of the best-known examples of reductive activation is for the antineoplastic agent mitomycin C, which is used in the treatment of bladder and lung cancer (Scheme 19).[25–28] Mitomycin C contains a quinone functionality that undergoes reduction to give a hydroquinone. This is important due to the differential effect of the quinone and hydroquinone on the electron pair of the nitrogen. While the quinone had an electron-withdrawing effect on this electron pair, the hydroquinone has an electron-releasing effect, which allows these electrons to participate in the expulsion of methoxide and, subsequently, the loss of the carbamate to generate a reactive species, which may alkylate DNA.

The cascade of events that leads to an alkylating active drug species is initiated by the reduction of the quinone functionality in mitomycin C. The ease with which this occurs is determined by the reduction potential of the quinone, which can be influenced by the substituents attached to the ring. In an effort to modify the reduction potential of mitomycin C, various analogs have been prepared and tested



Scheme 18.



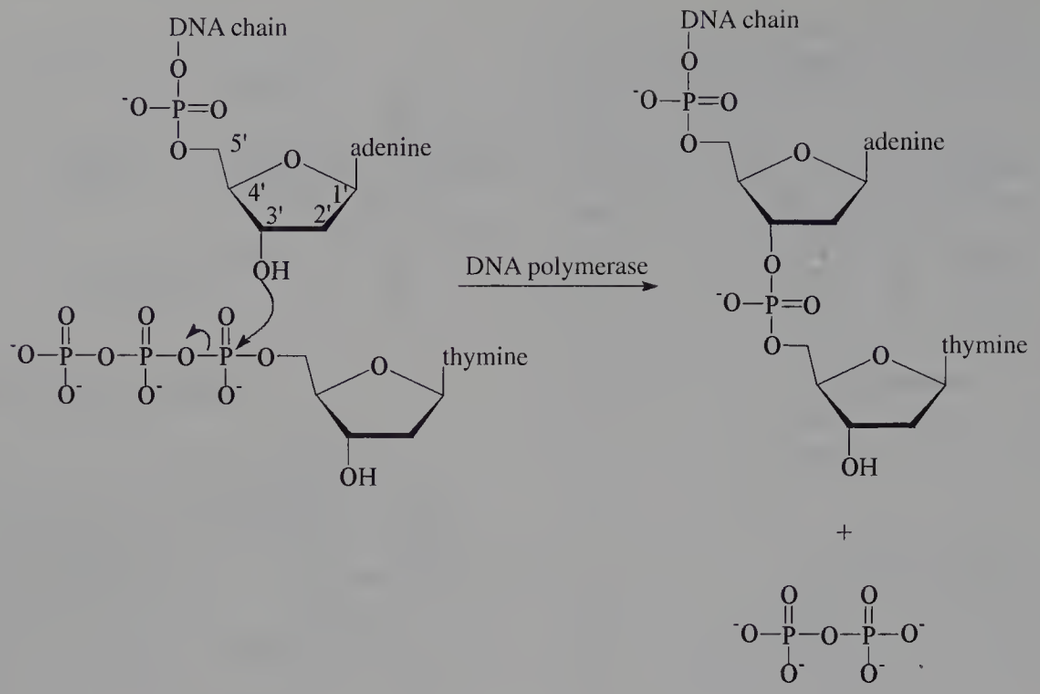
Scheme 19.

for antineoplastic activity. It was hoped that the reduction potential could be altered so that the analogs would only be activated in hypoxic conditions, such as those found in slow-growing solid tumors that are poorly vascularized. In these tissues with a low oxygen content, it was thought that reductive metabolism might be more prevalent than in normal tissues, and the agents would be selectively activated and therefore selectively toxic.

Phosphorylation is a common metabolic function of the body, which is used as a means of producing high-energy phosphodiester bonds such as those present in ATP and GTP. The body then typically uses these molecules to phosphorylate other molecules and, in the process of doing so, activates these molecules. The type of activation achieved is dependent upon the molecule phosphorylated, but, in many cases, phosphorylation introduces a leaving group, which can be displaced by an incoming nucleophile. This is seen for example in the synthesis of DNA and RNA, in which nucleotides are added to the 3′ end of a growing chain of DNA or RNA (Scheme 20).

Phosphorylation is commonly required for the bioactivation of antiviral agents. These agents are commonly nucleosides, which must be converted to the nucleotides to have activity. Most often, antiviral agents disrupt the synthesis or function of DNA or RNA, and this is generally accomplished by conversion to the triphosphate. Since normal cells are also involved in the synthesis of DNA and RNA, compounds have been sought that would be converted to the triphosphates, the active form, in greater amounts in the infected cells than in normal cells. Therefore, nucleosides that have higher affinity for the viral kinase enzymes than the mammalian kinase enzymes are desirable and have a greater selective toxicity.

This can be seen in the prodrug iodoxuridine, which was the first agent to show clinical effectiveness against viruses (Scheme 21).[29] The nucleoside enters the cell, where it is phosphorylated. In virally infected cells, this phosphorylation is accomplished preferentially by viral thymidine kinase, the iodoxuridine being a better substrate for the viral enzyme than for the corresponding mammalian enzyme.



Scheme 20.

Therefore, the drug is activated to a greater extent in the virally infected cells and achieves some level of selective toxicity, although this selectivity is rather low and significant toxicities to normal cells also occur. Once the drug has been phosphorylated to the triphosphate stage, it can inhibit DNA synthesis in a number of ways, including inhibition of viral DNA polymerase and incorporation into DNA, resulting in incorrect base pairing, which disrupts the ability of DNA to function as a template for DNA and RNA synthesis.

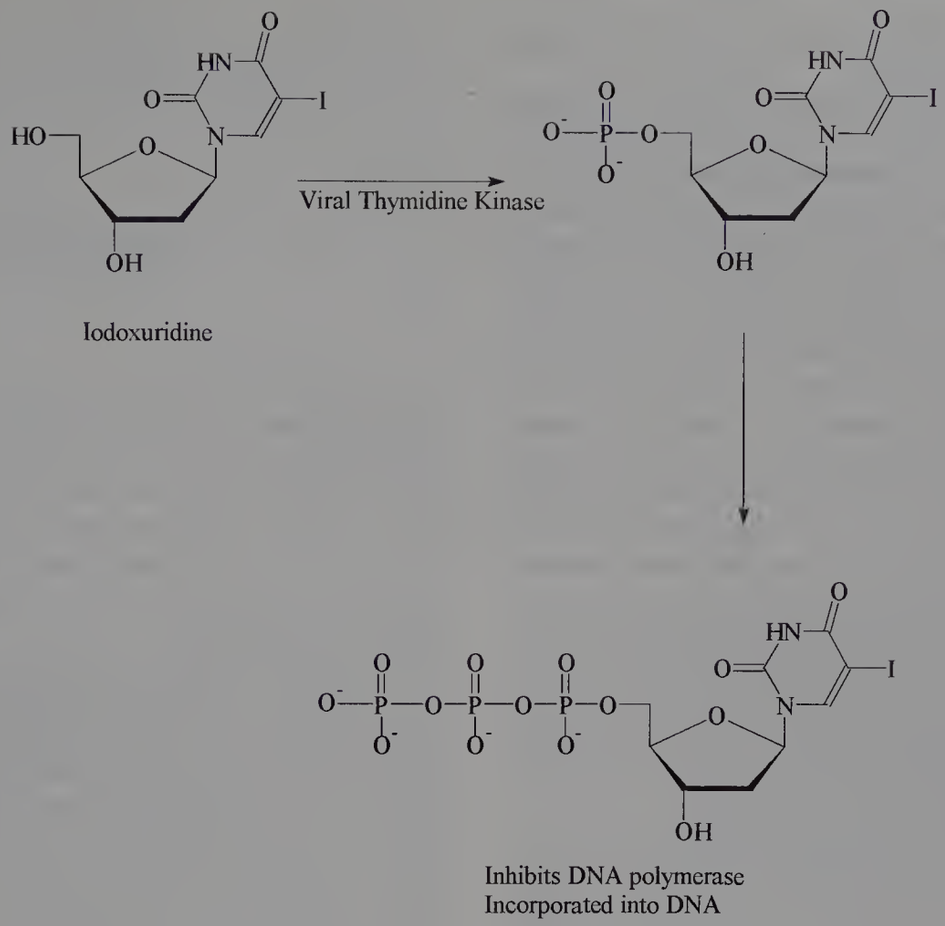
In addition to the selective toxicity mentioned, the prodrug approach offers the additional advantage of increased cell penetration. The prodrug can easily enter the cell via active transport mechanisms, whereas the active nucleotides are unable to utilize this process and are too polar to cross the membrane via passive diffusion.

CHEMICAL DELIVERY SYSTEMS

The knowledge gained from drug metabolism and prodrug studies may be utilized to target a drug to its site of action. Site-specific chemical delivery systems take advantage of higher levels of activity in a metabolic or chemical pathway at the target site. A prodrug form of the active drug is designed to serve as a substrate in that specific pathway, thus yielding a high concentration of active drug at the target site. Site-specific chemical delivery requires that the prodrug reach the target site and that the enzymatic or chemical process exists at the target site for conversion of the prodrug to the active drug. Many factors are involved in the relative success of site-specific drug delivery, including extent of target organ perfusion, rate of conversion of prodrug to active drug in both target and nontarget sites, and input/output rates of prodrug and drug from the target sites.

Site-specific chemical delivery systems represent but one approach to the selective delivery of drug molecules to their site of action for increased therapeutic effectiveness and limited side effects. Other than chemical drug delivery, many carrier systems have been evaluated for drug delivery, including proteins, polysaccharides, liposomes, emulsions, cellular carriers (erythrocytes and leukocytes), magnetic control targeting, and implanted mechanical pumps.[30] As the fate of drugs in the human body has become more clearly understood, research activity to improve the delivery of active drug to the target site has increased. The basic goal of these efforts is to protect the drug from the nonspecific biological environment and to protect the nonspecific biological environment from the drug in order to achieve some degree of site-specific drug delivery. Site-specific drug delivery has been extensively evaluated for the delivery of drugs with narrow therapeutic windows such as many of the anticancer drugs.

The site-specific delivery of the active drug via its prodrug counterpart requires that the prodrug be readily transported to the site of action and rapidly absorbed at the site. Upon arrival at the target site, the prodrug should be selectively converted to drug relative to its rate of conversion at nontarget sites. Since high metabolic activity occurs in highly perfused tissues such as liver and kidney, delivery to these organs has a natural advantage. Unfortunately, prodrug delivery of active drug to other organs or tissues is disadvantaged for the same reasons. Furthermore, it is highly desirable that the active drug, once formed, migrate from the target site at a slow rate. Based upon all these requirements,

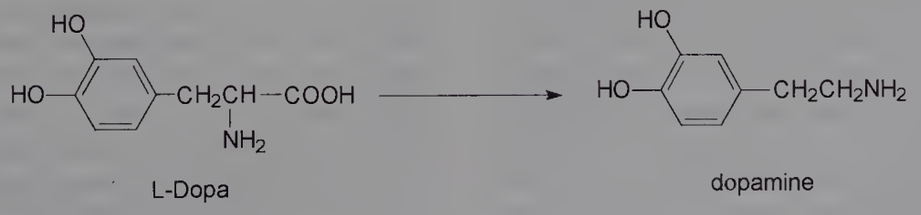


Scheme 21.

it becomes quite clear that the site-specific delivery of a drug to the target by a prodrug chemical delivery system represents a far more complex undertaking than just the design of a prodrug to improve one aspect of its overall properties. Yet there are several excellent examples of site-specific chemical delivery systems in use in modern drug therapy. The target sites include cancer cells, the gastrointestinal tract, the kidney and urinary tract, bacterial cells, viral material, ocular tissue, and the blood-brain barrier.

The prodrug methenamine, already described in this chapter (Scheme 17), can be considered a site-specific chemical delivery system for the urinary tract antiseptic agent formaldehyde.[30] The low pH of the urine promotes the hydrolysis of methenamine to formaldehyde, the active antibacterial agent. The rate of hydrolysis increases with an increase in acidity (decreased pH), and this can be promoted by administration of urinary pH-lowering agents or diet. The pH of the plasma is buffered to ~7.4, and the rate of hydrolysis is low, preventing systemic toxicity from formaldehyde. As mentioned previously, this compound is administered in enteric-coated tablets, which prevent dissolution and therefore premature hydrolysis in the highly acidic environment of the stomach.

A number of prodrugs for cancer chemotherapy have been designed to selectively deliver active drug to the tumor tissue based upon higher levels of activating enzyme in the tumor cell relative to normal tissue.[31] Many enzymatic systems show higher levels of activity in tumor cells than in normal tissue; these enzyme systems are more active because of the higher growth rates associated with the tumor tissue. Peptidases and proteolytic enzymes are among those systems showing higher activity in and near tumor cells. Derivatization of a drug molecule with an amino acid or peptide fragment has been used as a means of attempting to produce



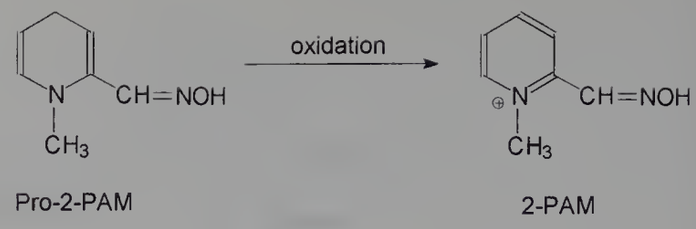
Scheme 22.

higher rates of drug incorporation into tumors as compared to surrounding normal tissue.

An interesting example of site-specific chemical delivery is an antiviral drug such as iodoxuridine (Scheme 21).[32] Antiviral drugs serve as a substrate for phosphorylating enzymes found in viruses, and the phosphorylated species is the active antiviral agent. The active phosphorylated species is incorporated into viral DNA, disrupting viral replication and thus producing the antiviral effect. These drugs do not undergo phosphorylation by mammalian cells, and thus the prodrug is specific for those sites at which it serves as a substrate for phosphorylation enzymes. One of the requirements for site-specific chemical delivery discussed earlier was the proper input/output ratios for prodrug and active drug species at the target. The relative physicochemical properties of prodrug and its phosphorylated derivative would suggest an appropriate input/output ratio for site specificity. The prodrug is readily able to penetrate into the virus, and the increased polarity of the phosphorylated derivative would serve to retain that active species inside the virus. The increased polarity and viral retention of the active phosphorylated species likely reduce any human toxicity that might be associated with this active species.

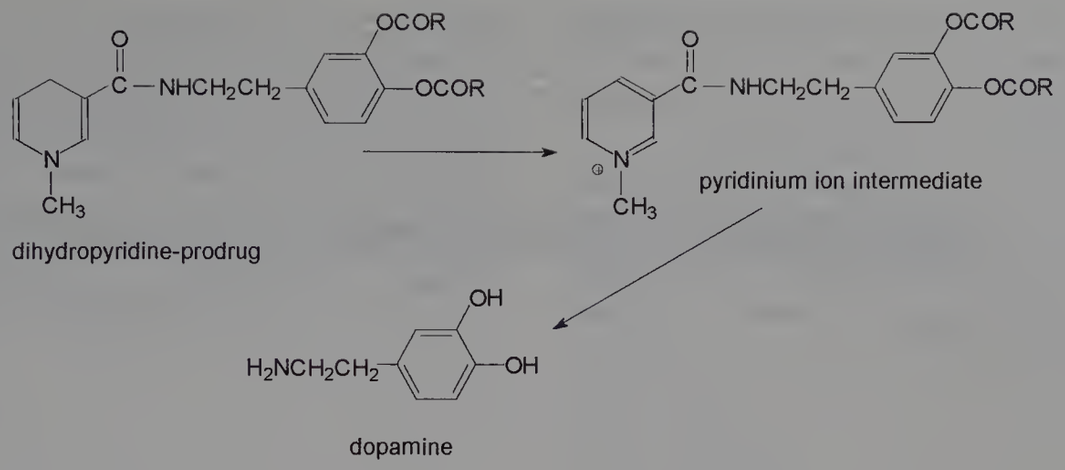
The amino acid drug L-Dopa can be considered a site-specific chemical delivery system delivering the drug dopamine to the brain. The brain has an active transport system that operates to incorporate L–amino acids into the central nervous system, and L-Dopa is transported into the brain in this manner. Once across the blood-brain barrier, the L-Dopa undergoes decarboxylation as shown in Scheme 22 to yield the active metabolite, dopamine. Direct systemic administration of dopamine does not produce significant brain levels of the drug due to its high polarity and poor membrane permeability as well as its facile metabolic degradation by oxidative deamination. However, dopamine formed on the inside of the blood-brain barrier is held there due to the poor membrane permeability of this drug. While some specificity for brain tissue is achieved by this delivery method, peripheral side effects of L-Dopa are the direct result of decarboxylation to dopamine in other organ systems. In this case, the enzyme-activating system is not localized at the target site, and its presence in other tissues and organs leads to the undesirable side effects.

Another example of the chemical delivery of a drug to the brain and central nervous system is the prodrug form of 2-PAM (Pro-2-PAM), an important antidote for the phosphate and carbamate acetylcholinesterase inhibitors used in insecticides and nerve gases.[32] The polar properties of 2-PAM, a permanent cationic species, prevent this drug from being absorbed following oral administration and restrict the drug from access to the brain even after intravenous administration. Pro-2-PAM is a dihydropyridine derivative that undergoes metabolic and chemical oxidation to yield the active drug 2-PAM (Scheme 23). The nonionic Pro-2-PAM can easily cross the blood-brain barrier, and the oxidation to 2-PAM within the brain essentially traps the active cationic drug species inside the brain. The oxidation of the dihydropyridine ring of Pro-2-PAM occurs throughout the mammalian system and not just in the brain, and the levels of the resulting 2-PAM are approximately the same in peripheral tissue as in the brain. However, intravenous administration of Pro-2-PAM yields brain levels of 2-PAM ~10 times higher than those achieved by intravenous administration of the parent drug.



Scheme 23.

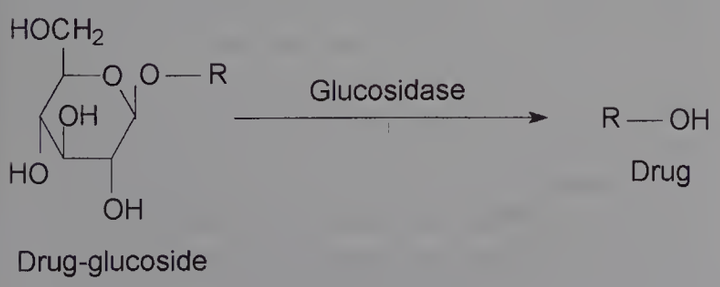
The delivery of drugs across the blood-brain barrier has been a significant issue in the design of many therapeutic compounds. Only very lipophilic drugs are capable of crossing into the brain without the aid of some active uptake process such as that which operates to incorporate essential amino acids into the central nervous system. The facile oxidation of the dihydropyridine ring system has been extensively investigated as a general process for the chemical delivery of a number of drugs to the central nervous system. The approach has been described as a chemical delivery system and not just a prodrug designed to be able to penetrate the blood-brain barrier.[33] This process is a multistep procedure involving delivery of the drug dihydropyridine derivative to the brain via facile diffusion across the blood-brain barrier followed by oxidation to the quaternary pyridine cation, which is trapped in the brain. The drug is then released from the pyridine cation by a second metabolic/chemical event. A number of functional groups can be added to the dihydropyridine to facilitate the derivatization of various functional groups found in central nervous system drugs. Since many central nervous system drugs are amines, amides of dihydropyridine carboxylic acids are often prepared, and these amides are used to deliver the drugs across the blood-brain barrier into the brain. Additionally, these amide derivatives often serve to protect the amines from metabolic degradation before they reach the target site. Primary amines such as dopamine and norepinephrine are readily metabolized and degraded by oxidative deamination before reaching the central nervous system. The dihydropyridine derivative of a dopamine ester shown in Scheme 24 has access to the central nervous system via passive absorption of the tertiary amine, which upon oxidation restricts the resulting pyridinium amide to the brain. Amide hydrolysis then delivers the active form of the drug to or near its site of action. The amide hydrolysis step may be slower than the dihydropyridine oxidation step, and thus a reservoir of pyridinium amide precursor may be available for conversion to the active drug species.



Scheme 24.

The use of prodrug concepts has been very successful in the delivery of active drug species to the human eye following local application. Lipophilic esters of epinephrine such as the dipivaloyl ester previously described (Scheme 6) show improved cornea penetration following direct application to the eye when compared to application of the more polar parent drug epinephrine.[32] The esterases necessary for the hydrolysis of the prodrug are readily available in the eye and skin. The more polar drug species, epinephrine, is then localized within the lipophilic membrane barriers of the eye, and the drug remains available at the target site to produce its antiglaucoma effects. The local application of the prodrug species to the skin or eye allows metabolic processes to activate the drug without concern for competitive reactions at other tissues or sites of loss.

The delivery of drugs to the colon and lower gastrointestinal tract has been accomplished by taking advantage of the unique enzymatic processes found in colon bacteria. The glucosidase activity of these bacteria allows for the hydrolysis of glucoside derivatives of drugs in the colon and provides higher concentrations of active drug.[31] A number of steroid drugs (Scheme 25) have shown increased effectiveness in the lower gastrointestinal tract following administration as their glucoside derivatives. The polar glucoside derivatives of the steroids are not well absorbed into the bloodstream from the gastrointestinal tract and remain available to serve as substrates for the bacteria found primarily in the human colon.



Scheme 25.

The prodrug approach for the delivery of anticancer drugs to the site of action has been used in a number of cases in an effort to increase effectiveness and lower side effects. Several enzyme systems that show higher activity in and near cancer cells have been evaluated for their ability to activate the prodrug species. In most cases, the enzyme activity level is simply higher near the faster-growing cancer cells, but the presence of the enzymes in normal tissue prevents the possibility of complete site specificity in these agents.

It is clear from this brief discussion of site-specific drug delivery that in some cases the prodrug was in use before its mechanism of delivery and specificity were discovered. Thus, some compounds were discovered to represent site-specific drug delivery well after they were placed into therapeutic use. An evaluation of the properties of these agents has produced the framework for the design of other prodrugs having target sites in specific tissues. This process is really no different from the general drug discovery process, in which a unique substance is observed to have desirable pharmacological effects and studies of its properties lead to the design of better drugs.

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CHAPTER 5

Biotechnology and Drug Discovery

John W. Regan

In February 1975, a remarkable meeting took place at the Asilomar Conference Center near Monterey, California, in which scientists came up with recommendations for experiments involving the use of recombinant DNA technology.[1] What was remarkable about this meeting is that it was held to discuss the potential hazards of such research essentially before the actual tools of recombinant DNA technology were in place. In place or not, however, it was clear to these men and women that we were about to enter a new era of biotechnology that would forever affect the world in which we live. In the 20-plus years since this historic meeting, we came to speak of cloned human genes, transgenic animals, and gene therapy not as abstractions, but as patentable products and opportunities for investment.

A reflection of the impact of biotechnology is the growth of a data base known as GenBank. GenBank is an electronic repository of sequence information, specifically the nucleotide sequence of cDNA and genomic clones that have been isolated and sequenced by scientists around the globe.[2] As can be appreciated in Fig. 5-1, the growth of this data base has been exponential since it was founded in 1982, and at its current rate it is doubling every 2 years. Another indication of the impact of biotechnology is the Human Genome Project, which is an international effort whose goal it is to obtain complete genetic and physical maps, including nucleotide sequence, of each of the 24 chromosomes that make up the human genome. These 24 chromosomes, which consist of roughly 3 billion (10^9) nucleotides, essentially define our species. It is estimated that the project will take 15 years at a cost of 3 billion and will provide a basis for a better understanding of human biology and medicine.[3]

As it concerns medicine, biotechnology is having a significant impact in terms of the process by which drugs are discovered, and there are several ways this impact is being felt. The first is that the cloning of genes and their expression can provide a new way of preparing essentially old drugs. For example, instead of purifying a protein from a biological source, it is often possible to prepare the protein using high-yield cell culture systems. This can provide significant advantages in terms of both cost and the quality of the product. A second manner in which biotechnology is being broadly applied is in the discovery of new biological targets for traditional drug development and in the discovery of biological substances with therapeutic activity. Thus, the molecular cloning of a previously unknown receptor can provide a new target for drug development, while the cloning of a novel growth factor can provide a potentially new therapeutic agent. Biotechnology is also being used in the screening of compounds for biological activity. With cloned and expressed genes, it is frequently possible to screen drugs with a cell culture system instead of using whole animals or preparations derived thereof. Again, there are several potential advantages of using biotechnology in this context, including lower cost, higher throughput, and the fact that the information obtained is from cloned human genes instead of animal models. Finally, biotechnology is being applied in totally new approaches to the treatment of human disease, including the use of antisense oligonucleotide and gene replacement therapies.

This chapter is devoted to expanding on the different areas in which biotechnology is being applied to the drug discovery process. It is assumed that the reader has had undergraduate courses in biology and biochemistry and is familiar with some of the basic terms used in molecular biology. For the reader who may be having difficulty recalling some of these terms, or who would simply like a more thorough treatment of the technology itself, *Recombinant DNA*, by Watson et al., is highly recommended.[4]

CLONING DNA

At the core of biotechnology is the ability to clone DNA and to manipulate it using recombinant DNA techniques.

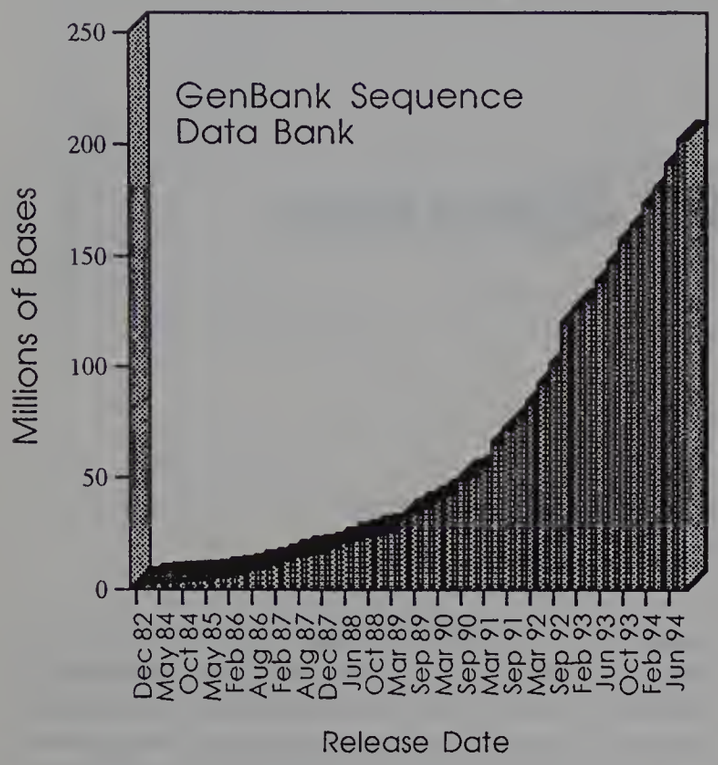

FIG. 5-1. *Growth of the GenBank Sequence Data Base.*

Cloning DNA involves the isolation of fragments of DNA using specialized vectors that can be used to amplify and propagate the cloned DNA in cells. The cloned DNA can also be removed or altered in these vectors using appropriate restriction enzymes. Restriction enzymes comprise one of the most important tools of biotechnology and are enzymes that recognize and cleave specific combinations of nucleotides that are present in DNA. In combination with DNA ligase, an enzyme that can join fragments of DNA, it is possible to selectively rearrange, or bioengineer, genetic information in a manner that effectively bypasses natural selection.

Genomic and cDNA libraries serve as the main sources of DNA fragments and, respectively, represent either the chromosomal DNA of a particular organism or the cDNA prepared from the messenger RNA (mRNA) present in a given cell, tissue, organ, etc. cDNA is generated using an enzyme called "reverse transcriptase," and it is important to realize that, because it is prepared from mRNA, it only represents the genes actually being expressed in a given cell or tissue at a given time. In contrast, genomic DNA represents all the genes present, expressed or not, which are present in the chromosomal DNA of a given animal species. In principle, for the preparation of a genomic library the cellular origin of the DNA is not an issue, whereas the cellular origin of mRNA is central to the preparation of a cDNA library. Thus, genomic libraries will vary from species to species but not from tissue to tissue within a species. cDNA libraries, on the other hand, will vary both from tissue to tissue and from species to species and on the stage of development of the cell, tissue, or species. Another important distinction between genomic and cDNA libraries is the fact that fragments of genomic DNA from eukaryotic organisms (e.g., mammals) will contain both the exons (the protein-coding sequence) and the introns (the noncoding chromosomal sequence between exons), whereas cDNA fragments will have the introns spliced out. Thus, genomic DNA fragments represent the actual genes themselves, whereas cDNA fragments represent the genes after they have been transcribed into mRNA and are ready for translation into protein. These and other characteristics of genomic and cDNA libraries are summarized in Table 5-1.

Fragments of DNA are cloned with the help of so-called vectors, which are the vehicles that give recombinant DNA mobility. Vectors are genetic elements such as plasmids or viruses that are capable of being propagated and that have been engineered so that they can accept fragments of foreign DNA known as inserts. Depending on the vector, they may have many other features as well, including the presence of a multiple cloning site, which is a region containing multiple restriction enzyme sites into which an insert can be put in or taken out; selection markers, which can be used to identify cells containing the vector (e.g., antibiotic resistance); and transcriptional promoters for the expression of the insert. As illustrated in Fig. 5-2, there are two main classes of vectors that have been used for the purpose of cloning fragments of DNA: those that have been derived from the bacterial plasmid pBR322 and those that have been derived from the bacteriophage lambda (λ). In addition, there are hybrid vectors known as phagemids and various specialized vectors such as baculovirus (for high-yield expression in insect cells); vaccinia and adenovirus (for expression in mammalian cells); and yeast artificial chromosomes or YACs (for cloning and selection in yeast). Some of the main differences between these vectors concern the size of the insert they will accept, the methods used for their selection, and how they are propagated.

The methods of isolating or cloning a fragment of DNA comprise perhaps one of the most creative aspects of biotechnology, and the approaches are as varied and imaginative as

TABLE 5-1

CHARACTERISTICS OF GENOMIC VS. cDNA LIBRARIES

Characteristic	*Genomic*	*cDNA*
Source of genetic material	Genomic DNA	Cell or tissue mRNA
Complexity (independent recombinants)	>100,000	5,000–20,000
Size range of recombinants	1,000–50,000 bp[a]	30–10,000 bp[a]
Presence of introns	Yes	No
Presence of regulatory elements (promoters, enhancers, etc.)	Yes	Maybe
Suitable for heterologous expression	Maybe	Yes

[a] bp, basepairs.

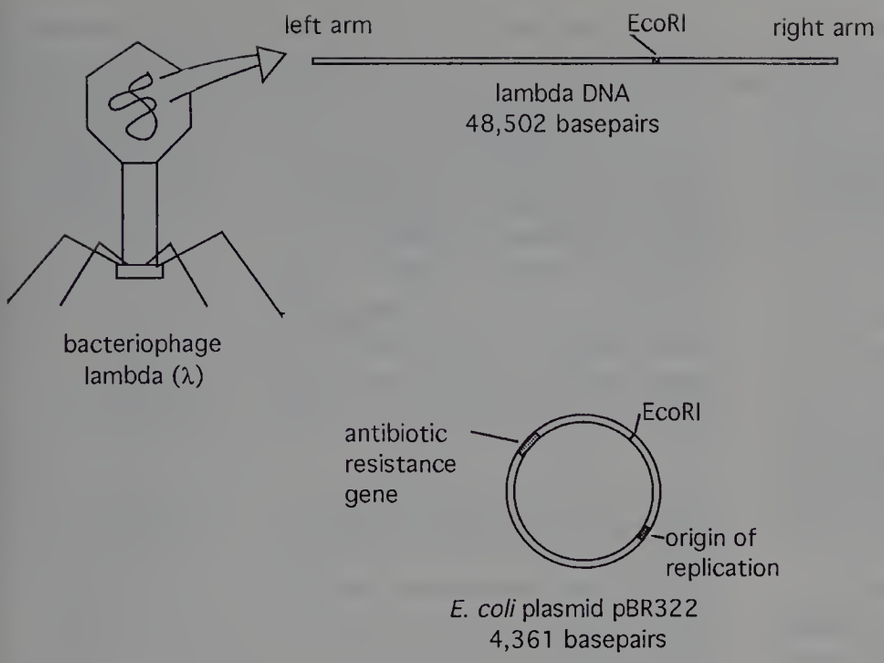

FIG. 5-2. *Features of plasmid and phage vectors.*

the practitioners of the art. Thus, it can be as straightforward as expressed sequence tagging or as roundabout as functional expression cloning. In the former, clones from a cDNA library are simply isolated and sequenced with the goal of obtaining at least partial identifying sequences (i.e., tags) from as many different cDNAs as possible. It relies on very high throughput, computational technology, and the hope that someday someone will be able to figure out what the cDNAs represent. Although the latter issue can be problematic, expressed sequence tagging has the advantage of identifying novel biological targets for drug discovery and for identifying patterns of gene expression.

Functional expression cloning is an approach that is directed toward getting a specific cDNA of known function. There are many variations of this approach, but they all rely on the ability to search for and isolate cDNAs based on a functional activity that can be measured, for example, the electrophysiological measurement of ion conductances following expression of cDNAs in frog oocytes. Thus, a cDNA library, containing perhaps 10,000 different cDNA clones, can be subdivided into 20 different pools, each representing ~500 different cDNAs. If the functional activity can be identified in one of these pools, then that pool can be further subdivided into 20 pools, each representing ~25 different cDNAs. By repeating this process as needed, it is possible to obtain a single cDNA clone encoding the protein that is responsible for the functional activity. This approach has the powerful advantage of yielding a functional clone, i.e., one that is likely to have the complete sequence encoding the protein. It also does not rely on knowledge of the primary amino acid sequence of the protein, a clear advantage for the cloning of low abundance proteins.

As listed in Table 5-2, other strategies have been used to clone fragments of DNA. These include positional cloning, homology-based cloning, cloning on the basis of known protein sequence, and cloning by antibody recognition. The two latter methods both require some degree of purification of the protein of interest. Thus, if it is possible to obtain an antibody that specifically recognizes a given protein, it is possible to use the antibody as a probe to identify and isolate clones that encode the amino acid sequences (epitopes) that are recognized by the antibody. To use such a strategy, it is necessary to prepare the cDNA or genomic library in a vector such that it can be expressed as protein.

The cloning of genes following the purification of proteins and the determination of their amino acid sequence is made possible by knowledge of the genetic code. Thus, if the primary amino acid sequence of a protein is known, then the codons that potentially encode this sequence are also known. It is possible, therefore, to synthesize single-stranded oligonucleotides that can be used as probes that will hybridize to complementary sequences that may be present in a genomic or cDNA library.

Positional cloning is a genetic approach that can be used to clone fragments of DNA encoding genes. A dramatic example of this approach was demonstrated with the cloning of the gene responsible for cystic fibrosis.[5] By studying the pattern of inheritance of this disease and by comparing it with the inheritance of known chromosomal markers (linkage analysis), it was possible, without knowing the function of this gene, to characterize its location (i.e., position) on human chromosome 7. Then by using a technique known as gene-walking it was possible to further characterize the localization of this gene to a DNA sequence that encoded a protein now known as the cystic fibrosis transmembrane conductance regulator (CFTR). This protein, previously unknown, was shown to be mutated and defective in patients with cystic fibrosis and could account for many of the symptoms of this disease. Like functional cloning, positional cloning has the advantage that specific knowledge of the protein

TABLE 5-2

CLONING STRATEGIES

Strategy	Advantages	Disadvantages	Example
Positional	Provides information underlying the genetic basis of known diseases	3.3×10^9 bp[a]; difficult to use with diseases caused by multiple interacting alleles	The cystic fibrosis gene product
Protein purification	Yields genetic information encoding proteins of known structure/function	Protein purification, especially low abundance proteins; availability of appropriate libraries; incomplete coding sequences	β_2-adrenergic receptor
Antibody based	Yields genetic information encoding proteins of known structure/function	Involves protein purification; unrecognized cross-reactivity; incomplete coding sequences	Vitamin D receptor
Functional expression	Yields genetic information encoding a functionally active protein; does not require protein purification	Function must be compatible with existing library screening technology	Substance-K receptor
Homology based	Identification of related genes/gene families; relatively easy	Depends on preexisting gene sequence; can yield incomplete genes or genes of unknown function	Muscarinic cholinergic receptor
Expressed sequence tagging	High throughput; identification of novel cDNAs	Incomplete coding sequences or genes of unknown function	
Total genomic sequencing	Knowledge of total genome; identification of all potential gene products	3.3×10^9 bp[a]; labor intensive; genes of unknown function	*H. influenzae*

[a] This is approximate size of the human genome in basepairs (bp).

is not required. It is also directly relevant to understanding human disease, and it can provide important new biological targets for drug development and the treatment of the disease.

A final cloning strategy involves the use of previously cloned genes to identify and clone evolutionary-related genes. This approach, referred to as homology-based cloning, takes advantage of the fact that nucleotide sequences encoding important functional domains of proteins tend to be conserved during the process of evolution. Thus, nucleotide sequences encoding regions involved with ligand binding or enzymatic activity can be used as probes that will hybridize to complementary nucleotide sequences that may be present on other genes that bind similar ligands or have similar enzymatic activity. This approach can be combined with the use of the polymerase chain reaction (PCR), which is a very powerful method for the amplification of DNA sequences. The use of homology-based cloning has the advantages that it can be used to identify families of related genes, does not rely on the purification or functional activity of a given protein, and can provide novel targets for drug discovery. Its utility is offset by the possibility that the isolated fragment may not encode a complete or functional protein or that, in spite of the knowledge of the shared sequence, the actual function of the clone may be difficult to identify.

EXPRESSION OF CLONED DNA

Once cloned there are essentially endless possibilities for the expression and manipulation of DNA sequences. As it concerns the use of cloned genes in the process of drug discovery and development, there are many obvious ways in which the expression of DNA sequences can be applied. One of the most obvious is in the replacement of older technologies that involve the purification of proteins for human use from either animal sources or human by-products, such as blood. A dramatic example of this is factor VIII, a clotting protein used for the treatment of the genetically linked bleeding disorder hemophilia. Until recently, the only source of factor VIII was its purification from human blood, and, tragically, before the acquired immunodeficiency syndrome (AIDS) was fully appreciated, stocks of factor VIII had become contaminated with the human immunodeficiency virus (HIV-1), resulting in the infection of as many as 75% of the patients receiving this product. The gene encoding factor VIII has since been cloned, and recombinant factor VIII is now available as a product purified from cultured mammalian cells. Other recombinant clotting factors, including factors VIIa and IX, are under development and, together with recombinant factor VIII, will eliminate the risk of exposure to human pathogens.

Other examples of the expression of cloned human genes that offer alternatives to previously existing products include human insulin, which is now a viable replacement for purified bovine and porcine insulin for the treatment of diabetes, and human growth hormone, which is used for the treatment of growth hormone deficiency in children (dwarfism). Unlike insulin, growth hormones from other animal species are ineffective in humans; therefore, until human recombinant growth hormone became available, the only source of human growth hormone was from the pituitaries of dead people. This obviously limited the supply of human growth hormone and, like factor VIII, exposed patients to potential contamination by human pathogens. Recombinant human growth

hormone can now be produced by expression in bacterial cells, which has solved these problems but in doing so has created new ethical concerns. These concerns center around the use of growth hormone in situations that do not involve true growth hormone deficiencies. For example, athletes may take recombinant growth hormone with the hope that it will provide a competitive advantage. In addition, parents may administer this product to their children to make them bigger rather than to correct a medical problem.

The expression of cloned genes can also be integrated into rational drug design by providing detailed information about the structure and function of the sites of drug action.[6] Thus, with the cloning of a gene comes knowledge of the primary amino acid sequence of the encoded protein. This can be used to model its secondary structure and as an initial attempt to define the protein's functional domains such as its ligand binding site. Such a model can then serve as a basis for the design of experiments, which can be used to test the model and allow further refinement. Of particular utility are mutagenesis experiments in which recombinant DNA techniques are used to change the primary amino acid sequence so that the consequences can be studied. In addition, expression of the cloned target protein can be used to generate material for various biophysical determinations such as x-ray crystallography. This technique, which can provide detailed information about the three-dimensional molecular structure of a protein, frequently requires large amounts of protein, which in some cases is only feasible with the use of recombinant expression systems.

Like the many strategies used to clone genes, there are many strategies for their expression, most of which involve the use of either bacterial or eukaryotic cells and specialized vectors compatible with expression in the host cells. Since these cells usually do not express the recombinant protein of interest, this methodology is often referred to as heterologous expression. It is also possible to prepare cRNA from recombinant DNA, which can then be used for either in vitro expression or for injection directly into cells. In the former situation, purified ribosomes are used in the test tube for the conversion of cRNA into protein, whereas for the latter situation, the endogenous cellular ribosomes make the protein. A relatively new development for the expression of cloned genes is the use of farm animals in which the cloned gene has been stably integrated into the genome of the animal. Such transgenic animals have the potential to make very large amounts of recombinant protein, which can be harvested from the milk, blood, ascites fluid, etc.

The choice of a particular expression system depends on many factors and is dictated by such things as yield, requirements for biological activity, compatibility of the expressed protein with the host organism, etc. For example, bacteria do not process proteins in exactly the same ways as do mammalian cells so that the expression of human proteins in bacteria will not always yield an active product or any product at all. The choice of an expression system also reflects the available vectors and corresponding host organisms. The basic requirements for the heterologous expression of a cloned gene is the presence of a promoter that can function in the host organism and a mechanism for getting the cloned gene into the organism. As in gene cloning, the vectors are generally either plasmids or viruses that have been engineered to accept recombinant DNA and that contain promoters that direct the expression of the recombinant DNA. The promoter is the specific site at which RNA polymerase binds to initiate transcription and is usually specific for the host organism. The techniques for getting the vector into the organism vary widely and depend upon whether one is interested in transient expression of the cloned gene or stable expression. In the latter case, integration into the host genome is usually required, whereas for transient expression simply getting the vector into the host cell is all that is needed.

MANIPULATION OF DNA SEQUENCE INFORMATION

Perhaps the greatest impact of recombinant DNA technology is the ability to alter DNA sequence and create entirely new molecules, which, if placed back into the genome, can be inherited and propagated in perpetuity. The ability to alter DNA sequence, literally in a test tube, at the discretion of an individual, corporation, or nation, brings with it important questions about ownership, ethics, and social responsibility. There is no question, however, that potential benefits to the treatment of human disease are great.

There are three principal reasons for using recombinant DNA technology to alter DNA sequence. The first is simply to allow it to be cloned and to facilitate subsequent manipulation. The second is to intentionally introduce mutations so that the effect on protein structure and function can be understood. The third reason is to add or remove sequences to obtain some desired attribute in the recombinant protein. For example, recent studies with factor VIII show that it contains a small region of amino acids that are a major determinant for the generation of anti-factor VIII antibodies. This autoimmune response inhibits the activity of factor VIII, which can be a serious complication for patients who are using factor VIII for the treatment of hemophilia. By altering the DNA sequence encoding this determinant, however, the amino acid sequence can be changed to both reduce the antigenicity of this new factor VIII molecule and to make it transparent to any existing anti-factor VIII antibodies (i.e., changing the epitope eliminates the existing antibody recognition sites).

Many other changes are also possible such as combining elements of two proteins into one new recombinant protein. The resulting protein, which is sometimes referred to as a chimeric or fusion protein, may then have some of the functional properties of both original proteins. This is illustrated in Fig. 5-3 for two receptors labeled A and B. For each receptor, there are functional domains that are responsible for ligand binding, integration into the plasma membrane, and activation of intracellular signalling pathways. Using

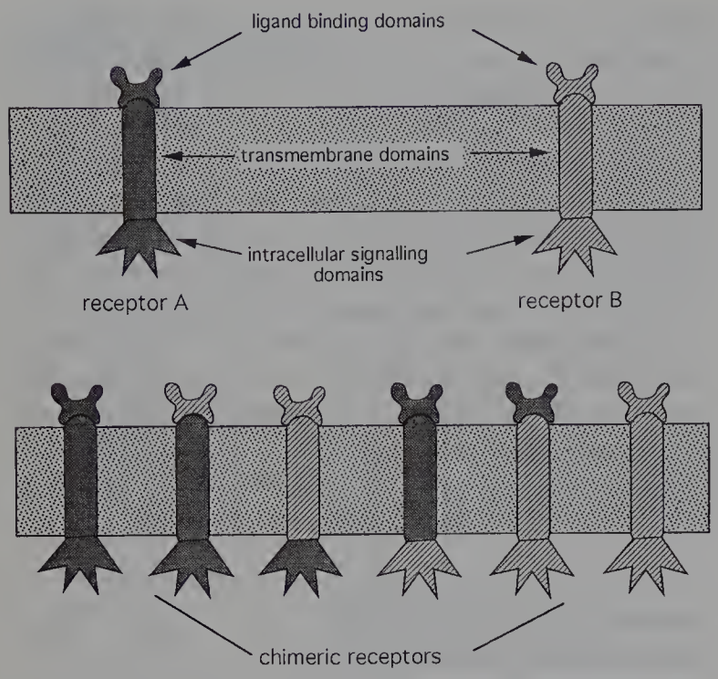

FIG. 5-3. *Chimeric receptors.*

recombinant DNA techniques, it is possible to exchange these functional domains to create chimeric receptors that, for example, contain the ligand binding domain of receptor B but the transmembrane and intracellular signalling domains of receptor A. The application of this strategy is discussed further in connection with the human growth hormone receptor (see *Novel Drug-Screening Strategies*).

Another reason for combining elements of two proteins into one recombinant protein is to facilitate its expression and purification. For example, recombinant glutathione-S-transferase (GST), cloned from the parastic worm *Schistosoma japonicum*, is strongly expressed in *Escherichia coli* and has a binding site for glutathione. It has been found that heterologous sequences encoding the functional domains from other proteins can be fused, in-frame, to the carboxy terminus of GST and that the resulting fusion protein is often expressed at the same levels as GST itself. In addition, the resulting fusion protein still retains the ability to bind glutathione, which means that affinity chromatography, using glutathione that has been covalently bonded to agarose, can be used for a single-step purification of the fusion protein. The functional activity of the heterologous domains that have been fused to GST can then be studied either as part of the fusion protein or separately following treatment of the fusion protein with specific proteases that cleave at the junction between GST and the heterologous domain. Purified fusion proteins can also be used for the generation of antibodies to the heterologous domains and for other biochemical studies. Sometimes fusion proteins are made to provide a recombinant protein that can be easily identified. An example of this is a technique called "epitope-tagging," in which well-characterized antibody recognition sites are fused with recombinant proteins. The resulting recombinant protein can then be identified by immunofluorescence or can be purified with antibodies that recognize the epitope.

NEW BIOLOGICAL TARGETS FOR DRUG DEVELOPMENT

One of the outcomes of the progress that has been made in the identification and cloning of genes is that many proteins encoded by these genes represent entirely new targets for drug development. In some cases, the genes themselves may represent the ultimate treatment of a disease in the form of gene therapy. The cloning of the cystic fibrosis gene is an example of both a new drug target and a gene that could potentially be used to treat the disease. The protein encoded by this gene, CFTR, is a previously unknown integral membrane protein that functions as a channel for chloride ions. Mutations in the CFTR underlie the pathophysiology of cystic fibrosis, and, in principle, replacement of the defective gene with the healthy, nonmutated gene would cure the disease. It is also possible, however, that by understanding the structure and function of the healthy CFTR, drugs could be designed to interact with the mutated CFTR and improve its function.

A dramatic example of the identification of new drug targets has been the recognition that many traditional targets, such as various enzymes and receptors, are considerably more heterogeneous than previously thought. Thus, instead of one enzyme or receptor, there may be several closely related subtypes, or isoforms, each with the potential of representing a separate drug target. This can be illustrated with the enzyme cyclooxygenase (COX), which is pivotal to the formation of prostaglandins and which is the target of aspirin and the nonsteroidal anti-inflammatory agents (NSAIDs). Until recently, this was considered one enzyme, but through pharmacological and gene cloning studies it has been found that there are at least two, named COX-1 and COX-2. Interestingly, they appear to be differentially regulated such that COX-1 is constitutively expressed in many tissues, whereas the expression of COX-2 is induced. This could be very important, especially if the expression of COX-2 is induced by such things as the overzealous consumption of ethanol, exercise, or inflammation. Thus, the development of COX-2 selective agents could yield NSAIDs with the same efficacy as existing (nonselective) agents but with fewer side effects, such as those on the gastric mucosa.

The family of adrenergic receptors is another example in which molecular cloning studies have revealed previously unknown heterogeneity with the consequence of providing new targets for drug development. The adrenergic receptors mediate the physiological effects of the catecholamines epinephrine and norepinephrine. They are also the targets for many drugs used in the treatment of such conditions as congestive heart failure, asthma, hypertension, glaucoma, benign prostatic hypertrophy, and others. Prior to the purifi-

cation and molecular cloning of adrenergic receptors, the pharmacological classification of this family of receptors consisted of four subtypes: β_1, β_2, α_1, and α_2. With the initial cloning of the β-adrenergic receptor in 1986 and subsequent gene cloning studies, it is now recognized that there are at least nine subtypes: β_1, β_2, β_3; α_{1A}, α_{1B}, α_{1D}; and α_2A, α_2B, α_2C.

The evidence that there are nine subtypes of adrenergic receptors is very important in terms of understanding the physiology of these receptors and in terms of developing drugs that can selectively interact with these subtypes. For example, in the case of the α_2-agonist, *p*-aminoclonidine, an agent used to lower intraocular pressure (IOP) in the treatment of glaucoma, it may now be possible to explain some of its pharmacological side effects (e.g., bradycardia and sedation) by interactions with the additional α_2-adrenergic receptor subtypes. Of considerable interest is the possibility that each of these pharmacological effects (i.e., lowering of IOP, bradycardia, and sedation) are each mediated by one of the three different α_2-receptor subtypes. If true, it might be possible to develop a subtype-selective α_2-agonist that lowers IOP but does not cause bradycardia or sedation. Likewise, it might even be possible to take advantage of the pharmacological "side effects" and develop α_2-adrenergic agents that selectively lower heart rate or produce sedation.

The discovery of subtypes of receptors and enzymes by molecular cloning studies seems to be the rule rather than the exception and is offering a plethora of potential new drug targets (Table 5-3). To note just a few: five dopamine receptor subtypes have been cloned (replacing two defined pharmacologically); seven serotonin receptor subtypes have been cloned (replacing three); four genes encoding receptors for prostaglandin-E2 have been isolated, including twelve additional alternative mRNA splice variants; and three receptors for nerve growth factor have been cloned (replacing one).

NOVEL DRUG-SCREENING STRATEGIES

The combination of the heterologous expression cloned DNA, the molecular cloning of new biological targets, and the ability to manipulate gene sequence has created powerful new tools that can be applied to the process of drug discovery and development. In its most straightforward application, the ability to simply express newly identified biological targets offers a novel means of obtaining information that may be difficult, or even impossible, to obtain from more complex native biological systems. There are two reasons for this. One is that the newly identified protein can be expressed in isolation. Thus, even for closely related enzyme or receptor subtypes, heterologous expression of the individual subtype will provide data that are specific for the subtype being expressed, whereas the data from native biological systems will reflect the summation of the individual subtypes that may be present.

The potential advantage of heterologous expression is illustrated in Fig. 5-4 for the interaction of a drug with multiple binding sites. In panel A, which could represent the data obtained from a native biological system, the data are complex and reflect interactions of the drug with two populations of receptors: one with high affinity, representing 50% of the total receptor population and one with low affinity, representing the remaining 50%. The individual contributions of these two populations of receptors are indicated in panel B, which could also reflect the data obtained if recombinant DNA encoding these two receptors were expressed individually in a heterologous expression system. Although in some cases the data, as in panel A, can be analyzed with success, frequently they cannot, especially if more than two subtypes are present or if any one subtype makes up $<10\%$ of the total receptor population or if the affinities of the drug for the two receptor populations differ by <10 fold.

A second important reason for integrating heterologous expression into drug screening strategies is that data can

TABLE 5-3

SELECT EXAMPLES OF RECEPTOR SUBTYPE HETEROGENEITY

Receptor Superfamily	*Original Subtypes*	*Present Subtypes*
G-protein coupled		
Adrenergic	β_1, β_2, α_1, α_2	β_1, β_2, β_3, α_{1A}, α_{1B}, α_{1D}, α_2A, α_2B, α_2C
Dopamine	D_1, D_2	D_1, D_2[a], D_3, D_4, D_5
Prostaglandin E_2	EP_1, EP_2, EP_3	EP_1, EP_2, EP_3[a], EP_4
Receptor tyrosine kinase neurotrophins	Nerve growth factor receptor	TrkA[a], TrkB, TrkC[a]
DNA binding		
Estrogen	Estrogen receptor	ERR1, ERR2
Thyroid hormone	Thyroid hormone receptor	TRα, TRβ
Retinoic acid	Retinoic acid receptor	RARα, RARβ
Ligand-activated channels		
Glycine	Glycine/strychine receptor	(Multi-subunit)[b] α1, α2, α3
$GABA_A$	GABA/benzodiazepine receptor	(Multi-subunit)[b] α1, α2, α3, α4, α5, α6

[a] Alternative mRNA splicing creates additional receptor heterogeneity.

[b] Only the heterogeneity of the ligand-binding subunit is listed; a multi-subunit structure combined with heterogeneity of the other subunits creates a very large number of potential subtypes.

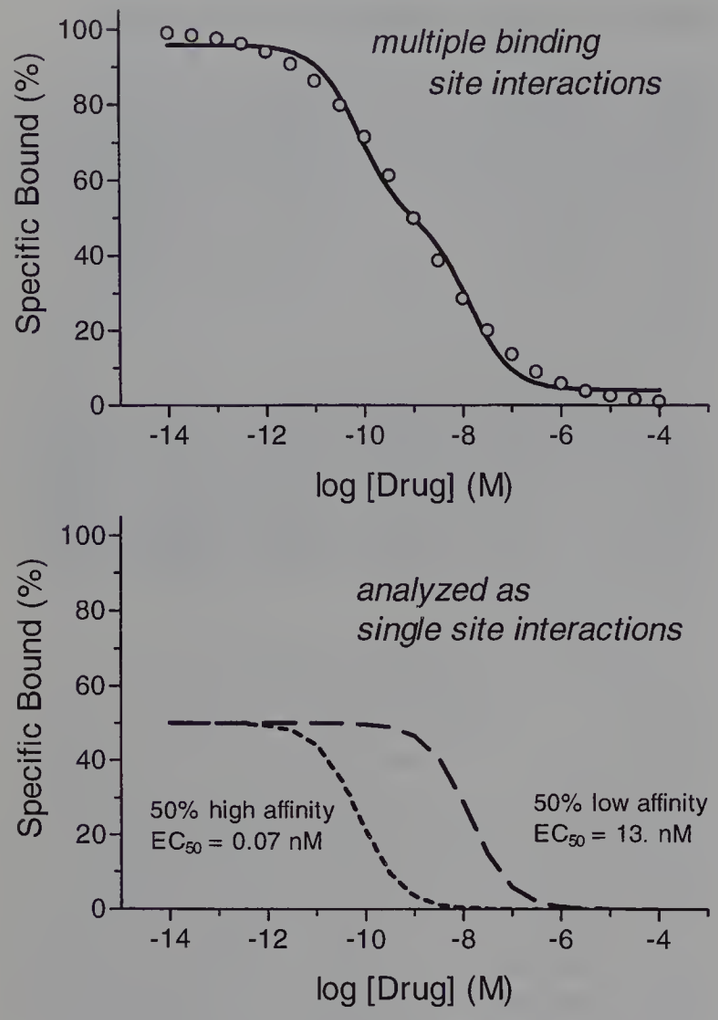

FIG. 5-4. *Convoluted data from binding to multiple receptor subtypes versus classic mass action.*

usually be obtained for the human target protein rather than an animal substitute. This does not mean that organ preparations or animal models will be totally replaced. However, for the purposes of the identification of lead compounds and the optimization of selectivity, affinity, etc., the use of recombinant expression systems provides some obvious advantages.

By combining heterologous expression with novel functional assays, it is frequently possible to obtain a major increase in both specificity and throughput, i.e., the number of compounds that can be screened per unit time. For example, reporter genes have been developed that respond to changes in a variety of intracellular second messengers, such as the activation of guanine nucleotide binding proteins (G-proteins), cAMP, calcium, etc. One approach to the development of novel functional assays involves the use of promoter regions in DNA that control the transcription of genes and is exemplified by the *c*AMP *r*esponse *e*lement (CRE). This is a specifically defined sequence of DNA that is a binding site for the *c*AMP *r*esponse *e*lement *b*inding (CREB) protein. In the unstimulated condition, the binding of CREB to the CRE prevents the transcription and expression of genes that follow it (Fig. 5-5). When CREB is phosphorylated by cAMP-dependent protein kinase (PKA), however, the conformation of CREB changes, which permits the transcription and expression of the downstream gene. Thus, increases in intracellular cAMP, such as those caused by receptors that activate adenylyl cyclase (e.g., β-adrenergic, vasopressin, and many others), will stimulate the activity of PKA, which, in turn, will result in the phosphorylation of CREB and the activation of gene transcription.

In nature, there are a limited number of genes whose activity is regulated by a CRE. In the world of biotechnology, however, the expression of almost any gene can be regulated in a cAMP-dependent fashion if it is placed downstream of a CRE using recombinant DNA techniques. If the products of the expression of the downstream gene can be easily detected, then it can serve as a reporter for any receptor or enzyme that can modulate the formation of cAMP in the cell. The genes encoding chloramphenicol acetyl transferase (CAT), luciferase, and β-galactosidase are three examples of potential "reporter genes" whose products can be easily detected. Sensitive enzymatic assays have been developed for all of these enzymes such that any changes in their transcription will be quickly reflected by changes in enzyme activity. By co-expressing the reporter gene along with the genes encoding receptors and enzymes that modulate cAMP formation, it is possible to get very sensitive functional measures of the activation of the co-expressed enzyme or receptor.

Another example of the use of a reporter gene for high throughput drug screening is the *r*eceptor *s*election and *a*mplification *t*echnology (R-SAT) assay. This assay takes advantage of the fact that the activation of several different classes of receptors can cause cellular proliferation. If such receptors are co-expressed with a reporter gene, such as β-galactosidase, then the activity of the reporter will be increased as the number of cells increase as a consequence of receptor activation. Initially, a limitation of this assay was

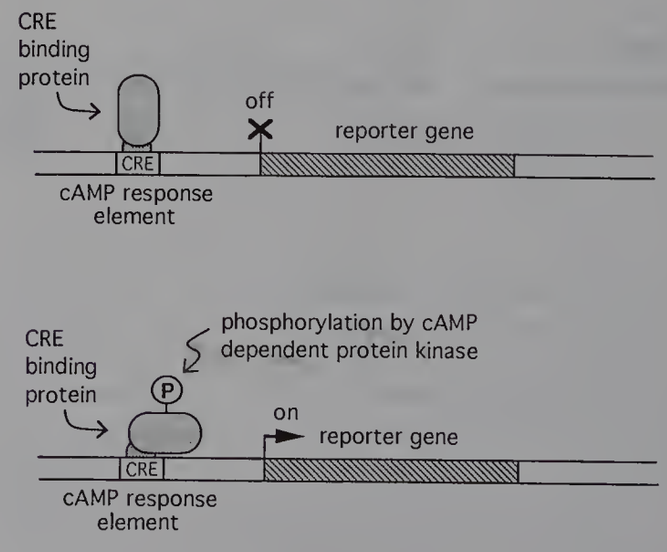

FIG. 5-5. *Activation of transcription by a cAMP response element.*

that it only worked with receptors normally coupled to cellular proliferation; however, by making a mutation in one of the second messenger proteins involved with the proliferative response, it was possible to get additional receptors to work in this assay. This second messenger protein, known as G_q, was cloned and a recombinant chimera was made, which included a part of another second messenger known as G_i. In native cells, receptors that activate G_i are not known for their stimulation of cell proliferation, but, when such receptors are co-expressed in the R-SAT assay with the chimeric Gq, their activity can be measured.

A similar strategy involving chimeric proteins has been used for receptors whose second messenger signalling pathways are not clearly understood. For example, the development of potential therapeutic agents acting on the human growth hormone receptor has been difficult because of a lack of a good signalling assay for this receptor. However, the functional activity of other receptors that are structurally and functionally related to the growth hormone receptor can be measured in a cell proliferation assay. One such receptor that has been cloned is the murine receptor for granulocyte colony-stimulating factor (G-CSF). By making a recombinant chimeric receptor containing the ligand binding domain of the human growth hormone receptor with the second messenger coupling domain of the murine G-CSF receptor, it was possible to stimulate cellular proliferation using human growth hormone.[7]

In addition to providing a useful pharmacological screen for human growth hormone analogues, the construction of this chimeric receptor also provided considerable insight into the mechanism of agonist-induced growth hormone receptor activation. Thus, the growth hormone binding domain was clearly localized to the extracellular amino terminus of the receptor while the transmembrane and intracellular domains were implicated in the signal transduction process. It was also determined that successful signal transduction required receptor dimerization by the agonist, i.e., the simultaneous interaction of two receptor molecules with one molecule of growth hormone. On the basis of this information, a mechanism-based strategy was used for the design of potential antagonists. Thus, human growth hormone analogues were prepared that were incapable of producing receptor dimerization and were found to be potent antagonists.

NOVEL BIOLOGICAL AGENTS

The progress that has been made in gene cloning has not only provided possible replacements for the preparation of existing biological agents, such as insulin and factor VIII, it is now also providing totally new agents whose potential therapeutic use is just beginning to be explored. Nowhere is this more evident than for cellular growth factors and cytokines, several of which have been approved or are being developed for human use. Erythropoietin is an example of a growth factor that was previously known for its ability to stimulate erythropoiesis, i.e., the production of red cells, but was simply unavailable for human use. With the cloning of the human gene, however, recombinant erythropoietin became commercially available and in 1989 was approved for the treatment of the anemia associated with chronic renal failure. In this application, recombinant erythropoietin has been tremendously beneficial because the only previous treatment was through blood transfusions and the administration of anabolic steroids. With its availability, however, other indications have been found. Thus, recombinant erythropoietin is now approved for anemia in AIDS patients treated with zidovudine and for anemia caused by chemotherapy in patients with nonmyeloid malignancies. In addition, recombinant erythropoietin is being investigated for sickle cell anemia and other conditions.

Recombinant insulin-like growth factor-1 (ILGF1), epidermal growth factor (EGF), fibroblast growth factor (FGF), and nerve growth factor (NGF) are other examples of growth factors that are either approved or under development as new therapeutic agents. In each case, their initial therapeutic potential is related to their known biological activity: i.e., ILGF-1 is being investigated for the treatment of several growth disorders, while EGF, FGF, and NGF have potential to facilitate wound repair following damage to the eye, vasculature, and nerves, respectively. Like erythropoietin, however, once these products become available, their potential indications may increase as well as their potential for misuse, as in the case of human growth hormone.

The cytokines represent another large family of proteins in which molecular cloning has allowed a better understanding of both their biology and their potential therapeutic use. With respect to their biology, the cytokines are proteins that are involved with cellular communication, particularly those of the immune system. They include such agents as the colony-stimulating factors, the interleukins, and the interferons as well as some other substances such as tumor necrosis factor-α and transforming growth factor-β. Specific indications for many of these agents are still being explored, and considering that there are four colony-stimulating factors that have been identified and cloned, 12 interleukins, and three interferons, there is a lot of exploration yet to be done. In most cases, the potential indications involve the modulation of immune function as it relates to cancer and/or viral infections. For example, recombinant interleukin-2 is approved for the treatment of renal cell carcinoma, and recombinant interferon-α is approved for the treatment of hairy-cell leukemia, AIDS-related Kaposi's sarcoma, and genital warts. In addition, recombinant granulocyte colony-stimulating factor is approved for use as an adjunct to bone marrow transplantation and chemotherapy to increase the impaired production of neutrophils that occurs during these procedures.

The recent cloning of a gene involved with obesity is another interesting example of how molecular genetics is contributing to our understanding of biology and is providing entirely new possibilities for treatment of disease.[8] It has

been suspected for some time that a satiety signal exists that is released from adipose tissue and decreases the urge to eat, thus helping to regulate the total fat content of the body. If true, this satiety signal is defective in a strain of mice known as *obob*, which have an inherited predisposition to overeat and to get very fat. Using molecular genetics, the *ob* gene was recently cloned, and it encodes a protein with many of the expected properties of the presumed satiety signal. Thus, the encoded protein, known as leptin, is unique to adipose tissue, is secreted, and is mutated in the *obob* mouse. Cloning of the gene from normal mice and administration of recombinant leptin to obese *obob* mice decreased feeding and resulted in weight loss. This gene is also present in humans, and the commercial possibilities of using recombinant human leptin for the treatment of human obesity is tantalizing. It is possible, however, that obesity in humans may have other causes besides decreased leptin secretion. For example, there may be psychological causes or other biochemical lesions, such as a defect in the cellular receptor for leptin. Whatever the cause, however, mechanisms underlying the development of obesity are starting to be understood, and both a potential biological target (the leptin receptor) and potential therapeutic agents (leptin or a recombinant analogue) have been identified.

ANTIBODIES

The use of antibodies or antisera for the treatment of disease and the induction of an immune response through vaccination are well-established medical strategies that are being influenced by the application of biotechnology. Two ways this is being felt are in the cloning and identification of potential antigens and in the use of monoclonal antibody technology. The use of recombinant antigens for the development of vaccines has recently been realized for hepatitis B and for *Hemophilus influenzae*. In addition, there are possibilities for the development of an AIDS vaccine as well as vaccines for many other diseases. For hepatitis B, the identification and cloning of a viral coat protein allowed it to be expressed and purified. The use of this recombinant protein as a vaccine for hepatitis B is a significant advance because the use of the killed or attenuated virus was precluded by its potential to cause hepatocellular carcinoma.

The use of monoclonal antibodies for diagnostic procedures and for therapeutics is recognized as one of the major applications of biotechnology that actually preceded the application of recombinant DNA technology. It involves the use of an antigen to generate an immune response in mice, which is followed by removal of the spleen and the isolation of lymphocytes, the actual antibody-producing cells. By physically fusing the lymphocytes with cancer cells, it is possible to get a hybridoma, which is a cell line that expresses the antibodies originally present in the lymphocytes. Since the hybridoma is essentially immortal, it can be propagated ad infinitum in tissue culture. By using specialized screening techniques, it is possible to isolate individual hybridomas that represent clonal populations of cells expressing a single antibody (i.e., monoclonal). The use of recombinant DNA techniques now allows the use of recombinant proteins as antigens, and, more importantly, it now permits the isolation of the cDNAs that encode the antibodies so that they can be modified in various ways.

There are several reasons why it is often desirable to modify monoclonal antibodies. These include making them less immunogenic, increasing their affinity, and/or incorporating parts of other antibodies or proteins to increase their potential cytotoxicity. Making monoclonal antibodies less immunogenic is important for applications involving human use. This is because monoclonal antibodies are usually derived from mouse cells and, therefore, have the potential to generate an immune response in humans. This limits their use. However, by cloning the cDNAs encoding monoclonal antibodies, it is possible to alter their sequence to make them less immunogenic in humans. Another problem with using mouse antibodies in humans is that the mouse antibodies lack the determinants that activate the human complement system and induce cytotoxicity. This is a problem if the goal of using a monoclonal antibody is to recognize and kill cancer cells; however, it is again possible to use recombinant DNA techniques to introduce human antibody sequences into mouse monoclonals so that these activities will be present.

ANTISENSE OLIGONUCLEOTIDE THERAPY

One area of biotechnology getting a lot of attention is the possible use of antisense oligonucleotides in the treatment of human disease. Recall that most DNA in the cell is double-stranded while the mRNA is single-stranded. mRNA, however, has the potential to be double-stranded if its complementary sequence is present (in fact, mRNA can be thought of as being briefly double-stranded during the binding of transfer-RNAs). This potential of mRNA to be double-stranded is exploited in antisense oligonucleotide therapies. Antisense oligonucleotides are nucleic acid sequences (DNA, RNA, or chemically modified derivatives thereof) that are complementary to disease-producing mRNA molecules (Fig. 5-6). By hybridizing to these disease-producing mRNAs, the antisense oligonucleotides will interfere with translation and possibly interfere with the pathology of the disease.

There are a number of attractive features of antisense therapy, a major one being its potential specificity. Thus, for an oligonucleotide of just 20 bases, the probability of randomly encountering its exact complementary sequence is four (the number of different bases in DNA) to the 20th power or once in 1×10^{12} bases (or ~1,000 times the total number of bases in the human genome). Also, because of the hydrogen bonding that takes place, the affinity of oligonucleotide for its complementary sequence is very high. The idea, there-

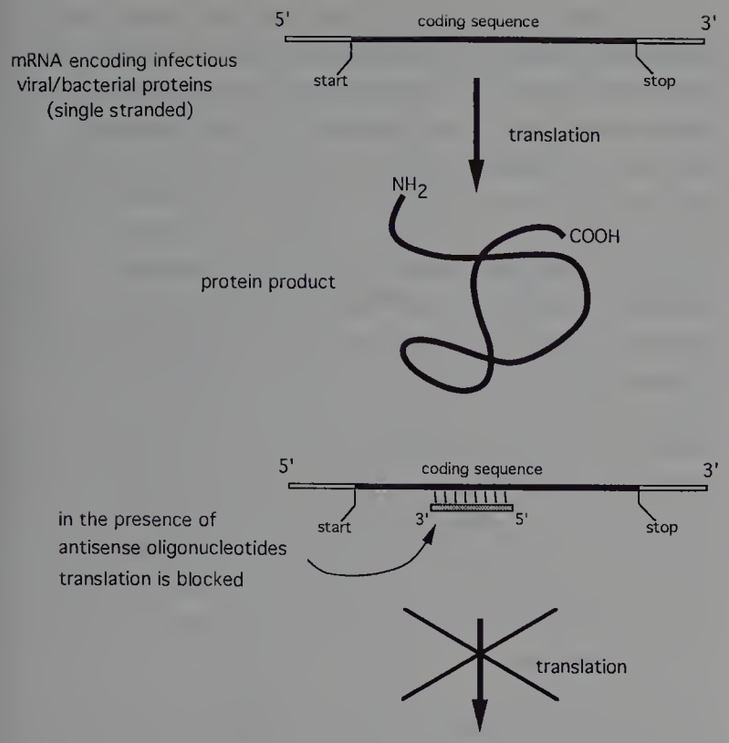

FIG. 5-6. *Antisense oligonucleotide strategy.*

fore, behind antisense oligonucleotide therapy is to identify a potential target mRNA sequence, perhaps a sequence encoding an important viral or cancer protein, and to introduce the antisense oligonucleotide to the infected or cancerous cell so that the synthesis of the protein will be blocked.

There is no question that the potential of this approach is real and that antisense oligonucleotides can definitely interfere with protein synthesis. However, there are problems related to the delivery of the oligo to the infected cell and in the identification of the specific mRNA targets that will halt viral replication or the growth of cancer cells. The delivery problem is significant because oligonucleotides are large molecules (~6,500 g/mole for an oligonucleotide consisting of 20 bases), are hydrophilic, and, unless they are modified chemically, are relatively unstable in biological fluids. Oligonucleotides can, however, be chemically modified so that the stability problems can be overcome, but getting them to cross cellular membranes is still a challenge. Another strategy being explored is the use of recombinant vectors to introduce antisense genes into cells. These recombinant vectors are generally viruses, such as the vaccinia and adeno viruses, which are still capable of getting into cells but which have been genetically modified so that they do not propagate or cause disease. Once in the cell the vector containing the antisense gene will be expressed, but the product of transcription will be an antisense mRNA that will be capable of hybridizing to the target mRNA.

GENE THERAPY

Gene therapy represents the ultimate use of recombinant DNA technology for the treatment of disease, and it represents either the replacement of a defective gene with a normal gene or the addition of a gene whose product can help fight a viral infection or cancer. The great appeal of gene therapy is that in the former case, i.e., the replacement of a defective gene, it can represent an actual cure for the disease as opposed to a treatment of the symptoms. For example, in the case of cystic fibrosis, the defective gene has been clearly identified as the cause of the disease, and, thus, its replacement could be expected to bring about a cure. Similar expectations exist for other inherited genetic disorders such as insulin-dependent diabetes, growth hormone deficiency, hemophilia, sickle cell anemia, etc.

The ability to transfer genes into other organisms has other important applications, including the heterologous production of recombinant proteins (previously discussed) and the development of animal models to study human diseases. Another area being explored is the introduction of recombinant genes as biological response modifiers as, for example, in organ transplantation. Thus, a serious problem with tissue and organ transplantation is the immune-mediated rejection by the host. If genes can be introduced into transplanted cells, it may be possible to introduce host-cell determinants so that the transplanted tissue is recognized as self. It may also be possible to introduce genes, such as transforming growth factor-β, that will decrease local cell-mediated immune responses. An opposite strategy is being considered for the treatment of cancer whereby transplanted cells expressing recombinant genes will be used to help differentiate cancer cells and increase local cell-mediated immune responses.

The transfer of genes from one organism to another is called transgenics, and an animal that has received such a transgene is referred to as a transgenic animal. It is important to understand that, if the transgene is incorporated into the germ cells (e.g., sperm, eggs), the transgene will be inherited and passed on to successive generations. If the transgene is incorporated into other cells of the body (somatic cells), it will only be present as long as the newly created transgenic cells and/or their daughter cells are alive. Thus, it makes a big difference if a terminally differentiated, postmitotic cell receives a transgene as opposed to an undifferentiated stem cell; i.e., the former will not undergo further division, whereas the latter will continuously give rise to new cells.

There are two basic strategies with respect to the introduction of transgenes. In the first, the transgene is introduced at a very early stage of embryonic development or into a germline cell, with the ultimate goal of obtaining a stable inherited transgene. In the second, the transgene is introduced either directly into the organism or cells are removed from the organism, made transgenic, and then injected back into the organism (autologous cell transplantation). In the second case, the transgene will be expressed but is unlikely to be inherited. With respect to human gene therapy, there are serious ethical concerns about germline transplantation, especially if it goes beyond the replacement of defective genes to the replacement of "less desirable" genes; there-

fore, efforts are solely concerned with the introduction of transgenes that cannot be inherited.

Further considerations with respect to the introduction of transgenes concern the nature of the condition to be treated. For example, in the case of cystic fibrosis, the defective CFTR gene is widely expressed in epithelial tissues, with cells of the lung and gastrointestinal tract being most seriously affected. To effectively treat this condition, it is desirable to replace the defective gene in as many of the affected cells as possible. On the other hand, for a condition like insulin-dependent diabetes, simply having cells present that are capable of making insulin may be sufficient for controlling the disease. In either event, it is necessary to get the replacement gene into the cells of interest. It is also generally desirable to get the transgene into stem cells so that it will be continuously replenished as the cells divide, which will reduce the need for reintroduction of the transgene.

At present, the main approaches to introducing transgenes into cells involve the use of viral vectors and the use of liposomes. Liposomes are spherical aggregates of phospholipids that can be used to encapsulate DNA. The liposomes can then be injected into the circulation or delivered by aerosol or lavage into the nasal mucosa, lungs, gastrointestinal tract, etc. Following adsorption on the cell surface, the liposomes may fuse and deliver their content of DNA into the cell, where it can recombine with the chromosomal DNA and be expressed. The advantages of liposomes are that they are relatively nonimmunogenic and are nonviral so that there is minimal risk of accidental transfer to a pathogenic organism. The disadvantages are that they are inefficient, only ~5% of primary epithelial cells become transgenic, and they are rapidly cleared by the reticuloendothelial system. Efforts are being made to increase their stability and to target them, perhaps, through the use of monoclonal antibodies, to specific cell populations.

The use of so-called disabled viral vectors represents the major approach being used for the creation of transgenic cells. Being disabled means that critical sections of the original viral genome have been removed so that the recombinant virus cannot replicate even though it can be used to infect cells. The adenoviruses and the retroviruses represent two major classes of viruses being explored for this purpose. Perhaps the most important difference between these two classes of viruses is that infection with the retroviruses is limited to actively dividing cells, whereas infection with the adenovirus does not require cellular proliferation but does require tropism which is the ability of a cell to be recognized by the virus. Based on this difference, therefore, both adeno- and retroviruses could be potentially used for the infection of stem cells, whereas the infection of postmitotic cells would be limited to the use of adenoviral vectors.

The use of viral vectors for the creation of transgenic cells, therefore, involves the construction of the recombinant virus containing the gene of interest and the infection of the target cells. In a condition such as cystic fibrosis, the infection would be done on the whole animal. For example, since cells of the airway represent a target tissue, the virus could be introduced directly by inhalation or lavage. The problem here is that unless the stem cells become transgenic the procedure would have to be repeated as the differentiated cells die. For other conditions, such as insulin-dependent diabetes and growth hormone deficiency, it has been demonstrated in mice that it is possible to use liver or muscle cells to introduce the replacement gene. This is advantageous in that these cells can be removed from the body, infected in the laboratory, and then reintroduced. This virtually eliminates the possibility that germline cells might be infected, and it allows the use of protocols that give higher yields of transgenic cells. For other conditions, such as sickle cell anemia, gene replacement therapy would probably require the introduction of normal genes into the stem cells responsible for the production of red blood cells. This would require the isolation of an appropriate population of stem cells, their transfection, and then reintroduction, perhaps, after killing the remaining stem cells by chemotherapy.

There are still hurdles to be overcome, but, ready or not, human gene replacement therapy is rapidly becoming a reality. There is great promise in gene therapy for the amelioration of many devastating genetic and acquired diseases. There is also a very real potential for misuse. It will be the obligation of everyone involved to try to understand these procedures, and their potential risks and liabilities, and to make intelligent decisions about their use.

PRODUCTS

GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR

GM-CSF is a growth factor that stimulates neutrophil and monocyte production by the bone marrow. cDNAs encoding GM-CSF have been cloned, and recombinant GM-CSF (sargramostim, Leukine, Prokine) is produced commercially in yeast. Sargramostim is approved for use in the treatment of autologous bone marrow transplantation in patients with Hodgkin's disease and in non-Hodgkin's lymphoma or acute lymphoblastic leukemia.

ERYTHROPOIETIN

Erythropoietin is a growth factor that is made in the kidneys and stimulates erythropoiesis (red blood cell production) by the bone marrow. A cDNA encoding human erythropoietin has been cloned, and recombinant erythropoietin (epoetin alfa, Epogen, Procrit) is produced commercially in mammalian cells that have been stably transfected with the cloned gene. Epoetin alfa is approved for use in the treatment of the anemia of chronic renal failure, in the treatment of the anemia of zidovudine-treated HIV patients, and in the treatment of anemia in cancer patients on chemotherapy. It

should be noted that epoetin alfa is not indicated for the *immediate* correction of severe anemia; i.e., it is not a substitute for an emergency transfusion.

FACTOR VIII

Factor VIII is a plasma protein that is necessary for the proper coagulation of blood. A deficiency of factor VIII is responsible for the inherited bleeding disorder hemophilia A, which can be treated by the administration of purified factor VIII. cDNAs encoding human factor VIII have been cloned, and recombinant factor VIII (KoGENate, Recombinate) is produced by mammalian cells in tissue culture. In the case of Recombinate, factor VIII is initially co-expressed with von Willebrand factor, which increases the yield, but is then purified so that the final product consists only of factor VIII. In another case, the recombinant factor VIII has been engineered so that the glycosylated B domain of the molecule has been deleted. The resulting product, however, retains all of the functional characteristics of native factor VIII. Factor VIII is approved for use in the treatment of hemophilia A.

GRANULOCYTE COLONY-STIMULATING FACTOR

G-CSF is a growth factor that stimulates the production of neutrophils by the bone marrow. cDNAs encoding human G-CSF have been cloned, and recombinant G-CSF (filgrastim, Neupogen) is produced commercially in bacteria. Unlike endogenous G-CSF, filgrastim is not glycosylated and bears an additional methionine on the amino terminus. Filgrastim is approved for use in patients to reduce the incidence of infection associated with the treatment of nonmyeloid malignancies with myelosuppressive antineoplastic drugs. It is also approved for the treatment of congenital neutropenia and idiopathic or cyclic neutropenia.

GROWTH HORMONE

Growth hormone is an anterior pituitary hormone important for normal skeletal and soft tissue growth in children and adolescents. Deficiencies of growth hormone are responsible for dwarfism, whereas an excess may lead to gigantism or acromegaly. cDNAs encoding human growth hormone have been cloned, and recombinant growth hormone (somatropin, Humatrope; and somatrem, Protropin) are now produced commercially. Somatropin is identical to endogenous growth hormone, whereas somatrem has an additional methionine at the amino terminus of the protein. Both agents are approved for use in the treatment of children with growth-hormone deficiency. Antibodies to somatrem develop in 30% to 40% of patients receiving this drug whereas, the incidence in patients receiving somatropin is ~2%.

INSULIN

Insulin is a hormone that is made in the β-cells of the pancreas and is essential for the regulation of blood glucose concentrations and the proper metabolism of glucose. The human gene encoding insulin has been cloned, and insulin is produced commercially either in bacteria (Humulin) or in yeast (Novolin) that have been transfected with the cloned gene. Recombinant human insulin is approved for use in the treatment of diabetes mellitus.

INTERFERON ALFA-2A

Interferon alfa-2a is a cytokine with antiviral activity and other modulatory effects on the immune system. A cDNA encoding human interferon alfa-2a has been cloned, and recombinant interferon alfa-2a (Roferon-A) is produced commercially by bacterial fermentation. Roferon-A is approved for use in the treatment of hairy-cell leukemia and AIDS-related Kaposi's sarcoma.

INTERFERON ALFA-2B

Interferon alfa-2b is a cytokine with antiviral activity and other modulatory effects on the immune system. A cDNA encoding human interferon alfa-2b has been cloned, and recombinant interferon alfa-2b (Intron A) is produced commercially by bacterial fermentation. Intron A is approved for use in the treatment of hairy-cell leukemia, AIDS-related Kaposi's sarcoma, genital warts, hepatitis C, and hepatitis B.

INTERFERON ALFA-N3

Interferon alfa-n3 is a cytokine with antiviral activity and other modulatory effects on the immune system. A cDNA encoding human interferon alfa-n3 has been cloned, and recombinant interferon alfa-n3 (Alferon) is produced commercially by bacterial fermentation. Alferon is approved for use in the treatment of genital warts.

INTERFERON BETA-1B

Interferon beta-1b is a cytokine, normally produced by fibroblasts, with antiviral activity and other modulatory effects on the immune system. A cDNA encoding human interferon beta-1b has been cloned, and recombinant interferon beta-1b (Betaseron) is produced commercially by bacterial fer-

mentation. The recombinant product differs from the wild type by substitution of a serine for a cysteine at position 17, and it is not glycosylated. Betaseron is approved for use in the treatment of patients with relapsing-remitting multiple sclerosis.

INTERFERON GAMMA-1B

Interferon gamma-1b is a cytokine related to the interleukins, which is produced by antigen-stimulated T-lymphocytes. Interferon gamma-1b has less antiviral activity than other classes of interferons but more immunomodulating effects, especially on the activation of macrophages. A cDNA encoding human interferon gamma-1b has been cloned, and recombinant interferon gamma-1b (Actimmune) is produced commercially by bacterial fermentation. Actimmune is approved for use in the treatment of chronic granulomatous disease.

INTERLEUKIN-2

IL-2 is a cytokine that can stimulate the proliferation and differentiation of T helper and T cytotoxic cells. A cDNA encoding human IL-2 has been cloned, and recombinant IL-2 (aldesleukin, Proleukin) is produced commercially by bacterial fermentation. Aldesleukin differs from native IL-2 in that the alanine at position 1 has been deleted, serine has been substituted for cysteine at position 125, and it is not glycosylated. Aldesleukin has all of the known biological activity of IL-2 and is approved for use in the treatment of renal cell carcinoma.

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CHAPTER 6

Fundamentals of Immunology and Immunizing Biologicals (Vaccines and Toxoids)

John M. Beale, Jr.

DISEASE PREVENTION: AN HISTORICAL PERSPECTIVE

Early in the 20th century, there were no vaccines, no antibiotics, and in fact few direct treatments of any kind for cases of infectious disease. Disease was a part of everyday life; it was understood that illness was bound to strike sooner or later and people did what they could to prevent it. Treatment of an infectious disease was largely palliative and consisted of support for the patient and an attempt to control the spread of the disease from the host through the community.[1-3] For support, Grandma often had special remedies that were applied with the great trust of the sick person. The greatest success in preventing the spread of disease was a result of physically separating infected from healthy people, and hence preventing transmission by droplet or surface contact. Some of the traditional methods used to isolate people were exotic. The disease-preventing "power" of the asafetida (devil's dung) bag worn around the neck of a healthy individual lay in the herb's incredible stench; people simply stayed away from someone wearing the magic bag of smell. As long as one could tolerate wearing asafetida on one's person, everyone, including diseased individuals, gave a wide berth.

As recently as the early to mid-20th century persons with tuberculosis (TB) were isolated in a sanatorium, a special hospital for long-term TB care. Sanatoria were said to be hospitals for bed rest and the medical treatment of TB, but were really just isolation wards for TB patients. There was no effective treatment against *Mycobacterium tuberculosis*, and patients whose immune systems were strong enough might eventually be discharged. In those days, TB patients rarely emerged quickly. This type of isolation treatment was not new; it was used in biblical times, when persons with leprosy were concentrated in colonies that people feared and knew to avoid. A different approach was used in the United States from the 1930s to the early 1950s, when frightening epidemics of polio, influenza, smallpox, and scarlet fever, among others, were rampant. To prevent patient contact with healthy individuals, the legal tactic of the quarantine was used. The health department affixed quarantine signs to all entrances of homes where disease-infected individuals were known to reside (Fig. 6-1). The signs were bright red and stated boldly in black the name of the disease that infected the residents inside. Signs warning of tuberculosis, salmonella, smallpox, polio, venereal disease, and many others were used as necessary. Cities, counties, and states took quarantines very seriously. The legal penalties for violating quarantine were stiff, and included a large monetary fine, imprisonment in the county jail, or both.

Incidences of morbidity and mortality decreased dramatically from the 1930s to near the end of World War II with the discovery and introduction of antibacterials and antibiotics. The beginning of the antibiotic era heralded an entirely new dimension in the treatment of bacterial diseases. With antibiotics a patient could be treated and even cured quickly and effectively. The excitement engendered among the medical profession was only beginning.[4-6] Still to come was an even more revolutionary new technology: drugs that not only cured infectious diseases but prevented them altogether. These miracle drugs are called vaccines, toxoids, and antisera, and their value and success throughout their history are undeniable. It is often thought that vaccine technology is very new. In point of fact, the process of inoculation with variola (smallpox) virus, called variolation,[5] had been used since 600 A.D. and continued into the 1700s, and a variety of other similar techniques for rabies, diphtheria, and typhus were developed in the 1800s. Edward Jenner's controlled experiments with cowpox and cross-resistance with smallpox of 1796 were the first truly scientific investigations, and are often held to be the official beginning of vaccination. Louis Pasteur coined the term *vaccine* from *vacca*, the Latin word for cow, at about the same time. These early vaccines were dirty, crude, and dangerous. Often others who came

FIG. 6-1. *Poliomyelitis quarantine sign, circa 1940.*

into contact with the variolated patient developed full-blown smallpox. Adverse reactions like fever and convulsions were very common. In the 1940s research into clean, dependable vaccines was successful, and the end of a frightening period in health care was in sight. Scientists spoke boldly of eradicating diseases like smallpox and polio from the planet. These predictions would prove to be nearly correct in some cases, but fell short in most others for a variety of reasons that will be discussed later. Nevertheless, vaccines have largely controlled many serious infectious diseases by preventing outbreaks. A major success has been with polio (infantile paralysis), which was greatly feared by society until the 1960s. The iron lung, a 1950s supportive measure for polio victims, is today a specter too horrible to contemplate. With the advent of polio vaccine and other new immunological agents, physicians and parents rested easier and stopped worrying about diseases like polio, diphtheria, and smallpox.[6] The public and health care professions, however, were too complacent to foresee the potential disaster that lay just ahead.

TOO GREAT A FEELING OF SECURITY?

With vaccination and antibiotic treatment preventing or quickly curing infectious disease, the public saw the incidence of many afflictions like polio decline until they seemed to be essentially eradicated. Unfortunately, what had really been eradicated were society's memories of the occurrences of many of the infectious diseases of children. Today, normal childhood diseases like chickenpox, mumps, and measles are considered by most people to be rare and of low risk. The phrase "out of sight, out of mind" applies perfectly to the situation in which we find ourselves in the late 1990s. Physicians have treated patients (often without identification of the causative organism, a culture and sensitivity test, or even an office visit) aggressively with the most powerful of antibiotics. Antibiotics have sometimes been prescribed over the telephone without examining the patient, and the most specific and powerful antibiotics have been used inappropriately in the treatment of simple infections. Antibiotic misuse has led to rampant antibiotic resistance.[7] Yet, as the widespread incidence of severe infectious disease has waned, many parents no longer see a given disease as a threat and many see no need to have their children (or themselves) properly immunized. They feel that an antibiotic can take care of it. Now, as antibiotic resistance is the rule rather than the exception, it is common to speak of the postantibiotic era, when antibiotics will no longer be effective. As we approach these times vaccination will be more important than ever. Unfortunately, complacency and misinformation about immunization are common among the populace. Indeed, vaccines are often erroneously viewed as being dangerous and able to produce disease in healthy people.[8,9] Minor side effects are often incorrectly described as allergies or reactions, or as occurrences of the active disease that the vaccine is designed to prevent. Some people believe that they will contract the human immunodeficiency virus (HIV-1) by getting a vaccine, as they have heard about HIV infection occurring among intravenous (IV) drug users who share hypodermic needles, and they avoid immunization because of this fear. Others feel that they cannot afford to purchase the immunizations. Because of complacency and misinformation, diseases such as polio, which were once spoken of as being eradicated, have reemerged and threaten people in all nations of the world. The problem of overall low immunization compliance rate is highest in some of the largest cities in the United States.[10] Governments can address these problems by assuring that all children, regardless of their family income, receive the proper doses of required vaccines on the recommended schedule. Most states and communities provide free vaccination services for children of low-income families. Complacency and misinformation can be reduced by education of the public by pharmacists and physicians, by responsible media reporting, and by government educational programs, so that parents are not fearful of having their children (and themselves) immunized.

FUNDAMENTAL IMMUNOLOGICAL NOMENCLATURE[12-14]

A discussion of the fundamentals of immunology requires the mention of four common terms that are central to the topic.[11]

1. An *antigen* (Ag) is a substance that induces antibody formation, and then reacts with that antibody. Inherent in the definition is a chemical reaction. Antigens are usually thought of as protein or carbohydrate substances of molecular weight $\geq$ 6,000.
2. An *antibody* (Ab) is a modified serum protein elicited by

an antigen. Antibodies are often referred to as globulins because they are insoluble from the globulin fraction of the centrifuged serum.

3. The *antigenic determinant* is that part of the complete antigen that provides the three-dimensional molecular structure that is responsible for the specificity of the Ab that reacts with it. The determinant is exactly like a receptor ligand, and reacts with the antibody receptor to produce a complex.
4. A *hapten* is a substance that cannot, by itself, elicit antibody formation because its molecular weight is too low and it can't be detected by the immune system. To become a complete immunogen, a hapten must bind covalently to a serum protein such as albumin to increase its molecular weight. In the three-dimensional structure of the complete immunogen, the hapten serves as the antigenic determinant. After Ab to the immunogen forms for the first time, the hapten itself is recognized and elicits its Ab, without having to bind to the protein. A good example of a hapten reaction is the penicillin allergy (Fig. 6-2). Penicillin is too small to trigger the immune system. After a dose of the antibiotic, a covalent bond forms between penicillin and serum albumin. This complex is the immunogen, with penicillin as the determinant. The complex sensitizes the immune system for the first time. After the initial sensitization, each subsequent challenge with penicillin triggers a full-blown immunological reaction with a rash, itching, and redness.

THE CHEMICAL NATURE OF AN ANTIGEN

Antigens of bacteria and viruses are distinct substances, yet we consider them to be the same in one respect. They are all *cell surface antigens*. This means that the molecules making up the antigens are those normally involved in the microbe's intercellular communication, adhesion to a host membrane, and cellular integrity.[15]

BACTERIAL ANTIGENS

Motile bacteria utilize two kinds of appendages for translocation through a medium. These appendages are *flagella* and *cilia*. Both structures are composed of protein, and since their parent structures are responsible for motion we refer to the flagella or cilia antigens as H antigens (German *hauch*, to march). In the case of flagella the antigenic protein is called *flagella*. The amino acid sequences of flagella proteins are highly variable, and a host of three-dimensional shapes is possible. Hence, protein from flagella or cilia is capable of encoding a large variety of immunological information. Examples of motile pathogens are *Escherichia coli, Pseudomonas aeruginosa, Clostridium botulinum*, and *Proteus mirabilis*. A second bacterial antigen is the O antigen, or somatic cell antigen. The O antigen (German *ohne hauch*, not marching) is a surface antigen composed of the KDO core polysaccharide region and lipopolysaccharide. The va-

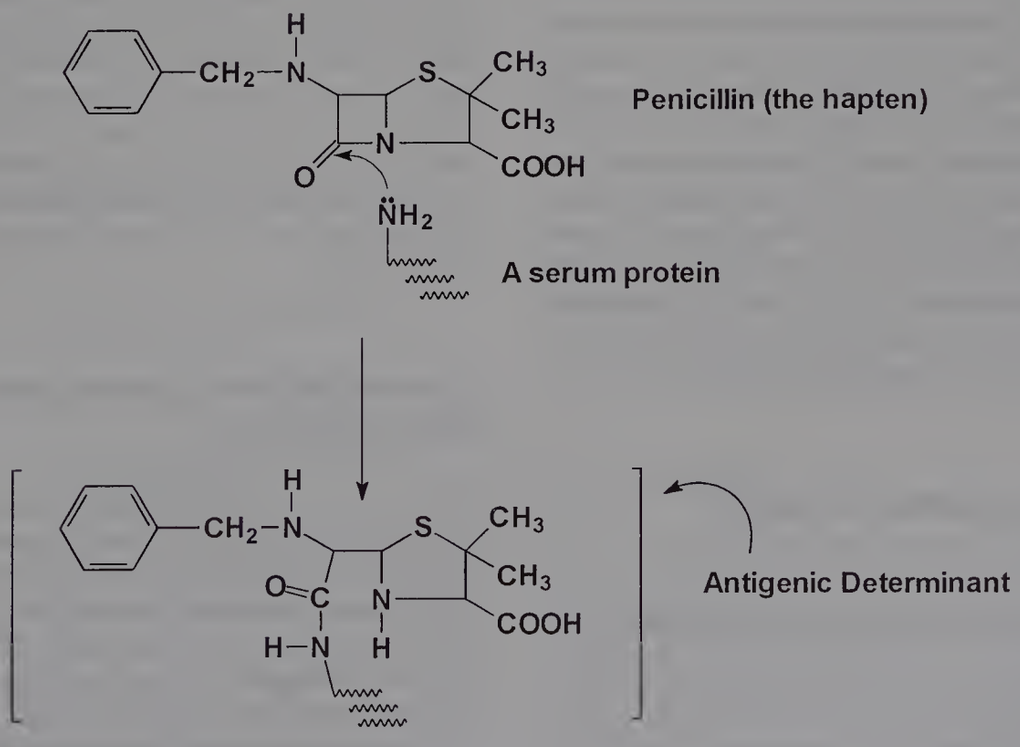


FIG. 6-2. The conversion of the hapten penicillin into a complete immunogen.

riety of antigenic structures available from lipid-linked carbohydrates is large; this feature explains the great antigenic variability among gram-negative bacteria. Some bacteria, such as *Pneumococcus* or mucoid *P. aeruginosa*, protect themselves from the host or adverse conditions by surrounding themselves with a surface carbohydrate capsule. The three-dimensional structures of these carbohydrate polymers are highly variable, making them extremely versatile immunogens. *Pili* are thread-like projections that facilitate adhesion to mucosal surfaces as a first step in bacterial infection of the respiratory tract, intestinal mucosa, and urogenital tract. Examples of such microbes are *Proteus mirabilis* and *Neisseria gonorrhea*. Pili are composed of variable protein subunits called pilin. Pilin is a powerful immunogen, and is extremely variable. In its pili, a single strain of an organism, such as a population of *N. gonorrhea,* may possess more than 1500 immunological phenotypes. A separate immunological response is required to combat each one. All of the above types of antigenic cellular subunit can be removed from their host bacterial cells by mechanical shearing. When this is done, each subunit retains its immunogenicity and hence is a potential candidate for a new acellular vaccine.

VIRAL ANTIGENS

Like those in bacteria, viral antigens are surface molecules attached to the viral particle. Viral surface proteins (capsid proteins) are composed of many smaller subunits (capsomers), which may or may not be identical. Each capsid protein exists primarily to organize and protect the viral DNA, and is highly antigenic. Some viruses possess a lipid-bilayer envelope structure that surrounds the particle. By analogy with cell membranes, the envelope bilayer contains transmembrane or surface proteins with specialized carbohydrate ends which may protrude from the viral particle surface. Observed microscopically, these often resemble spikes projecting from the particle surface. The spikes are involved in the binding of the particle to a host cell, and some allow the virus to stimulate cell lysis. The host may build an immune response to any of these surface molecules.

FUNDAMENTAL IMMUNOLOGICAL CONCEPTS[16-17]

OVERVIEW: HOST DEFENSE MECHANISMS

A human host possesses two different types of defense mechanisms: physical/chemical and immunological. These can be distinguished by two parameters: whether or not they are innate to the host and whether or not they exhibit specificity toward a bacterium or virus.

Physical and Chemical Defense Mechanisms (Nonspecific Defenses)

The intact skin has the primary role in human physical defenses, by acting as a barrier to the entry of foreign agents. As long as the epidermis is intact, an efficient barrier is formed. Burns, abrasions, cuts, and surgical wounds breach the barrier and increase the likelihood of infection. Dry skin as well allows the barrier function to fail. Intact skin is the ultimate first line of defense against invading pathogens. The gastrointestinal tract possesses mechanisms to prevent infection by organisms ingested with food or by contact with surfaces. The low stomach pH (about 2.0), salivary enzymes, and mucopolysaccharides coating the mucosa destroy or immobilize potentially infectious microbes. Protection against organisms inhaled during breathing is conferred by the innate functions of the respiratory tract. Here, cilia beat automatically to mechanically move foreign matter up toward the oral cavity, where it is swallowed or expectorated. Mucus coating the linings of the air passageways immobilizes organisms, and the coughing reflex expels microbes complexed with either saliva or mucus. The eyes and mouth are natural ports of entry for microbes, and are heavily protected. Here, lysozyme in tears and saliva degrades bacterial cell walls so that an organism lyses. The normal flora of the lower gastrointestinal tract, the skin, and the vaginal mucosa provides a competitive pressure that prevents colonization of these regions by foreign microbes. Drugs that can alter the populations of flora (oral contraceptives, antibiotics) can compromise the normal flora functions. Even the urinary tract is protected; the pH of the urine (about 5.5) and normal urine flow inhibit growth and flush invaders from the body. All of the above innate functions are very effective as long as they are intact. All of them can be compromised by medical procedures, injuries, drug therapy (e.g., histamine receptor antagonists, steroids, antineoplastics), antimicrobial therapy, aging, catheterization, and so on. When compromise occurs and a pathogen enters, a secondary defense system may become engaged.

Immunological Defense Mechanisms (Specific Defenses)

The specific defense mechanisms consist of two branches: cell-mediated immunity and humoral immunity. Both systems employ specifically targeted populations of cells to destroy invading organisms. Some of these responses are innate, whereas some are acquired only after birth, although the innate and acquired systems are interrelated. In general, these mechanisms are specific for a given type of infectious or invading agent, although some of the first-line innate systems lack absolute specificity. Systems such as these dispose of bacteria and viruses entering the body without triggering the immune system. These systems are discussed later. In

discussing vaccines, we will be concerned primarily with the humoral immune system.

Components of the Innate Active Immune Response

The innate active immune response develops in humans early in life. Certainly the earliest instance of innate active immunity develops in a neonate from placental transfer of maternal antibodies. This immunity is short term, and is designed to last until the child is self-immunocompetent. Innate immunity involves no preprogramming and serves to protect the host should pathogens penetrate the nonspecific defenses. The innate active system can be thought of as a cellular mechanism that becomes activated and sends to the site of infection destructive cells that deal directly but nonspecifically with infectious agents. The cells of the innate immune system possess no specific antigenic targeting receptors, but do have surface receptors that specifically recognize structural components of antibodies (Fc receptors) and/or the complement protein (C). These receptors are used to tag foreign bodies to make them stronger immunological targets. The cells of the innate active immune response utilize gradients of chemical attractants to direct first-line immune cells to the site of infection. This process is called chemotaxis. Most innate immune cells can physically engulf the foreign agent in the process of phagocytosis. Intracellular bodies of digestive enzymes, sequestered in storage granules, are released to chemically degrade the agent to small particles that can be rendered soluble or eliminated by the spleen. To stimulate this process, complexes of complement and specialized Abs coat infectious organisms and particles in a process termed *opsonization*, which tags them, identifying them as prime targets for phagocytosis. Figure 6-3 shows a diagram of the operation of a phagocyte. Because the intracellular degradative enzymes are sequestered in storage granules that are visible microscopically, the cells bearing them are called granulocytes.

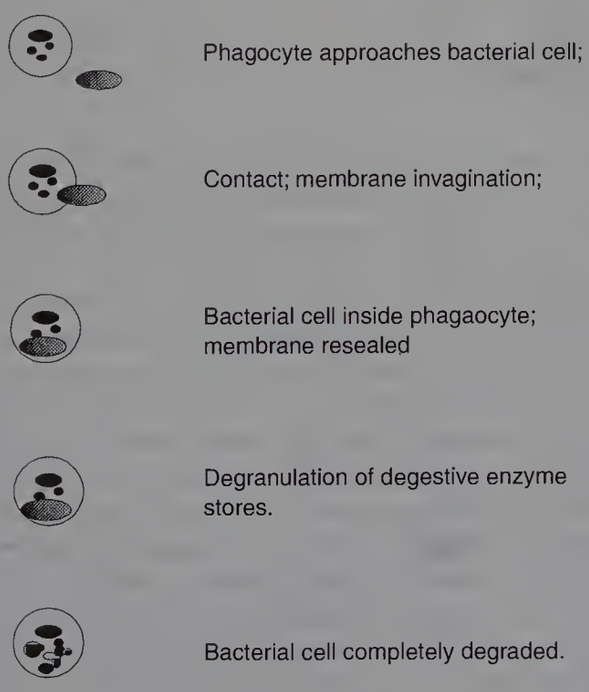


FIG. 6-3. *The process of phagocytosis.*

Complement[15]

Complement is a system of at least 20 separate proteins and cofactors that continuously circulate in the bloodstream. It complements the action of Ab in killing cells, thereby obtaining its name. Complement is the principal means by which Abs defend vertebrates against most bacterial infections. Some complement fragments also behave as chemoattractants to direct phagocytic cells to the site of infection and others enhance their ability to ingest and destroy microorganisms. Complement proteins remain inactive unless Ag and Ab are both present as complexes in the cellular fluids or are bound to cell surfaces. Components of complement are highly labile, and typically are stabilized by association with a cell membrane. When complement is activated, Ag and Ab bind to the cell surface along with complement, initiating a classical biological cascade reaction. The first component of complement is actually an enzyme that is activated by Ag and Ab binding. The enzyme acts upon components of the complement system itself, adding new fragments and splitting off others. Some of the split-off fragments are biologically active. Under Ca^{2+} and Mg^{2+} catalysis, components of the protein cascade bind sequentially to the complex. The pivotal component is the third protein in the cascade, C3. Its activation by its own cleavage is the central reaction in the entire complement activation sequence. C3 is cleaved by an enzyme, C3 convertase, into C3 and C3b. C3b goes on into the complement cascade; C3a stimulates the release of anaphylatoxin. There are two complement pathways, the classical pathway activated by Ag-Ab complexes, and the alternative pathway activated directly by microorganisms. In either case, the buildup of complement proteins on the bacterial cell membrane forms a toroid-shaped molecule that causes loss of membrane integrity and lysis, much like a polyene antibiotic. A diagram of the complement cascade is shown in Fig. 6-4.

Granulocytes[14]

If one views a granulocyte under the microscope, it is possible to observe dense intracytoplasmic granules. The granules contain inflammatory mediators and digestive enzymes that directly destroy invading pathogens, control the rate and pathway of migration of other cells by chemotaxis, and cause dilation of blood vessels at the infected site. The increased blood flow ensures that an ample supply of granulocytes and inflammatory mediators reaches the site of infection. There is a family of granulocytic cells, each member with its own specialized function. Under microscopic examination, some

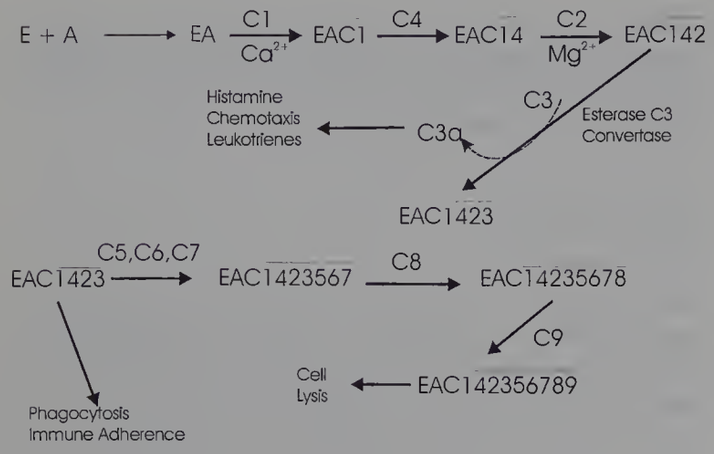


FIG. 6-4. *An overview of the complement activation sequence. E, bacterial cell; A, antibody; C, complement; bar over number, activated state of component that has enzymatic or other activity; bar over sequence, active state of the sequence with biological activity.*

granulocytes are seen to be multinuclear and some mononuclear. The configuration of the nuclear region provides a way of classifying the granulocytic cells. The entire group will be discussed below.

Neutrophils[14]

Polymorphonuclear cells (PMNs) or *polymorphonuclear leukocytes* are our primary innate defense against pathogenic bacteria. PMNs make up the majority of the leukocyte fraction in the bloodstream. Microscopically, PMNs have multiple nuclei. They respond to chemical motility factors such as complement mediators released from infected or inflamed tissues, and migrate to the site of infection by a process known as chemotaxis. There, they recognize, adhere to, and phagocytose bacteria and viruses. The phagocytic process is initiated by contact of the invader with the phagocyte cell membrane. Then, invagination of the membrane engulfs the particle, and the membrane is resealed with the particle inside the phagocyte. The contents of the granular bodies are then released within the phagocyte, killing the engulfed pathogen and enzymatically cleaving its remains into smaller pieces.

Macrophages and Monocytes[14-15]

Macrophages and monocytes are mononuclear cells that are capable of phagocytosis. In addition to the phagocytic capabilities, they biosynthesize and release soluble factors possessing inflammatory properties. Monocytes occur in the bloodstream, whereas macrophages are found within tissues. Sometimes, macrophages are restricted to a single type of tissue, and hence can be said to possess a true anatomic distribution. Special macrophages are found in tissues such as the liver, lungs, spleen, gastrointestinal (GI) tract, lymph nodes, and brain. These specific macrophages are either called histiocytes (generic term) or by certain specialized names (Kupffer cells in liver, Langerhans cells in skin, alveolar macrophages in lung, etc.). In the reticular connective tissues we call them the "mononuclear phagocyte system." Other macrophages exist free in the tissues, where they carry out more nonspecific functions. Macrophages are thought to have evolved from monocytes that once migrated into the tissues, then evolved to gain many more Fc Ab and complement receptors. Macrophages kill more slowly than neutrophils, but have a much broader spectrum. It has been estimated that more than 100 soluble inflammatory substances are produced by macrophages. These substances account for macrophages' prolific abilities to direct, modulate, stimulate, and retard the immune response.

Demonstrating one very specialized function, macrophages act as *antigen presenting cells* (APCs) (Fig. 6-5). APCs are responsible for the preprocessing antigens, amplifying the numbers of antigenic determinant units, and presenting these determinant structures to the programming cells of the immune system. APCs internalize an organism or particle, digest it into small fragments, and conjugate the fragments with molecules from the major histocompatibility complex (MHC). These are known as human leukocyte antigens (HLAs) in humans, and are responsible for self/nonself cell recognition. Once the APC has formed the Ag/MHC complex, it is placed on the macrophage's cell surface, allowing B lymphocytes and helper T cells to recognize the Ag via the B- and T-cell receptors. It is this step that transfers specificity and memory information from the determinant into the immune system. Under the regulatory influence of the helper T cells, B cells are stimulated to differentiate into plasma cells, that produce Ab. The helper T cells accelerate and retard the process as necessary.

Eosinophils[15]

Eosinophils are granulocytes that are capable of functioning as phagocytes, but in this capacity are much less efficient than neutrophils. Their name derives from their intense staining reaction with the dye eosin. Clues to the functions of eosinophils come from their behavior in different disease states. Eosinophil counts are elevated above normal in the tissues in many different diseases, but are recognized primarily for their diagnostic role in parasitic infections and in allergies. Eosinophils have a unique mode of action that leads to their extreme importance. Unlike neutrophils, eosinophils need not phagocytose a parasite to kill it. Indeed, some parasites are too large to undergo phagocytosis. Eosinophils physically surround the large parasite, forming a cell coat around the invader. Eosinophil granules release oxidative substances capable of destroying even large multicellular parasites. Hence, even when phagocytosis fails, a mechanism exists to destroy large parasites.

Mast Cells and Basophils

Mast cells and basophils release the inflammatory mediators commonly associated with allergy. Mast cells are especially prevalent in the skin, lungs, and nasal mucosa; their granules contain histamine. Basophils also contain histamine granules, but they are found circulating in the blood and not isolated in connective tissue. Both mast cells and basophils have high-affinity immunoglobulin E (IgE) receptors. A complex of an Ag molecule with a molecule of IgE binds to the high-affinity receptors on the mast cells. The binding causes the mast cell to degranulate, releasing mediators of the allergic response. Due to its association with hypersensitivity, IgE has been called ''reagin'' in the allergy literature. Diagnostically, IgE is elevated in allergy, systemic lupus erythematosus, and rheumatoid arthritis. In an interesting application of rational drug design, cromolyn sodium was synthesized and found to prevent mast cell degranulation and therefore block the allergic response.

Adaptive Immunity

If an invading pathogen escapes both the nonspecific and innate defenses, the *adaptive immune response* takes control. The adaptive immune response utilizes cells including the B and T lymphocytes, which possess surface receptors specific for each invading organism. Unlike the familiar one gene–one protein hypothesis, which could not possibly predict all possible permutations of antigenic structure, natural and synthetic, the adaptive immune system utilizes genetic recombination of DNA as a way of encoding its Abs. Lymphocytes have the ability to recognize an estimated 10^{16} different types of Ags through this genetic recombination mechanism, far more than a person is likely to encounter during one lifetime. Adaptive immunity is Ab-mediated immunity, based on circulating pools of Abs that react with and inactivate Ags. These Abs are found in the globulin fraction of the serum. Consequently, Abs are also referred to as immunoglobulins (Ig). The adaptive immune response has the unique property of ***memory***, by which the sensitivity, specificity, and memory for a particular antigen is retained so that future exposures will stimulate an enhanced response. Hence, the adaptive immune response differs from the innate in two respects: specificity and memory.

The adaptive immune response can be divided into two branches: humoral immunity and cell-mediated immunity (CMI). Humoral immunity is mediated by B lymphocytes

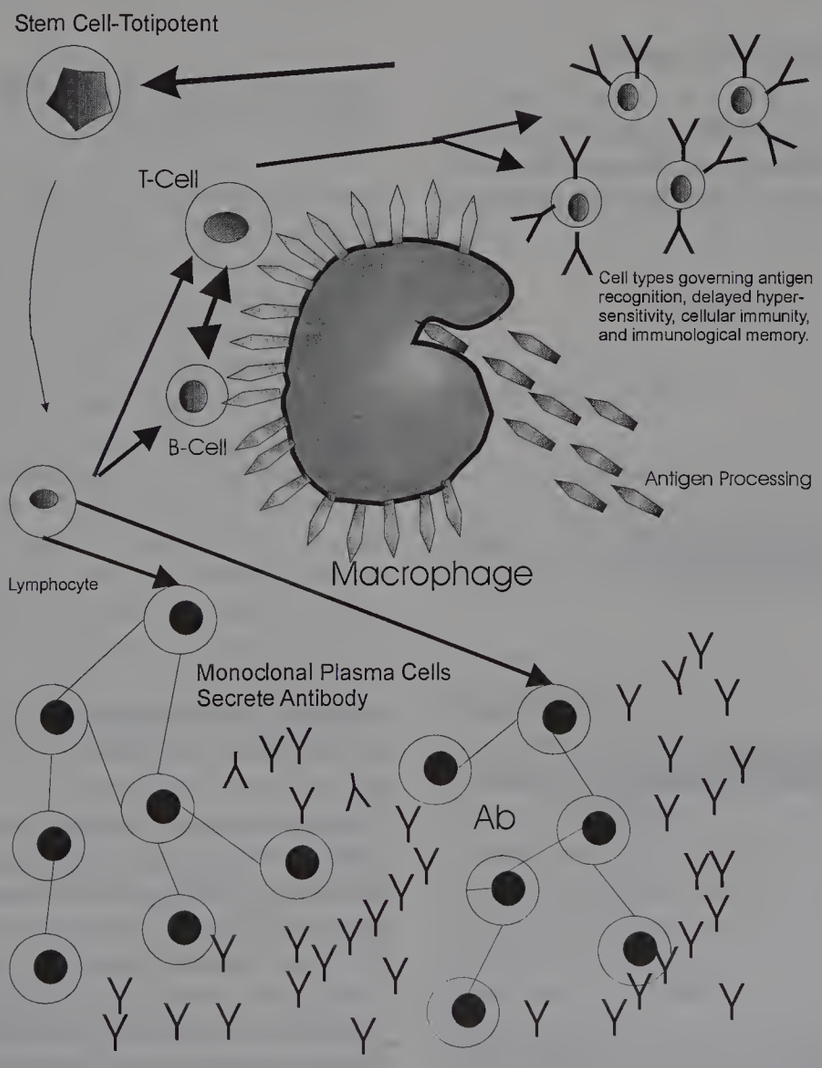


FIG. 6-5. *Antigen presenting cell and antibody formation.*

and activated B lymphocytes known as plasma cells. CMI is controlled by the T lymphocytes. The immune function of the T lymphocytes cannot be transferred by serum alone; it is necessary to actually have the T cells present, whereas the immunity of the humoral system can be isolated from the serum and transferred. T cells are specially tailored to deal with infections that are intracellular (such as virally infected cells), whereas B cells secrete soluble Abs that can neutralize pathogens prior to their entry into host cells. Both B and T cells possess specific receptors on their surfaces to recognize unique stimulatory Ags. When B cells are stimulated they express specific Igs that are capable of binding to the Ag. A fraction of the B-cell population does not differentiate into Ab-producing cells, but forms a pool of cells that retain immunological memory. T cells express a specific Ag receptor, the T-cell receptor, similar to the surface immunoglobulin receptor of B cells. This receptor is activated by a piece of processed Ag (presented with MHC). Activated T cells release soluble factors such as interleukins, cytokines, interferons, lymphokines, and colony-stimulating factors, all of which regulate the immune response. Interactions with some of these help to regulate the B-cell activity, directing the innate immune response.

Immunoglobulin Structure and Function

An Ab, or Ig, is composed of peptide chains with carbohydrate pendant groups. A schematic of the antibody IgG is shown in Fig. 6-6. The peptide chains form the quaternary structure of the immunoglobulin, while the carbohydrate moieties serve as antigen recognition groups and probably as conformation-stabilizing units. The general structure of the Ig looks something like a Y, with the Ag-binding regions at the bifurcated end. In this area are peptide sequences that are "programmable" by the immune system to allow the Ig to recognize a large number of Ags. These regions are also referred to as "hypervariable."

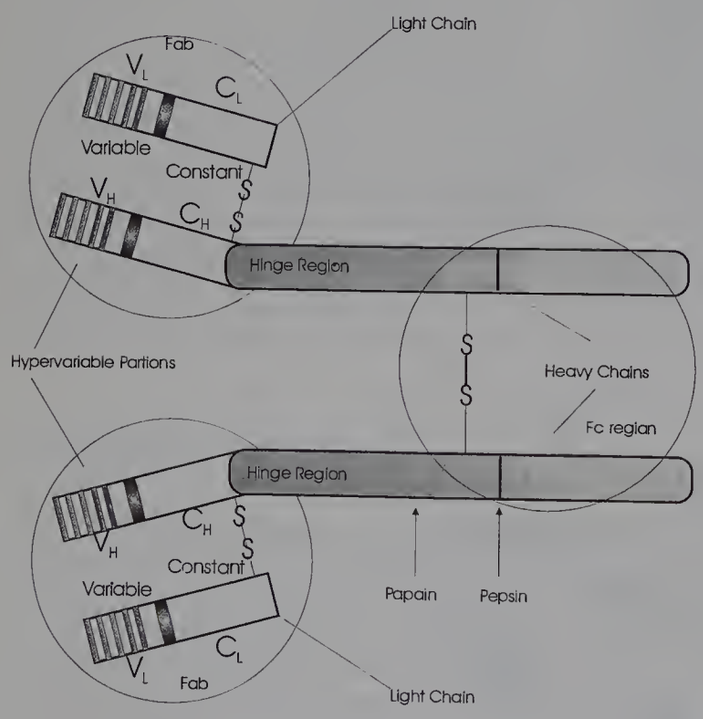


FIG. 6-6. *Diagram showing the peptide chains, variable regions, and enzyme cleavage points of Immunoglobulin G (IgG). The hypervariable regions are programmable.*

It has been known for many years that treatment with either of two enzymes, papain or pepsin, digests an Ab into fragments that are useful in understanding its molecular structure. Papain clips the Ab into two fragments that contain the Ag-binding regions. These fragments have been termed the Fab, or Ag-binding, fragment. The remaining part of the Ab after papain digestion contains two peptide chains linked by a disulfide bond. Treatment of the same Ab with pepsin yields the two Fab units joined by the disulfide bond, plus two of the distal peptide chains. These distal units have been crystallized, and hence are termed the Fc fragment for "crystallizable." The disulfide bond therefore provides a demarcation between the two molecular regions. The nomenclature of an Ab includes a high-molecular-weight, or heavy, chain on the inside and a low-molecular-weight, or light, chain on the outside.

Important Features of Antibody Molecular Structure[12,14]

As stated previously, the tip end of the Fab region is for Ag binding. There are two of these, so we say that the antibody is *bivalent* and can bind two Ag molecules. The overall amino acid sequence of the Ab dictates the conformation of the Ab. The peptide sequence for most Abs is similar, except for the hypervariable regions. The amino acid sequence at the end of the heavy chain (Fc) determines the class of the Ig (i.e., IgG, IgM, etc.). All Abs resemble each other in basic shape, but each has a unique amino acid sequence that is complementary to the antigen in a "lock and key" interaction (Ag/Ab specificity). Some, such as IgM, are pentamers of IgG. In reality, the lock-and-key model is too simplistic, and an induced fit model is preferred.

Antibody Production and Programming of the Immune System

The main element of the programming portion of the immune system is the macrophage. There are many types of macrophages throughout the body, some associated with specific tissues (e.g., dust cells in the lungs) and some free in the circulation. A common property of macrophages is *phagocytosis*, the capacity to engulf a particle or cell through invagination and sealing off of the cell membrane. The macrophages involved in the immune response set in motion a unique amplification process so that a large response is

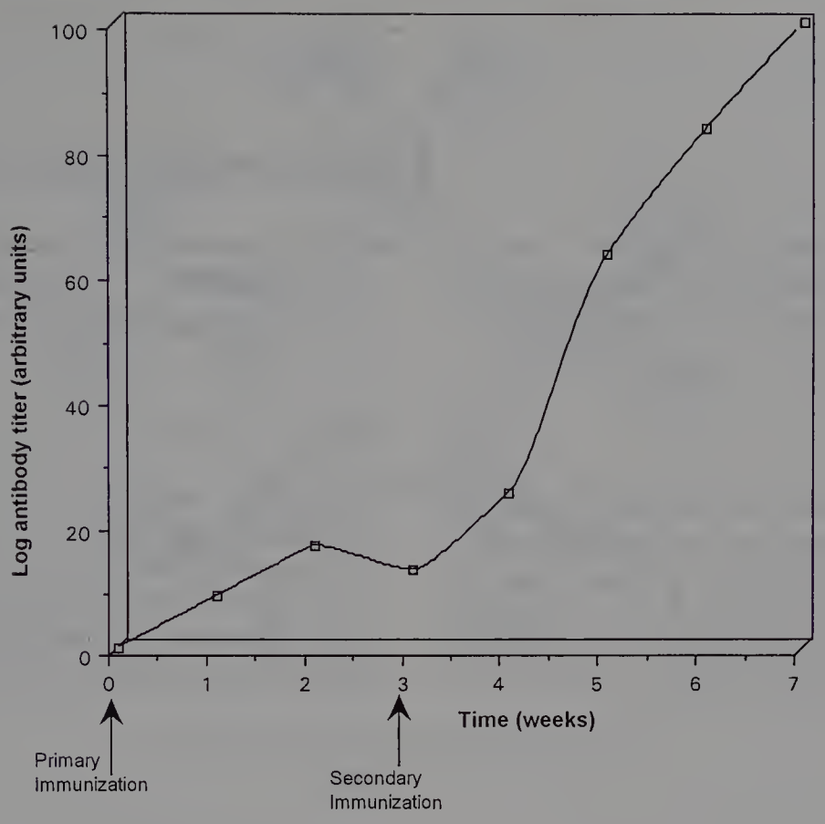


FIG. 6-7. *Serum antibody response to primary and secondary immunization doses.*

obtained relative to the amount of Ag processed. The macrophages engulf antigenic particles and take them into their cytoplasm, where the Ags are fragmented and made into multiple copies. The fragments are then combined with MHC displayed on the cell membrane of the macrophage and presented to the immune system. This process makes the macrophage an APC. The presented Ags interact with B cells and T cells, programming the former to differentiate into the actual Ab-producing cell, the plasma cell. Plasma cells are monoclonal (genetically identical) and produce monoclonal Ab. The process, from the totipotent stem cell, to the B and T cells, to the plasma cells, is depicted in Fig. 6-5.

Antibody Formation[15]

Figure 6-5 also indicates the actual Ab-producing steps. Plasma cells are programmed as cloned of Ab producers, which then amplify and produce copious amounts of Ab. The plasma cells can easily be regenerated if called upon to do so by the memory functions. At the bottom of the diagram a population of plasma cells is shown. These are identical and amplify and produce large quantities of Ab—proportionally much greater than the amount of Ag that was initially processed.

Anamnestic Response[15]

The programmed immune system has the property of memory, so that subsequent exposures to Ag are immediately countered. The actual memory response is referred to as the anamnestic response, a secondary response of high Ab production to a particular Ag due to ''memory cell'' formation as a result of the initial Ag stimulus (sensitization or immunization). The anamnestic response is demonstrated in Fig. 6-7.

Ag/Ab REACTIONS[14]

An Ab is bivalent, and an Ag is multivalent, so we can get lattice formation. The complex may be fibrous, particulate, matrix-like, soluble or insoluble, dictating how it must be disposed of. Four basic reactions describe these processes.

Neutralization

Neutralization is an immunological disposal reaction only for toxins (which are small and soluble). Once they bind the antibody they are no longer toxic, because their active site structures are covered and cannot bind their targets. Examples of toxins are tetanus toxin (*Clostridium tetani*) or *Clostridium botulinum* toxin. Both react with specific receptors in the inhibitory interneurons of the nervous system, causing spastic paralysis or flaccid paralysis, respectively. When an Ab covers the toxin's receptor-binding region, it can no longer bind to the neural receptors. The toxin is rendered

harmless. The toxin–Ab complex is then soluble and requires no further processing.

Precipitation

Often when a soluble Ag reacts with an Ab it forms an insoluble particulate precipitate. The complex cannot remain in the tissues and must be removed by phagocytosis.

Agglutination

Bacteria may be aggregated by binding to Abs that cover negative ionic surface charges and cross-linking cell structures (Fig. 6-8). The bacteria are thus immediately immobilized, limiting their ability to maintain an infection. This complex also must be removed by phagocytosis because of its particulate nature.

Bacteriolysis

Bacteriolysis is a complement-mediated reaction. As stated previously, the last five proteins in the activation cascade produce a "doughnut-shaped" molecule that punches holes in the cell membranes of the bacteria, acting like bacitracin or amphotericin B. The cell membranes lose integrity, cell contents leak out, membrane transport systems fail, and the cell dies.

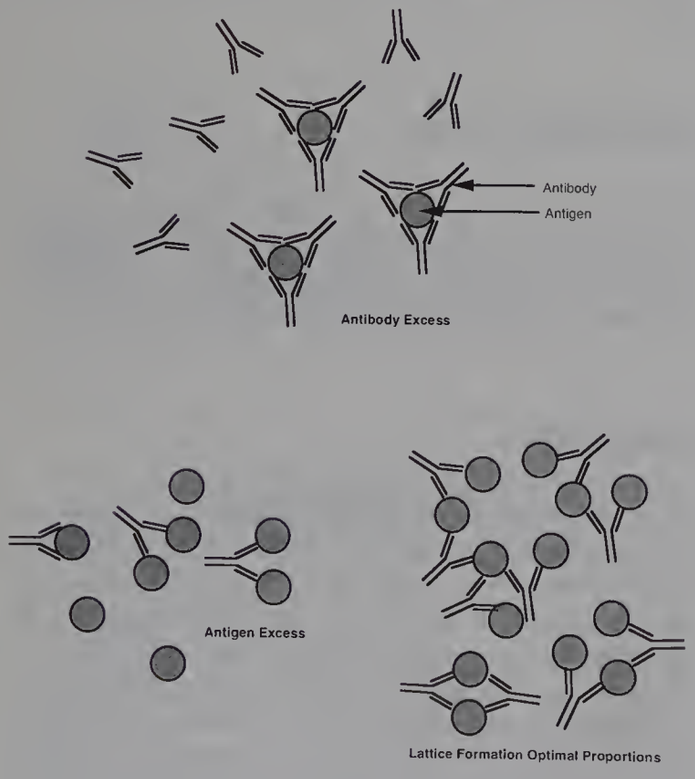


FIG. 6-8. Diagrammatic representation of possible complexes between antigen and antibody.

ANTIBODY TYPES AND REACTIONS

Ab types and reactions are classified based on a variation in a common section of the Fc fragment, which governs the biological activity in a general way.

IgG

IgG (Fig. 6-6) participates in precipitation reactions, toxin neutralizations, and complement fixation. IgG is the major (70%) human Ig. The Fab end fixes Ag, and the Fc fragment can fix complement to yield agglutination or lysis. IgG is the only Ig that crosses the transplacental barrier, so it provides maternal protection. IgG also causes Rh factor problems.

IgM

IgM (Fig. 6-9) participates in opsonization, agglutination reactions, and complement fixation. Opsonization, as stated before, is a "protein coating" or tagging of a bacterium that renders it more susceptible to phagocytosis. A complex of IgM plus complement is that protein. IgM is the first immunoglobulin formed during immunization, but it wanes and gives way to IgG. Since IgM is a pentamer, its agglutination potency is about 1,000 times that of IgG. IgM is also responsible for the A, B, and O blood groups (Fig. 6-9).

IgA

IgA is found in exocrine gland secretions (milk, saliva, tears), where it protects mucous membranes (e.g., in the respiratory tract). IgA therefore has a true anatomically specific distribution, unlike all other Abs. Once an IgA to Ag complex forms at the mucosal surface, other proteins are added to allow transport across mucosal surfaces, where other Abs are elicited. Recent research evidence shows that IgA can form the basis of an influenza vaccine administered by nasal spray; IgA is also the mediator of oral polio vaccination (the mucosal reaction gives way to systemic protection).

IgD

IgD is the putative "trigger Ab." It is thought to be the Ag receptor on Ab-producing cells that activates immunoglobulin production. There is a major problem in defining and characterizing its function since so little is present in the immune system.

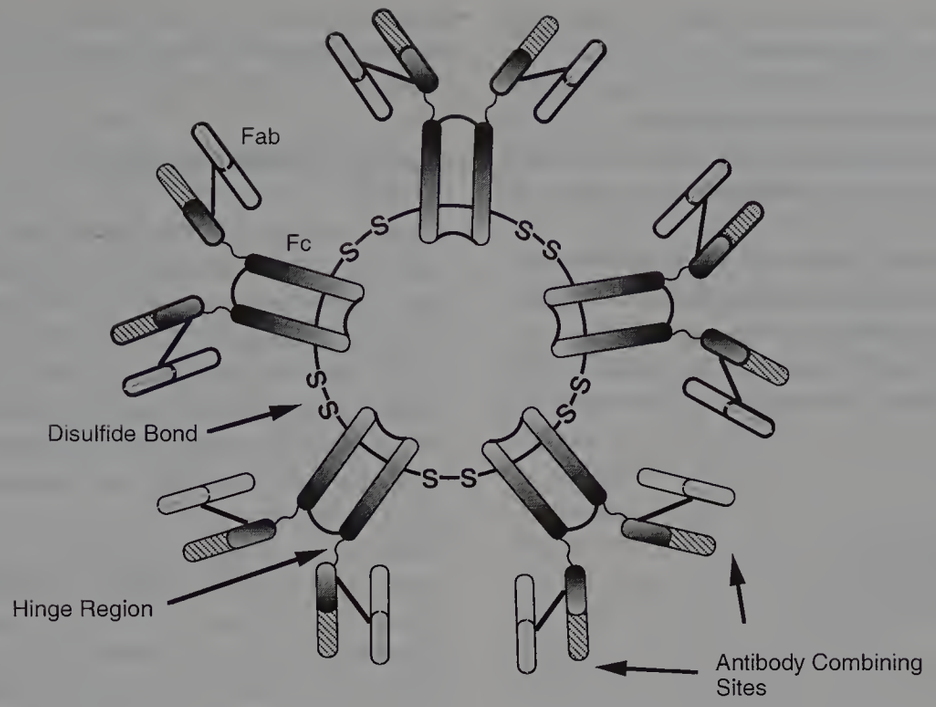


FIG. 6-9. *Diagram of the IgM molecule, showing the monomer subunit that resembles IgG.*

IgE

IgE is the Ab responsible for hypersensitivity reactions. IgE complexes have a high affinity for host cell surfaces, and can damage the host. High levels of IgE are found in persons with allergies of various types, as well as in autoimmune diseases. IgE is often called "reagin" in the allergy literature. The Fc fragment is responsible for the Ig-cell reactivity. An Ab + Ag reaction yields the typical Ab–Ag complex. The Fc portion of the antibody part of the complex binds to mast cells and stimulates them to release histamine, which causes bronchial constriction, itching, redness, anaphylaxis, and so on. An important observation is that isolated IgE Fc fragments can coat mast cell receptors, protecting them from activation. This discovery may suggest a novel allergy therapy for the future.

ACQUISITION OF IMMUNITY

We need to consider several cases of immunity when describing vaccines. Some are artificial and some are natural. *Natural immunity* is endowed by phagocytic white blood cells, lysozyme in tears, the skin, and so on. *Acquired immunity* is acquired after birth (or by passage from mother to fetus).

- *Active acquired immunity*: the host produces his or her own Ab

- *Naturally acquired active immunity*: occurs upon recovery from a disease (or from Ag exposure)
- *Artificially acquired active immunity*: occurs as a response from a sensitization by a vaccine or a toxoid
- *Passive acquired immunity*: the subject receives Ab from an outside source, such as a gamma-globulin injection, or by transplacental transfer
- *Naturally acquired passive immunity*: the temporary neonatal protection from maternal IgG that passes to the fetus in utero; this type of immunity is not long-lasting
- *Artificially acquired passive immunity*: an Ab is given by injection, for example, by an antitoxin or a gamma-globulin injection

IMMUNOBIOLOGICALS (VACCINES AND TOXOIDS)[11-13,16,17]

A vaccine may be defined as a solution or suspension of killed or live/attenuated virus, killed rickettsia, killed or live/attenuated bacteria, or Ags derived from these sources, which are used to confer active, artificially acquired immunity against that organism or related organisms. When administered, the vaccine represents the initial exposure, resulting in the acquisition of immunity. A subsequent exposure or challenge (a disease) results in the anamnestic, or memory, response.

METHODS OF VACCINE PRODUCTION

Vaccine production methods have varied greatly over the years, and are best discussed according to a parallel chronological and sophistication approach.

Killed (Inactivated) Pathogen

In this method, the normal pathogen is treated with a strong, denaturing disinfectant like formaldehyde or phenol. The process causes denaturation of the proteins and carbohydrates that are essential for the organism to live and infect a host, but if treated properly the surface Ags are left intact. The process must be done carefully to control the unwinding of proteins or carbohydrates by denaturation, since the preparation must be recognized as the original antigen. The main problems with killed pathogen vaccines are (1) if the vaccine is not inactivated totally, disease will result; (2) if the preparation is overtreated, vaccine failure usually occurs because denaturation occurs; (3) the production laboratory must grow the pathogen in large quantities in order to be commercially useful, putting lab technicians at risk; and (4) in the patient, abnormal and harmful responses, such as fever, convulsions, and death, may result. These vaccines typically are viewed as ''dirty'' vaccines, and some, like the pertussis vaccine, have been associated with problems serious enough to warrant their removal from the market.

Live/Attenuated Pathogens

The word *attenuated* for our purposes simply means ''low virulence.'' The true pathogen is altered phenotypically so that it cannot invade the human host, and it cannot get ahead of the host's immune system. Low pathogenicity strains such as these were originally obtained by passage of the microbes through many generations of host animals. The idea was that the animal and the pathogen, if both were to survive, needed to adapt to live with each other without either partner being killed. Poliovirus is attenuated in this fashion in monkey tissue. In the live/attenuated strain, antigenicity is still required, as is infectivity (polio vaccine yields an infection), but the host's immune system must be able to stay ahead of the infection. The key problems are (1) the vaccine cannot be used if the patient is immunocompromised, or if fever, malignancy, or immunosuppressive drug use are present; (2) these vaccines should not be used during pregnancy; (3) it used to be common for the attenuated organism to revert to the virulent strain, which was the reason for the failure of some early polio vaccines. Today, biological quality control is very stringent and these problems have been eliminated.

Live/Attenuated Related Strain

The live/attenuated related strain is antigenically related so that it can provide cross-immunity to the pathogen. For example, cowpox virus can be used in place of smallpox virus. The strains are antigenically similar enough that the host's immune system reacts to the related strain to provide protection against the normal pathogen. The main advantage is that we are not using a true pathogen, and the chance of contracting the actual disease is zero. The problem with such vaccines is that they cause an infection. Cowpox is known to spread to the central nervous system in $1/10^5$ cases, causing a potentially fatal form of meningitis.

Cellular Antigen from a Pathogen

In this method, the surface Ag (since that's what is recognized as foreign), is harvested from the pathogen, purified, and reconstituted into a vaccine preparation. These antigens can take a number of forms, including the carbohydrate capsule, as in *Neisseria meningitidis*; pili, as in *N. gonorrhea*; flagella from motile bacteria (the basis for an experimental cholera vaccine); or the viral protein coat, as in the vaccine for hepatitis B. Advantages of the method are that there is virtually no chance of disease, contamination, or reversion, and there are no storage problems. This method is currently

as close to a "perfect approach" as we have. A problem is that we need to grow the pathogen ourselves under careful control or we must rely on an unsure source. For example, hepatitis B vaccine was originally prepared from the serum of a controlled population of human carriers. Imagine the impact if one of the carriers developed another blood-borne disease. Additionally, these antigens are strain-specific (e.g., *N. gonorrhea* may require 1500 different pilar antigens). Acellular vaccines may be of lower antigenicity in the very young, and may require several injections for full immunological competence. To be safe and consistent, we need to identify the antigenic component. Given the complex nature of biological materials, this is not always easy or even possible.

Genetically Engineered Pathogens[15]

The techniques of genetic engineering have allowed the pharmaceutical industry to prepare absolutely pure surface Ags while totally eliminating the pathogenic organism from the equation. As shown in Fig. 6-10, the virus contains surface Ags designated by filled circles. Inside the viral capsule is a circular piece of DNA, containing genes for the various biological molecules of the virus. The diagram shows, at about 3 o'clock, a small piece of DNA that is designated to code for the surface Ag. The strategy is to isolate this piece of DNA and insert it into a rapidly growing expression vector for production of the surface protein. In this case, *E. coli* serves as an excellent vector. It contains a plasmid that can be removed, clipped open, and used as a cassette to carry the viral DNA. Additionally, *E. coli* can be grown in batch to produce the viral surface Ag. To begin, the DNA is removed from the virus and the plasmid is removed from the vector. Viral and bacterial nucleic acid is treated with a restriction endonuclease, which cleaves the DNA and plasmid at designated restriction sites. The viral DNA is cleaved into a number of fragments, each of which is ligated into the *E. coli* plasmid with a ligase enzyme. Plasmids are inserted into *E. coli* and the organism is grown in batch fermentation. The organisms containing the gene for the viral surface protein can be separated by screening and purified to serve as the ultimate Ag producer—free of contamination or pathogenic viral particles. The pure Ag may then be constituted into a vaccine and used in human hosts.

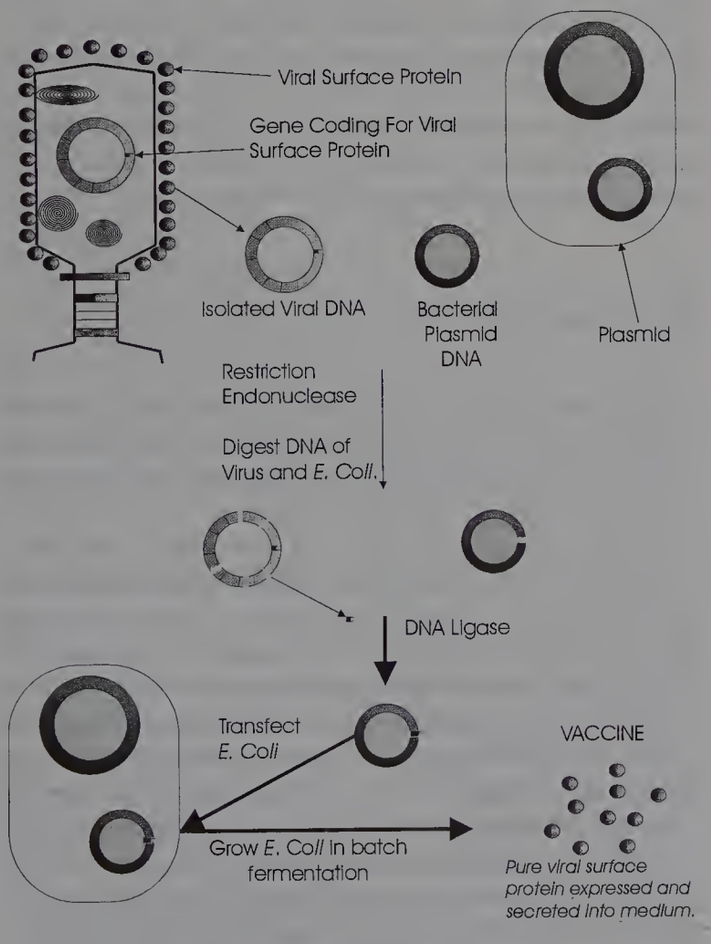


FIG. 6-10. *Simple schematic of genetic engineering of a vaccine.*

USE OF VACCINES IN COMBINATION: DOSING[4,15]

Types of Vaccine

There are three basic types of vaccine preparations that are used clinically: (1) A *simple* vaccine contains one strain of a disease-causing organism (e.g., plague vaccine, *Pasteurella pestis* and smallpox vaccine). (2) A *multivalent* vaccine is prepared from two or more strains of an organism that cause the same disease (e.g., polio is trivalent). Administration of the multiple strains are required for full protection because their Ags are not cross-immunizing. The immune system must mount a separate immune response to each strain. (3) A *polyvalent* vaccine is prepared from two or more organisms that cause different diseases. Polyvalent vaccines are given for convenience, primarily so that a child can be given one shot rather than several. The measles/mumps/rubella (MMR) vaccine is of the polyvalent type.

Types of Dosing

Vaccines can be administered according to a variety of dosing regimens, depending on the vaccine type and the purpose of the injection: (1) A *single-dose vaccine* is usually assumed to confer, with one shot, "lifetime immunity." The smallpox vaccine was a single-dose vaccine. (2) In a *multiple dosing* regimen, several doses are given spaced weeks or months apart to get maximum immunogenicity. Multiple dosing is usually done with inactivated vaccines since these are less antigenic. Multiple dosing is not the same as a booster dose. (3) A *booster dose* is administered years after the initial immunization schedule (regardless of single or multiple first dose). As a patient ages, Ab levels may wane. A booster is

used to bolster immunity. Also, boosters are used if a patient is known or suspected to have been exposed to a pathogen (e.g., tetanus). (4) Coadministration of a vaccine can be done only if it has been demonstrated that one vaccine does not interfere with another. (5) There are two physical forms of vaccines: a *fluid vaccine* is a solution or a suspension of the vaccine in saline of an aqueous buffer; the solution or suspension in an *adsorbed vaccine* is adsorbed on a matrix of aluminum or calcium phosphate. Like a sustained release dosage form, in theory there is longer exposure via a depot injection. The higher surface area of the matrix will be exposed to the immune system. Generally, adsorbed vaccines are preferred.

Pharmaceutical Principles of Vaccines

As expected for a live biological preparation, heat destroys live viral and bacterial vaccines. If the agent is not killed, the Ag may be altered. Like many biologicals, lyophilized vaccines are unstable after reconstitution. Ice crystals formed inside the protein structure during freeze-drying expand during thawing and disrupt the structure of the vaccine. Live vaccines can be inactivated by minute amounts of detergent. Detergent residue adhering to glassware is concentrated enough to act as a disinfectant. It is safe to use only plastic implements specified for the vaccine. Concentrated Ab suspensions (gamma globulins) are typical amphiphilic proteins and aggregate on storage. If injected, the particulates may cause anaphylaxis.

VIRAL VACCINES[5]

Smallpox Vaccine (Dryvax)

Smallpox vaccine is live *Vaccinia* (cowpox) virus grown on the skin of a bovine calf. Smallpox is a highly lethal and disfiguring disease that was common throughout history. Smallpox vaccine was used routinely in the United States, but today is no longer recommended. (There have been no reported cases of smallpox since the 1940s.) In 1982 smallpox was declared eradicated worldwide. With smallpox, the risks of the vaccine outweigh the benefits; the vaccine penetrates the central nervous system and potentially fatal encephalitis occurs in $1/10^5$ patients. After exposure to smallpox, the vaccine can be injected to lessen the severity of the disease.

Influenza Vaccine[19,20,22,23]

Influenza vaccine is a multivalent inactivated influenza virus or viral subunits (split vaccine). The virus is grown on chick embryo and inactivated by exposure to ultraviolet (UV) light or to formaldehyde. *Influenza* is a respiratory tract infection with a 2-day incubation period. The disease is devastating and may lead to pneumonia. Without the vaccine, influenza is common in epidemics and pandemics. To clarify, the *flu* is a gastrointestinal infection with diarrhea and vomiting. This infection requires weeks of incubation. Influenza is caused by two main genetic strains each year (A and B): type A is most common in humans; B is less common but more serious. The virus mutates very rapidly, and vaccines must be tailored yearly. The World Health Organization and the Centers for Disease Control monitor the migration of the disease from Southeast Asia, type the strains causing the occurrences, and order a vaccine to counter the organisms most likely to enter the United States. Each year's vaccine contains at least three different strains. Types A and B are further typed as N (neuraminidase) and H (hemagglutinin). According to this naming, a vaccine might be composed of A_N Texas–A_H Johannesburg–B_H Beijing.

Influenza vaccine is recommended yearly for high-risk groups—adults over 65 years of age, chronically ill people, immunosuppressed patients (AIDS), people with cardiopulmonary disease, and as of 1986 nursing home and hospital staff. As of about 1992, the vaccine has been recommended for college populations who live and study in close quarters. Influenza vaccine is safe and effective. The only side effects may be local pain and tenderness at the injection site, with low-grade fever in 3% to 5% of patients. Aspirin and acetaminophen are effective in combating these symptoms. Allergic reactions are rare, but may be seen in people allergic to eggs. Immunity to influenza vaccine takes 2 weeks to develop. Some people fear the vaccine because of reports of a strange paralysis and lack of nerve sensation associated with the 1976 swine flu vaccine. This problem, Guillain-Barré syndrome, was associated only with this 1976 vaccine and has not been associated with vaccines since.[23]

Polio Vaccines[24-26]

Polio is a dangerous viral infection that affects both muscle mass and the spinal cord. Some children and adults who contract polio become paralyzed, and some may die due to respiratory paralysis. Polio was the cause of the "infantile paralysis" epidemic of 1950–1953, which led to many paralyzed children and the specter of patients spending their lives in an iron lung. Serious cases of polio cause muscle pain and may make movement of the legs and/or arms difficult or impossible, and as stated above may make breathing difficult. Milder cases last a few days and may cause fever, sore throat, headache, and nausea. There has been an increase in interest in polio due to recent local outbreaks; large numbers of people are unimmunized. There are no drugs or special therapies to cure polio; treatment is only supportive. The symptoms of polio may reappear 40 to 50 years after a severe infection. This phenomenon is known as postpolio muscle atrophy (PPMA). PPMA is not a reinfection or reactivation

of the virus, but is probably a form of rapid aging in polio survivors. There are two types of polio vaccines.[27,28]

Inactivated Polio Vaccine (IPV; Salk Vaccine, 1954). IPV is a trivalent (strains 1, 2, 3) vaccine, grown in monkey kidney culture and subjected to elaborate precautions to ensure inactivation (typically formaldehyde is used). The vaccine is injected to give systemic immunity (typically four doses over 6 to 12 months), and boosters may be needed. IPV provides immunity from polio but does not stop polio carriers, who shed the virus from the oral and nasal cavities. Commonly today, the vaccine is given as a highly purified preparation called enhanced IPV (eIPV).

Trivalent Oral Polio Vaccine (TOPV; Sabin Vaccine, 1960). TOPV is a live attenuated vaccine containing polio strains 1–3. The culture is grown on monkey kidneys using an elaborate attenuation protocol. Oral administration of the vaccine yields a local GI infection, and the initial immune response is via IgA (mucosal, local to the GI tract). The immunity then becomes systemic as IgM and IgG form. A major caution with TOPV is that it is a live vaccine and must never be injected. TOPV is given to children as part of the routine immunization schedule. Clinics should avoid winter and spring administration because enteroviruses are common during these times and may interfere with the acquisition of immunity. Multiple dosing is necessary because interference among the three viral types could prevent acquisition of immunity and because the initial doses are given to very young children.

Advantages of TOPV over IPV are many. TOPV provides longer-lasting immunity and stronger immunity, both factors beneficial in children. The oral route of administration is simply easier. TOPV is cheaper than IPV, and stops carriers of polio. For healthy children and teenagers up to 18 years old, TOPV is the recommended form of the vaccine. In cases of neighborhood outbreaks or for those who travel to countries where polio is endemic, an extra dose is recommended. A good statistic to remember is that 90 out of every 100 people who get three or more doses of polio vaccine (either IPV or TOPV) will be protected against polio. The World Health Organization has advocated giving children eIPV instead of TOPV to prevent exposure of others by shedding of the virus through the nose and mouth.[29,30]

Rubella Vaccine (German Measles Vaccine)[26,30]

German measles is a disease that was once called the "3-day measles" and was considered a normal childhood illness. It is a mild disease with few consequences, except in the first trimester of pregnancy. In these mothers, rubella causes birth defects in 50% of cases. Defects may include heart disease, deafness, blindness, learning disorders, and spontaneous abortion of the fetus. Symptoms of rubella are a low-grade fever, swollen neck glands, and a rash that lasts for about 3 days. About one out of every ten women of childbearing age in the United States are not protected against rubella. Also, 20% of all adults escaped this normal childhood disease or are not vaccinated.

Rubella vaccine is a live/attenuated rubella virus produced in human diploid cell culture. It is administered as part of the normal immunization schedule at 15 months. Side effects are minimal but there may be some soreness and pain at the site of injection and stiffness of the joints.

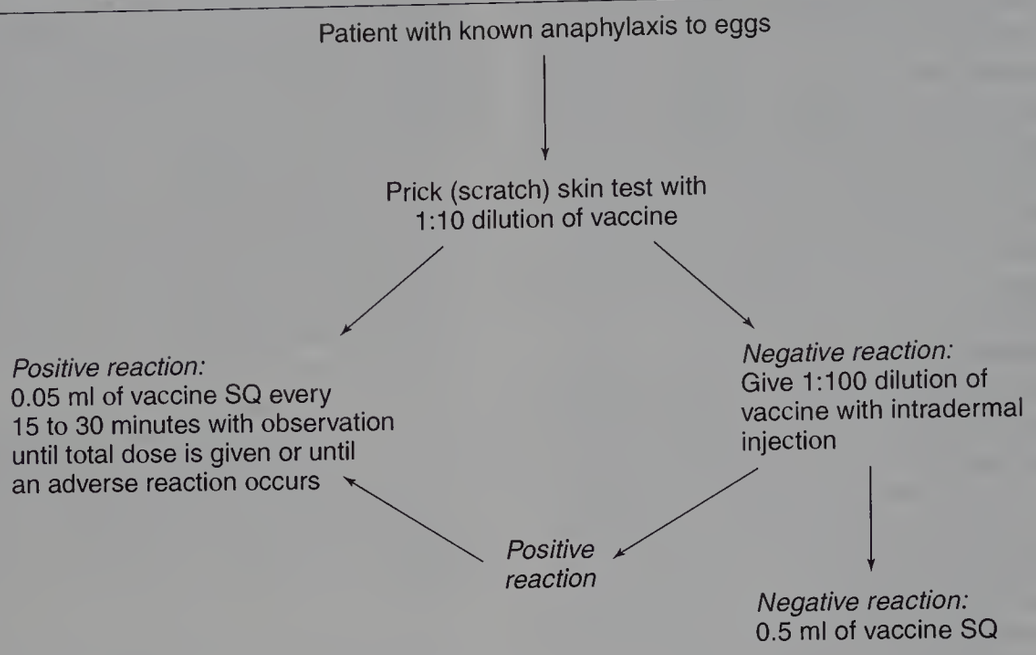
A problem with the vaccine is that administration of a live virus is contraindicated in pregnancy. Recommendations for handling rubella vaccination in women of childbearing age are as follows: on the first routine visit to the obstetrician–gynecologist the immune status should be checked. If the woman is not immunized against rubella, administer the vaccine and stress avoiding pregnancy for 3 months. If the patient is already pregnant, do not administer the vaccine. If exposure is suspected, the cord blood should be monitored for the presence of rubella antibodies. All unimmunized women should be vaccinated immediately after delivery of the baby.

Measles Vaccine (Rubeola Vaccine)[26,30]

Measles is a very serious, highly contagious disease. It causes a high fever, rash, and a cough lasting 1 to 2 weeks. In some patients extreme sensitivity to light occurs. The rash may occur inside the eyelids, producing a very painful condition. In the United States between 3000 and 28,000 cases occur each year, depending on factors such as weather and localized outbreaks. Outbreaks are very common in neighborhoods and schools. One out of ten children contracting measles will develop an ear infection or pneumonia. Measles may infect the brain (encephalitis) and lead to convulsions, hearing loss, and mental disability. In the United States, one out of every 500 to 10,000 children contracting measles die from it. Severe sickness and death is more common in babies and adults than in elementary schoolchildren or teenagers. Measles has been linked to multiple sclerosis. In 1977 a severe epidemic occurred in the United States, and 50,000 cases were reported. Only 60% of the population was vaccinated.

Measles vaccine is composed of live/attenuated measles virus that is grown on chick embryo culture with an attenuation protocol. The vaccine is required by law at 15 months and again at 11 to 12 years of age. It is useful to know that the vaccine can be administered after exposure to measles to lessen the disease severity. This is because Ab to the vaccine develops in 7 days, while the incubation period for the disease is 11 days. The vaccine should not be administered in pregnancy, and should be administered always with great care in women of childbearing age. Because measles vaccine is cultivated in egg medium, care must be used in patients who are allergic to eggs and egg products. For this reason, a test dose regimen is used. The regimen is shown in Table 6-1.

TABLE 6-1
PROTOCOL FOR USING MEASLES AND/OR MUMPS VACCINE IN A PATIENT ALLERGIC TO EGGS



Mumps Vaccine[41,42]

Mumps virus causes fever, headache, and a painful swelling of the parotid glands under the jaw. Mumps can be serious and is highly contagious. Prior to the vaccine the disease was passed from child to child with ease. The disease runs its course over several days. Between 4500 and 13,000 mumps cases occur as outbreaks in the United States every year. In severe cases, mumps may cause inflammation of the coverings of the brain and spinal cord (meningitis); this occurs in about 10% of infected persons. Swelling of the brain itself occurs in 1:200 patients. Men may experience a painful swelling of the testicles (orchitis), which may presage sterility. Women may experience a corresponding infection of the ovaries. Male teens are often sicker than other age groups of either sex. Mumps early in childhood has been linked to the development of juvenile diabetes.

The mumps vaccine is a live, attenuated virus grown on chick embryo culture with attenuation protocols. The vaccine is normally administered to children at 15 months of age and again at 11 to 12 years. Because mumps vaccine is cultivated in egg medium, care must be used in patients who are allergic to eggs and egg products. For this reason, a test dose regimen is used. The regimen is shown in Table 6-1.

Combination Products (Polyvalent Viral Vaccines)

If two or more vaccines have been demonstrated to be free of interference with each other, they can be administered as a mixture (polyvalent) for convenience. Examples of the polyvalent viral vaccines are measles/rubella (MR), rubella/mumps (RM), and measles/mumps/rubella (MMR). MMR is indicated for routine immunization at 15 months (not given at less than 1 year unless exposed or lacking immunocompetence). This is because maternal Abs interfere with development of vaccine immunity in small children. If the MMR is given at < 1 year, revaccination must be done at 15 months of age.

Chickenpox Vaccine[31-35,43]

Chickenpox is caused by the *Varicella zoster* virus. Every year, about 3.5 million people in the United States, mostly children, contract chickenpox. The incidence peaks between 3 and 9 years of age. Chickenpox is caused by a generalized rash, with 300 to 500 blister-like lesions occurring on the scalp, face, and trunk. Symptoms include loss of appetite, malaise, and headache. The disease is usually benign, but can lead to bacterial superinfection, pneumonia, encephalitis, and Reye's syndrome. About 50 to 100 previously healthy children die of the disease. About 2% of all cases occur in adults, who have more serious symptoms than children.

Varicella vaccine (Varivax) is derived from live virus from a child with natural varicella. The virus has been attenuated by passage through a series of guinea pig and human cell cultures. The final preparation is a lyophilized live, attenuated virus. The vaccine is well tolerated, with pain and redness at the injection site as the only side effects. The vaccine has shown tremendous success in reducing infections. It is recommended for children 12 months to 12 years old as a single dose. Adults who are exposed to chickenpox

TABLE 6-2

CRITERIA FOR USE OF *VARICELLA ZOSTER* (VZ) IMMUNE GLOBULIN[a]

1. Susceptible to VZ infection
2. Significant exposure within 96 hours
   a. Household contact
   b. Playmate contact (more than 1 hour play indoors)
   c. Hospital contact (in adjacent beds or same two to four bed room)
   d. School contact (adjacent desks in same classroom or same carpool)
   e. Transplacental contact (newborn born to mother who developed varicella less than 5 days prior or 48 hours after delivery)
3. Age <15, with administration to immunocompromised adolescents, adults, and other older patients on an individual basis
4. One of the following underlying illnesses or conditions
   a. Leukemia or lymphoma
   b. Congenital or acquired immunodeficiency
   c. Immunosuppressive treatment
   d. Newborn of mother with varicella (2e, above)
   e. Premature infant (>28 weeks' gestation) whose mother lacks a prior history of chickenpox
   f. Premature infants (<28 weeks' gestation or ≤1000 g) regardless of maternal history

[a] Patients should meet all four criteria.

should continue to receive varicella zoster immune globulin (VZIG). Table 6-2 gives the protocols for VZIG use in adults and children. In public health terms, it is estimated that varicella vaccine could save $348 million a year in health care costs and lost work time.

Hepatitis Vaccines[36-40,44-49]

Hepatitis is a complex of diseases that causes fever, nausea, abdominal pain, jaundice, liver failure, and death. There are four clinically recognized types (A, B, C, and E).

Hepatitis A virus (HAV; infectious hepatitis) is an acute disease with an abrupt onset. About 15 to 50 days of incubation are required before the disease becomes clinically noticeable. The disease lasts several weeks and is followed by complete recovery. Hepatitis A is transmitted when the virus is taken in by mouth. The fecal/oral route and close contact, unwashed food, and contaminated water account for most routes of transmission. The sexual anal/oral route is also a route of spread. Children under the age of 3 frequently have no symptoms but transmit the disease to adults in child-care centers. An injection of hepatitis A immune globulin is one way of preventing the disease, but is effective only for about 30 days. The hepatitis A vaccine (Havrix) is an inactivated preparation that is produced by propagation of the virus in cultured human diploid cells, and then inactivating with formalin. The course of immunization involves two injections over a 4-week period and a booster 12 months after the first injection. The vaccine is recommended for persons traveling outside the United States, except to Australia, Canada, Japan, New Zealand, and Western Europe; for those with chronic liver disease; for persons living in an outbreak zone; for people who inject medications; and for persons engaging in high-risk sexual activity. Child-care workers caring for children under 2 years should also receive Havrix. It is also used heavily in developing countries with poor sanitation. Side effects are minor and usually are limited to soreness at the injection site and fever.

Hepatitis B virus (HBV), the cause of serum hepatitis, is a much more insidious, chronic disease, transmitted by needles, mucosal contact, blood, or high-risk sexual activity. The highest risk for contraction of hepatitis B is among IV drug abusers. The disease is linked to cirrhosis and liver cancer. There are about 200,000 new cases reported per year in the United States; of these, 10% become carriers, one-fifth die from cirrhosis, and 1000 die from liver cancer. The hepatitis B vaccine was first introduced in 1981. Initially, it was prepared as an inactivated vaccine from the plasma of carefully screened human, high-titer carrier/donors. In 1986 the recombinant DNA (rDNA) vaccine (Engerix B, Recombivax) was introduced to the market. The rDNA vaccine contains only viral subunits, and may be used with hepatitis B immune globulin in a postexposure setting to boost the ability of the host to resist the infection. In adults, three doses should be given, at 0, 1, and 6 months. In children, the vaccine is given at birth, 1 month, and 9 months. Administration may be delayed in premature infants, whose immune systems are not fully developed. If not immunized at birth, a child should receive three doses by 18 months. If the mother tests positive for hepatitis B, the vaccine plus the immune globulin must be given at or shortly after birth. The vaccine is 95% effective and is typically without side effects. A number of high-risk groups have been identified: health care workers, student health care workers, people living in high-risk environments, and dentists. They should receive a three-dose course of the vaccine. In most other cases, a physician can make the judgment if a patient is high risk or not. Side effects of the vaccine are minor.

Hepatitis C virus (HCV) was once called hepatitis non-A, non-B, but has been recognized as a separate entity. HCV infection is spread primarily by the parenteral route (transfusions), and unlike HBV maternal–fetal and sexual transmissions are uncommon. Acute infection may show no symptoms; fewer than 25% of patients develop full-blown hepatitis. Unfortunately, 50% to 60% of those with HCV infection develop chronic hepatitis. This is often manifested by periodic increases in hepatic enzyme levels. Cirrhosis develops in 20% of chronic infectees; this usually requires 15 to 20 years to develop. Patients with HCV are at risk for hepatocellular carcinoma. Estimates are that there are 150,000 to 170,000 new cases in the United States per year. IV drug users, transfusion patients, and health care workers are at highest risk.

Development of an HCV vaccine proved difficult, but was accomplished in 1998. There are 15 genotypes, and the virus has the ability to change within the host's body. A new ap-

proach using genetic material from the virus, analogous to the approach to the influenza vaccine, is said to be promising.[54]

Hepatitis E virus (HEV) causes disease clinically indistinguishable from hepatitis A. Symptoms include malaise, anorexia, abdominal pain, arthritis-like symptoms, and fever. Distinguishing HEV from HAV must be done genetically. The incubation period is 2 to 9 weeks. The disease is usually mild and resolves in 2 weeks, with no sequelae. The fatality rate is 0.1% to 1%, except in pregnant women where the rate soars to 20%. No outbreaks have been reported in the United States as of 1996. There is no vaccine against HEV.

BACTERIAL VACCINES[50-53]

Pertussis Vaccine

Pertussis, also known as whooping cough, is a highly communicable infection caused by *Bordetella pertussis*. *B. pertussis* produces an endotoxin that causes a spectrum of symptoms in a host. Pertussis occurs mainly in children, and there is no effective treatment once the disease becomes manifest. *Bordetella* endotoxin attacks the tracheal mucosa and causes extreme irritation. The inflammatory responses produce the characteristic "whooping inspiration" associated with pertussis. The swollen and irritated tissues may lead to choking in children. The cough may last for months, and is often called the "hundred-day cough." About 4200 cases of pertussis occur yearly in the United States. Pertussis is most dangerous to babies (<1 year old). Even with the best supportive medical care, complications occur. At least 50% of pertussis patients must be hospitalized, 16% get pneumonia, 2% develop convulsions, and one in 200 babies will die or have lifelong complications.

Pertussis vaccine has been highly controversial in recent years. The original vaccine consisted of killed pertussis bacilli (*B. pertussis*) and was considered somewhat "dirty." Side effects such as fever and convulsions were common, and it was decided by health authorities in the United States, Japan, and the United Kingdom that the risk of the vaccine outweighed the risk of contracting the disease. In all three of these countries pertussis vaccine was removed from the routine immunization schedules. Almost immediately pertussis, which had been held in check, began to occur in epidemics. In 1992 a new vaccine was developed that consists of bacterial fractions. This vaccine, called Acell-Immune, is safe and highly effective, and has been added to the routine immunization schedule. The vaccine is adsorbed, and is used for routine immunization as the polyvalent preparation diphtheria–tetanus–pertussis (DTP) (at 2, 4, 6, and 15 months, and at 4 to 6 years). Pertussis vaccination is recommended for most children.

Haemophilus Influenzae Conjugate Vaccine (Hib Conjugate)

H. influenzae type B (Hib) causes the most common type of bacterial meningitis and is a major cause of systemic disease in children less than 6 years old. The chances of contracting the disease are about 1:200. Of these contractees, 60% of all patients develop meningitis while 40% display systemic signs. Hib is a tremendous problem in day-care centers, where the risk of contracting the disease is 400 times greater than in the general population. Hib has approximately a 10% mortality rate, and one-third of all survivors have some sort of permanent damage such as hearing loss, blindness, or impaired vision. Hib can also cause a throat inflammation that results in fatal choking, or ear, joint, and skin infections.

Hib conjugate vaccine (HibCV) is a sterile, lyophilized capsular polysaccharide from *H. influenzae* type B. The polysaccharide capsular material is covalently linked to diphtheria toxoid or another bacterial polysaccharide. The conjugation produces a stronger, longer-lasting response through the adjuvant effect. HibCV is safe and almost completely effective, and is a mandatory part of the childhood immunization schedule.

Tuberculosis Vaccine

Tuberculosis (TB) is a serious disease caused by *Mycobacterium tuberculosis*. The organism becomes established in the lungs and forms walled-off abscesses that shield the bacterium from the immune system. The disease is diagnosed by a chest x-ray. Until the 1940s people with TB were sent to sanatoria, special hospitals to isolate TB patients. The vaccine is referred to as the bacillus Calmette-Guerin (BCG) vaccine, and is a live/attenuated strain of *Mycobacterium bovis*. The vaccine is of questionable efficacy, and has been judged to be only 50% to 77% effective. The duration of protection is highly questionable. The incidence of TB in the United States is so low that the vaccine is not indicated in most cases. The vaccine is recommended for health care workers and persons in close contact with infected patients.

An adverse effect of the BCG vaccine includes a positive TB skin test. A red blister forms within 7 to 10 days, then ulcerates and scars within 6 months. BCG is a live vaccine, so it cannot be administered to immunosuppressed patients, burn patients, or pregnant women unless exposed (and even then not in the first trimester).

TOXOIDS

Toxoids are detoxified toxins used to initiate active immunity (i.e., create an antitoxin). They are typically produced by formaldehyde treatment of the toxin. They are safe and unquestionably efficacious.

Disease States

All of these diseases are produced not by a bacterium, but by an exotoxin produced by that organism. For example,

powerful exotoxins are produced by *Corynebacterium diphtheriae* and *Clostridium tetani*. The exotoxins are the most serious part of the disease. In both of the above disease states, survival does not confer immunity to subsequent infections, so lifelong vaccine boosters are needed.

In diphtheria, the exotoxin causes a pseudomembrane to be produced in the throat; the membrane then adheres to the tonsils. The organism releases a potent exotoxin that causes headache, weakness, fever, and adenitis. Severe diphtheria carries a 10% fatality rate. Only a few cases per year are reported in the United States.

Tetanus is caused by a skin wound with anaerobic conditions at wound site. A potent exotoxin (tetanospasmin) is produced that attacks the nervous system. The first sign of disease is jaw stiffness; eventually the jaw becomes fixed (lockjaw). The disease is essentially a persistent tonic spasm of the voluntary muscles. Fatality from tetanus is usually through asphyxia. Even with supportive treatment, tetanus is about 30% fatal in the United States. Recovery requires prolonged hospitalization. There have been 50 to 90 cases reported per year in the United States since 1975. There is no natural immunity to the exotoxin. The general rule of thumb is to follow the childhood immunization schedule carefully and immunize all persons of questionable immunization status. Adults require a booster every 10 years; patients who cannot remember their last one are due for another.

Clinically Used Toxoids

Diphtheria toxoid: A fluid preparation that is rarely used (designated "D").

Adsorbed diphtheria toxoid: In an identified household case of diphtheria, treat all asymptomatic, unimmunized household contacts with (1) the toxoid and (2) benzathine penicillin G or erythromycin. Some previously immunized persons are very sensitive to the toxoid; exercise care.

Tetanus toxoid: A fluid, designated "T."

Adsorbed tetanus toxoid (T, adsorbed): This toxoid lasts approximately 10 years. A booster is recommended if injured or every 5 years. Reactions other than pain at the site of injection are rare. Table 6-3 shows the tetanus prophylaxis protocols for routine wound management.

Diphtheria and tetanus toxoid: A fluid toxoid.

Adsorbed diphtheria and tetanus toxoid: For children less than 7 years old and who should not get pertussis vaccine (designated DT).

Adsorbed tetanus and diphtheria toxoid for adults (designated Td): For children older than 7 years and for adults. It has a lower level of diphtheria toxoid (1/15) because older children are much more sensitive to "D." Used for immunization of schoolchildren.

DTP: D and T toxoids with pertussis vaccine.

DTP adsorbed: Used for early vaccination of infants in repeated doses, starting at 2 to 3 months.

TABLE 6-3

SUMMARY GUIDE TO TETANUS PROPHYLAXIS IN ROUTINE WOUND MANAGEMENT

History of Tetanus Immunization	Clean Minor Wounds		All Other Wounds	
	TD[a]	TIG	TD[a]	TIG
Uncertain	Yes	No	Yes	Yes
0–1 dose	Yes	No	Yes	Yes
2 doses	Yes	No	Yes	No[b]
3 or more doses	No	No	No[d]	No

TD, tetanus-diphtheria; TIG, tetanus immunoglobulin.

[a] For persons aged 7 years and older, combined tetanus-diphtheria toxoid is preferred to tetanus toxoid alone.

[b] Yes, if the wound is more than 24 hours old.

[c] Yes, if more than 10 years has passed since the last dose.

[d] Yes, if more than 5 years has passed since the last dose. More frequent boosters are not needed and can add to side effects.

Facts About DTP

Three or more DTP shots will

- keep 70% to 90% of children from getting pertussis if exposed to it, and if pertussis develops, it is milder.
- protect 85% of children from contracting diphtheria for at least 10 years.
- protect 95% of children from getting tetanus for 10 years.

TABLE 6-4

RECOMMENDED IMMUNIZATION SCHEDULE FOR ROUTINE IMMUNIZATIONS IN HEALTH FACILITIES IN THE UNITED STATES, 1997

Age	Immunization/Procedure Needed
Birth	Hepatitis B—dose 1 (0–2 months acceptable)
1 week	First office examination
1 month	Newborn screening exam, hepatitis B—dose 2 (1–4 months acceptable)
2 months	DTP, IPV, Hib conjugate
4 months	DTP, IPV, Hib conjugate
6 months	DTP, TOPV (opt.), Hib conjugate
9 months	Hepatitis B—dose 3 (6–18 months acceptable)
12 months	Tuberculosis tine test, varicella (12–18 months acceptable)
15 months	MMR, Hib conjugate (12–15 months acceptable)
18 months	DTP (15–18 months); TOPV (12–18 months acceptable)
2 years	Complete physical examination
4–6 years	DTP, TOPV, MMR (or alternatively MMR @ 11–12 years)
11–12 years	MMR, Td (11–16 years), VAR*, HepB*
Over 16 years	Td every 10 years

* Catch-up vaccine for previously unimmunized.

TB tine test, Mantoux tuberculosis skin test; MMR, measles/mumps/rubella; DTP, diphtheria–tetanus–pertussis; TOPV, trivalent oral polio vaccine; IPV, inactivated polio vaccine; Hib conjugate, *Haemophilus influenzae* type B conjugate vaccine.

All of these recommendations of the Advisory Committee on Immunization Practices are given in the *Morbidity and Mortality Weekly Report* (*MMWR*) 46(2):35, 1997.

- There are few problems or side effects from DTP. There may be a mild fever, soreness, and redness for 1 to 2 days, and a child may be cranky, drowsy, and have little appetite for 1 to 2 days.
- In 1:100 to 1:1000 cases a temperature of 105° or more develops, along with prolonged, unusually high-pitched crying. Less often (1:750), convulsions or shock develop.
- The benefits outweigh the risks in most people.

Table 6-4 shows the Routine Childhood Immunization Schedule formulated by the Advisory Committee on Immunization Practices (1997). This schedule should be followed for all children and young adults regardless of economic circumstances.[55-61]

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CHAPTER 7

Anti-Infective Agents

Arnold R. Martin

Selective toxicity, the property of certain chemicals to destroy one form of life without harming another, is the cornerstone of modern antimicrobial chemotherapy. This concept is largely credited to Paul Ehrlich, who discovered the selective-staining properties of certain antibacterial dyes and the antiparasitic activity of organic arsenicals shortly after the turn of this century. Although the compounds discovered by Ehrlich have largely been replaced by safer and more effective agents, his ideas paved the way for the advent of the sulfonamides and penicillin and the elucidation of the mechanisms for their selective toxicity. The local antimicrobial properties of phenol and iodine were discovered before the beginning of the century, but the only selective systemically useful chemotherapeutic agents known before Ehrlich's time were herbal remedies, such as cinchona for malaria and ipecac for amebic dysentery.

During the first quarter of the 20th century, the development of useful chemotherapeutic agents was largely confined to organic compounds containing heavy metals, such as mercury, arsenic, and antimony; dyes, such as gentian violet and methylene blue; and a few modifications of the quinine molecule. Although some of these discoveries represented significant advances, there were many drawbacks to them as well. The second quarter of this century ushered in the period of greatest progress in antimicrobial chemotherapy. The sulfonamides and sulfones (Chap. 8), improved phenolic compounds such as hexachlorophene, synthetic antimalarial agents (Chap. 9), the surfactants and, of greatest importance, numerous antibiotics (Chap. 10) were introduced into medicine during this time. The first indication that certain antineoplastic agents might exhibit selective toxicity to tumor cells also began to appear toward the end of this period.

Chemotherapeutic agents may be classified according to their chemical type, their biologic properties, or their therapeutic indications. A combination of these classification systems is used to organize the chapters covering chemotherapeutic agents in this book. Where several chemically divergent compounds are indicated for a specific disease or group of diseases, the medical classification is used, and the drugs are subclassified according to chemical type. On the other hand, when the information is best unified and presented in a chemical or biologic classification system, as for the sulfonamides or antibacterial antibiotics, then one of these systems is used.

This chapter covers a broad range of anti-infective drugs, including local anti-infective agents (alcohols, phenols, oxidizing agents, halogen-containing compounds, cationic surfactants, dyes, and mercury compounds), preservatives, antifungal agents, synthetic antibacterial agents, antitubercular agents, antiprotozoal agents, and anthelmintics. A separate chapter is devoted to antiviral agents (Chap. 11). Those groups of antibiotics employed for the specific treatment of tuberculosis (cycloserine, viomycin, capreomycin, and rifampin) and fungal infections (griseofulvin and the polyenes) are also covered in this chapter. A separate chapter is devoted to antibacterial antibiotics (Chap. 10), whereas antineoplastic antibiotics are presented in Chap. 12.

LOCAL ANTI-INFECTIVE AGENTS

Local anti-infectives, or germicides, may be classified as antiseptics and disinfectants and constitute an important, if underappreciated, group of drugs. Antiseptics are compounds that kill (*-cidal*) or prevent the growth of (*-static*) microorganisms when applied to living tissue. An ideal antiseptic would exert a rapid and sustained lethal action against microorganisms (the spectrum may be narrow or broad depending on the use); have a low surface tension; retain activity in the presence of body fluids, including pus; be nonirritating to tissues; be nonallergenic; lack systemic toxicity when applied to skin or mucous membranes; and not interfere with healing. It is doubtful that any antiseptic available today fully meets all of these criteria.

A few antibiotics, generally ones that are poorly absorbed through the skin and mucous membranes, have been used topically for the treatment of localized infections, for which they are uniquely effective. In general, the topical use of antibiotics has been limited because of the possibility that allergic reactions may result or that resistant strains of microorganisms may emerge, thus reducing their usefulness for the treatment of more serious, systemic infections.

A *disinfectant* is an agent that prevents infection by the destruction of pathogenic microorganisms when applied to inanimate objects. The ideal disinfectant exerts a rapidly lethal action against all potentially pathogenic microorganisms and spores, has good penetrating power into organic matter, is compatible with organic compounds (especially soaps), is not inactivated by living tissue, is noncorrosive, and is esthetically desirable (nonstaining, odorless, and such).

Local anti-infective drugs continue to be widely used by the lay public and by members of the medical profession even though the effectiveness of most such agents has not been firmly established. In certain situations, the use of a disinfectant or antiseptic may actually be harmful. Because of the availability of over-the-counter (OTC) germicides, the pharmacist is in a unique position to advise the public concerning the rational use of disinfectants and antiseptics. Although the effectiveness for most germicides is either lacking or yet to be established, there are a few compounds that have been shown to be effective in controlled studies. A major problem is that adequate standardized methods for evaluating antiseptics, in particular, have been established only very recently.

Numerous classes of chemically divergent compounds possess local anti-infective properties.

ALCOHOLS AND RELATED COMPOUNDS

Various alcohols and aldehydes have been used as antiseptics and disinfectants. Ethyl and isopropyl alcohols are still widely used for these purposes.

ANTIMICROBIAL ACTION AND CHEMICAL STRUCTURE

The antibacterial potencies of primary alcohols (against *Staphylococcus aureus*) increase with molecular weight up to C_8, where the "cutoff" is reached. Beyond this point, water solubility is less than the minimum effective concentration, and the apparent potency deceases with molecular weight. Branching decreases antibacterial potency; hence, the isomeric alcohols follow the order of primary > secondary > tertiary. Nonetheless, isopropyl alcohol is used commercially instead of normal propyl alcohol, because it is cheaper. Isopropyl alcohol is slightly more active than ethyl alcohol against vegetative bacterial growth, but both alcohols are largely ineffective against spores.

Alcohol, USP (Ethanol, *Spiritus vini rectificatus*, Wine Spirit

Ethanol is a clear, colorless, volatile liquid having a burning taste and a characteristic pleasant odor. It is flammable and miscible with water and most organic solvents. The commercial product contains ~95% ethanol by volume because this concentration forms an azeotrope that distills at 78.2°C. Alcohol has been known for centuries as a fermentation product from grain and other carbohydrate sources. It can also be prepared synthetically by the sulfuric acid-catalyzed hydration of ethylene.

Control of the use of alcohol in the United States is exercised by the Treasury Department, which has provided the following definition:

> The term "alcohol" means that substance known as ethyl alcohol, hydrated oxide of ethyl, or spirit of wine, from whatever source or whatever process produced, having a proof of 160 or more, and not including the substances commonly known as whiskey, brandy, rum or gin.

Denatured alcohol is ethanol that has been rendered unfit for use in intoxicating beverages by the addition of other substances. Completely denatured alcohol contains added wood alcohol (methanol) and benzene and is unsuitable for either internal or external use. Specially denatured alcohol is ethanol treated with one or more substances so that its use may be permitted for a specialized purpose. Examples are iodine in alcohol for tincture of iodine, methanol and other substances in mouthwashes and after-shave lotions, and methanol in alcohol for preparing plant extracts.

The primary medicinal uses of alcohol are external: as an antiseptic, preservative, mild counterirritant, or solvent. Rubbing alcohol is employed as an astringent, rubefacient, refrigerant, and mild local anesthetic. Ethanol has been injected near nerves and ganglia to alleviate pain of neuralgias. It has low narcotic potency and has been used internally in diluted forms as a mild sedative, as a weak vasodilator, as a carminative, and as a source of energy.

Alcohol is, by far, the most widely abused of all recreational drugs. It is metabolized in the body by a series of oxidations, first to acetaldehyde, then to acetic acid (or active acetate), and finally to carbon dioxide and water. A widely employed form of aversion therapy for the prevention of alcohol abuse by alcoholics utilizes the aldehyde dehydrogenase inhibitor disulfiram, whose effectiveness results from the accumulation of acetaldehyde and its attendant toxicity.

Alcohol is widely employed in the practice of pharmacy for the preparation of a variety of pharmaceutical preparations, including spirits, tinctures, and fluid extracts. Spirits are liquid pharmaceutical preparations containing alcohol as the sole solvent, whereas tinctures are hydroalcoholic mixtures. Many fluid extracts also contain some alcohol.

The widely accepted optimal bactericidal concentration of 70% alcohol is not supported by a study that found that the kill rates of microorganisms suspended in alcohol concentrations between 60% and 95% were not significantly different.[1] Concentrations of <60% are also effective, but longer contact times are necessary. Concentrations of >70% can be used safely for preoperative sterilization of the skin.[2]

Dehydrated Ethanol, USP

Dehydrated ethanol, absolute alcohol contains not less than 99% by weight of C_2H_5OH. It is prepared commercially by azeotropic distillation of an ethanol and benzene mixture. It has a very high affinity for water and must be stored in tightly sealed containers. Absolute alcohol is used primarily as a chemical agent, but it has also been injected for the local relief of pain in carcinomas and neuralgias.

Isopropyl Alcohol, USP

2-Propanol is a clear, colorless, volatile liquid having a characteristic odor and a slightly bitter taste. It is considered a suitable substitute for ethyl alcohol for most external uses, but it must not be taken internally.

$$CH_3CH{=}CH_2 \xrightarrow[H_2SO_4]{H_2O} CH_3CHOHCH_3$$

Isopropyl Alcohol

Isopropyl alcohol is prepared commercially by the sulfuric acid–catalyzed hydration of propylene. It forms a constant-boiling mixture with water, containing 91% by volume of 2-propanol. It is primarily used to disinfect the skin and surgical instruments. Isopropyl alcohol is rapidly bactericidal in the concentration range of 50% to 95%. A 40% concentration is considered equal in antiseptic power to a 60% ethanol concentration. Azeotropic isopropyl alcohol, USP is used by diabetics for the sterilization of hypodermic needles and syringes. Isopropyl alcohol also finds use in pharmaceuticals and toiletries as a solvent and preservative.

Ethylene Oxide

C_2H_4O is a colorless flammable gas that liquefies at 12°C. It has been used to sterilize temperature-sensitive medical equipment and certain pharmaceuticals that cannot be autoclaved. Ethylene oxide readily diffuses through porous material and effectively destroys all forms of microorganisms at ambient temperatures.[3]

Ethylene oxide forms explosive mixtures in air in concentrations ranging from 3% to 80% by volume. The hazard is eliminated when the gas is mixed with sufficient concentrations of carbon dioxide. Carboxide is a product consisting of 10% ethylene oxide and 90% carbon dioxide, by volume, that can be released in air without danger of explosion.

The mechanism of germicidal action of ethylene oxide involves alkylation of functional groups in nucleic acids and proteins by nucleophilic ring opening. Owing to its nonselective action, ethylene oxide is potentially very toxic and possibly carcinogenic. Personnel using the gas mixture should be cautioned to use a gas mask and avoid exposure to skin and mucous membranes.

Formaldehyde Solution, USP

Formalin (formol) is a colorless aqueous solution containing not less than 37% of formaldehyde (CH_2O) with methanol added to retard polymerization. It is miscible in water and alcohol and is characteristically pungent. Formaldehyde readily undergoes oxidation and polymerization, leading to formic acid and paraformaldehyde, respectively. It should be preserved in tightly closed containers and stored at temperatures above 15°C to prevent the cloudiness that occurs at lower temperatures.

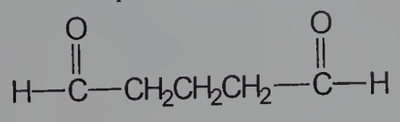
Formaldehyde solution exerts a slow, but powerful germicidal action. The mechanism of this effect is believed to involve the direct, nonspecific alkylation of nucleophilic functional groups (amino, hydroxyl, and sulfhydryl) in proteins to form carbinol derivatives. The action of formaldehyde is not confined to microorganisms. The compound is irritating to mucous membranes and causes hardening of the skin. Oral ingestion of the solution leads to severe gastric distress.

Formalin, diluted in water, had been employed to harden the skin, to prevent excessive perspiration, and to disinfect the hands before surgery. The gas has been employed to disinfect rooms, clothing, and surgical instruments. A high frequency of allergic reactions associated with formaldehyde and formaldehyde-based products and the designation of formaldehyde as a suspect carcinogen suggest that it should not be used for the indicated foregoing purposes.

Glutarol Disinfectant Solution, USP

Glutaraldehyde (Cidex), a dialdehyde, is used as a dilute sterilizing solution for equipment and instruments that cannot be autoclaved. The commercial product (Cidex) is a stabilized alkaline glutaraldehyde solution, which actually consists of two components that are mixed together immediately before use. The activated solution thus prepared contains 2% glutaraldehyde buffered to pH 7.5 to 8.0. Stabilized solutions retain 86% of their original activity 30 days after preparation,[4] whereas the nonstabilized alkaline solutions lose 44% of their activity after 15 days. At high pH (>8.5) glutaraldehyde rapidly polymerizes. Nonbuffered solutions of the compound are acidic, possibly owing to the existence of the

cyclic hydrated (hemiacetal) form. Such acidic solutions are very stable, but lack sporicidal activity.



Glutaraldehyde

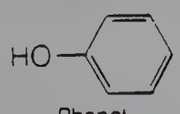
PHENOLS AND THEIR DERIVATIVES

The standard by which most germicidal substances are compared is the activity of phenol USP. The *phenol coefficient* is defined as the ratio of a dilution of a disinfectant to the dilution of phenol required to kill to the same extent a given strain of the bacterium *Salmonella typhi* (*Eberthella typhosa*), under carefully controlled conditions over a given time period. If, for example, the dilution of a test compound is 10 times as great as the dilution of phenol, then the phenol coefficient (PC) is 10. The PC of phenol is, of course, unity. There are many shortcomings of this testing method. Different microorganisms differ in their sensitivities to phenols, as compared with other germicides, and, therefore, different phenol coefficients would be expected. Also, the conditions used to conduct the test are difficult to reproduce exactly; hence, a high degree of variability between different laboratories is common.

Several phenols are more bactericidal than phenol itself. Substitution with alkyl, aryl, and halogen (especially *para*) groups increases bactericidal activity. Straight-chain alkyl groups are more effective than branched ones. Alkylated phenols and resorcinols are actually less toxic than the parent compounds. Phenols are believed to precipitate bacterial proteins at low concentrations. Lysis of bacterial cell membranes occurs at higher concentrations.

PHENOL (CARBOLIC ACID)

Phenol occurs as a colorless to pale pink crystalline solid with a characteristic "medicinal" odor. It is soluble 1:15 in water, very soluble in alcohol, and soluble in menthol and solol.



Phenol

Phenol was introduced as a surgical antiseptic by Sir Joseph Lister in 1867. In addition to its germicidal activity, phenol has caustic and local anesthetic actions. It is a general protoplasmic poison that is toxic to all cells. Phenol is corrosive to the skin and must be diluted to avoid tissue destruction and dermatitis.

Phenol is still occasionally used as an antipruritic in phenolated calamine lotion in 0.1% to 1% concentrations. A 4% glycerin solution has been used to cauterize small wounds. Small amounts of pure phenol have also been used for this purpose. The use of phenol as either an antiseptic or disinfectant is largely obsolete.

LIQUEFIED PHENOL, USP

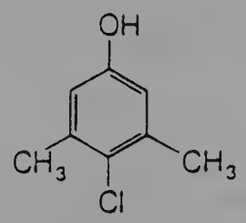
Liquefied phenol is phenol containing 10% water. The liquid form provides a convenient method for using phenol in a variety of pharmaceutical preparations. However, the water content precludes its use in fixed oils or liquid petrolatum.

P-CHLOROPHENOL

P-Chlorophenol is used in combination with camphor in liquid petrolatum. It has a phenol coefficient of ~4.

P-CHLORO-M-XYLENOL

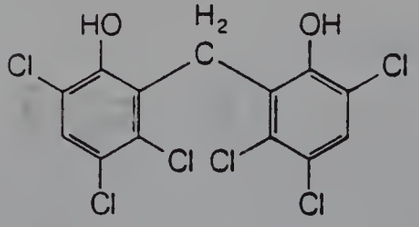
P-Chloro-*m*-xylenol (PC-MX, Metasep) is a relatively nonirritating antiseptic agent with broad-spectrum antibacterial and antifungal properties. It is available in 2% concentration as a medicated shampoo. It has also been used in topical preparations for the treatment of tinea infections, such as athlete's foot and jock itch.



P-Chloro-*m*-xylenol

HEXACHLOROPHENE, USP

2,2′-Methylenebis(3,4,6-trichlorophenol), or 2,2′dihydroxy-3,5,6,3′,5′,6′-hexachlorodiphenylmethane (Gamophen, Surgicon, pHisoHex, Hex-O-San, Germa-Medica), occurs as a white to light tan, crystalline powder that is insoluble in water, but soluble in alcohol and most other organic solvents.



Hexachlorophene

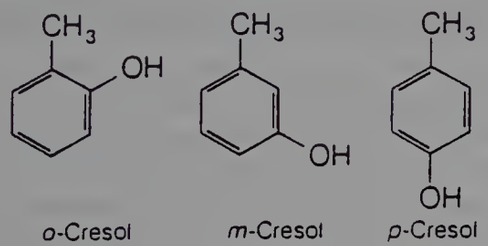
Biphenolic compounds, such as hexachlorophene, are generally more potent than the corresponding monophenolic counterparts. Moreover, the chlorine content of hexachlorophene further increases its potency. The physical properties

of this substance are such that it is readily deposited on the skin and in sebaceous glands. Therefore, it creates a prolonged antiseptic effect in low concentrations when applied topically. Hexachlorophene is employed in concentrations of 2% to 3% in soaps, detergent creams, lotions, and other forms for a variety of antiseptic uses. It is generally very effective against gram-positive bacteria, but many gram-negative organisms are resistant to its action.

Although the systemic toxicity of hexachlorophene in animals following oral and parenteral administration had been known for some time, it was not until the late 1960s and the early 1970s that reports of neurologic toxicity in infants and burn patients prompted the Food and Drug Administration (FDA) to ban its use in OTC antiseptic and cosmetic preparations.[5]

CRESOL, NF

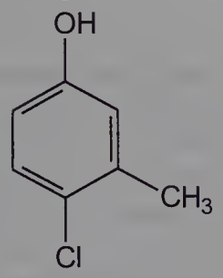
This is actually a mixture of the three isomeric cresols. It occurs as a yellowish to brownish-yellow liquid that has a characteristic creosote odor. Cresol is obtained from coal tar or petrolatum by alkaline extraction, acidification, and fractional distillation. Cresol is an inexpensive antiseptic and disinfectant with a phenol coefficient of 2.5. It is sparingly soluble in water.



o-Cresol *m*-Cresol *p*-Cresol

CHLOROCRESOL, NF

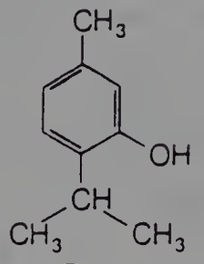
4-Chloro-3-methylphenol occurs as a colorless crystalline powder with a characteristic odor. Chlorocresol is slightly soluble in water. It is employed as a preservative.



Chlorocresol

THYMOL, NF

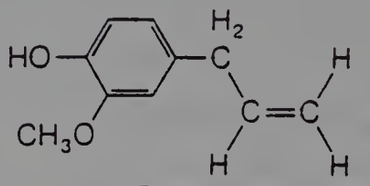
Isopropyl *m*-cresol is obtained from oil of thyme by alkaline extraction followed by acidification. Thymol occurs as large colorless crystals, with an aromatic thymelike odor. It is sparingly soluble in water, but soluble in alcohol and organic solvents. Thymol has fungicidal properties and is used in alcoholic solutions and in dusting powders for the treatment of tinea infections.



Thymol

EUGENOL, USP

4-Allyl-2-methoxyphenol is obtained from clove oil and other volatile oils. It occurs as a pale yellow liquid having an aromatic odor of cloves and a pungent taste. It is slightly soluble in water and miscible in alcohol and other organic solvents.

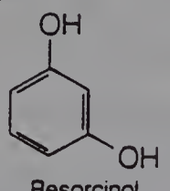


Eugenol

Eugenol has local anesthetic, as well as antiseptic, activity and is used to relieve toothaches. It also finds use in mouthwashes because of these properties. The phenol coefficient of eugenol is 14.4.

RESORCINOL, USP

m-Dihydroxybenzene (resorcin), or resorcinol, is synthetically prepared. It occurs as white needle-shaped crystals or powder that is soluble in water and alcohol. Resorcinol is light-sensitive and readily oxidizes. It should be stored in light-resistant containers. It is much less stable in solution, especially at alkaline pH.

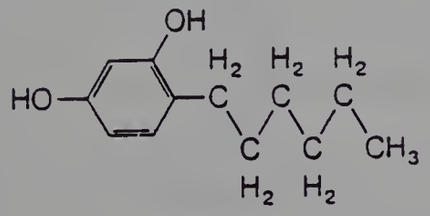


Resorcinol

Despite its weak antiseptic properties (phenol coefficient, 0.4), resorcinol is used in 1% to 3% solutions and in ointments and pastes in concentrations of 10% to 20% for the treatment of skin conditions such as ringworm, eczema, psoriasis, and seborrheic dermatitis. Resorcinol possesses keratolytic properties to augment its antiseptic action.

HEXYLRESORCINOL, USP

4-Hexylresorcinol (Crystoids), or hexylresorcinol, is found as a white, needle-like crystalline solid with a faint phenolic odor and an astringent taste. When placed on the tongue, it produces a sensation of numbness. It is freely soluble in alcohol, but sparingly soluble in water (1:20,000).

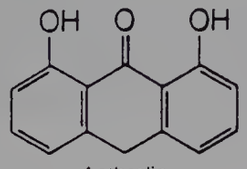


Hexylresorcinol

Hexylresorcinol is an effective antiseptic, having bactericidal and fungicidal properties. It has a phenol coefficient of 98 against *Staphylococcus aureus*. As with other alkylated phenols and resorcinols, hexylresorcinol has surface-active properties. It also has local anesthetic activity. These properties may contribute to its effectiveness as an anthelmintic for the treatment of ascaris and hookworm infestations. However, more effective and better-tolerated anthelmintics are currently available for these purposes. Hexylresorcinol is also found in throat lozenges because of its local anesthetic and antiseptic properties. Such preparations are of dubious value and may be harmful because the local concentration of hexylresorcinol is probably not bactericidal, and the larynx may be anesthetized, causing temporary laryngitis.

ANTHRALIN, USP

1,8,9-Anthracenetriol occurs as a yellowish-brown crystalline powder that is insoluble in water, slightly soluble in alcohol, and soluble in most nonpolar organic solvents. It finds application in the treatment of psoriasis and other chronic skin conditions because of its antiseptic, irritant, and keratolytic properties.



Anthralin

OXIDIZING AGENTS

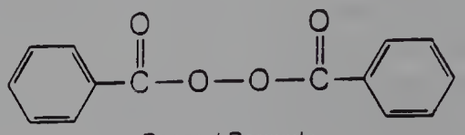
Most oxidizing agents that are of value as germicides depend upon the liberation of oxygen in the tissues. Many are inorganic compounds and include hydrogen peroxide, various metal peroxides, and sodium perborate. Other oxidizing agents, such as potassium permanganate, denature proteins through a direct oxidant action. Oxidizing agents are particularly active against anaerobic bacteria and find use in the cleansing of contaminated wounds. The effectiveness of these agents is somewhat limited by their poor penetrability into tissues and organic matter and their transient action.

CARBAMIDE PEROXIDE TOPICAL SOLUTION, USP

Carbamide peroxide is a stable complex of urea and hydrogen peroxide, having the formula $H_2NCONH_2 \cdot H_2O_2$. The solution contains 12.6% of the complex in anhydrous glycerin. It releases hydrogen peroxide when the solution is mixed with water. It is used as an antiseptic and disinfectant.

HYDROUS BENZOYL PEROXIDE, USP

Hydrous benzoyl peroxide (Benoxyl, Oxy-5, Oxy-10, Persadox, Vanoxide) is a white, granular powder with a characteristic odor. It contains ~30% water to make it safer to handle.



Benzoyl Peroxide

Benzoyl peroxide is employed in concentrations of 5% to 10% as a keratolytic and keratogenic agent for the control of acne. Similar to other peroxides, it is chemically unstable and can explode when heated. Nonstabilized aqueous solutions slowly decompose to hydrogen peroxide and benzoic acid. Lotions containing benzoyl peroxide are stabilized with the addition of two parts of dicalcium phosphate.

The value of benzoyl peroxide in acne treatment is believed to derive from its irritant properties.[6] It induces proliferation of epithelial cells, leading to sloughing and repair.

HALOGEN-CONTAINING COMPOUNDS

IODOPHORS

Iodine (I_2) is one of the oldest known germicides in use today. It was listed in 1830 in *USP II* as a tincture and as a liniment. Iodine tincture (a 2% solution of iodine in 50% alcohol with sodium iodide), strong iodine solution (Lugol's solution, 5% iodine in water with potassium iodide), and iodine solution (2% iodine in water with sodium iodide) are currently official in the *USP*. Inorganic iodide salts are present to solubilize the iodine and reduce its volatility. Iodine remains one of the most effective and useful germicides available today. It is believed to inactivate proteins by iodination (phenylalanyl and tyrosyl residues) and oxidation (sulfhydryl groups).

Various nonionic and cationic surfactants have been found to act as solubilizers for iodine by forming complexes that

retain the germicidal properties of iodine. These complexes also reduce the volatility of iodine and essentially remove its irritant properties. It is estimated that ~80% of the dissolved iodine remains as available bacteriologically active iodine in the more effective, nonionic surfactant complexes.[7] Such complexes, called iodophors, are bactericidal and fungicidal.

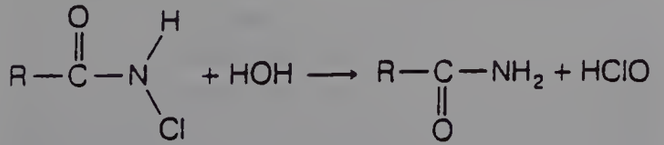
POVIDONE-IODINE, USP

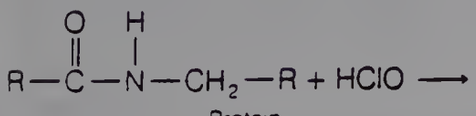
Povidone-iodine (Betadine, Isodine) is a complex with the nonionic surfactant polymer polyvinylpyrrolidone. This water-soluble complex releases iodine slowly. It provides a nontoxic, nonirritating, nonvolatile, and nonstaining form of iodine. Approximately 10% of available iodine is present in the complex.

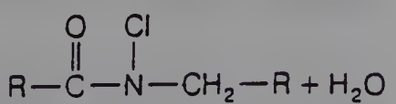
Povidone-iodine is used as an aqueous solution as an antiseptic for application to the skin before surgery and injections, for the treatment of infected wounds and lacerations, and for local bacterial and fungal infections. Several other topical preparations containing providone-iodine are available, including aerosols, ointments, surgical scrubs, antiseptic gauze pads, sponges, whirlpool concentrates, and mouthwashes.

CHLORINE-CONTAINING COMPOUNDS

Chlorine and its derivatives have been used to disinfect water for more than a century. The discovery that hypochlorous acid (HClO) was the active germicidal species formed when chlorine was dissolved in water led to the use of the first inorganic hypochlorite salts such as NaOCl and $Ca(OCl)_2$ and, later, *N*-chloro organic compounds as disinfectants. These compounds release hypochlorous acid when dissolved in water, especially in the presence of acid. Two equally plausible mechanisms have been proposed for the germicidal action of hypochlorous acid—the chlorination of amide nitrogen atoms in proteins (see diagram) and the oxidation of sulfydryl groups in proteins:



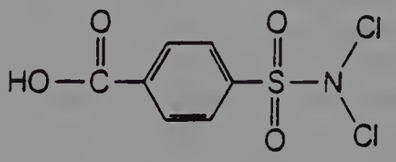




Organic compounds that form reasonably stable *N*-chloro derivatives include amides, imides, and amidines. *N*-Chloro derivatives slowly release HOCl in water, forming the parent amide in the process. The antiseptic power of these compounds is optimal at pH 7.

HALAZONE, USP

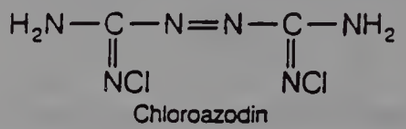
P-Dichlorosulfamoylbenzoic acid is a white, crystalline, light-sensitive compound with a faint chlorinelike odor. It is slightly soluble in water, but very soluble in alkaline solution. The sodium salt is used to disinfect drinking water.



Halazone

CHLOROAZODIN

N,N-Dichlorodicarbonamidine (Azochloramid) is a bright yellow crystalline solid with a faint odor of chlorine. It is not very soluble in water or in most organic solvents and is unstable to light or heat. Chloroazodin is reported to explode when heated above 155°C.

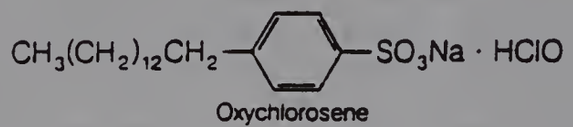


Chloroazodin

Dilute solutions are used to disinfect wounds, as a packing for cavities, and for lavage and irrigation. A glyceryltriacetate solution is used as a dressing. The antiseptic action of chloroazodin is prolonged because of its relatively slow reaction with water.

OXYCHLOROSENE SODIUM

Chlorpactin is a complex of the sodium salt of dodecylbenzene sulfonic acid and hypochlorous acid. The complex slowly releases hypochlorous acid in solution. It is available as an amorphous white powder with a faint chlorinelike smell.



Oxychlorosene

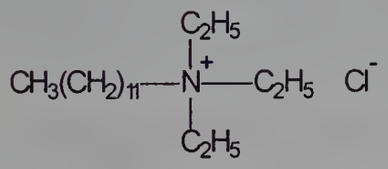
Oxychlorosene combines the germicidal properties of hypochlorous acid with the emulsifying, wetting, and keratolytic properties of an anionic detergent. The preparation has a marked and rapid cidal action against most microorganisms, including both gram-positive and gram-negative bacteria, molds, yeasts, viruses, and spores. It is used to treat localized infections (particularly when resistant organisms are present), to remove necrotic tissue in massive infections or from

radiation necrosis, to counteract odorous discharges, as an irritant, and to disinfect fistulas, empyemas, and wounds.

Oxychlorosene is supplied as a powder for solution. Typical applications use 0.1% to 0.5% concentrations in water. Dilutions of 0.1% to 0.2% are used in urology and ophthalmology.

CATIONIC SURFACTANTS

The cationic surfactants are quaternary ammonium compounds that ionize in water and exhibit surface-active properties. The surface activity of these compounds, exemplified by lauryl triethylammonium sulfate, results from two structural features: (1) the cationic head, which has a high affinity for water, and (2) a long hydrocarbon tail, which has a high affinity for lipids and nonpolar solvents. These disparate solvent affinities cause molecules of lauryl trimethylammonium chloride to concentrate at the interface between immiscible solvents, such as water and oil, with the cationic group in the aqueous phase and the hydrocarbon group in the oil phase.



Lauryl Triethylammonium Chloride

The synthesis and antimicrobial actions of members of this class of compounds were first reported in 1908, but it was not until after the pioneering work of Gerhard Domagk in 1935[8] that attention was directed to their usefulness as antiseptics, disinfectants, and preservatives.

The cationic surfactants exert a bactericidal action against a broad spectrum of gram-positive and gram-negative bacteria. They are also active against several pathogenic species of fungi and protozoa. Spore-forming microorganisms, on the other hand, are resistant.

Mechanisms by which the cationic surfactants exert their bactericidal actions center around their surface-active properties. One suggestion is that they are adsorbed onto the surface of the bacterial cell, where they cause lysis by interfering with enzymes in the cell wall and cell membrane.

The cationic surfactants possess several other desirable properties, in addition to their broad-spectrum antimicrobial activity, that are advantageous for germicidal use. They are highly water-soluble, relatively nontoxic, stable in solution, nonstaining, and noncorrosive. Their surface-active properties provide a keratolytic action and relatively good tissue penetration.

Despite all of the foregoing advantages, the cationic surfactants have numerous serious disadvantages. They are inactivated by soaps and other anionic detergents. All traces of soap must be removed from skin and other surfaces before they are applied. Tissue constituents, blood, serum, and pus tend to reduce the effectiveness of these substances. Cationic surfactants are also adsorbed on glass, talc, and kaolin to reduce or prevent their action. The bactericidal action of cationic surfactants is slower than that of iodine, and these compounds are not active against spores. Solutions of cationic surfactants intended for disinfecting surgical instruments, gloves, and the like should never be reused because reused solutions have been reported to be a source of infection (especially by Pseudomonas and Enterobacter species).

BENZALKONIUM CHLORIDE, NF

Alkylbenzyldimethylammonium chloride (Zephiran, Germicin, Benza) is a mixture of alkylbenzyldimethylammonium chlorides having the general formula $[C_6H_5CH_2N(CH_3)_2R]^+Cl^-$, where R represents a mixture of alkyl members beginning with C_8H_{17} and extending to higher homologous, with $C_{12}H_{25}$, $C_{14}H_{29}$, and $C_{16}H_{33}$ composing the major portion. Although variations in physical and antimicrobial properties exist between individual members of the mixture, they are of little importance for the usefulness of the mixture.

Benzalkonium chloride occurs as a white gel that is soluble in water, alcohol, and most organic solvents. Aqueous solutions are colorless, slightly alkaline to litmus paper, and highly foamy.

Benzalkonium chloride possesses detergent, emulsifying, and wetting actions. It is employed as an antiseptic for skin and mucosa in concentrations of 1:750 to 1:20,000. For irrigation, 1:20,000 to 1:40,000 concentrations are employed. For storage of surgical instruments, 1:750 to 1:5,000 concentrations are used with 0.5% sodium nitrate added as a preservative.

METHYLBENZETHONIUM CHLORIDE, USP

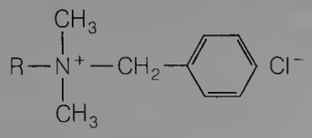
Benzyldimethyl[2-[2-[[4-(1,1,3,3-tetramethylbutyl)tolyl]oxy]-ethoxy]ethyl]ammonium chloride (Diaparene), a mixture of methylated derivatives of methylbenzethonium chloride, is used for the specific control of diaper rash in infants caused by the intestinal bacterium *Bacterium ammoniagenes*, which causes the liberation of ammonia in decomposed urine. It is also employed as a general antiseptic. Its properties are virtually identical with those of phemerol chloride.

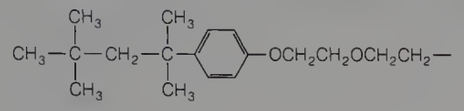
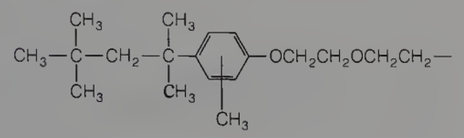
BENZETHONIUM CHLORIDE, USP

Benzyldimethyl[2-[2-[*p*-(1,1,3,3-tetramethylbutyl)phenoxy]-ethoxy]ethyl]ammonium chloride (Phemerol Chloride) is a colorless crystalline powder that is soluble in water, alcohol, and most organic solvents. The structure of this

TABLE 7-1

ANALOGUES OF DIMETHYLBENZYLAMMONIUM CHLORIDE



Compound	*R*
Benzalkonium chloride	n—C_8H_{17} to $C_{16}H_{33}$
Benzethonium chloride	
Methylbenzethonium chloride	

agent and its relationship to other analogues of the class are shown in Table 7-1.

The actions and uses of this agent are similar to those of benzalkonium chloride. It is used as a 1:750 concentration for skin antisepsis. For the irrigation of mucous membranes, a 1:5,000 solution is employed. A 1:500 alcoholic tincture is also available.

CETYLPYRIDINIUM CHLORIDE, USP

1-Hexadecylpyridinium chloride (Ceepryn) occurs as a white powder that is very soluble in water and alcohol. In this quaternary ammonium compound, the quaternary ammonium nitrogen atom is part of the aromatic pyridinium ring.

Cetylpyridinium Chloride

The cetyl derivative is the most active of a series of alkylpyridinium derivatives. It is used as general antiseptic solutions in concentrations of 1:100 to 1:1,000 for intact skin, 1:1,000 for minor lacerations, and 1:2,000 to 1:10,000 for irrigation of mucous membranes. Ceepryn is also available in the form of throat lozenges and a mouthwash in 1:20,000 concentration.

CHLORHEXIDINE GLUCONATE, USP

1,6-Di(4′-chloro-phenyldiguanido)hexane gluconate (Hibiclens) is the most effective of a series of antibacterial biguanides originally developed in Great Britain.[9] The antimicrobial properties of the biguanides were discovered as a result of earlier investigations of these substances as potential antimalarial agents (Chap. 9). Although the biguanides are technically not bisquaternary ammonium compounds and, therefore, should perhaps be separately classified, they share many physical, chemical, and antimicrobial properties with the cationic surfactants. The biguanides are strongly basic compounds that exist as di-cations at physiologic pH. Similar to cationic surfactants, they are inactivated by anionic detergents and by complex anions such as phosphate, carbonate, and silicate.

Chlorhexidine has broad-spectrum antibacterial activity, but it is not active against acid-fast bacteria, spores, or viruses. It has been employed for such topical antiseptic uses as preoperative skin disinfection, wound irrigation, bladder irrigation, mouthwashes, and general sanitation. Chlorhexidine is not absorbed through skin or mucous membranes and does not cause systemic toxicity.

DYES

Before the advent of the sulfonamides and the antibiotics, the organic dyes were used far more extensively than they are today. Only a handful of cationic dyes still find limited use as anti-infective agents. They include the triphenylmethane dyes—gentian violet and basic fuchsin—and the thiazine dye, methylene blue. These dyes form colorless leucobase forms under alkaline conditions.

Cationic dyes are active against gram-positive bacteria and many fungi; gram-negative bacteria are generally resistant. The difference in susceptibility is thought to be related to the cellular characteristics that underlie the differential gram stain.

GENTIAN VIOLET, USP

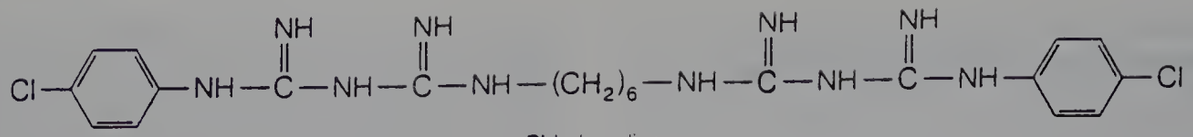
Also known as hexamethyl-*p*-rosaniline chloride, crystal violet, methyl violet, and methylrosaniline chloride (Genepax), gentian violet occurs as a green powder or green flakes with a metallic luster. It is soluble in water (1:35) and alcohol (1:10), but insoluble in nonpolar organic solvents.

Gentian violet is available as vaginal suppositories for the treatment of yeast infections. It is also used as a 1% to 3% solution for the treatment of tinea and yeast infections.

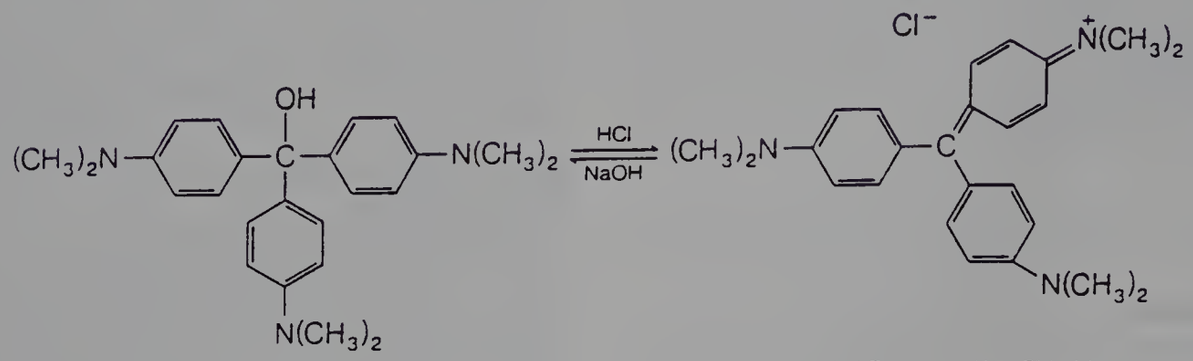
Gentian violet has also been used orally as an anthelmintic for strongyloidiasis (threadworm) and oxyuriasis.

BASIC FUCHSIN, USP

Basic fuchsin is a mixture of the chlorides of rosaniline and *p*-rosaniline. It exists as a metallic green crystalline powder that is soluble in water and in alcohol, but insoluble in ether.



Chlorhexidine



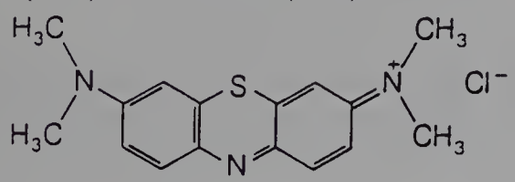
Leucobase

Hexamethyl Pararosaniline Chloride

Basic fuchsin is an ingredient of carbol-fuchsin solution (Castellani's paint), which is used topically in the treatment of fungal infections, such as ringworm and athlete's foot.

METHYLENE BLUE, USP

3,7-Bis(dimethylamino)-phenazathionium chloride occurs as a lustrous dark green crystalline powder that is soluble in water (1:25) and in alcohol (1:65).



Methylene Blue

The redox properties of methylene blue provide the basis of its use as an antidote in cyanide poisoning. In high concentrations, it promotes the conversion of hemoglobin to methemoglobin, which, because of its high affinity for cyanide ion, diverts it from inactivating cytochrome C. In low concentrations, methylene blue has the opposite effect and has been used to treat drug-induced methemoglobinemia.

Methylene blue has weak antiseptic properties that make it useful for the treatment of cystitis and urethritis. Its action is considered bacteriostatic. It colors the urine and sometimes the stool blue green. Methemoglobinemia and anemia may occur with prolonged use.

MERCURY COMPOUNDS

Mercury and its derivatives have been used in medicine for centuries. Elemental mercury incorporated in ointment bases was used topically for the treatment of local infections and syphilis. Several inorganic salts of mercury, such as mercuric chloride ($HgCl_2$) and mercurous chloride (Calomel, Hg_2Cl_2), were at one time widely used as antiseptics. Ammoniated mercury [$Hg(NH_2)Cl$] is still occasionally used for skin infections such as impetigo, psoriasis, and ringworm. Mercuric oxide is sometimes used to treat inflammatory infections of the eye. Although the potential interaction of mercuric ion with the tissues is greatly reduced by the low water solubility of these agents, they can be irritating to the tissues and can cause hypersensitivity reactions; therefore, their use is not recommended.

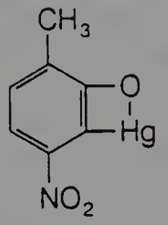
The comparatively few organic mercurials still in use are employed as antiseptics, preservatives, or diuretics (Chap. 18). Organic mercurials are of 2 general types: (1) compounds with at least one carbon-mercury bond, which does not generally ionize readily, and (2) compounds with mercury bonded to heteroatoms, such as oxygen, nitrogen, or sulfur, that ionize partially or completely. In addition to its effect on ionization, the organic moiety may increase the lipid solubility of an organomercurial compound, thereby facilitating its penetration into microorganisms and host tissues.

The antibacterial action of mercury compounds is believed to result from their reaction with sulfhydryl (SH) groups in enzymes and other proteins to form covalent compounds of the type R-S-Hg-R′. This action is reversible by thiol-containing compounds, such as cysteine and dimercaprol (BAL); thus, organomercurials are considered largely bacteriostatic. The antibacterial activity of mercurial antiseptics is greatly reduced in the presence of serum because of the presence of proteins that inactivate mercury compounds. Organomercury antiseptics are not particularly effective against spores.

The disadvantages of mercurials for antiseptic and disinfectant use far outweigh any possible advantages they might have. Hence, other more effective and less potentially toxic agents are now preferred.

NITROMERSOL, USP

3-(Hydroxymercuri)-4-nitro-*o*-cresol inner salt (Metaphen) occurs as a yellow powder that is practically insoluble in water and is sparingly soluble in alcohol and in most organic solvents. The somewhat improbable formula for the neutral form of nitromersol shown below is given in the *USP*. The sodium salt presumably has the "inner salt" structure.

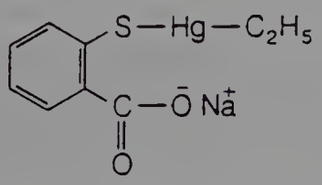


Nitromersol

Nitromersol is nonirritating to mucous membranes and is nonstaining. Therefore, at one time, it was a very popular topical antiseptic for skin and ocular infections. However, it has largely been replaced by more effective agents.

THIMEROSAL, USP

Sodium[(*o*-carboxyphenyl)-thio]ethylmercury (Merthiolate) occurs as a cream-colored, water-soluble powder. It is nonstaining and nonirritating to tissues. Thimerosal is a weakly bacteriostatic antiseptic that is applied topically as in aqueous solutions or an ointment.



Thimerosal

PRESERVATIVES

Preservatives are added to various liquid dosage forms and cosmetic preparations to prevent microbial contamination. In parenteral and ophthalmic preparations, preservatives are used to maintain sterility in the event of accidental contamination during use.

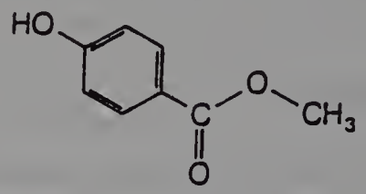
The ideal preservative would be effective at low concentrations against all possible microorganisms, nontoxic, compatible with other constituents used in the preparation, and stable for the shelf life of the preparation. The ideal preservative does not exist, but there is extensive experience with some of them. Sometimes combinations of preservative agents are employed.

P-HYDROXYBENZOIC ACID DERIVATIVES

Esters of *p*-hydroxybenzoic acid (parabens) have antifungal properties. Their toxicity is generally low, owing to rapid hydrolysis in vivo to *p*-hydroxybenzoic acid, which is rapidly conjugated and excreted. These properties make parabens useful as preservatives for liquid dosage forms. The preservative effect tends to increase with molecular weight, but the methyl ester is more effective against molds, whereas the propyl ester is more effective against yeasts. The more oil-soluble propyl ester is the preferred preservative for oils and fats.

Methylparaben, NF

Methyl *p*-hydroxybenzoate, or methylben, occurs as a white crystalline powder. It is soluble in water and alcohol, but only slightly soluble in nonpolar organic solvents. It is used as a preservative primarily to protect against molds.



Methylparaben

Propylparaben, NF

Propyl *p*-hydroxybenzoate, or propylben, occurs as a white crystalline powder that is only slightly soluble in water but soluble in most organic solvents. It is used as a preservative, primarily against yeasts. Propylparaben sodium is the water-soluble sodium salt of the 4-phenolic group. It occurs as a white powder that is fully soluble in water. The pH of solutions of propyl paraben sodium are alkaline (pH ~ 10).

Ethylparaben, NF

Ethyl-*p*-hydroxybenzoate is a white crystalline powder that is slightly soluble in water, but soluble in alcohol and most organic solvents.

Butylparaben, NF

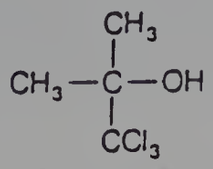
Butyl-*p*-hydroxybenzoate occurs as a white crystalline powder that is sparingly soluble in water but very soluble in alcohols and in nonpolar organic solvents.

OTHER PRESERVATIVES

Chlorobutanol, NF

1,1,1-Trichloro-2-methyl-2-propanol (Chloretone) is a white crystalline solid with a camphorlike odor. It occurs in an

anhydrous form and a hemihydrate form, both of which sublime at room temperature and pressure. Chlorobutanol is slightly soluble in water and soluble in alcohol and in organic solvents.

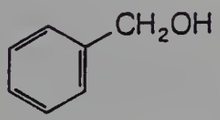


Chlorobutanol

Chlorobutanol is employed as a bacteriostatic agent in pharmaceuticals for injection, ophthalmic use, and intranasal administration. It is unstable when heated in aqueous solutions, especially at a pH of >7. It undergoes a haloform reaction. Solutions of pH ≤5 are reasonably stable at 25°C. It is stable in oils and organic solvents.

Benzyl Alcohol, NF

Benzyl alcohol (phenylcarbinol, phenylmethanol, benzyl alcohol) is found unesterified in oil of jasmine and as esters of acetic, cinnamic, and benzoic acids in gum benzoin, storax resin, Peru balsam, tolu balsam, and some volatile oils. It is soluble in water and alcohol. It is a clear liquid with an aromatic odor.



Benzyl Alcohol

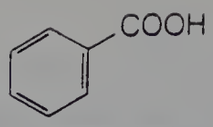
Benzyl alcohol is commonly used as a preservative in vials of injectable drugs in concentrations of 1% to 4% in water or saline solution. It has the added advantage of having a local anesthetic action. Benzyl alcohol is also used in ointments and lotions as an antiseptic in the treatment of various pruritic skin conditions.

Phenylethyl Alcohol, USP

Phenylethyl alcohol (2-phenylethanol, orange oil, rose oil, $C_6H_5CH_2CH_2OH$) is a clear liquid that is sparingly soluble in water (2%). It occurs naturally in rose oil, pine needle oil, and Neroli. It is used primarily in perfumery.

Benzoic Acid, USP

Benzoic acid and its esters occur naturally in gum benzoin and in Peru and tolu balsams. It is found as a white crystalline solid that slowly sublimes at room temperature and is steam distillable. It is slightly soluble in water (0.3%), but more soluble in alcohol and other polar organic solvents. It has a pK_a of 4.2.



Benzoic Acid

Benzoic acid is employed externally as an antiseptic in lotions, ointments, and mouthwashes. It is more effective as a preservative in foods and pharmaceutical products at low pH (below the pK_a). When used as a preservative in emulsions, its effectiveness depends upon both pH and distribution into the two phases.[10]

Sodium Benzoate, NF

Sodium benzoate is a white crystalline solid that is soluble in water and alcohol. It is used as a preservative in acidic liquid preparations in which benzoic acid is released.

Sodium Propionate, NF

Sodium propionate occurs as transparent colorless crystals that are soluble in water and alcohol. It is an effective antifungal agent that is used as a preservative. Sodium propionate is most effective at low pH.

Sorbic Acid, NF

2,4-Hexadienoic acid is an effective antifungal preservative. It is sparingly soluble in water and has a pK_a of 4.8. Sorbic acid is used to preserve syrups, elixirs, ointments, and lotions containing components, such as sugars, that support mold growth.

$$CH_3CH{=}CHCH{=}CHCOOH$$

Sorbic Acid

Potassium Sorbate, NF

Potassium sorbate occurs as a white crystalline powder that is soluble in water and alcohol. It is used in the same way as sorbic acid when greater water solubility is required.

Phenylmercuric Nitrate, NF

Phenylmercuric nitrate is a mixture of phenylmercuric nitrate and phenylmercuric hydroxide. It occurs as a white crystalline powder that is sparingly soluble in water and slightly soluble in alcohol. It is used in concentrations of 1:10,000 to 1:50,000 to preserve injectables against bacterial contamination. Organomercurials have the disadvantage of having their bacteriostatic effectiveness reduced in the presence of serum.

Phenylmercuric Acetate, NF

Acetoxyphenylmercury occurs as white prisms that are soluble in alcohol but only slightly soluble in water. It is used as a preservative.

ANTIFUNGAL AGENTS

Most fungal infections (mycoses) involve superficial invasion of the skin or the mucous membranes of body orifices. These diseases, which can usually be controlled by local application of an antifungal agent, are conveniently divided into 2 etiologic groups: (1) the dermatophytoses (tinea infections), which are contagious superficial epidermal infections caused by various Epidermophyton, Microsporum, and Trichophyton species; and (2) mycoses caused by pathogenic saprophytic yeasts, which are contagious and usually superficial infections involving the skin and mucous membranes. Some species of saprophytic yeasts (Aspergillus, Blastomyces, Candida, Coccidioides, Paracoccidiodes, Cryptococcus, Histoplasma, Sporothrix, and Torulopsis) under certain conditions are capable of invading deeper body cavities and causing systemic mycoses. Such infections may become serious and occasionally life-threatening, and they are frequently difficult to treat. The treatment of systemic mycoses has acquired increased importance in recent years as a result of the increased incidence of opportunistic yeast infections in immunocompromised patients. The widespread use of immunosuppressants following organ transplant operations and the AIDS epidemic have been major contributors to this situation.

Fatty acids in perspiration have been found to be fungistatic, and this discovery has led to the introduction of fatty acids in therapy. The use of copper and zinc salts provides the added antifungal activity of the metal ion. Aromatic acids, especially salicylic acid, which also has a useful keratolytic action, and its derivatives, are employed for their topical fungistatic effect. A variety of alkylated or halogenated phenols and their derivatives are useful for the treatment of local fungal infections. The antifungal activity of the aforementioned compounds is largely confined to local dermatophytic infections. Deep dermatophytic infections, resistant to topical therapy, may be treated systemically with the antibiotic griseofulvin.

Several years ago, it was discovered that local and gastrointestinal yeast infections, which became prevalent as superinfections that resulted from the misuse of broad-spectrum antibiotics, such as the tetracyclines, could be combated effectively with the polyene antibiotic nystatin. Later, two polyene antibiotics, amphotericin B and pimaricin, were introduced for the treatment of topical yeast infections. Research at Janssen Laboratories in Belgium during the 1960s led to the discovery that certain highly substituted and lipophilic imidazoles possessed useful broad-spectrum antifungal activity. Since this discovery, numerous similar imidazoles and analogous isosteric 1,2,4-triazoles have been introduced throughout the world for the treatment of fungal infections. Naftifine and terbinafine are examples of a new class of allylamine antifungal agents that have been introduced recently for the topical treatment of local infections.

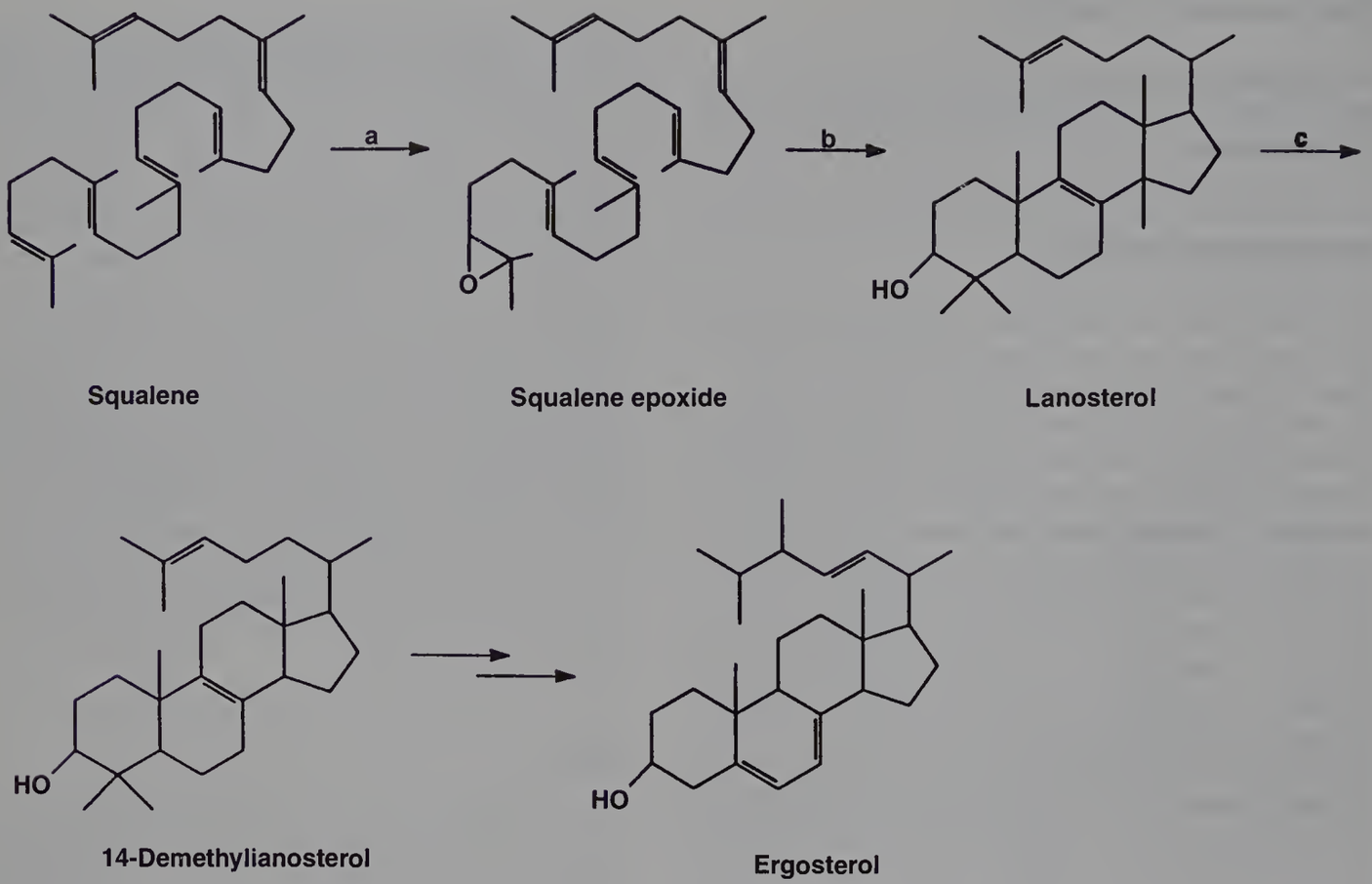
Despite the progress that has been made in antifungal drug discovery, relatively few agents have been found that combine the properties required for the treatment of systemic yeast infections, namely, effectiveness against the causative organisms and a reasonable margin of safety. For many years, amphotericin B (an intravenously administered polyene antibiotic) and flucytosine were the only agents available for the treatment of systemic yeast infections. Although injectable amphotericin B is effective against a broad range of pathogenic yeasts in vivo, it is highly toxic, in part due to its poor solubility properties. Oral flucytosine is relatively nontoxic, but has a very narrow spectrum of activity. More recently, the azole antifungal drugs miconazole, ketoconazole, itraconazole, and fluconazole have been introduced for the treatment of systemic infections, thus greatly expanding the choices available to the physician.

AZOLES

The azoles represent a class of versatile antifungal agents with an apparently unique mechanism of action. Early members of the class, such as clotrimazole and miconazole, were highly substituted imidazoles. However, structure-activity studies revealed that the imidazole ring could be replaced with the isosteric 1,2,4-triazole ring without adversely affecting the antifungal properties of the molecule. Hence, the more general term azoles is applied to designate this class of antifungal agents.

In general, the azoles are effective against most fungi that cause superficial infections of the skin and mucous membranes, including the dermatophytes, such as Trichophyton, Epidermophyton, and Microsporum spp., and yeasts, such as *C. albicans*. They also exhibit activity against yeasts that cause systemic infections, including *Coccidioides immitis, Cryptococcus neoformans, Paracoccioides brasiliensis, Petriellidium boydii, Blastomyces dermatitidis*, and *Histoplasma capsulatum*.

The actions of the azoles on mycotic biochemistry and physiology have been studied extensively. Nonetheless, the mechanisms by which they exert their antifungal effects remain to be fully elucidated.[11] At high concentrations (micromolar) the azoles are fungicidal; at low concentrations (nanomolar) they are fungistatic. The fungicidal effect is associated with damage to the cell membrane, with the loss of essential cellular constituents, such as potassium ion and amino acids. The fungistatic effects of the azoles have been correlated with the inhibition of membrane-bound enzymes by low concentrations of the azoles. Cytochrome P450 enzymes involved in fungal sterol biosynthesis, in particular lanosterol 14α-demethylase, have been implicated.[12] This enzyme is essential for the biosynthesis of egosterol, the principal sterol compo-



Squalene

Squalene epoxide

Lanosterol

14-Demethylianosterol

Ergosterol

Enzymes:
- **a Squalene epoxidase**
- **b Squalene epoxide cyclase**
- **c Lanosterol 14α-demethylase**

FIG. 7-1.

nent of fungal cell membranes (Fig. 7-1). Lanosterol 14α-demethylase is also required for the mammalian biosynthesis of cholesterol. The azoles are known to inhibit cholesterol biosynthesis in experimental animals, and to inhibit other cytochrome P450 oxidases involved in mammalian steroid biosyntheses.[13] In general, higher concentrations are required for the inhibition of the mammalian enzymes, as compared with the fungal 14α-demethylase. The 1,2,4-triazoles appear to cause a lower incidence of endocrine effects and hepatotoxicity than the corresponding imidazoles, possibly because of a lower affinity for the mammalian cytochrome P450-requiring enzymes involved.[14]

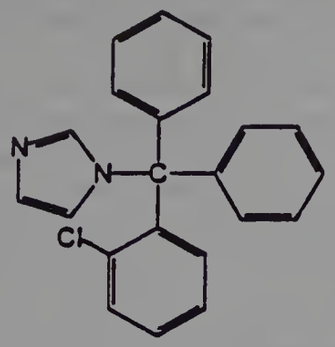
The primary structural requirement for members of the azole class is a weakly basic imidazole or 1,2,4-triazole ring (pK_as in the range of 6.5 to 6.8) bonded by a nitrogen-carbon linkage to the rest of the structure. At the molecular level, the amidine nitrogen atom (N-3 in the imidazoles; N-4 in the triazoles) is believed to bind the heme iron of enzyme-bound cytochrome P450 to inhibit activation of molecular oxygen and prevent oxidation of steroidal substrates by the enzyme. The more potent antifungal azoles possess two or three aromatic rings, at least one of which is halogen-substituted (for example, 2,4-dichlorophenyl-, 4-chlorophenyl-, or 2,4-difluorophenyl), and other nonpolar functionality. Presumably the extensive nonpolar portion of these molecules mimics the correspondingly nonpolar steroidal portion of the substrate for lanosterol 14α-demethylase, lanosterol, in shape and size.

The nonpolar functionality confers a high degree of lipophilicity to the antifungal azoles. The free bases are generally insoluble in water, but soluble in most organic solvents, while the acid salts (for example, the nitrates) are only sparingly soluble in water, but soluble in polar organic solvents such as ethanol. Fluconazole, which contains two polar triazole moieties, is an exception. It is sufficiently water soluble to be injected intravenously as a solution of the free base.

Clotrimazole, USP

1-(*o*-Chloro-α,α-diphenylbenzyl)imidazole (Lotrimin, Gyne-Lotrimin Mycelex) is a broad-spectrum antifungal

agent used topically for the treatment of tinea infections and candidiasis. It occurs as a white crystalline solid that is sparingly soluble in water, but soluble in alcohol and most organic solvents. It is a weak base that is solubilized by dilute mineral acids.



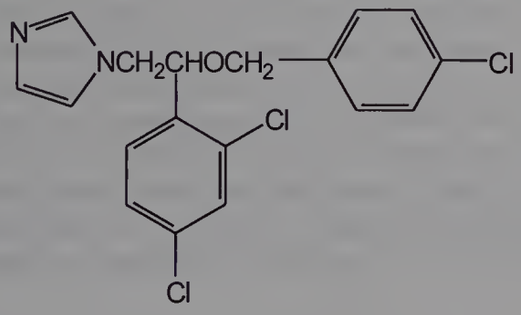
Clotrimazole

Clotrimazole is supplied as a solution in polyethylene glycol 400, a lotion, and a cream in a concentration of 1% for the treatment of tinea pedis, tinea cruis, tinea capitis, tinea versicolor, or cutaneous candidiasis. A 1% vaginal cream and tablets of 100 and 500 mg are available for vulvo-vaginal candidiasis. The compound is very stable, having a shelf life of >5 years.

Although clotrimazole is effective against a variety of pathogenic yeasts and is reasonably well absorbed orally, it causes severe gastrointestinal disturbances and is thus not considered suitable for the treatment of systemic infections.

Econazole Nitrate, USP

1-[2-[(4-Chlorophenyl)methoxy]-2-(2,4-dichlorophenyl)-ethyl]-1*H*-imidazole (Spectrazole) is a white crystalline nitric acid salt of econazole. It is only slightly soluble in water and most common organic solvents.

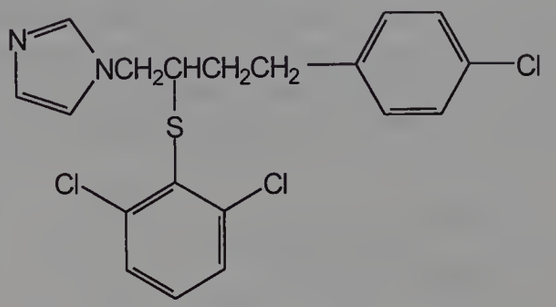


Econazole

Econazole is used as a 1% cream for the topical treatment of local tinea infections and cutaneous candidiasis.

Butoconazole Nitrate, USP

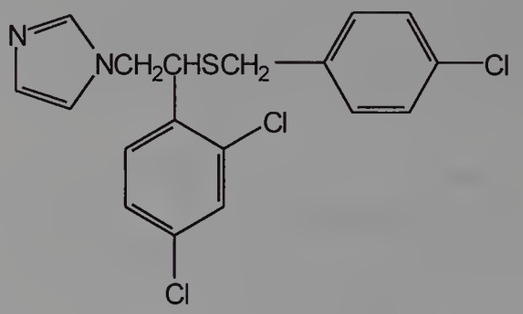
1-[4-(4-Chlorophenyl)-2-[(2,6-dichlorophenyl)-thio]butyl]-1*H*-imidazole (Femstat) is a broad-spectrum antifungal agent that is particularly effective against *C. albicans*. It is supplied as a vaginal cream containing 2% of the salt and is intended for the treatment of vaginal candidiasis.



Butoconazole

Sulconazole Nitrate, USP

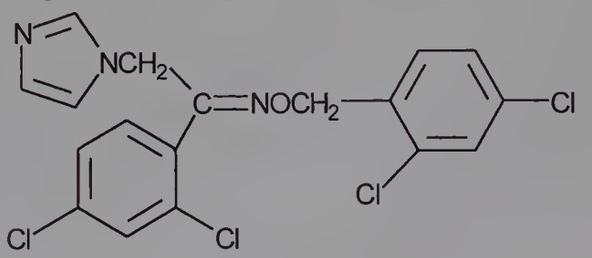
1-[2,4-Dichloro-β-[(ρ-Chlorobenzyl)thio]phenethyl]imidazole mononitrate (Exelderm) is the white crystalline nitric acid salt of sulconazole. It is sparingly soluble in water, but soluble in ethanol. The salt is employed in a solution and a cream in 1% concentration for the treatment of local tinea infections, such as athlete's foot, jock itch, and ringworm.



Sulconazole

Oxiconazole Nitrate, USP

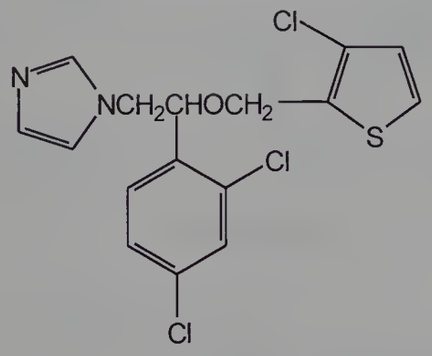
(Z)-1-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-yl)ethanone O-[2,4-dichlorophenyl)methyl]oxime mononitrate (Oxistat) is a white crystalline nitric acid salt. It is employed in cream and lotion forms in a concentration of 1% for the treatment of tinea pedis, tinea corporus and tinea capitis.



Oxiconazole

Tioconazole, USP

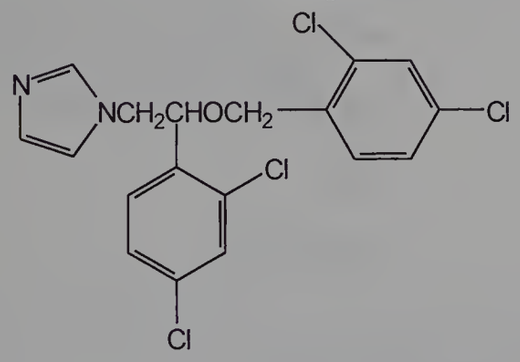
1-[2-[(2-chloro-3-thienyl)methoxy]-2-(2,4-dichlorophenyl) ethyl]-1*H*-imidazole (Vagistat) is employed for the treatment of vulvo-vaginal candidiasis. A vaginal ointment containing 6.5% of the free base is available. Tioconazole is more effective against *Torulopsis glabrata* than other azoles.



Tioconazole

Miconazole Nitrate, USP

1 - [2 - (2,4 - Dichlorophenyl) - 2 - [2,4 - dichlorophenyl] methoxy]ethyl]-1*H*-imidazole mononitrate (Monistat, Micatin Monistal IV) is a weak base with a pK_a of 6.65. The nitric acid salt occurs as a white crystalline salt that is sparingly soluble in water and most organic solvents.



Miconazole

The free base is available in an injectable form, solubilized with polyethylene glycol and castor oil, and intended for the treatment of serious systemic fungal infections, such as candidiasis, coccidioidomycosis, cryptococcosis, petriellidiosis, and paracoccidioidomycosis. It may also be used for the treatment of chronic mucocutaneous candidiasis. Although serious toxic effects from the systemic administration of miconazole are comparatively rare, thrombophlebitis, pruritus, fever, and gastrointestinal upset are relatively common.

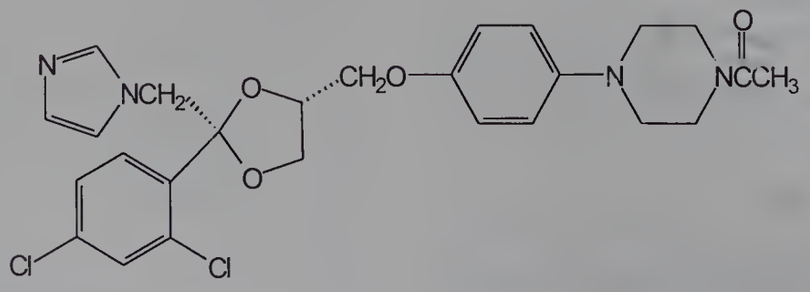
Miconazole nitrate is supplied in a variety of dosage forms (cream, lotion, powder, and spray) for the treatment of tinea infections and cutaneous candidiasis. Vaginal creams and suppositories are also available for the treatment of vaginal candidiasis. A concentration of 2% of the salt is used in most topical preparations.

Ketoconazole, USP

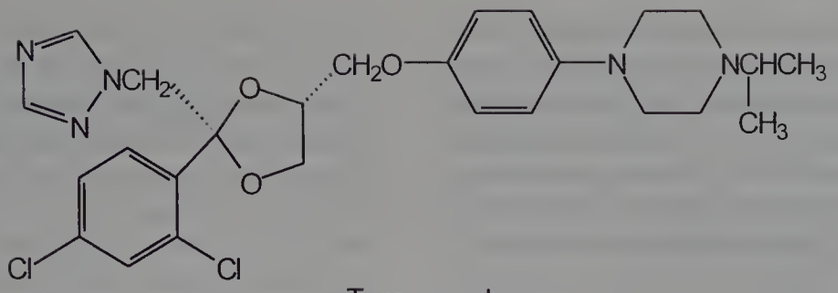
1-Acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2(1*H*-imidazole-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazine (Nizoral) is a broad-spectrum antifungal agent that is administered orally for the treatment of systemic fungal infections. It is a weakly basic compound that occurs as a white crystalline solid that is very slightly soluble in water.

The oral bioavailability of ketoconazole depends upon an acidic pH for dissolution and absorption. Antacids and drugs, such as H_2-histamine antagonists and anticholinergics that inhibit gastric secretion, interfere with its oral absorption. Ketoconazole is extensively metabolized to inactive metabolites, and the primary route of excretion is enterohepatic. It is estimated to be 95% to 99% bound to protein in the plasma.

Hepatotoxicity, primarily of the hepatocellular type, is the most serious adverse effect of ketoconazole. Ketoconazole is known to inhibit cholesterol biosynthesis,[13] suggesting that lanosterol 14α-demethylase is inhibited in mammals as well as in fungi. High doses have also been reported to lower testosterone and corticosterone levels, reflecting the inhibition of cytochrome P450-requiring enzymes involved in human steroid hormone biosynthesis.[13] Cytochrome P450 oxidases responsible for the metabolism of various drugs may also be inhibited by ketoconazole to cause enhanced effects. Thus, ketoconazole causes clinically significant increases in plasma concentrations of cyclosporine, phenytoin, and terfenidine. It may also enhance responses to sulfonylurea hypoglycemic and coumarin anticoagulant drugs.



Ketoconazole



Terconazole

Ketoconazole is a racemic compound, consisting of the cis-2S, 4R and cis-2R, 4S isomers. An investigation of the relative potencies of the four possible stereoisomers of ketoconazole against rat lanosterol 14α-demethylase[15] indicated that the 2S, 4R isomer was 2.5 times more active than its 2R, 4S enantiomer. The trans isomers, 2S, 4S and 2R, 4R are much less active.

Ketoconazole is recommended for the treatment of the following systemic fungal infections: candidiasis (including oral thrush and the chronic mucocutaneous form), coccidioidomycosis, blastomycosis, histoplasmosis, chromomycosis, and paracoccidioidomycosis. It is also used orally to treat severe refractory cutaneous dermatophytic infections not responsive to topical therapy or oral griseofulvin. The antifungal actions of ketoconazole and the polyene antibiotic amphotericin B are reported to antagonize each other.

Ketoconazole is also employed topically in a concentration of 2% in a cream and in a shampoo for the management of cutaneous candidiasis and tinea infections.

Terconazole, USP

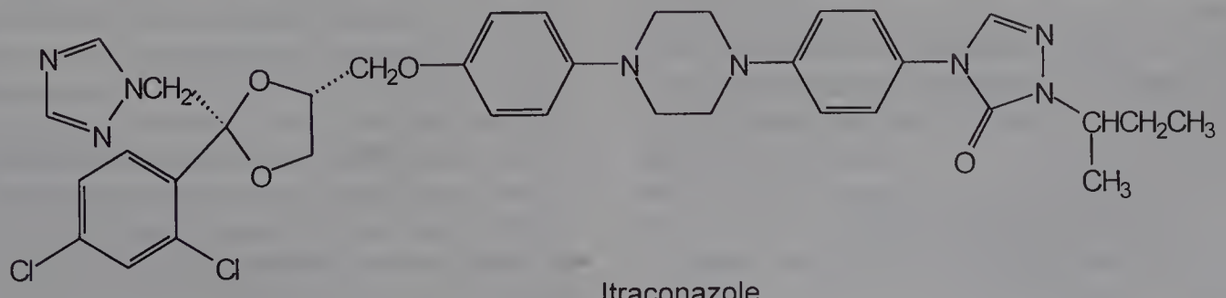
cis-1-[4-[[2-(2,4-Dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl)methoxy]-phenyl]-4-(1-methylethyl)-piperazine (Terazol), or terconazole, is a triazole derivative that is used exclusively for the control of vulvo-vaginal moniliasis caused by *Candida albicans* and other Candida species. It is available in creams containing 0.4% and 0.8% of the free base intended for 7-day and 3-day treatment periods, respectively. Suppositories containing 80 mg of the free base are also available.

Itraconazole, USP

4-[4-[4-[[2-(2,4-Dichlorophenyl)-2-1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-2-(1-methylpropyl)-3H-1,2,4-triazol-3-one (Sporonox) is a unique member of the azole class that contains two triazole moieties in its structure, a weakly basic 1,2,4-triazole and a nonbasic 1,2,4-triazol-3-one.

Itraconazole is an orally active, broad-spectrum antifungal agent that has become an important alternative to ketoconazole. An acidic environment is required for the optimum solubilization and oral absorption of itraconazole. Drugs such as H_2-histamine antagonists and antacids, which reduce stomach acidity, reduce its gastrointestinal absorption. Food greatly enhances the absorption of itraconazole, nearly doubling its oral bioavailability. The drug is avidly bound to plasma proteins (nearly 99% at clinically effective concentrations) and extensively metabolized in the liver. Only one of the numerous metabolites, namely 1-hydroxyitraconazole, has significant antifungal activity. Virtually none of the unchanged drug is excreted in the urine. Thus, the dose need not be adjusted in patients with renal impairment. The terminal elimination half-life of itraconazole ranges from 24 to 40 hr.

The primary indications for itraconazole are for the treatment of systemic fungal infections including blastomycosis, histoplasmosis (including patients infected with human immunodeficiency virus), nonmeningeal coccidioidomycosis, paracoccidioidomycosis, and sporotrichosis. It may also be effective in the treatment of pergellosis, disseminated and deep organ candidiasis, coccidioidal meningitis, and cryptococcosis.

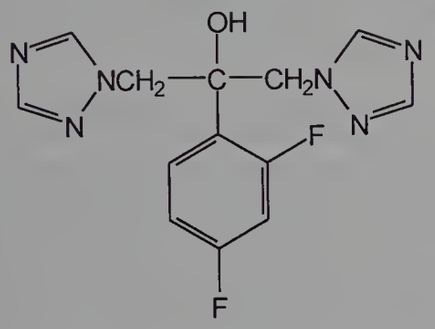


Itraconazole

In general, itraconazole is more effective and better tolerated than is ketoconazole. Unlike ketoconazole, it is not hepatotoxic and does not cause adrenal or testicular suppression in recommended therapeutic doses.[14] Nonetheless, itraconazole can inhibit cytochrome P450 oxidases involved in drug and xenobiotic metabolism and is known to increase plasma levels of the antihistaminic drugs terfenadine and astemizole.

Fluconazole, USP

α-(2,4-Difluorophenyl)-α-(1*H*-1,2,4-triazol-1-ylmethyl)-1*H*-1,2,4-triazole-1-ethanol or 2,4-difluoro-α,α-bis(1*H*-1,2,4-triazol-1-ylmethyl)benzyl alcohol (Diflucan) is a water-soluble bis-triazole with broad-spectrum antifungal properties that is suitable for both oral and intravenous administration as the free base. Intravenous solutions of fluconazole contain 2 mg of the free base in 1 ml of isotonic sodium chloride or 5% dextrose vehicle.



Fluconazole

The oral bioavailability of fluconazole, following administration of either tablet or oral suspension dosage forms, is excellent. Apparently the presence of two weakly basic triazole rings in the molecule confers sufficient aqueous solubility to balance the lipophilicity of the 2,4-difluorophenyl group. The oral absorption of fluconazole, in contrast to the oral absorption of ketoconazole or itraconazole, is not affected by alteration in gastrointestinal acidity or the presence of food.

Fluconazole has a relatively long elimination half-life, ranging from 27 to 34 hr. It penetrates well into all body cavities, including the cerebrospinal fluid. Plasma protein binding of fluconazole is <10%, and it is efficiently removed from the blood by hemodialysis. Fluconazole experiences little or no hepatic metabolism and is excreted substantially unchanged in the urine. A small amount of unchanged fluconazole (~10%) is excreted in the feces. Side effects of fluconazole are largely confined to minor gastrointestinal symptoms. Inhibition of cytochrome P450 oxidases by fluconazole can give rise to clinically significant interactions involving increased plasma levels of cyclosporine, phenytoin, and the oral hypoglycemic drugs (tolbutamide, glipizide, and glyburide). Fluconazole does not appear to interfere with corticosteroid or androgen biosynthesis in doses employed to treat systemic fungal infections.

Fluconazole is recommended for the treatment and prophylaxis of disseminated and deep organ candidiasis. It is also employed to control esophageal and oropharyngeal candidiasis. Because of its excellent penetration into cerebrospinal fluid, fluconazole is an agent of choice for the treatment of cryptococcal meningitis and for prophylaxis of cryptococcosis in AIDs patients. Although fluconazole is generally less effective than either ketoconazole or itraconazole against nonmeningeal coccidiomycosis, it is preferred therapy for coccidioidal meningitis.

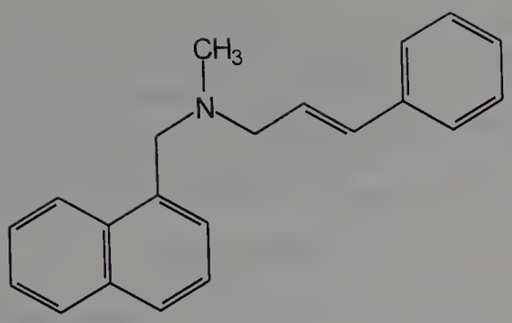
ALLYLAMINES AND RELATED COMPOUNDS

The allylamine class of antifungal agents was discovered as a result of random screening of a chemical inventory for antifungal activity.[16] Naftifine, the first member of the series to be introduced, was actually synthesized by accident.[17] Structure-activity studies in the series subsequently led to the discovery of compounds with enhanced potency and potential oral activity, such as terbinafine. Investigation of the mechanism of action of the allylamines revealed that they interfere with fungal ergosterol biosynthesis at an early stage, namely the epoxidation of squalene catalyzed by the enzyme squalene epoxidase[18] (Fig. 7-1). Inhibition of the enzyme causes accumulation of squalene which, in turn, damages the fungal cell membrane. The allylamines exert a fungicidal action against dermatophytes and other filamentous fungi, but their action against pathogenic yeasts such as Candida is largely fungistatic. Mammalian squalene epoxidase is very weakly inhibited by the allylamines, which do not appear to significantly affect cholesterol biosynthesis.

Two allylamines, naftifine and terbinafine, have been approved as topical agents for the treatment of tinea pedis, tinea cruris, and tinea corposis caused by *Trichoplyton rubrum*, *Trichophyton mentagrophytes*, or *Epidermophyton floccosum*. The topical antifungal agent tolnaftate, although it is not an allylamine, is an inhibitor of squalene epoxidase[19] and has a similar spectrum of antifungal action to the allylamines, and is therefore classified with the allylamines. The allylamines are weak bases that form hydrochloride salts that are slightly soluble in water.

NAFTIFINE HYDROCHLORIDE, USP

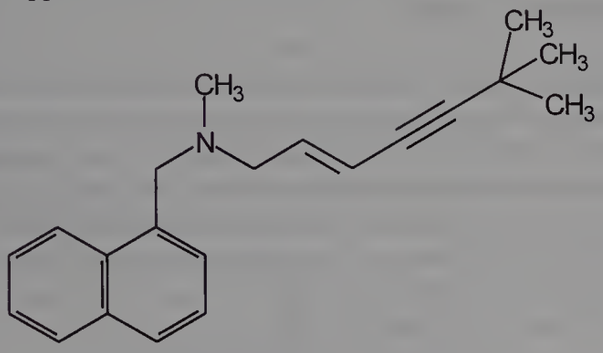
N-Methyl-N-(3-phenyl-2-propenyl)-1-naphthalene methanamine hydrochloride (Naftin) is a white crystalline powder that is soluble in polar organic solvents such as ethanol and methylene chloride. It is supplied in 1% concentration in a cream and in a gel for topical treatment of ringworm, athlete's foot and jock itch. Unapproved uses for which naftifine has been shown to be effective include the treatment of tinea barbae (ringworm of the beard), tinea capitis (ringworm of the scalp), and tinea versicolor (pityriasis versicolor or "sun fungus").



Naftifine

TERBINAFINE HYDROCHLORIDE, USP

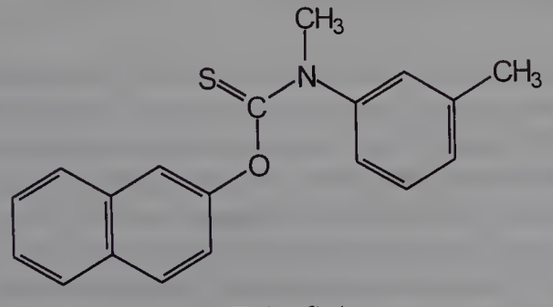
(E)-N-(6,6-dimethyl-2-hepten-4-ynyl)-N-methyl-1-naphthalenemethanamine hydrochloride (Lamsil) is an off-white crystalline powder that is soluble in polar organic solvents such as methanol, ethanol, and methylene chloride, but only slightly soluble in water. The highly lipophilic free base is insoluble in water. Terbinafine hydrochloride is available in 1% concentration as a cream for topical administration for the control of tinea pedis, tinea corporus, and tinea cruris. Terbinafine is more potent than naftifine and also has demonstrated oral activity that may be useful in the treatment of onychomycoses (ringworm of the nails). It has not, however, been approved in the United States for oral administration.



Terbinafine

TOLNAFTATE, USP

O-2-Naphthyl *m, N*-dimethylthiocarbanilate (Tinactin, Aftate, Footwork, Fungatin, NP-27) occurs as a white crystalline solid that is insoluble in water, sparingly soluble in alcohol, and soluble in most organic solvents.



Tolnaftate

This compound, which is a thioester of β-naphthol, is fungicidal against dermatophytes, such as Trichophyton, Microsporum, and Epidermophyton species that cause superficial tinea infections. It is available in a concentration of 1% in creams, powders, aerosols, gels, and solutions for the treatment of ringworm, jock itch, and athlete's foot.

Tolnaftate has been shown to be an inhibitor of squalene epoxidase in susceptible fungi.[19] It is therefore classified with the allylamines. Despite its lack of basic functionality, tolnaftate bears structural resemblance to the allylamine antifungal agents.

FATTY ACIDS

All fatty acids have fungicidal properties. The higher-molecular-weight members have the advantage of having lower volatility. The salts of fatty acids are also fungicidal and provide nonvolatile forms for topical administration. Because of their availability, the fatty acids and salts thereof that are the most widely used are propionic, caprylic, and undecylenic.

Propionic Acid

Propionic acid is a readily available fungicide that is nonirritating and nontoxic. It is present in sweat in low concentrations (~0.01%). Various salts, such as those of sodium, potassium, calcium, ammonium, and zinc are also fungicidal.

Propionic acid is a clear, corrosive liquid, with a characteristic odor. It is soluble in water and in alcohol. The salts are generally used, instead of the free acid, because they are nonvolatile and odorless.

Zinc Propionate

Zinc propionate occurs as both an anhydrous form and a monohydrate. It is very soluble in water, but only sparingly soluble in alcohol. The salt is unstable to moisture, forming zinc hydroxide and propionic acid. It is employed as a fungicide, particularly on adhesive tape.

Sodium Caprylate

Sodium caprylate is prepared from caprylic acid, which is found in coconut and palm oils. The salt occurs as cream-colored granules that are soluble in water and sparingly soluble in alcohol.

$CH_3(CH_2)_5CH_2COO^-Na^+$
Sodium Caprylate

Sodium caprylate is used topically to treat superficial fun-

gal infections caused by *Candida albicans* and Trichophyton, Microsporum, and Epidermophyton species. It is found in solution, powder, and ointment forms.

Zinc Caprylate

Zinc caprylate is a fine white powder that is virtually insoluble in water or alcohol. It is used as a topical fungicide. The copper salt is also found in various proprietary antifungal preparations. These salts must be kept in tightly closed containers to protect them from moisture.

Undecylenic Acid, USP

10-Undecenoic acid (Desenex, Cruex, Decylenes) has the formula $CH_2 = CH(CH_2)_8CO_2H$. It is obtained from the destructive distillation of castor oil. Undecylenic acid occurs as viscous yellow liquid having a characteristic odor. The acid is nearly insoluble in water, but soluble in alcohol and most organic solvents.

Undecylenic acid is one of the better fatty acids available as a fungicide. It may be used in concentrations up to 10% in solutions, ointments, emulsions, and powders for topical administration. It is considered too irritating, however, to be applied to mucous membranes. Several salts, including those of sodium, potassium, zinc, and copper, are also used, some in combination with the acid.

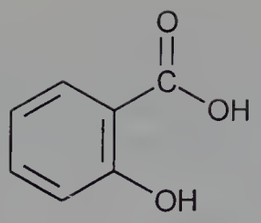
Triacetin, USP

Glyceryl triacetate (Enzactin, Fungacetin) is a colorless oily liquid, with a slight odor and a bitter taste. It is soluble in water and miscible with alcohol and most organic solvents.

The fungicidal properties of triacetin are due to acetic acid formed by enzymatic hydrolysis by esterases of the skin. The rate of acetic acid formation is self-limiting because the esterases are inactivated below a *p*H of 4.

Salicyclic Acid, USP

o-Hydroxybenzoic acid is a strong acid ($pK_a = 2.5$) with antiseptic and keratolytic properties. It occurs as white, needle-like crystals or as a fluffy, crystalline powder. It is only slightly soluble in water, but soluble in most organic solvents. The greater acidity of salicylic acid and its lower solubility in water, as contrasted with *p*-hydroxybenzoic acid, are the consequence of intramolecular hydrogen bonding.



Salicylic Acid

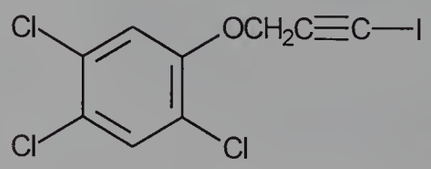
Salicylic acid is used externally in ointments and solutions for its antiseptic and escharotic properties. Many foot remedies for corns and athlete's foot contain salicylic acid.

PHENOLS AND THEIR DERIVATIVES

A number of phenols and their derivaties are known to possess antifungal properties. Some of them, such as hexylresorcinol and parachlorometaxylenol, have been employed topically for the treatment of tinea infections in the past. Two compounds, clioquinol and haloprogin, remain official in the USP. A third agent, ciclopirox, is not a phenol, but has properties similar to those of phenols. All of these agents appear to interfere with cell membrane integrity and function in susceptible fungi.

Haloprogin, USP

3-Iodo-2-propynyl 2,4,5-trichlorophenyl ether (Halotex) occurs as white to pale yellow crystals that are sparingly soluble in water and easily soluble in ethanol. It is used as a 1% cream or solution for the treatment of superficial tinea infections. Formulations of haloprogin should be protected from the light owing to the photosensitivity of the compound. It is available as a solution and as a cream, both containing 1% of the fungicide.



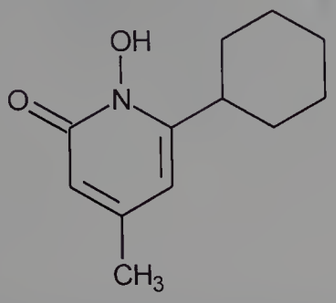
Haloprogin

Ciclopirox Olamine, USP

6-Cyclohexyl-1-hydroxy-4-methyl-2-(1*H*)-pyridinone ethanolamine salt (Loprox) is a broad-spectrum antifungal agent intended for topical use. It is active against dermatophytes, as well as pathogenic yeasts, such as *C. albicans*, that cause superficial fungal infections. Ciclopirox is considered a primary agent for the treatment of cutaneous candidiasis, tinea corporis, tinea cruris, tinea pedis, and tinea versicolor. It is a secondary agent for the treatment of onychomycosis

(ringworm of the nails). It is supplied as a cream and a lotion, each containing 1% of the water-soluble ethanolamine salt.

Ciclopirox is believed to act on the cell membranes of susceptible fungi at low concentrations to block the transport of amino acids into the cell. At higher concentrations, it disrupts membrane integrity, causing loss of cellular constituents.[20]

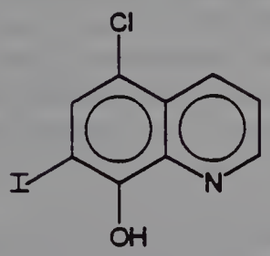


Ciclopirox Olamine

Clioquinol, USP

5-Chloro-7-iodo-8-quinolinol, 5-chloro-8-hydroxy-7-iodoquinoline, or iodochlorhydroxyquin (Vioform) is found as a spongy, voluminous, light-sensitive, yellowish-white powder that is virtually insoluble in water.

This compound was originally introduced as an odorless substitute for iodoform in the belief that it liberated iodine in the tissues. It has been used as powder for a variety of skin conditions, such as atopic dermatitis, eczema, psoriasis, and impetigo. It has also been applied vaginally as a 3% ointment or cream for the treatment of *T. vaginalis* vaginitis. However, its principal current use is in the local treatment of fungal infections, such as athlete's foot.

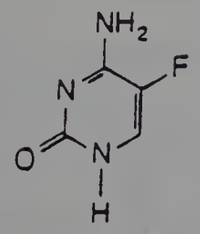


Clioquinol

NUCLEOSIDES

Flucytosine, USP

5-Fluorocytosine, 5-FC, 4-amino-5-fluoro-2(1*H*)-pyrimidinone, or 2-hydroxy-4-amino-5-fluoropyrimidine (Ancobon) is an orally active antifungal agent having a narrow spectrum of activity. It is indicated for the treatment of serious systemic infections caused by susceptible strains of Candida and Cryptococcus.



Flucytosine

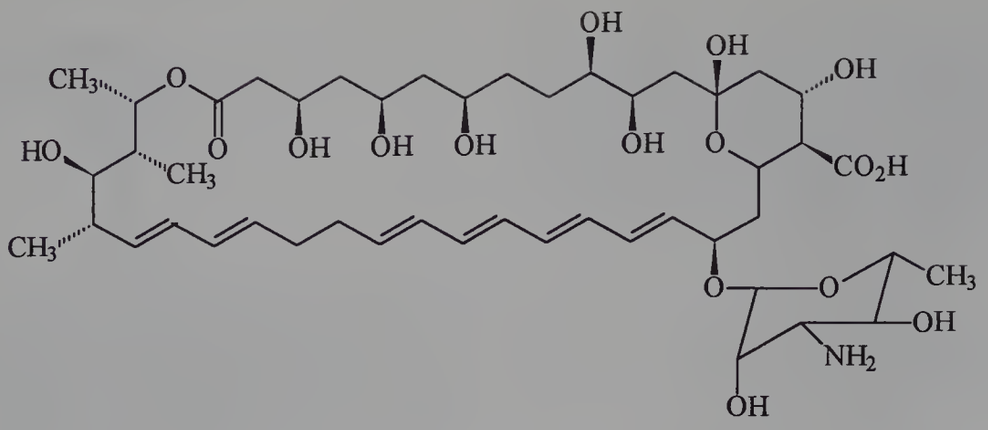
The mode of action of 5-flucytosine in susceptible fungi and mechanisms of resistance to the drug in nonsusceptible strains have been studied in some detail.[21] Incorporation of fluorinated pyrimidine into RNA following selective deamination to 5-fluorouracil (5-FU) in the fungus appears to be required for antifungal activity. Resistant strains appear either to be deficient in enzymes required for the bioactivation of 5-FC (e.g., uridine monophosphate pyrophosphorylase or cytosine deaminase) or to have a surplus of de novo pyrimidine-synthesizing capacity. The comparative lack of toxicity of 5-FC in humans is apparently because it is not deaminated to the toxic antimetabolite 5-FU in cells of the host after oral administration. The half-life of flucytosine, which is excreted largely unchanged, is 3 to 6 hr.

ANTIFUNGAL ANTIBIOTICS

POLYENES

A number of structurally complex antifungal antibiotics isolated from soil bacteria were discovered to contain a conjugated system of double bonds in large lactone rings. They differ from the antibacterial macrocyclic lactones (macrolides; see Chap. 10) of the erythromycin type in the size of the lactone ring and the presence of the conjugated ene system and, hence, are referred to as the polyenes. These antibiotics fall into two groupings, based on the size of the macrolide ring: the 26-membered ring polyenes, such as natamycin (pimaricin) and the 38-membered ring polyenes such as nystatin and amphotericin B. A glycosidically linked deoxyaminohexose sugar, mycosamine, is common to the currently available polyenes. They also differ in the number of double bonds present in the lactone ring: natamycin is a pentaene; nystatin a hexaene; and amphotericin B a heptaene.

The polyenes are broad-spectrum antifungal agents with potent activity against pathogenic yeasts, molds, and dermatophytes. Important pathogenic fungi inhibited by low concentrations of these agents in vitro include Candida sp., *Coccidioides immitis, Cryptococcus neoformans, Histoplasma capsulatum, Blastomyces dermatitidis,* Rhodotorula sp., *Sporothrix schenckii, Mucor mucedo, Aspergillis fumigatus,* Cephalosporium sp., and Fusarium sp. Polyenes are without effect against bacteria, rickettsia, or viruses. They do, however, possess activity against certain protozoa, such as Leishmania sp. Less expensive compounds are used to treat tinea infections. The usefulness of the polyenes for the



Nystatin A_1

treatment of systemic infections is limited by their toxicities, low water solubilities, and poor chemical stabilities. Thus, amphotericin B, the only polyene available for the treatment of serious systemic infections, must be solubilized with the aid of an emulsifying agent. The use of other members of the class is confined to the topical treatment of superficial fungal infections.

The mode of action of the polyene antibiotics has been extensively investigated.[11,22] They appear to bind with sterols in the cell membrane to cause disorganization and loss of cell constituents, especially potassium ion. The action may be fungistatic or fungicidal, suggesting an initial effect on membrane-bound enzymes (such as ATPase) at low concentrations and a generalized membrane disruption at higher concentrations.

Amphotericin B, USP (Fungizone)

A potent antifungal substance with a polyene structure was isolated in 1956 by Gold et al.[23] from an actinomycete, *Streptomyces nodosus*, found in a soil sample from Venezuela. The antibiotic material was a mixture of two closely related compounds designated amphotericins A and B. The B compound was more active and is consequently the one employed therapeutically. Its structure and absolute stereochemistry have been determined[24–26] as shown.

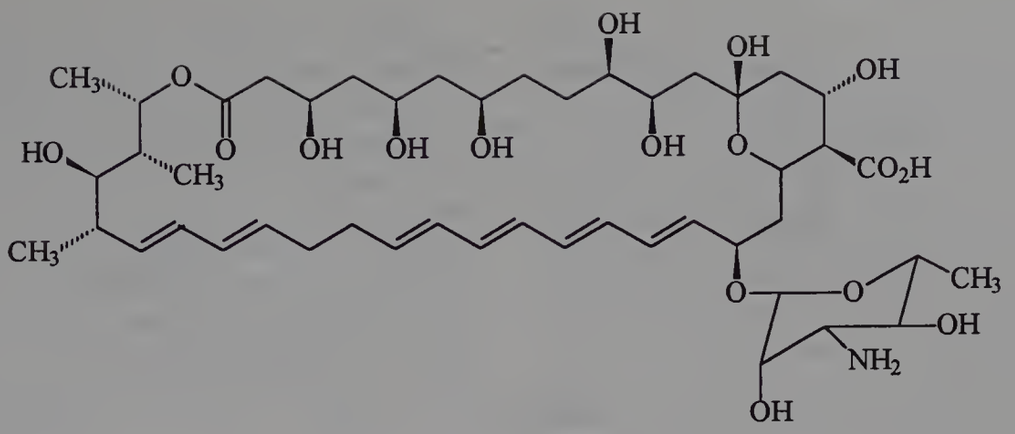
As the name implies, amphotericin B is an amphoteric substance containing a primary amino group in the sugar moiety, mycosamine, and a carboxyl group attached to the macrolide ring. It occurs as a deep yellow crystalline solid that is sparingly soluble in polar organic solvents, but insoluble in water. Although it forms salts with both acids and alkalis, these salts are only slightly soluble in water (~0.1 mg/ml) and thus are not suitable for systemic administration. The parenteral dosage form is an aqueous colloidal dispersion stabilized with sodium deoxycholate. The compound is light- and heat-sensitive.

Parenterally, amphotericin B is indicated for the treatment of serious, potentially life-threatening fungal infections, including disseminated forms of coccidioidomycosis and histoplasmosis, sporotrichosis, North American blastomycosis, cryptococcosis (torulosis), mucormycosis, and aspergillosis. It may be beneficial in the treatment of American mucocutaneous leishmaniasis, but it is not a drug of choice.

A high prevalence of adverse reactions limits the usefulness of amphotericin B. Some form of nephrotoxicity occurs in nearly 80% of the patients. Generalized toxic reactions, such as fever, headache, anorexia, gastrointestinal distress, muscle and joint pain, and malaise, are also common. Pain at the injection site and thrombophlebitis frequently occur when the drug is injected intravenously. It must never be administered intramuscularly. The hemolytic action of amphotericin B may be the result of its affinity for membrane lipids and cholesterol.

Amphotericin B for injection is supplied as a sterile lyophylized cake or powder containing 50 mg of active ingredient with 41 mg of sodium deoxycholate to be dispersed in 10 ml of water. The infusion solution, providing 0.1 mg/ml, is then obtained by further dilution (1:50) with 5% dextrose injection. Normal saline should not be used because it will destroy the suspension. The suspension should be freshly prepared and used within 24 hr. The drug powder should be refrigerated and protected from light.

A variety of sterile preparations wherein amphotericin B is formulated with a lipid carrier have been developed with the goal of counteracting the dose-limiting toxicity of the drug following intravenous administration. These include: amphotericin B colloidal dispersion (Amphocil, Amphocyte), which contains nearly equal parts of the drug and cholesterol sulfate in a suspension of disk-like particles; Abelcet, a 1:1 combination of amphotericin B with L-α-dimyristoylphosphatidylcholine (7 parts) and L-α-dimyristoylphosphatidylglycerol (3 parts) to create a suspension of ribbon-like sheets; and liposomal amphotericin B (Ambisome), a small laminar vesicular preparation consisting of an approximately 1:10 mol ratio of amphotericin B and lipid (made up of hydrogenated soy phosphatidyl choline, cholesterol and distearoylphosphatidylcholine in a 10:5:4 ratio) for an aqueous suspension.



Nystatin A_1

The rationale for the use of these lipid formulations is based on the idea that amphotericin B will complex more avidly with the lipid vehicle than with cholesterol in cell membranes and thereby the toxicity of the drug would be reduced. Additionally, lipid-associated amphotericin B will tend to distribute to reticuloendothelial tissue and thus concentrate in the lymphatic system, spleen, liver and lungs where invading fungi tend to locate. Fungal and host lipases are expected to release the drug from its lipid carrier, making it available to bind ergosterol in fungal cell membranes and exert its fungistatic and fungicidal effects. Reduced renal toxicity has been demonstrated with the clinical use of each of the approved amphotericin B lipid preparations. Liposomal amphotericin B has been approved specifically for the treatment of pulmonary aspergillosis, because of its demonstrated superiority to the sodium deoxycholate stabilized suspension.

Amphotericin B is also used topically to treat cutaneous and mucocutaneous mycotic infections caused by *C. albicans*. It is supplied in a variety of topical forms, including a cream, a lotion, and an ointment. The concentration of antibiotic in these preparations is 3%.

Nystatin, USP

Nystatin (Mycostatin, Nilstat, Mykinac, Nystex) is a polyene antibiotic first isolated in 1951 from a strain of *S. noursei* by Hazen and Brown.[27] It occurs as a yellow to light tan powder with a cereal-like odor. Nystatin is only very slightly soluble in water and sparingly soluble in organic solvents. It is unstable to moisture, heat, and light.

Nystatinolide, the aglycon portion of nystatin, consists of a 38-membered lactone ring with single tetracene and diene chromophores isolated from each other by methylene group, one carboxyl, one keto, and eight hydroxyl groups. It is glycosidically linked to the amino sugar mycosamine. The complete structure of nystatin has been determined by x-ray crystallographic and chemical degradation procedures.[28,29] A minor revision of the originally proposed structure[26] shows a hemiacetal formed between the keto group at C-13 and the hydroxyl group at C-17.

Nystatin has been a valuable agent for the treatment of local and gastrointestinal monilial infections caused by *C. albicans* and other Candida species for more than three decades. For the management of cutaneous and mucocutaneous candidiasis, it is supplied as a cream, an ointment, and a powder. Vaginal tablets are available for the control of vaginal candidiasis. Oral tablets and trochees are used in the treatment of gastrointestinal and oral candidiasis. The systemic absorption of nystatin following oral administration is practically nil. Combinations of nystatin with tetracyclines have been long employed to prevent monilial overgrowth caused by destruction of the bacterial microflora of the intestine. Most informed medical opinion now appears to favor treatment of intestinal candidiasis only after it occurs secondary to tetracycline therapy.

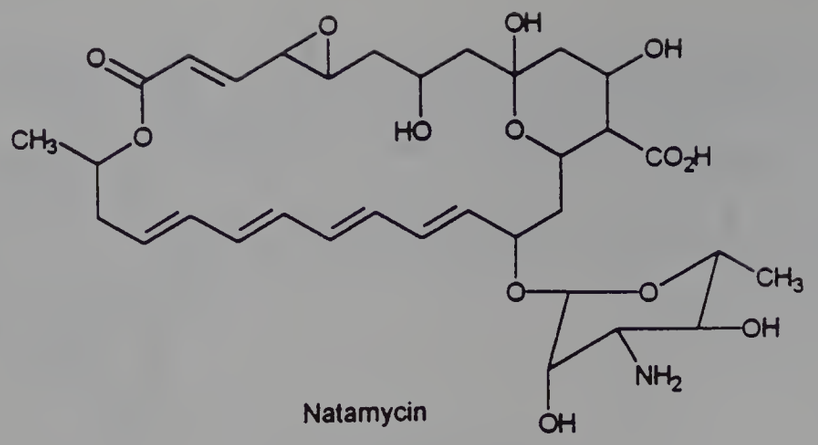
Although nystatin is a pure compound of known structure, its dosage is still expressed in terms of units. One milligram of nystatin contains not less than 2,000 USP units.

Natamycin, USP

Natamycin (pimaricin; Natacyn) is a polyene macrolide antibiotic obtained from *S. natalensis*. It was first isolated by Struyk et al.,[30] and its structure was elucidated 8 years later.[31] A minor revision of the originally proposed structure has been subsequently reported.[26]

The natamycin structure consists of a 26-membered lactone ring containing a tetraene chromophore, a double bond conjugated with the lactone carbonyl group, three hydroxyl groups, one keto group forming a hemiacetal with one of the hydroxyl groups,[26] an epoxide, and a carboxyl group. The sugar mycosamine, common to all of the polyenes, is present. Of course, natamycin is an amphoteric substance.

Studies on the mechanism of action of the polyene antibiotics indicate differences in their effects on fungal membranes between smaller-ring macrolides, such as natamycin, and the larger-ring macrolides, such as amphotericin B and nystatin. The 26-membered ring polyenes cause both potassium ion leakage and cell lysis at the same concentration, whereas the 38-membered ring polyenes cause potassium ion leakage at low, fungistatic concentrations and cell lysis



Natamycin

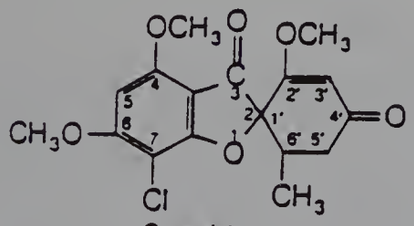
at high, fungicidal concentrations. The smaller-ring polyenes are fungistatic and fungicidal within the same concentration range.

Natamycin possesses in vitro activity against a variety of yeasts and filamentous fungi, including Candida, Aspergillis, Cephalosporium, Penicillium, and Fusarium species. It is supplied as a 5% ophthalmic suspension intended for the treatment of fungal conjunctivitis, blepharitis, and keratitis.

OTHER ANTIFUNGAL ANTIBIOTICS

Griseofulvin, USP

Griseofulvin (Grisactin, Fulvicin, Grifulvin, Gris-PEG) was first reported in 1939 by Oxford et al.[32] as an antibiotic obtained from the mold *Penicillium griseofulvum* (Dierckx). Although it had been used for its antifungal action in plants and animals for many years, it was not until 1959 that griseofulvin was introduced into human medicine in the United States for the systemic treatment of tinea infections.



Griseofulvin

The structure of griseofulvin was determined by Grove et al.[33] to be 7-chloro-2′,4,6-trimethoxy-6′,β-methylspiro[benzofuran-2(3*H*)-1′-[2]cyclohexene]-3,4′-dione. It occurs as a white, bitter, heat-stable powder or crystalline solid that is sparingly soluble in water, but relatively soluble in alcohol and in nonpolar organic solvents. It is very stable in the dry state.

Griseofulvin is recommended for the systemic treatment of refractory ringworm infections of the body, nails, hair, and feet (tinea corporus, tinea unguium, tinea capitis, and tinea pedis) caused by various species of dermatophytic fungi, including Trichophyton, Microsporum, and Epidermophyton. Following oral absorption, some griseofulvin is carried by the systemic circulation to the skin, hair, and fingernails, where it concentrates in keratin precursor cells, which are gradually exfoliated and replaced by new tissue. As the new tissue develops, the fungistatic action of the griseofulvin present prevents further infection within it. Because the old tissue may continue to support fungal growth, the treatment must be continued until all of the infected tissue has been exfoliated. Consequently, therapy of infections in slow-growing tissues, such as the nails, must be continued for several months.

Relatively few adverse effects have been reported for griseofulvin. The most common are mild allergic reactions, such as skin rashes and urticaria. Occasional gastrointestinal upset, headache, dizziness, or insomnia may also occur. Griseofulvin has no antibacterial activity, nor is it effective against pathogenic yeasts, including *Pityosporum obiculare (Malassezia furfur)*, the organism that causes tinea versicolor.

The oral bioavailability of griseofulvin is notoriously bad. It is a very lipophilic compound with very low water solubility. The most successful attempts to improve oral absorption have concentrated on improving the dissolution of the antibiotic in the gastrointestinal tract by reducing its particle size. Griseofulvin is supplied in "microsize" and "ultramicrosize" forms. The efficiency of gastrointestinal absorption of the ultramicrosize form is 1.5 times that of the microsize form, permitting a dosage reduction of one-third. The bioavailability of griseofulvin may also be increased by administering the drug with a fatty meal. Although several structural analogues have been synthesized, none of them has shown activity superior to that of griseofulvin.

Studies on the mechanism of action of griseofulvin have concentrated on its ability to arrest cell division in metaphase in vitro. The drug causes a rapid, reversible dissolution of the mitotic spindle apparatus, apparently by binding with the tubulin dimer required for microtubule assembly.[34] The basis for its selective toxicity is not known, but it may be related to the tendency of the drug to concentrate in tissues rich in keratin.

SYNTHETIC ANTIBACTERIAL AGENTS

A number of organic compounds obtained by chemical synthesis have useful antibacterial activity for the treatment of

local, systemic, and/or urinary tract infections. Some chemical classes of synthetic antibacterial agents include the sulfonamides (discussed separately in Chap. 8), certain nitroheterocyclic compounds (for example, the nitrofurans and metronidazole), and the quinolones. Some antibacterial agents that fail to achieve adequate concentrations in the plasma or tissues for the treatment of systemic infections following oral or parenteral administration are concentrated in the urine, where they can be effective for eradicating urinary tract infections. Nitrofurantoin (a nitrofuran), nalidixic acid (a quinolone), and methenamine are examples of such urinary tract antiinfectives.

QUINOLONES

The quinolones comprise a series of synthetic antibacterial agents patterned after nalidixic acid, a naphthyridine derivative introduced for the treatment of urinary tract infections in 1963. Isosteric heterocyclic groupings in this class include the quinolines (e.g., norfloxacin, ciprofloxacin, and lomefloxacin), the naphthyridines (e.g., nalidixic acid and enoxacin), and the cinnolines (e.g., cinoxacin). Up to the present time, the clinical usefulness of the quinolones has been largely confined to the treatment of urinary tract infections. For this, good oral absorption, activity against common gram-negative urinary pathogens, and comparatively higher urinary (compared with plasma and tissue) concentrations are useful properties. However, as a result of extensive structure-activity investigations leading to compounds with enhanced potency, extended spectrum of activity, and improved absorption and distribution properties, the class has evolved to the point that certain newer members have utility for the treatment of a variety of serious systemic infections. In fact, these more potent analogs are sometimes classified separately (from the urinary tract-specific agents) as the fluoroquinolones because all members of the group have a 6-fluoro substituent in common.

Structure-activity studies have shown that the 1,4-dihydro-4-oxo-3-pyridinecarboxylic acid moiety is essential for antibacterial activity. The pyridone system must be annulated with an aromatic ring. Isosteric replacements of nitrogen for carbon atoms at positions 2 (cinnolines), 5 (1,5-napthyridines), 6 (1,6-naphthyridines), and 8 (1,8-naphthyridines) are consistent with retained antibacterial activity. Although the introduction of substituents at position 2 greatly reduces or abolishes activity, positions 5, 6, 7 (especially), and 8 of the annulated ring may be substituted to good effect. For example, piperazinyl and 3-aminopyrrolidinyl substitutions at position 7 have been identified with the enhanced activity of members of the quinolone class against *Pseudomonas aeruginosa*. Fluorine atom substitution at position 6 is also associated with significantly enhanced antibacterial activity. Alkyl substitution at the 1-position is essential for activity, with lower alkyl (methyl, ethyl, cyclopropyl) compounds generally having progressively greater potency. Aryl substitution at the 1-position is also consistent with antibacterial activity, with the 2,4-difluorophenyl group providing optimal potency. Ring condensations at the 1,8-, 5,6-, 6,7-, and 7,8-positions also lead to active compounds.

The effective antibacterial spectrum of nalidixic acid and early members of the quinolone class (e.g., oxolinic acid and cinoxacin) is largely confined to gram-negative bacteria, including common urinary pathogens, such as *Escherichia coli*, Klebsiella, Enterobacter, Citrobacter, and Proteus species. Shigella, Salmonella, and Providencia are also susceptible. Strains of *P. aeruginosa, Neisseria gonorrhoeae*, and *Haemophilus influenzae* are resistant, as are the gram-positive cocci and anaerobes. Newer members of the class possessing 6-fluoro and 7-piperazinyl substituents exhibit an extended spectrum of activity that includes effectiveness against additional gram-negative pathogens (such as *P. aeruginosa, H. influenzae*, and *N. gonorrhoeae*) and gram-positive cocci (such as *S. aureus*), and some streptococci. The quinolones generally exhibit poor activity against most anaerobic bacteria, including most Bacteriodes and Clostridium species. In many cases, bacterial strains that have acquired resistance to the antibacterial antibiotics, such as penicillin-resistant gonococci, methacillin-resistant *S. aureus*, and aminoglycoside-resistant *P. aeruginosa* are susceptible to the quinolones.

The bactericidal action of nalidixic acid and its congeners is known to result from the inhibition of DNA synthesis. This effect is believed to be due to the inhibition of bacterial DNA gyrase (topoisomerase II), an enzyme responsible for introducing negative supercoils into circular duplex DNA.[35] Negative supercoiling relieves the torsional stress of unwinding helical DNA and, thereby, allows transcription and replication to occur. Although nalidixic acid inhibits gyrase activity, it binds only to single-stranded DNA and not to either the enzyme or double-helical DNA.[35] Bacterial DNA gyrase is a tetrameric enzyme consisting of two A and two B subunits, encoded by gyr A and gyr B genes. Bacterial strains resistant to the quinolones have been identified, with decreased binding affinity to the enzyme due to amino acid substitution in either A or B subunits resulting from mutations in either gyr A[36] or gyr B[37] genes.

The highly polar quinolones are believed to enter bacterial cells through highly charged porin channels in the outer membrane. Mutations leading to altered porin proteins can lead to decreased uptake of quinolones and resistance.[38] Also, there is evidence for energy-dependent efflux of quinolones by some bacterial species. A quantitative structure-activity relationship (QSAR) study of bacterial cellular uptake of a series of quinolones[39] revealed an inverse relationship of uptake versus Log P (a measure of lipophilicity) for gram-negative bacteria, on the one hand, but a positive correlation of quinolone uptake to Log P in gram-positive bacteria, on the other. This result probably reflects the observed differences in outer envelope structure of gram-negative bacteria as compared with gram-positive bacteria.[40]

The relatively low incidence (<1%) of CNS effects associated with the quinolones (for example, irritability, tremor, sleep disorders, vertigo, anxiety, agitation, convulsions, etc.)

TABLE 7-2

DISSOCIATION AND ISOELECTRIC CONSTANTS FOR ANTIBACTERIAL QUINOLONES

Quinolone	pK_1[a]	pK_2[a]	*pI*[a]	*QH#/QH⁰*
Nalidixic acid	6.03	—	—	—
Norfloxacin	6.39	8.56	7.47	118
Enoxacin	6.15	8.54	7.35	238
Ciprofloxacin	6.08	8.73	7.42	444
Ofloxacin	5.88	8.06	6.97	146
Lomefloxacin	5.65	9.04	7.35	3,018

[a] Each value represents an average of literature values. Data taken from ref. 41.

has been attributed to antagonism of gamma amino butyric acid (GABA) receptors in the brain by the quinolones. Only fluoroquinolones having a 1-piperidino, a 3-amino-1-pyrrolidino, or similar basic moiety at the 7-position appear to have this property.[40] The low incidence of CNS effects is for most quinolones apparently due to their poor penetration into the brain.

Another class property of the quinolones is phototoxicity or extreme sensitivity to sunlight. Quinolones possessing a halogen atom at the 8-position (for example, lomefloxacin) have the highest incidence of phototoxicity, while those having an amino (for example, sparfloxacin) or methoxy group at either the 5- or the 8-position have the lowest incidence.[40]

The antibacterial quinolones can be divided into two classes based on their dissociation properties in physiologically relevant conditions. The first class, represented by nalidixic acid, oxolinic acid (no longer marketed in the United States), and cinoxacin, possesses only the 3-carboxylic acid group as an ionizable functionality. The pK_a values for the 3-carboxyl group in nalidixic acid and other quinolone antibacterial drugs fall in the range of 5.6 to 6.4 (Table 6.2).[41] These comparatively high pK_a values, relative to the pK_a of 4.2 for benzoic acid, are attributed to the acid weakening effect of hydrogen bonding of the 3-carboxyl group to the adjacent 4-carbonyl group.[41] The extremely low pK_a values for various quinolones observed by Lee et al.[42] and attributed to the 3-carboxyl group ionization have obviously been misassigned.[41]

The second class of antibacterial quinolones embraces the broad-spectrum fluoroquinolones (namely, norfloxacin, enoxacin, ciprofloxacin, ofloxacin, lomefloxacin, and sparfloxicin), all of which possess, in addition to the 3-carboxylic acid group, a basic piperazino functionality at the 7-position and a 6-fluoro substituent. The pK_a values for the more basic nitrogen atom of the piperazino group fall in the range of 8.1 to 9.3 (Table 7-2).[41] At most physiologically relevant pH values, significant dissociation of both the 3-carboxylic acid and the basic 7-(1-piperazino)-groups occurs, leading to significant fractions of zwitterionic species. As an example, the dissociation equilibria for norfloxacin are illustrated in Fig. 7-2.[41] The tendency for certain fluoroquinolines (for example, norfloxacin and ciprofloxacin) in high doses to cause crystalluria in an alkaline urine is due, in part, to the predominance of the comparatively less-soluble zwitterionic form. Solubility data presented for ofloxacin in the 15th edition of the United States Pharmacopea dramatically illustrate the effect of *p*H on water solubility of compounds of the fluoroquinolone class. Thus, at *p*H values ranging from 2 to 5 the solubility of ofloxacin in water is 60 mg/ml, at *p*H 7 (near the isoelectric point, pI) the water solubility falls to 4 mg/ml, and at *p*H 9.8 the water solubility of ofloxacin rises to 303 mg/ml.

The excellent chelating properties of the quinolones provide the basis for their incompatibility with antacids, hematinics, and mineral supplements containing divalent or trivalent metals. Thus, the quinolones may form 1:1, 2:1, or 3:1 chelates with metal ions such as Ca^{+2}, Mg^{+2}, Zn^{+2}, Fe^{+2}, Fe^{+3}, and Bi^{+3}. The stoichiometry of the chelate formed will depend on a variety of factors, such as the relative concentrations of chelating agent (quinolone) and metal ion present, the valence (or charge) on the metal ion, and the pH. Since such chelates are often insoluble in water, coincidental oral administration of a quinolone with an antacid, a hematinic, or a mineral supplement can significantly reduce the oral bioavailability of the quinolone. As an example, the insoluble 2:1 chelate formed between ciprofloxacin and magnesium ion is shown in Fig. 7-3. The presence of divalent ions (such as Mg^{+2}) in the urine may also contribute to the comparatively lower solubility of certain fluoroquinolines in the urine as compared with the plasma.

Nalidixic Acid, USP

1-Ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid (NegGram) occurs as a pale buff crystalline powder that is sparingly soluble in water and ether, but soluble in most polar organic solvents.

Nalidixic Acid

Nalidixic acid is useful in the treatment of infections of the urinary tract in which gram-negative bacteria are predominant. The activity against indole-positive Proteus species is particularly noteworthy, and nalidixic acid and its congeners represent important alternatives for the treatment of urinary tract infections caused by strains of these bacteria resistant to other agents.

Nalidixic acid is rapidly absorbed, extensively metabolized, and rapidly excreted after oral administration. The 7-hydroxymethyl metabolite is significantly more active than the parent compound. Further metabolism of the active metabolite to inactive glucuronide and 7-carboxylic acid metabolites also occurs.

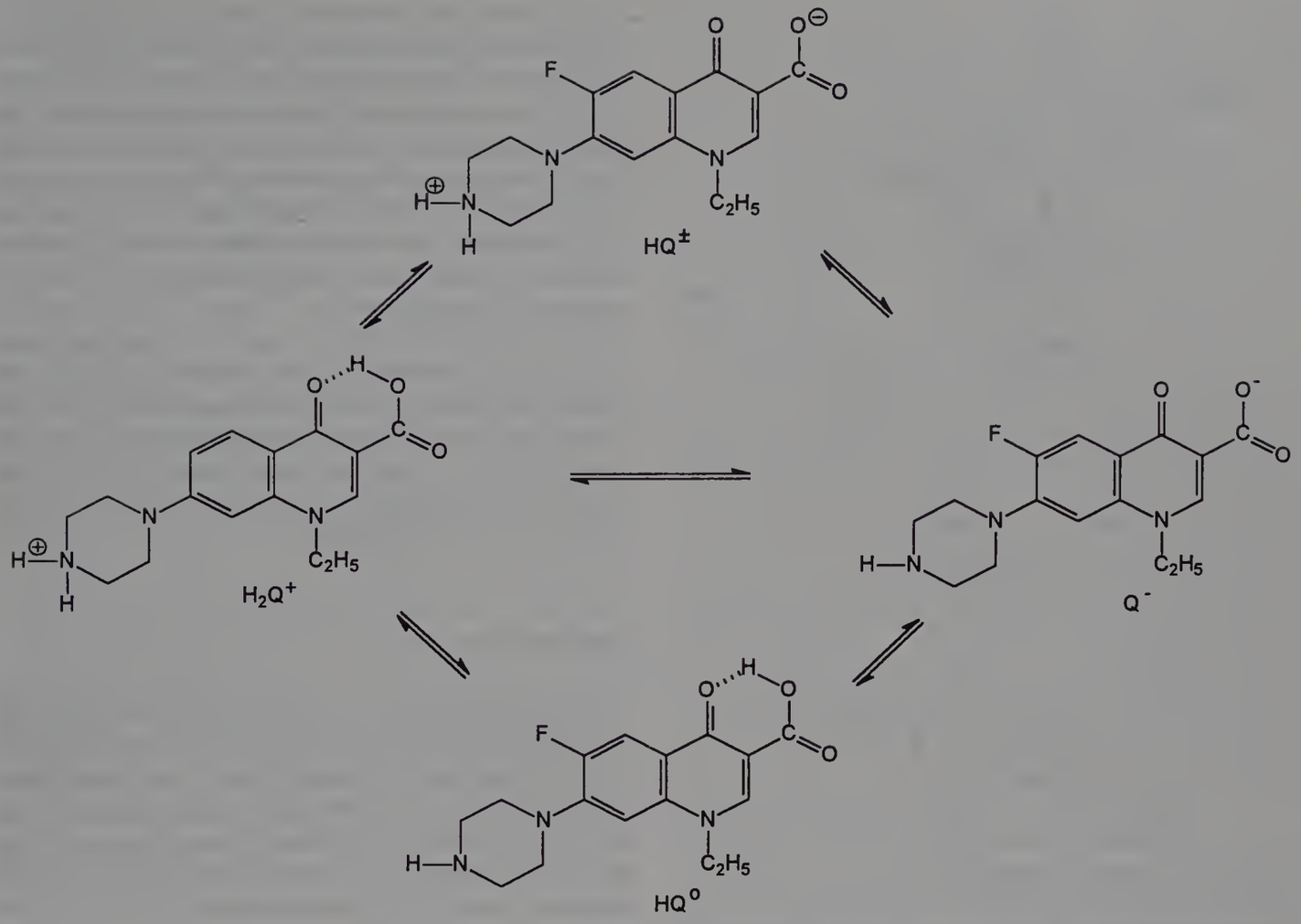
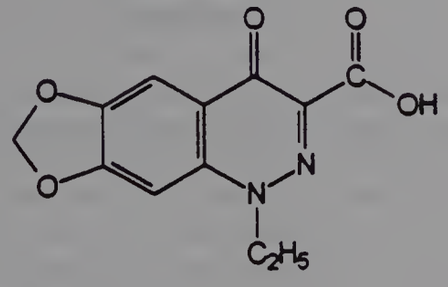


FIG. 7-2.

Cinoxacin, USP

1 - Ethyl - 1,4 - dihydro - 4 - oxo[1,3]dioxolo[4,5g]cinnoline - 3 - carboxylic acid (Cinobac) is a close congener (isostere) of oxolinic acid (no longer marketed in the United States) and



Cinoxacin

has antibacterial properties similar to those of nalidixic and oxolinic acids. It is recommended for the treatment of urinary tract infections caused by strains of gram-negative bacteria susceptible to these agents. Early clinical studies indicate that the drug possesses pharmacokinetic properties superior to those of either of its predecessors. Thus, higher urinary concentrations of cinoxacin are achieved following oral administration, compared with nalidixic acid or oxolinic acid. Cinoxacin appears to be more completely absorbed and less protein-bound than is nalidixic acid.

Norfloxacin

1 - Ethyl - 6 - fluoro - 1,4 - dihydro - 4 - oxo - 7 - (1 - piperazinyl) - 3 - quinoline carboxylic acid (Noroxin) is a pale yellow crystalline powder that is sparingly soluble in water. This quinoline has broad-spectrum activity against gram-negative and gram-positive aerobic bacteria. The fluorine atom provides increased potency against gram-positive organisms, whereas the piperazine moiety improves antipseudomonal activity. Norfloxacin is indicated for the treatment of urinary tract infections caused by *E. coli, K. pneumoniae, Enterobacter cloacae, Proteus mirabilis,* indole-positive Proteus species, including *P. vulgaris, Providencia rettgeri, Morganella morganii, P. aeruginosa, S. aureus, S. epidermidis,* and group D streptococci. It is generally not effective against obligate anaerobic bacteria. Norfloxacin has also been approved for the treatment of uncomplicated gonorrhea in a single 800-mg oral dose.

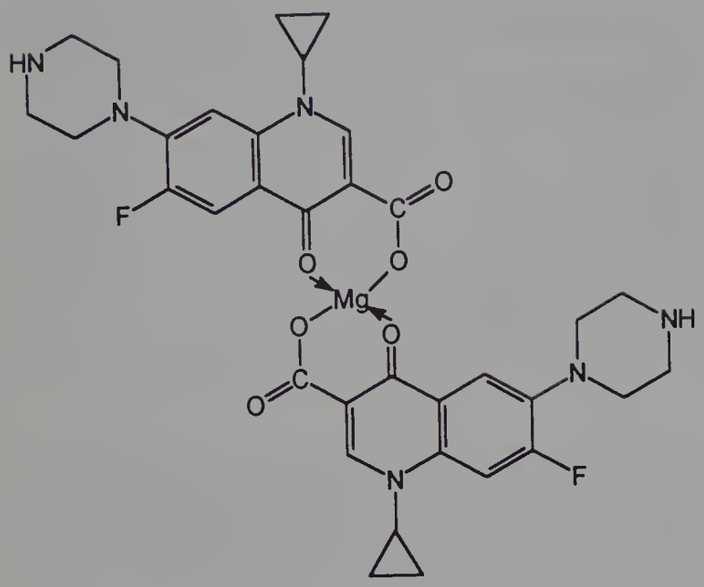
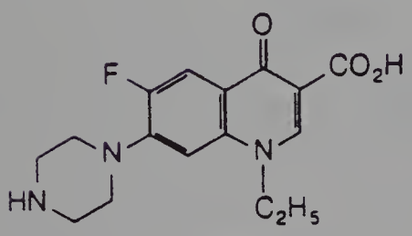


FIG. 7-3.

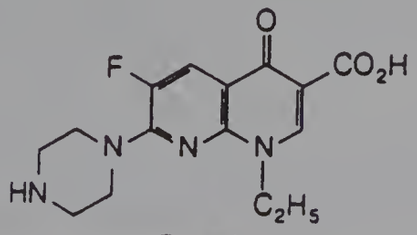


Norfloxacin

The oral absorption of norfloxacin is rapid and reasonably efficient. Approximately 30% of an oral dose is excreted in the urine in 24 hr, along with 5% to 8% consisting of less-active metabolites. There is significant biliary excretion with ~30% of the original drug appearing in the feces.

Enoxacin, USP

1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1,8-naphthyridine-3-carboxylic acid (Penetrex) is a quinolone with broad-spectrum antibacterial activity that is employed primarily for the treatment of urinary tract infections and sexually transmitted diseases. Enoxacin has been approved for the treatment of uncomplicated gonococcal urethritis and has also been shown to be effective in chanchroid caused by *Haemophilus ducreyi*. A single 400-mg dose is employed for these indications. Enoxacin is also approved for the treatment of acute (uncomplicated) and chronic (complicated) urinary tract infections.



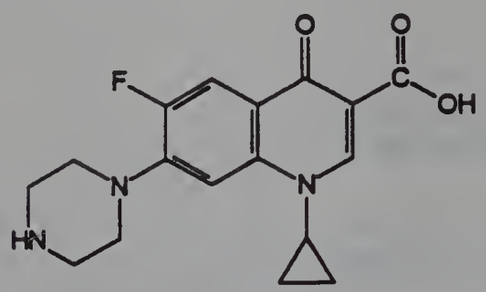
Enoxacin

Enoxacin is well absorbed following oral administration. Oral bioavailability approaches 90%. Concentrations of the drug in the kidneys, prostate, cervix, fallopian tubes, and myometrium typically exceed those in the plasma. More than 50% of the unchanged drug is excreted in the urine. Metabolism, largely catalyzed by cytochrome P450 enzymes in the liver, accounts for 15% to 20% of the orally administered dose of enoxacin. The relatively short elimination half-life of enoxacin dictates twice-a-day dosing for the treatment of urinary tract infections.

Some cytochrome P450 isozymes are inhibited by enoxacin, resulting in potentially important interactions with other drugs. For example, enoxacin has been reported to decrease theophylline clearance, causing increased plasma levels and increased toxicity. Enoxacin forms insoluble chelates with divalent metal ions present in antacids and hematinics, which reduce its oral bioavailability.

Ciprofloxacin, USP

1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid (Cipro, Cipro IV) is supplied in both oral and parenteral dosage forms. The hydrochloride salt is available in 250-, 500-, and 750-mg tablets for oral administration. Intravenous solutions containing 200 mg and 400 mg are provided in concentrations of 0.2% in normal saline and 1% in 5% dextrose solutions.



Ciprofloxacin

The bioavailability of ciprofloxacin following oral administration is good, with 70% to 80% of an oral dose being absorbed. Food delays, but does not prevent, absorption. Significant amounts (20% to 35%) of orally administered ciprofloxacin are excreted in the feces, in part due to biliary excretion. Biotransformation to less-active metabolites accounts for ~15% of the administered drug. Approximately 40% to 50% of unchanged ciprofloxacin is excreted in the urine following oral administration. This value increases to 50% to 70% when the drug is injected intravenously. Somewhat paradoxically, the elimination half-life of ciprofloxacin is shorter following oral administration ($t_{1/2}$, 4 hr) than it is following intravenous administration ($t_{1/2}$, 5 to 6 hr).

The oral dose of this quinolone is typically 25% higher than the parenteral dose for a given indication. Probenecid

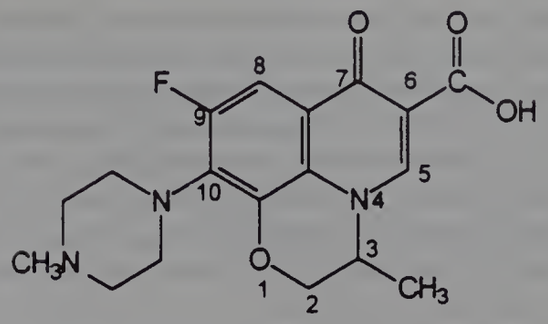
significantly reduces the renal clearance of ciprofloxacin, presumably by inhibiting its active tubular secretion. Ciprofloxacin is widely distributed to virtually all parts of the body, including the cerebrospinal fluid, and is generally considered to provide the best distribution of the currently marketed quinolones. This property, together with the potency and broad antibacterial spectrum of ciprofloxacin, account for the numerous therapeutic indications for the drug. Ciprofloxacin also exhibits higher potency against most gram-negative bacterial species, including *P. aeruginosa,* than other quinolones.

Ciprofloxacin is an agent of choice for the treatment of bacterial gastroenteritis caused by gram-negative bacilli, such as enteropathogenic *Escherichia coli*, Salmonella (including *S. typhi*), Shigella, Vibrio, and *Aeromonas hydrophilia.* It is widely employed for the treatment of respiratory tract infections, and is particularly effective for controlling bronchitis and pneumonia caused by gram-negative bacteria. Ciprofloxacin is also used for combating infections of the skin, soft tissues, bones, and joints. Both uncomplicated and complicated urinary tract infections caused by gram-negative bacteria can be effectively treated with ciprofloxacin. It is particularly useful for the control of chronic infections characterized by renal tissue involvement. The drug also has important applications in controlling venereal diseases. A combination of ciprofloxacin with the cephalosporin antibiotic ceftriaxone (Chap. 10) is recommended as the treatment of choice for disseminated gonorrhea, while gonococcal urethritis can usually be eradicated with single-dose treatment of ciprofloxacin plus doxycycline, a tetracycline antibiotic (Chap. 10). Ciprofloxacin has also been used for chancroid.

Injectable forms of ciprofloxacin are incompatible with drug solutions that are alkaline due to the reduced solubility of the drug pH 7. Thus intravenous solutions should not be mixed with solutions of ticarcillin sodium, mezlocillin sodium, or aminophylline. Ciprofloxacin may also induce crystalluria under the unusual circumstance that urinary pH rises above 7; for example, with the use of systemic alkalinizers or a carbonic anhydrase inhibitor or through the action of urease elaborated by certain species of gram-negative bacilli.

Ofloxacin, USP

9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-de]-1,4,-benzoxazine-6-carboxylic acid (Floxin, Floxin IV) is a member of the quinolone class of antibacterial drugs wherein the 1- and 8-positions are joined in the form of a 1,4-oxazine ring. The ring system is numbered beginning with the oxazine oxygen atom as shown below.



Ofloxacin

Ofloxacin resembles ciprofloxacin in its antibacterial spectrum and potency. Like ciprofloxacin, this quinolone is also widely distributed into most body fluids and tissues. In fact, higher concentrations of ofloxacin are achieved in cerebrospinal fluid than can be obtained with ciprofloxacin. The oral bioavailability of ofloxacin is superior (95% to 100%) to that of ciprofloxacin, and metabolism is negligible (~3%). The amount of an administered dose of ofloxacin excreted in the urine in a 24- to 48-hr period ranges from 70% to 90%. There is relatively little biliary excretion of this quinolone. Although food can slow the oral absorption of ofloxacin, blood levels following oral versus intravenous administration are comparable. The elimination half-life of ofloxacin ranges from 4.5 to 7 hr.

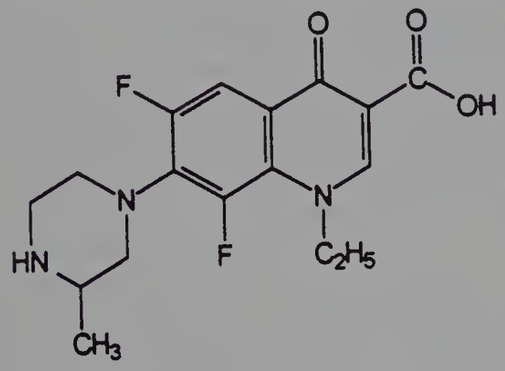
Ofloxacin has been approved for the treatment of infections of the lower respiratory tract, including chronic bronchitis and pneumonia, caused by gram-negative bacilli. It is also employed for the treatment of pelvic inflammatory disease (PID) and is highly active against both gonococci and chlamydia. In common with other fluoroquinolones, ofloxacin is not effective in the treatment of syphilis. A single 400-mg oral dose of ofloxacin in combination with the tetracycline antibiotic doxycycline is recommended by the Centers for Disease Control for the outpatient treatment of acute gonococcal urethritis. Ofloxacin is also employed for the treatment of urinary tract infections caused by gram-negative bacilli and for prostatitis caused by *E. coli.* Infections of the skin and soft tissues caused by staphylococci, streptococci, and gram-negative bacilli may also be treated using ofloxacin.

Because ofloxacin has an asymmetric carbon atom in its structure, it is obtained and supplied commercially as a racemate. The racemic mixture has been resolved and the enantiomers independently synthesized and evaluated for antibacterial activity.[43] The 3S(−)-isomer is substantially more active (eight to 125 times, depending on the bacterial species) than the 3R(+)-isomer and has recently been marketed as levofloxacin (levaquin) for the same indications as the racemate.

Lomefloxacin, USP

1-Ethyl-6,8-difluoro-1,4-dihydro-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid (Maxaquin) is a diflu-

orinated quinolone with a longer elimination half-life (7 to 8 hr) than other members of its class. It is the only quinolone for which once-daily oral dosing suffices. The oral bioavailability of lomefloxacin is estimated to be 95% to 98%. Food slows, but does not prevent, its oral absorption. The extent of biotransformation of lomefloxacin is only ~5%, and high concentrations of unchanged drug, ranging from 60% to 80%, are excreted in the urine. The comparatively long half-life of lomefloxacin is apparently due to its excellent tissue distribution and renal reabsorption and not due to plasma protein binding (only ~10%) or enterohepatic recycling (biliary excretion is estimated to be ~10%).



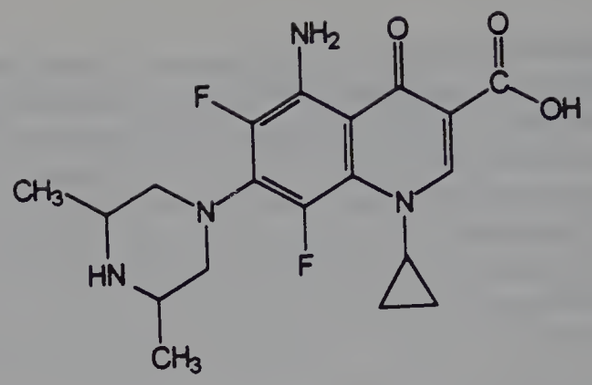
Lomefloxacin

Lomefloxacin has been approved for two primary indications. First, it is indicated for acute bacterial exacerbations of chronic bronchitis caused by *H. influenzae* or *Moraxella (Branhamella) catarrhalis*, but not if *Streptococcus pneumoniae* is the causative organism. Second, it is used for prophylaxis of infection following transurethral surgery. Lomefloxacin also finds application in the treatment of acute cystitis and chronic urinary tract infections caused by gram-negative bacilli.

Lomefloxacin reportedly causes the highest incidence of phototoxicity (photosensitivity) of the currently available quinolones. The presence of a halogen atom (fluorine, in this case) at the 8-position has been correlated with an increased chance of phototoxicity in the quinolones.[40]

Sparfloxacin

Sparfloxacin is a new fluoroquinolone that is currently marketed in Japan. This compound exhibits higher potency against gram-positive bacteria, especially staphylococci and streptococci, than the fluoroquinolones currently marketed. It is also more active against chlamydia and the anaerobe *Bacteriodes fragilis*. The activity of sparfloxacin against gram-negative bacteria is also very impressive, and it compares favorably with ciprofloxacin and ofloxacin in potency against Mycoplasma, Legionella, mycobacteria, and Listeria monocytogenes.



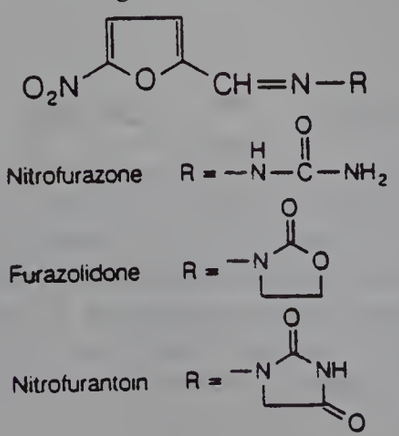
Sparfloxacin

Sparfloxacin has a long elimination half-life of 18 hr, which permits once-a-day dosing for most indications. The drug is widely distributed into most fluids and tissues. Effective concentrations of sparfloxacin are achieved for the treatment of skin and soft tissue infections, lower respiratory infections (including bronchitis and bacterial pneumonias), and PID caused by gonorrhea and chlamydia. Sparfloxacin has also been recommended for the treatment of bacterial gastroenteritis and colecystitis. The oral bioavailability of sparfloxacin is claimed to be good, and sufficient unchanged drug is excreted to be effective for the treatment of urinary tract infections. Nearly 20% of the orally administered dose is excreted as the inactive glucuronide.

The incidence of phototoxicity of sparfloxacin is the lowest of the fluoroquinolones, because of the presence of the 5-amino group, which counteracts the effect of the 8-fluoro-substituent.[40]

NITROFURANS

The first nitroheterocyclic compounds to be introduced into chemotherapy were the nitrofurans. Three of these compounds—nitrofurazone, furazolidone, and nitrofurantoin—have been used for the treatment of bacterial infections of various kinds for nearly 50 years. A fourth nitrofuran, nifurtimox, is used as an antiprotozoal agent to treat trypanosomiasis and leishmaniasis. Another nitroheterocycle of considerable importance is metronidazole, which is an amebicide (a trichomonicide), and is employed for the treatment of systemic infections caused by anaerobic bacteria. This important drug is discussed later in this chapter.



The nitrofurans are derivatives of 5-nitro-2-furaldehyde formed on reaction with the appropriate hydrazine or amine derivative. Antimicrobial activity is present only when the nitro group is in the 5-position.

The mechanism of antimicrobial action of the nitrofurans has been extensively studied, but it still is not fully understood. In addition to their antimicrobial actions, the nitrofurans are known to be mutagenic and carcinogenic under certain conditions. It is thought that DNA damage caused by metabolic reduction products may be involved in these cellular effects.

Nitrofurazone

5-Nitro-2-furaldehyde semicarbazone (Furacin) occurs as a lemon-yellow crystalline solid that is sparingly soluble in water and practically insoluble in organic solvents. Nitrofurazone is chemically stable, but moderately light-sensitive.

It is employed topically in the treatment of burns, especially when bacterial resistance to other agents may be a problem. It may also be used to prevent bacterial infection associated with skin grafts. Nitrofurazone has a broad spectrum of activity against gram-positive and gram-negative bacteria, but it is not active against fungi. It is bactericidal against most bacteria commonly causing surface infections, including *S. aureus*, Streptococcus sp., *E. coli, Clostridium perfringens, Enterobacter (Aerobacter) aerogenes*, and Proteus sp; however, *P. aeruginosa* strains are resistant.

Nitrofurazone is available in solutions, ointments, and suppositories in the usual concentration of 0.2%.

Furazolidone, USP

3-[(5-Nitrofurylidene)amino]-2-oxazolidinone (Furoxone) occurs as a yellow crystalline powder with a bitter aftertaste. It is insoluble in water or alcohol.

Furazolidone has bactericidal activity against a relatively broad range of intestinal pathogens, including *S. aureus, E. coli*, Salmonella, Shigella, Proteus, Enterobacter, and *Vibrio cholerae*. It is also active against the protozoan *Giardia lamblia*. It is recommended for the oral treatment of bacterial or protozoal diarrhea caused by susceptible organisms. The usual adult dosage is 100 mg four times daily.

Only a small fraction of an orally administered dose of furazolidone is absorbed. Approximately 5% of the oral dose is detectable in the urine in the form of several metabolites. Some gastrointestinal distress has been reported with its use. Alcohol should be avoided when furazolidone is being used because the drug can inhibit aldehyde dehydrogenase.

Nitrofurantoin, USP

1 - [(5 - Nitrofurfurylidene) - amino]hydantoin (Furadantin, Macrodantin) is a nitrofuran derivative that is suitable for oral use. It is recommended for the treatment of urinary tract infections caused by susceptible strains of *E. coli*, enterococci, *S. aureus*, Klebsiella, Enterobacter, and Proteus species. The most common side effects are gastrointestinal (anorexia, nausea, and vomiting); however, hypersensitivity reactions (pneumonitis, skin rashes, hepatitis, and hemolytic anemia) have occasionally been observed. A large crystalline form (Macrodantin) is claimed to improve gastrointestinal tolerance without interfering with oral absorption.

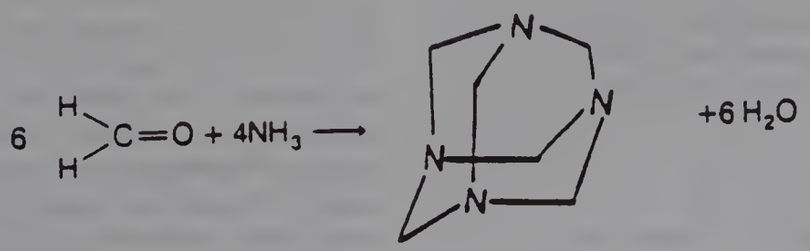
METHENAMINE AND ITS SALTS

Methenamine, USP

Hexamethylenetetramine (Urotropin, Uritone) depends upon the liberation of formaldehyde for its activity. It is manufactured by evaporating a solution of formaldehyde to dryness with strong ammonia water.

The free base exists as an odorless white crystalline powder that sublimes at ~260°C. It dissolves in water to form an alkaline solution and liberates formaldehyde when warmed with mineral acids. Methenamine is a weak base with pK_a of 4.9.

Methenamine is employed internally as a urinary antiseptic for the treatment of chronic urinary tract infections. The free base has practically no bacteriostatic power; acidification to release formaldehyde in the comparatively lower pH of the kidney is required. To optimize the antibacterial effect, the administration of methenamine is generally accompanied



Methenamine

by an acidifying agent such as sodium biphosphate or ammonium chloride.

Certain bacterial strains are resistant to the action of methenamine because they elaborate urease, an enzyme that hydrolyzes urea to form ammonia. The resultant high urinary pH prevents the activation of methenamine, rendering it ineffective. This problem can be overcome by the coadministration of the urease inhibitor acetohydroxamic acid (Lithostat).

Acetohydroxamic Acid

Methenamine Mandelate, USP

Hexamethylenetetramine mandelate (Mandelamine) is a white crystalline powder with a sour taste and practically no odor. It is very soluble in water and has the advantage of furnishing its own acidity, although in its use the custom is to carry out a preliminary acidification of the urine for 24 to 36 hrs before administration. It is effective with smaller amounts of mandelic acid and, thus, avoids the gastric disturbances attributed to the acid when used alone.

Methenamine Hippurate, USP

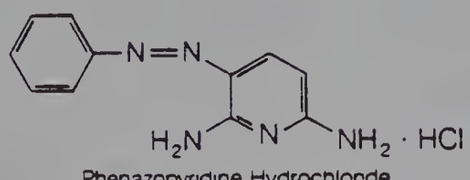
Methenamine hippurate (Hiprex) is the hippuric acid salt of methenamine. It is readily absorbed after oral administration and is concentrated in the urinary bladder, where it exerts its antibacterial activity. Its activity is increased in acid urine.

URINARY ANALGESICS

Pain and discomfort frequently accompany bacterial infections of the urinary tract. For this reason, certain analgesic agents, such as the salicylates (Chap. 22) or phenazopyridine, which concentrate in the urine due to their solubility properties, are combined with a urinary antiinfective agent.

Phenazopyridine Hydrochloride, USP

2,6 - Diamino - 3 - (phenylazo)pyridine monohydrochloride (Pyridium) is a brick-red fine crystalline powder. It is slightly soluble in alcohol, in chloroform, and in water.



Phenazopyridine Hydrochlonde

Phenazopyridine hydrochloride was formerly used as a urinary antiseptic. Although it is active in vitro against staphylococci, streptococci, gonococci, and *E. coli*, it has no useful antibacterial activity in the urine. Thus, its present utility lies in its local analgesic effect on the mucosa of the urinary tract.

Usually, it is now given in combination with urinary antiseptics. For example, it is available as Azo-Gantrisin, a fixed-dose combination with the sulfonamide antibacterial sulfisoxazole (Chap. 8), and as urobiotic, a combination with the antibiotic oxytetracycline (Chap. 10) and the sulfonamide sulfamethiazole. The drug is rapidly excreted in the urine, to which it gives an orange-red color. Stains in fabrics may be removed by soaking in an 0.25% solution of sodium dithionite.

ANTITUBERCULAR AGENTS

Ever since Koch identified the tubercle bacillus, *Mycobacterium tuberculosis*, there has been keen interest in the development of antitubercular drugs. The first breakthrough in antitubercular chemotherapy occurred in 1938 with the observation that sulfanilamide had weak bacteriostatic properties. Later, the sulfone derivative dapsone (4,4′-diaminodiphenylsulfone) was investigated clinically. Unfortunately, this drug, which is still considered one of the most effective drugs for the treatment of leprosy and which also has useful antimalarial properties (Chap. 8), was considered too toxic because of the high dosages used. The discovery of the antitubercular activity of the amino glycoside antibiotic streptomycin (Chap. 10) by Waksman et al. in 1944 ushered in the modern era of tuberculosis treatment. This development was quickly followed by discoveries of the antitubercular properties of first, *p*-aminosalicylic acid and then, in 1952, of isoniazid. Later, the usefulness of the synthetic drug ethambutol, and, eventually, of the semisynthetic antibiotic rifampin was discovered.

Combination therapy, with the use of two or more antitubercular drugs, has been well documented to reduce the emergence of strains of *M. tuberculosis* resistant to individual agents and has become standard medical practice. The choice of antitubercular combination is dependent on a variety of factors, including the location of the disease (pulmonary, urogenital, gastrointestinal, or neural); the results of susceptibility tests and the pattern of resistance in the locality; the physical condition and age of the patient; and the toxicities of the individual agents. For some time, a combination of isoniazid and ethambutol, with or without streptomycin, had been the preferred choice of treatment among clinicians in this country. However, the discovery of the tuberculocidal properties of rifampin resulted in its replacement of the more toxic antibiotic streptomycin in most regimens. The synthetic drug pyrazinamide, because of its sterilizing ability, is also considered a first-line agent and is frequently employed in place of ethambutol in combination therapy.

Second-line agents for tuberculosis include the antibiotics cycloserine, kanamycin (Chap. 10), and capreomycin and the synthetic compounds ethionamide and para-aminosalicylic acid (PAS).

A major advance in the treatment of tuberculosis was signaled by the introduction into therapy of the antibiotic rifampin. Clinical studies indicated that when rifampin is included in the regimen, particularly in combination with isoniazid and ethambutol (or pyrazinamide) a significant shortening of the period required for successful therapy is possible. Previous treatment schedules without rifampin required maintenance therapy for at least 2 years, whereas those based on the isoniazid-rifampin combination achieve equal or better results in 6 to 9 months.

Once considered to be on the verge of worldwide eradication, as a result of aggressive public health measures and effective chemotherapy, tuberculosis has made a comeback of alarming proportions in recent years.[44] A combination of factors has contributed to the observed increase in tuberculosis cases, including the worldwide AIDs epidemic, the general relaxation of public health policies in many countries, the increased overcrowding and homelessness in major cities, and the increased emergence of multiple drug-resistant strains of *M. tuberculosis*.

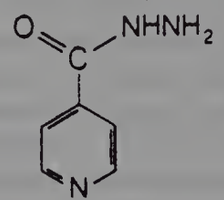
The development of drugs useful for the treatment of leprosy has long been hampered, in part, by the failure of the causative organism, *Mycobacterium leprae,* to grow in cell culture. However, the recent availability of animal models, such as the infected mouse footpad, now permits in vivo drug evaluations. The increasing emergence of strains of *M. leprae* resistant to dapsone (Chap. 8), long considered the mainstay for leprosy treatment, has caused public health officials to advocate combination therapy.

Mycobacteria other than *M. tuberculosis* and *M. leprae*, commonly known as "atypical" mycobacteria, were first established as etiologic agents of diseases in the 1950s. Atypical mycobacteria are primarily saprophytic species that are widely distributed in soil and water. Such organisms are not normally considered particularly virulent or infectious. However, diseases attributed to atypical mycobacteria are on the increase, in large part because of the increased numbers of immunocompromised individuals in the population resulting from the AIDs epidemic and the widespread use of immunosuppressive agents with organ transplantation.

The most common disease-causing species are *Mycobacterium avium* and *Mycobacterium intracellularae*, which have similar geographical distributions, are difficult to distinguish microbiologically and diagnostically, and are thus considered as a single complex (MAC). The initial disease attributed to MAC resembles tuberculosis, but skin and musculoskeletal tissues may also become involved. The association of MAC and HIV infection is dramatic. An overwhelming disseminated form of the disease occurs in severely immunocompromised patients, leading to high morbidity and mortality. Another relatively common atypical mycobacterium, *M. kansasii*, also causes pulmonary disease and can become disseminated in immunocompromised patients. Patients infected with *M. kansasii* can usually be treated effectively with combinations of antitubercular drugs. MAC infections, in contrast, are resistant to currently available chemotherapeutic agents.

ISONIAZID, USP

Isonicotinic acid hydrazide, isonicotinyl hydrazide, or INH (Nydrazid) occurs as a nearly colorless crystalline solid that is very soluble in water. It is prepared by reacting the methyl ester of isonicotinic acid with hydrazine.



Isoniazid

Isoniazid is a remarkably effective agent and continues to be one of the primary drugs (along with rifampin, pyrazinamide, and ethambutol) for the treatment of tuberculosis. It is not, however, uniformly effective against all forms of the disease. The frequent emergence of strains of the tubercle bacillus resistant to isoniazid during therapy was seen as the major shortcoming of the drug. This problem has been largely, but not entirely, overcome with the use of combinations.

The activity of isoniazid is manifested on the growing tubercle bacilli and not on resting forms. Its action, which is considered bactericidal, is to cause the bacilli to lose lipid content by a mechanism that has not been fully elucidated. The most generally accepted theory suggests that the principal effect of isoniazid is to inhibit the synthesis of mycolic acids,[45,46] high-molecular-weight, branched β-hydroxy fatty acids that constitute important components of the cell walls of mycobacteria.

It has been known for some time that a mycobacterial catalase/peroxidase enzyme complex is required for the bioactivation of isoniazid.[47] A reactive species, generated through the action of these enzymes on the drug, is believed to attack a critical enzyme required for mycolic acid synthesis in mycobacteria.[48] Resistance to INH, estimated to range from 25% to 50% of clinical isolates of INH-resistant strains, is associated with loss of catalase and peroxidase activities, both of which are encoded by a single gene, kat G.[49] The target for the action of INH has recently been identified as an enzyme that catalyzes the NADH-specific reduction of 2-trans-enoylacyl-carrier protein, an essential step in fatty acid elongation.[50] This enzyme is encoded by a specific gene, inhA, in *M. tuberculosis*.[51] Approximately 20% to 25% of INH-resistant clinical isolates display mutations in

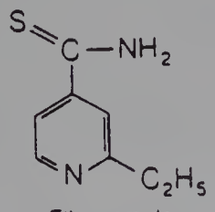
the inhA gene, leading to altered proteins with apparently reduced affinity for the active form of the drug. Interestingly, such INH-resistant strains also display resistance to ethionamide, a structurally similar antitubercular drug.[51] On the other hand, mycobacterial strains deficient in catalase/peroxidase activity are frequently susceptible to ethionamide.

Although treatment regimens generally require long-term administration of isoniazid, the incidence of toxic effects is remarkably low. The principal toxic reactions are peripheral neuritis, gastrointestinal disturbances (such as constipation, loss of appetite), and hepatotoxicity. Coadministration of pyridoxine is reported to prevent the symptoms of peripheral neuritis, suggesting that this adverse effect may be the result of antagonism of a coenzyme action of pyridoxal phosphate. Pyridoxine does not appear to interfere with the antitubercular effect of isoniazid. Severe hepatotoxicity rarely occurs with isoniazid alone. However, the incidence is much higher when it is used in combination with rifampin.

Isoniazid is rapidly and almost completely absorbed following oral administration. It is widely distributed to all tissues and fluids within the body, including the cerebrospinal fluid. Approximately 60% of an oral dose is excreted in the urine within 24 hr in the form of numerous metabolites as well as the unchanged drug. Although the metabolism of isoniazid is very complex, the principal path of inactivation involves acetylation of the primary hydrazine nitrogen. In addition to acetylisoniazid, the isonicotinyl hydrazones of pyruvic and α-keto-glutaric acids, isonicotinic acid and isonicotinuric acid, have been isolated as metabolites in humans.[52] The capacity to inactivate isoniazid by acetylation is an inherited characteristic in humans. Approximately half the population are fast acetylators (plasma half-life, 45 to 80 min) and the remainder slow acetylators (plasma half-life, 140 to 200 min).

ETHIONAMIDE, USP

2-Ethylthioisonicotinamide (Trecator SC) occurs as a yellow crystalline material that is sparingly soluble in water. This nicotinamide has weak bacteriostatic activity in vitro but, because of its lipid solubility, is effective in vivo. In contrast to the isoniazid series, 2-substitution enhances activity in the thioisonicotinamide series.



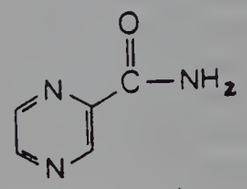
Ethionamide

Ethionamide is rapidly and completely absorbed following oral administration. It is widely distributed throughout the body and extensively metabolized to predominantly inactive forms that are excreted in the urine. Less than 1% of the parent drug appears in the urine.

Ethionamide is considered a secondary drug for the treatment of tuberculosis. It is used in the treatment of isoniazid-resistant tuberculosis or when the patient is intolerant to isoniazid and other drugs. Because of its low potency, the highest tolerated dose of ethionamide is usually recommended. Gastrointestinal intolerance is the most common side effect associated with its use. Visual disturbances and hepatotoxicity have also been reported.

PYRAZINAMIDE, USP

Pyrazinecarboxamide (PZA) occurs as a white crystalline powder that is sparingly soluble in water and slightly soluble in polar organic solvents. Its antitubercular properties were discovered as a result of an investigation of heterocyclic analogues of nicotinic acid, with which it is isosteric.



Pyrazinamide

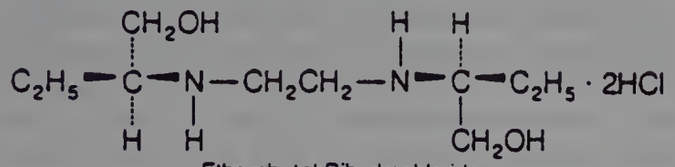
Pyrazinamide has recently been elevated to first-line status in short-term tuberculosis treatment regimens because of its tuberculocidal activity and comparatively low short-term toxicity. Since pyrazinamide is not active against metabolically inactive tubercle bacilli, it is not considered suitable for long-term therapy. Potential hepatotoxicity also obviates long-term use of the drug. Pyrazinamide is maximally effective in the low pH environment that exists in macrophages (monocytes). Evidence suggests that bioactivation of pyrazinamide to pyrazimoic acid by an amidase present in Mycobacteria occurs.[53]

Because bacterial resistance to pyrazinamide rapidly develops, it should not be used alone, but always in combination with other drugs. Cross-resistance between pyrazinamide and either isoniazid or ethionamide is relatively rare. The mechanism of action of pyrazinamide is not known. Despite its structural similarities to isoniazid and ethionamide, pyrazinamide apparently does not inhibit mycolic acid biosynthesis in Mycobacteria.

Pyrazinamide is well absorbed orally and widely distributed throughout the body. The drug penetrates inflamed meninges and is therefore recommended for the treatment of tuberculous meningitis. Unchanged pyrazinamide, the corresponding carboxylic acid (pyrazinoic acid), and the 5-hydroxy-metabolite are excreted in the urine. The elimination half-life ranges from 12 to 24 hr, which allows the drug to be administered on either once-daily or even twice-weekly, dosing schedules. Pyrazinamide and its metabolites are reported to interfere with uric acid excretion. The drug should therefore be employed with great caution in patients with hyperuricemia or gout.

ETHAMBUTOL, USP

(+)-2,2′-(Ethylenediimino)-di-1-butanol dihydrochloride, or EBM (Myambutol), is a white crystalline powder freely soluble in water and slightly soluble in alcohol.



Ethambutol Dihydrochloride

Ethambutol is active only against dividing mycobacteria. It has no effect on encapsulated or other nonproliferating forms. The in vitro effect may be bacteriostatic or bactericidal, dependent on the conditions. Its selective toxicity toward mycobacteria appears to be related to the inhibition of the incorporation of mycolic acids into the cell walls of these organisms.

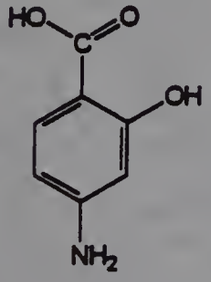
This compound is remarkably stereospecific. Tests have shown that, although the toxicities of the *dextro, levo*, and *meso* isomers are about equal, their activities vary considerably. The *dextro* isomer is 16 times as active as the *meso* isomer. In addition, the length of the alkylene chain, the nature of the branching of the alkyl substituents on the nitrogens, and the extent of *N*-alkylation all have a pronounced effect on the activity.

Ethambutol is rapidly absorbed after oral administration, and peak serum levels occur in ~ 2 hr. It is rapidly excreted, mainly in the urine. Up to 80% is excreted unchanged, with the balance being metabolized and excreted as 2,2′-(ethylenediimino)dibutyric acid and as the corresponding dialdehyde.

It is not recommended for use alone, but in combinations with other antitubercular drugs in the chemotherapy of pulmonary tuberculosis.

AMINOSALICYLIC ACID

4-Aminosalicylic acid (PAS) occurs as a white to yellowish-white crystalline solid that darkens on exposure to light or air. It is slightly soluble in water, but more soluble in alcohol. Alkali metal salts and the nitric acid salt are soluble in water, but the salts of hydrochloric acid and sulfuric acid are not. The acid undergoes decarboxylation when heated. An aqueous solution has a pH of ~3.2.



p-Amino Salicylic Acid

Aminosalicylic acid is administered orally in the form of the sodium salt, usually in tablet or capsule form. Symptoms of gastrointestinal irritation are common with both the acid and the sodium salt. A variety of enteric-coated dosage forms have been used in an attempt to overcome this disadvantage. Other forms that are claimed to improve gastrointestinal tolerance include the calcium salt, the phenyl ester, and a combination with an anion exchange resin (Rezi-PAS). An antacid, such as aluminum hydroxide, is frequently prescribed.

The oral absorption of PAS is rapid and nearly complete, and it is widely distributed into most of the body fluids and tissues, with the exception of the cerebrospinal fluid, in which levels are significantly lower. It is excreted primarily in theurine, both as unchanged drug and as metabolites. The *N*-acetyl derivative is the principal metabolite, with significant amounts of the glycine conjugate also being formed. When administered with isoniazid (which also undergoes *N*-acetylation), PAS increases the level of free isoniazid. The biologic half-life of PAS is ~ 2 hr.

The mechanism of antibacterial action of PAS is similar to that of the sulfonamides (Chap. 8). Thus, it is believed to prevent the incorporation of *p*-aminobenzoic acid (PABA) into the dihydrofolic acid molecule catalyzed by the enzyme dihydrofolate synthetase. Structure-activity studies have shown that the amino and carboxyl groups must be *para* to each other and free; thus, esters and amides must readily undergo hydrolysis in vivo to be effective. The hydroxyl group may be *ortho* or *meta* to the carboxyl group, but optimal activity is seen in the former.

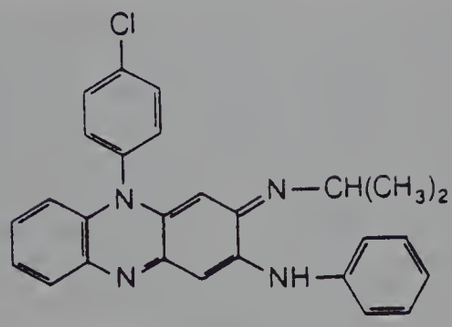
For many years, PAS was considered a first-line drug for the chemotherapy of tuberculosis and was generally included in combination regimens with isoniazid and streptomycin. However, the introduction of the more effective and generally better-tolerated agents, ethambutol and rifampin, have relegated it to an alternative drug status.

AMINOSALICYLATE SODIUM, USP

Sodium 4-aminosalicylate (sodium PAS), a salt, occurs in the dihydrate form as a yellow-white powder or crystalline solid. It is very soluble in water in the pH range of 7.0 to 7.5, at which it is the most stable. Aqueous solutions decompose readily and darken. Two pH-dependent types of reactions occur: decarboxylation (more rapid at low pH) and oxidation (more rapid at high pH). Therefore, solutions should be prepared within 24 hr of administration.

CLOFAZIMINE

Clofazimine (Lamprene) is a basic red dye that exerts a slow, bactericidal effect on *M. leprae*, the bacterium that causes leprosy. It occurs as a dark red crystalline solid that is insoluble in water.



Clofazimine

Clofazimine is used in the treatment of lepromatous leprosy, including dapsone-resistant forms of the disease. In addition to its antibacterial action, the drug appears to possess anti-inflammatory and immune-modulating effects that are of value in controlling neuritic complications and in suppressing erythema nodosum leprosum reactions associated with lepromatous leprosy. It is frequently used in combination with other drugs, such as dapsone (Chap. 8) or rifampin.

The mechanisms of antibacterial and anti-inflammatory actions of clofazimine are not known. The drug is known to bind to nucleic acids and concentrate in reticuloendothelial tissue. It can also act as an electron acceptor and may interfere with electron transport processes.

The oral absorption of clofazimine is estimated to be ~50%. It is a highly lipid-soluble drug that is distributed into lipoidal tissue and the reticuloendothelial system. Urinary excretion of unchanged drug and metabolites is negligible. Its half-life after repeated dosage is estimated to be ~70 days. Severe gastrointestinal intolerance to clofazimine is relatively common. Skin pigmentation, ichthyosis and dryness, rash, and pruritus also occur frequently.

Clofazimine has also been used to treat skin lesions caused by *M. ulcerans*.

ANTITUBERCULAR ANTIBIOTICS

RIFAMYCINS

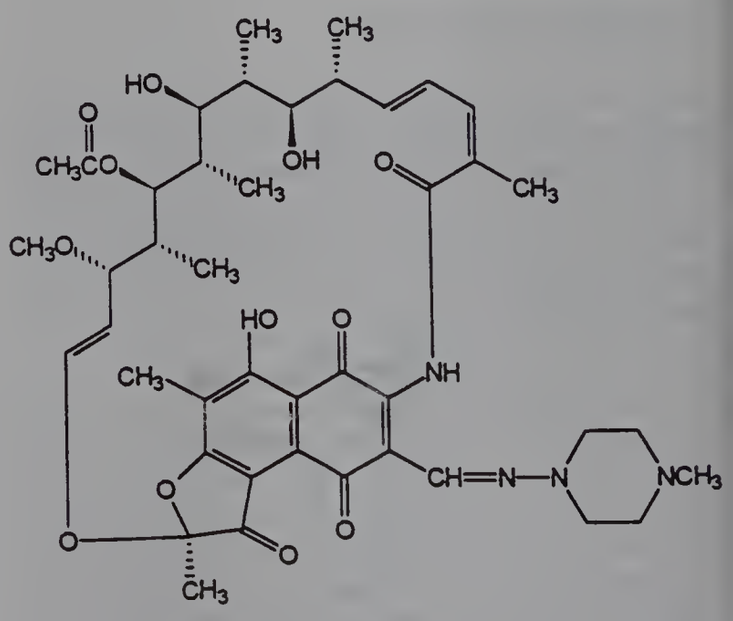
The rifamycins are a group of chemically related antibiotics obtained from *Streptomyces mediterranei*. They belong to a new class of antibiotics called ansamycins that contain a macrocyclic ring bridged across two nonadjacent (*ansa*) positions of an aromatic nucleus. The rifamycins and many of their semisynthetic derivatives have a broad spectrum of antimicrobial activity. They are most notably active against gram-positive bacteria and *M. tuberculosis*. However, they are also active against some gram-negative bacteria and many viruses. Rifampin, a semisynthetic derivative of rifamycin B, was released as an antitubercular agent in the United States in 1971. A second semisynthetic derivative, rifabutin, was approved in 1992 for the treatment of atypical mycobacterial infections.

The chemistry of rifamycins and other ansamycins has been reviewed.[54] All of the rifamycins (A, B, C, D, and E) are biologically active. Some of the semisynthetic derivatives of rifamycin B are the most potent known inhibitors of DNA-directed RNA-polymerase in bacteria,[55] and their action is bactericidal. They have no activity against the mammalian enzyme. The mechanism of action of rifamycins as inhibitors of viral replication appears to be different from that for their bactericidal action. Their net effect is to inhibit the formation of the virus particle, apparently by the prevention of a specific polypeptide conversion.[56] Rifamycins bind to the β-subunit of bacterial DNA-dependent RNA polymerases to prevent chain initiation.[57] Bacterial resistance to rifampin has been associated with mutations leading to amino acid substitution in the β-subunit.[57] A high level of cross-resistance between various rifamycins has been observed.

Rifampin, USP

Rifampin (Rifadin; Rimactane; Rifampicin) is the most active agent in clinical use for the treatment of tuberculosis. As little as 5 μg/ml is effective against sensitive strains of *M. tuberculosis*. Rifampin is also highly active against staphylococci, Neisseria, Hemophilus, Legionella, and Chlamydia. Gram-negative bacilli are much less sensitive to rifampin. However, resistance to rifampin develops rapidly in most species of bacteria, including the tubercle bacillus. Consequently, rifampin is used only in combination with other antitubercular drugs, and it is ordinarily not recommended for the treatment of other bacterial infections when other antibacterial agents are available.

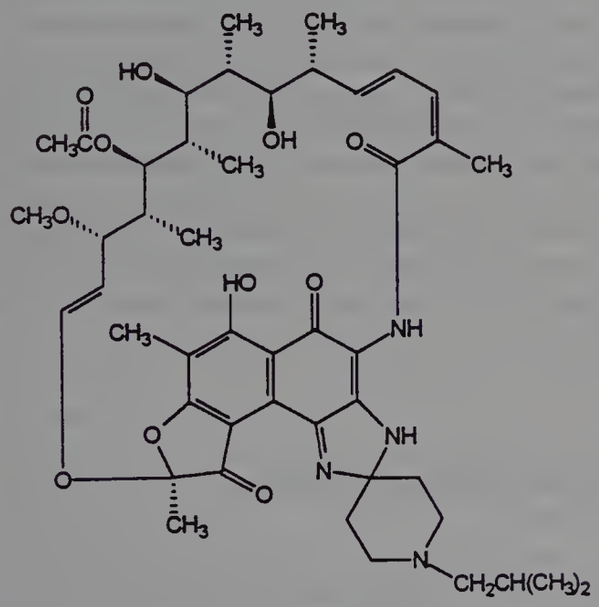
Toxic effects associated with rifampin are relatively infrequent. It may, however, interfere with liver function in some patients and should not be combined with other potentially hepatotoxic drugs, nor employed in patients with impaired hepatic function (e.g., chronic alcoholics). The incidence of hepatotoxicity was significantly higher when rifampin was combined with isoniazid than when



Rifampin

either agent was combined with ethambutol. Allergic and sensitivity reactions to rifampin have been reported, but they are infrequent and usually not serious. Rifampin is a powerful inducer of hepatic cytochome P450 oxygenases. It can markedly potentiate the actions of drugs that are inactivated by these enzymes. Examples include oral anticoagulants, barbiturates, benzodiazepines, oral hypoglycemic agents, phenytoin, and theophylline.

Rifampin is also employed to eradicate the carrier state in asymptomatic carriers of *Neisseria meningitidis* to prevent outbreaks of meningitis in high-risk areas such as military camps. Serotyping and sensitivity tests should be performed before its use because resistance develops rapidly. However, a daily dosage of 600 mg of rifampin for 4 days is sufficient to eradicate sensitive strains of *N. meningitidis*. Rifampin has also been very effective against *M. leprae* in experimental animals and in humans. When it is used in the treatment of leprosy, rifampin should be combined with dapsone or



Rifabutin

some other leprostatic agent to minimize the emergence of resistant strains of *M. leprae*.

Other, nonlabelled uses of rifampin include the treatment of serious infections such as endocarditis and osteomyelitis, caused by methicillin-resistant *S. aureus* or *S. epidermidis*, Legionnaires' disease resistant to erythromycin, and prophylaxis of *H. influenzae*–induced meningitis.

Rifampin occurs as an orange to reddish-brown crystalline powder that is soluble in alcohol, but only sparingly soluble in water. It is unstable to moisture, and a desiccant (silica gel) should be included with rifampin capsule containers. The expiration date for capsules thus stored is 2 years. Rifampin is well absorbed after oral administration to provide effective blood levels for ≥8 hr. However, food markedly reduces its oral absorption, and rifampin should be administered on an empty stomach. It is distributed in effective concentrations to all body fluids and tissues except the brain, despite the fact that it is 70% to 80% protein-bound in the plasma. The principal excretory route is through the bile and feces, and high concentrations of rifampin and its primary metabolite, deacetylrifampin, are found in the liver and biliary system. Deacetylrifampin is also microbiologically active. Equally high concentrations of rifampin are found in the kidneys, and although substantial amounts of the drug are passively reabsorbed in the renal tubules, its urinary excretion is significant. Patients should be made aware that rifampin causes a reddish-orange discoloration of the urine, stool, saliva, tears, and skin. It can also permanently discolor soft contact lenses.

Rifampin is also available as a parenteral dosage form consisting of a lyophilized sterile powder, which, when reconstituted in 5% dextrose or normal saline, provides 600 mg of active drug in 10 ml for slow intravenous infusion. The parenteral form may be employed for initial treatment of serious cases and for retreatment in patients who are unable to take the drug by the oral route. Parenteral solutions of rifampin are stable for 24 hr at room temperature. Although rifampin is stable in the solid state, it undergoes a variety of chemical changes in solution, the rates and nature of which are pH- and temperature-dependent.[58] In alkaline pH, it oxidizes to the quinone in the presence of oxygen; in acidic solutions, it hydrolyzes to 3-formyl rifamycin SV. Slow hydrolysis of ester functions also occurs, even at neutral pH.

Rifabutin, USP

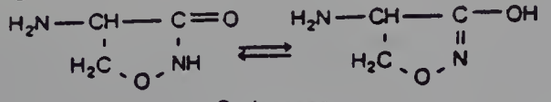
This spiroimidazopiperidyl derivative of rifamycin B has recently been approved in the United States for the prophylaxis of disseminated MAC in AIDS patients on the strength of clinical trials establishing its effectiveness. The activity of rifabutin against MAC organisms greatly exceeds that of rifamycin. However, this rifamycin derivative is not effective as monotherapy for existing disseminated MAC disease.

Rifabutin is a very lipophilic compound with a high affinity for tissues. Its elimination is distribution-limited with a half-life averaging 45 hr (range, 16 to 69 hr). Approximately 50% of an orally administered dose of rifabutin is absorbed, but the absolute oral bioavailability is only ~20%. Extensive first-pass metabolism and significant biliary excretion of the drug occur with ~30% and 53% of the orally administered dose excreted, largely as metabolites, in the feces and urine, respectively. The 25-O-desacetyl and 31-hydroxy metabolites of rifabutin have been identified. The parent drug is 85% bound to plasma proteins in a concentration-independent manner. Despite its greater potency against *M. tuberculosis* in vitro, rifabutin is considered inferior to rifampin for the short-term therapy of tuberculosis, because of its significantly lower plasma concentrations.

Although hepatotoxicity and the induction of cytochrome P450 enzymes are believed to be less with rifabutin, as compared with rifampin, these properties should be borne in mind when the drug is employed prophylactically. Rifabutin and its metabolites are highly colored compounds that can discolor skin, urine, tears, feces, etc.

Cycloserine, USP

D-(+)-4-Amino-3-isoxazolidinone (Seromycin) is an antibiotic that has been isolated from three different Streptomyces species: *S. orchidaceus, S. garyphalus*, and *S. lavendulus*. It occurs as a white to pale yellow crystalline material that is very soluble in water. It is stable in alkaline, but unstable in acidic solutions. The compound slowly dimerizes to 2,5-bis(aminoxymethyl)-3,6-diketopiperazine in solution or standing.



Cycloserine

The structure of cycloserine was reported simultaneously by Kuehl et al.[59] and Hidy et al.[60] to be D-(+)-4-amino-3-isoxazolidinone. It has been synthesized by Stammer et al.[61] and by Smrt et al.[62] Cycloserine is stereochemically related to D-serine. However, the L-form has similar antibiotic activity.

Cycloserine is presumed to exert its antibacterial action by preventing the synthesis of cross-linking peptide in the formation of bacterial cell walls.[63] Rando[64] has recently suggested that it is an antimetabolite for alanine, which acts as a suicide substrate for the pyridoxal phosphate-requiring enzyme alanine racemase. Irreversible inactivation of the enzyme thereby deprives the cell of the D-alanine required for the synthesis of the cross-linking peptide.

Although cycloserine exhibits antibiotic activity in vitro against a wide spectrum of both gram-negative and gram-positive organisms, its relatively weak potency and frequent toxic reactions limit its use to the treatment of tuberculosis. It is recommended for patients who fail to respond to other tuberculostatic drugs or are known to be infected with organisms resistant to other agents. It is usually administered orally in combination with other drugs, commonly isoniazid.

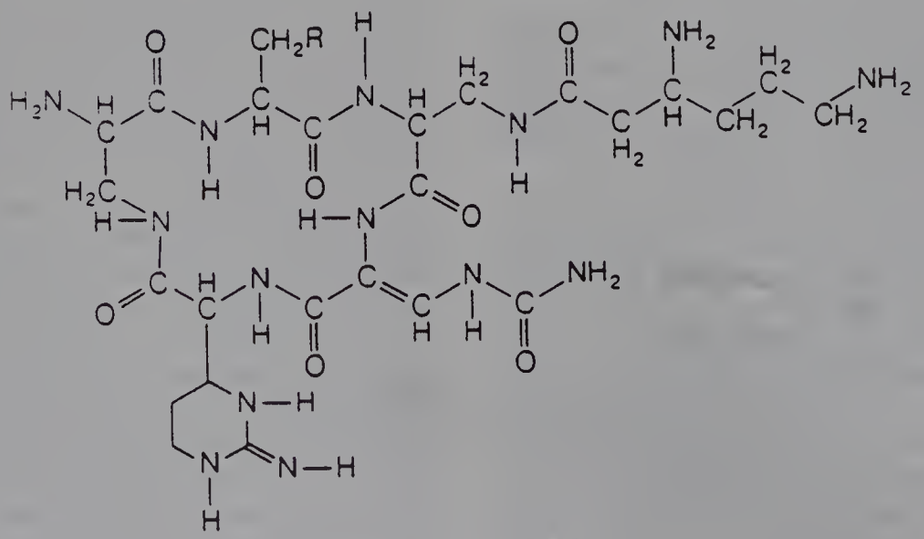
Sterile Capreomycin Sulfate, USP

Capastat sulfate, or capreomycin, is a strongly basic cyclic peptide isolated from *S. capreolus* in 1960 by Herr et al.[65] It was released in the United States in 1971 exclusively as a tuberculostatic drug. Capreomycin, which resembles viomycin (no longer marketed in the United States) chemically and pharmacologically, is a second-line agent employed in combination with other antitubercular drugs. In particular, it may be used in place of streptomycin when either the patient is sensitive to, or the strain of *M. tuberculosis* is resistant to, streptomycin. Similar to viomycin, capreomycin is a potentially toxic drug. Damage to the eighth cranial nerve and renal damage, as with viomycin, are the more serious toxic effects associated with capreomycin therapy. There is, as yet, insufficient clinical data with which to reliably compare the relative toxic potential of capreomycin with streptomycin. Cross-resistance among strains of tubercle bacilli is rare between capreomycin and streptomycin.

Four capreomycins, designated IA, IB, IIA, and IIB, have been isolated from *S. capreolus*. The clinical agent contains primarily IA and IB. The close chemical relationship between capreomycins IA and IB and viomycin was established,[66] and the total synthesis and proof of structure of the capreomycins later accomplished.[67] The structures of capreomycins IIA and IIB correspond to those of IA and IB, but lack the β-lysyl residue. The sulfate salts are freely soluble in water.

ANTIPROTOZOAL AGENTS

In the United States and other countries of the temperate zone, protozoal diseases are of minor importance, whereas bacterial and viral diseases are widespread and are the cause



Capreomycin 1A R = OH
1B R = H

of considerable concern. On the other hand, protozoal diseases are highly prevalent in tropical Third World countries, where they infect both human and animal populations, causing suffering, death, and enormous economic hardship. Common protozoal diseases in the United States are malaria, amebiasis, giardiasis, trichomoniasis, toxoplasmosis, and, as a direct consequence of the AIDs epidemic, pneumocystis pneumonia. Antimalarial agents are covered in Chap. 9.

Although amebiasis is generally thought of as a tropical disease, it actually has a worldwide distribution. In some areas with temperate climates, in which sanitation is poor, the prevalence of amebiasis has been estimated to be as high as 20% of the population. The causative organism, *Entamoeba histolytica*, can invade the wall of the colon, or other parts of the body (such as liver, lungs, or skin). An ideal chemotherapeutic agent would be effective against both the intestinal and extraintestinal forms of the parasite.

Amebicides that are effective against both intestinal and extraintestinal forms of the disease are limited to the somewhat toxic alkaloids emetine and dehydroemetine, the nitroimidazole derivative metronidazole, and the antimalarial agent chloroquine (Chap. 9). A second group of amebicides that are effective only against intestinal forms of the disease includes the aminoglycoside antibiotic paromomycin (Chap. 10), the 8-hydroxyquinoline derivative iodoquinolol, the arsenical compound carbarsone, and diloxanide.

Other protozoal species that colonize the intestinal tract and cause enteritis and diarrhea are *Balantidium coli* and the flagellates, *Giardia lamblia* and Cryptosporidium. Balantidiasis responds best to tetracycline (Chap. 10). Metronidazole and iodoquinol may also be effective. Giardiasis may be effectively treated with furazolidone, metronidazole, or the antimalarial drug quinacrine (Chap. 9). Cryptosporidosis is normally self-limiting in immunocompetent patients and is not normally treated. The illness can be a serious problem in AIDS patients because effective therapy is not currently available.

Trichomoniasis, a venereal disease caused by the flagellated protozoan *Trichomonas vaginalis*, is common in the United States and throughout the world. Although it is not generally considered serious, this affliction can cause serious physical discomfort and sometimes has a chilling effect on sexual relations. Oral metronidazole provides effective treatment against all forms of the disease. It is also employed to eradicate the organism from asymptomatic male carriers.

Pneumocystis carinii is an opportunistic pathogen that is found in the lungs of humans and other animals and, under the right conditions, can cause pneumonia. The organism has long been classified as a protozoa, but recent RNA evidence suggests that it may be more closely related to fungi. Only occasional cases of *Pneumocystis carinii* pneumonia (PCP) were known to occur in premature, undernourished infants and in patients receiving immunosuppressant therapy. The situation changed with the onset of the AIDS epidemic. It is estimated that at least 60% and possibly as high as 85% of patients infected with HIV develop PCP during their lifetimes.

The combination of the antifolate trimethoprim plus the sulfonamide sulfamethoxazole constitute the treatment of choice for PCP (Chap. 8). Other effective drugs include pentamidine, atovaquone, and a new antifolate, trimetrexate (Chap. 8).

Toxoplasma gondii is an obligate intracellular protozoan that is best known for causing blindness in neonates. Toxoplasmosis, the disseminated form of the disease, wherein the lymphatic system, skeletal muscles, heart, brain, eye, and placenta may be affected, has become increasingly prevalent in association with HIV infection. A combination of the antifolate pyrimethamine (Chap. 9) and the sulfa drug sulfadiazine (Chap. 8) constitute the most effective therapy for toxoplasmosis.

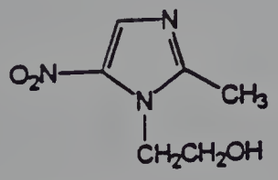
Various forms of trypanosomiasis, chronic tropical diseases caused by pathogenic members of the family Trypanosomidae, occur both in humans and in livestock. The principal disease in humans, sleeping sickness, can be broadly classified into two main geographic and etiologic groups: African sleeping sickness caused by *Trypanosoma gambiense* (West African), *T. rhodesiense* (East African), or *T. congolense*; and South American sleeping sickness (Chagas' disease) caused by *T. cruzi*. Of the various forms of trypanosomiasis, Chagas' disease is the most serious and generally the most resistant to chemotherapy. Leishmaniasis is a chronic tropical disease caused by various flagellate protozoa of the genus Leishmania. The more common visceral form caused by *L. donovani*, called kala-azar, is similar to Chagas' disease. Fortunately, although these diseases are widespread in tropical areas of Africa and South and Central American, they are of minor importance in the United States, Europe, and Asia.

The successful chemotherapy of trypanosomiasis and leishmaniasis remains somewhat primitive and often less than effective. In fact, it is doubtful that these diseases can be controlled by chemotherapeutic measures alone, without successful control of the intermediate hosts and vectors that transmit them. Heavy metal compounds, such as the arsenicals and antimonials, are sometimes effective, but frequently toxic. The old standby suramin appears to be of some value in long- and short-term prophylaxis. The nitrofuran derivative nifurtimox may be a major breakthrough in the control of these diseases. However, its potential toxicity remains to be fully assessed.

METRONIDAZOLE, USP

2-Methyl-5-nitroimidazole-1-ethanol (Flagyl, Protostat, Metro IV) is the most useful of a multitude of antiprotozoal nitroimidazole derivatives that have been synthesized in various laboratories throughout the world. Metronidazole was first marketed for the topical treatment of *T. vaginalis* vaginitis. It has since been shown to be effective orally against

both the acute and carrier states of the disease. The drug also possesses useful amebicidal activity and is, in fact, effective against both intestinal and hepatic amebiasis. It has also found use in the treatment of such other protozoal diseases as giardiasis and balantidiasis.



Metronidazole

More recently, metronidazole has been found to be effective against obligate anaerobic bacteria, but ineffective against facultative anaerobes or obligate aerobes. It is particularly active against gram-negative anaerobes, such as Bacteriodes and Fusobacterium species. It is also effective against gram-positive anaerobic bacilli (such as Clostridium sp.) and cocci (such as Peptococcus and Peptidostreptococcus sp.). Because of its bactericidal action, metronidazole has become an important agent for the treatment of serious infections (such as septicemia, pneumonia, peritonitis, pelvic infections, abscesses, meningitis, and others) caused by anaerobic bacteria.

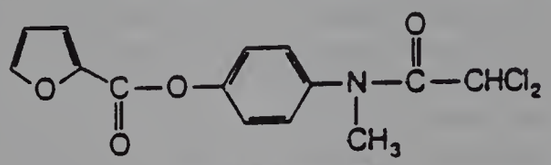
The common characteristic of microorganisms (bacteria and protozoa) sensitive to metronidazole is that they are anaerobic. It has been speculated that a reactive intermediate formed in the microbial reduction of the 5-nitro group of metronidazole covalently binds to the DNA of the microorganism triggering the lethal effect.[68] Potential reactive intermediates include the nitroxide, nitroso, hydroxylamine, and amine. The ability of metronidazole to act as a radiosensitizing agent is also related to its reduction potential.

Metronidazole is a pale yellow crystalline substance that is sparingly soluble in water. It is stable in air, but is light-sensitive. Despite its low water solubility, metronidazole is well absorbed following oral administration. It has a large apparent volume of distribution and achieves effective concentrations in all body fluids and tissues. Approximately 20% of an oral dose is metabolized to oxidized or conjugated forms. The 2-hydroxy metabolite is active; other metabolites are inactive.

Metronidazole is a weak base having a pK_a of 2.5. Although it is administered parenterally only as the free base by slow intravenous infusion, metronidazole for injection is supplied in two forms: a ready-to-inject 100-ml solution containing 5 mg of base per milliliter; and the hydrochloride salt as 500 mg of the sterile lyophilized powder. Metronidazole hydrochloride for injection must first be reconstituted with sterile water to give 5 ml of solution having a concentration of 100 mg/ml and a pH ranging from 0.5 to 2.0. The resulting solution must then be diluted with 100 ml of normal saline or 5% dextrose and neutralized with 5 mEq of sodium bicarbonate to provide a final solution of metronidazole base having an approximate concentration of 5 mg/ml and a pH of 6 to 7. Solutions of metronidazole hydrochloride are unsuitable for intravenous administration because of their extreme acidity. Reconstituted metronidazole hydrochloride solutions are stable for 96 hr at 30°C, while ready-to-use solutions of metronidazole base are stable for 24 hr at 30°C. Both solutions should be protected from light.

DILOXANIDE, USP

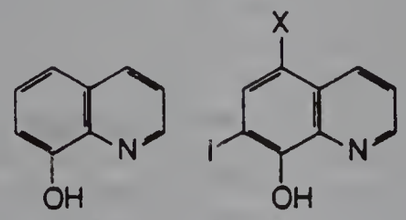
Furamide, or eutamide, is the 2-furoate ester of 2,2-dichloro-4′-hydroxy-N-methylacetanilide. It was developed as a result of the discovery that various α, α-dichloroacetamides possessed amebicidal activity in vitro. Diloxanide itself and many of its esters are also active, and drug metabolism studies indicate that hydrolysis of the ester is required for the amebicidal effect. Nonpolar esters of diloxanide are more potent than polar ones. Diloxanide furoate has been used in the treatment of asymptomatic carriers of *E. histolytica*. Its effectiveness against acute intestinal amebiasis or hepatic abscesses, however, has not been established. Diloxanide furoate is a white crystalline powder. It is administered orally only as 500-mg tablets and may be obtained in the United States from the Centers for Disease Control in Atlanta, Georgia.



Diloxanide Furoate

8-HYDROXYQUINOLINE

Oxine, quinophenol, or oxyquinoline is the parent compound from which the antiprotozoal oxyquinolines have been derived. The antibacterial and antifungal properties of oxine and its derivatives, which are believed to result from the ability to chelate metal ions, are well known. Aqueous solutions of acid salts of oxine, particularly the sulfate (Chinosol, Quinosol), in concentrations of 1:3,000 to 1:1,000, have been used as topical antiseptics. The substitution of an iodine atom at the 7-position of 8-hydroxyquinolines produces compounds with broad-spectrum antimicrobial properties.



Oxine

Clioquinol X = Cl
Iodoquinol X = I

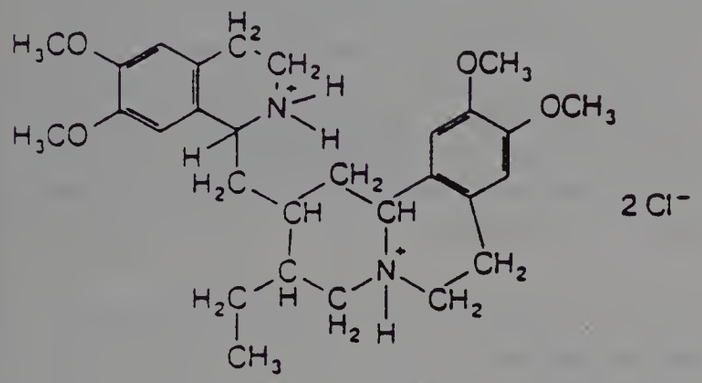
IODOQUINOL, USP

5,7-Diiodo-8-quinolinol, 5,7-diiodo-8-hydroxyquinoline, or diiodohydroxyquin (Yodoxin, Diodoquin, Diquinol) is a yel-

lowish to tan microcrystalline, light-sensitive substance that is insoluble in water. It is recommended for acute and chronic intestinal amebiasis, but is not effective in extraintestinal disease. Because a relatively high incidence of topic neuropathy has occurred with its use, iodoquinol should no longer be routinely used for traveler's diarrhea.

EMETINE AND DEHYDROEMETINE

The alkaloids emetine and dehydroemetine are obtained by isolation from ipecac. They occur as levorotatory, light-sensitive white powders that are insoluble in water. The alkaloids readily form water-soluble salts. Solutions of the hydrochloride salts intended for intramuscular injection should be adjusted to pH 3.5 and stored in light-resistant containers.



Emetine Hydrochloride

Emetine and dehydroemetine exert a direct amebicidal action on various forms of *E. histolytica*. They are protoplasmic poisons that inhibit protein synthesis in protozoal and mammalian cells by preventing protein elongation. Because their effect in intestinal amebiasis is solely symptomatic and the cure rate is only 10% to 15%, they should be used exclusively in combination with other agents. The high concentrations of the alkaloids achieved in the liver and other tissues after intramuscular injection provide the basis for their high degree of effectiveness against hepatic abscesses and other extraintestinal forms of the disease. Toxic effects limit the usefulness of emetine. It causes a high frequency of gastrointestinal distress (especially nausea and diarrhea), cardiovascular effects (hypotension and arrhythmias), and neuromuscular effects (pain and weakness). A lower incidence of cardiotoxicity has been associated with the use of dehydroemetine (Mebadin), available from the Centers for Disease Control, which is also amebicidal.

Emetine and dehydroemetine have also been used to treat balantidial dysentery and fluke infestations such as fasciliasis and paragonimiasis.

PENTAMIDINE ISETHIONATE, USP

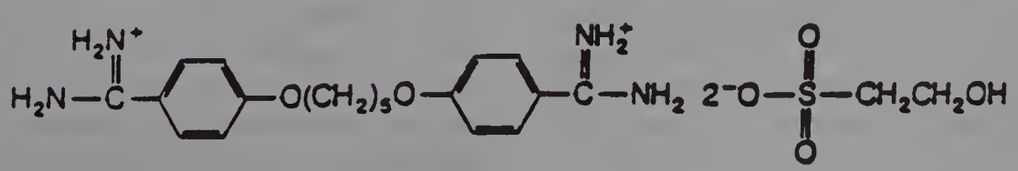
4,4′-(Pentamethylenedioxy)dibenzamidine diisethionate (NebuPent, Pentam 300) is a water-soluble crystalline salt that is stable to light and air. The principal use of pentamidine is for the treatment of pneumonia caused by the opportunistic pathogenic protozoan *Pneumocystis carinii*, a frequent secondary invader associated with AIDS. The drug may be administered by slow intravenous infusion or by deep intramuscular injection for PCP. An aerosol form of pentamidine is employed by inhalation for the prevention of PCP in high-risk patients infected with HIV who have a previous history of PCP infection or a low peripheral CD4 lymphocyte count.

Both the inhalation (aerosol) and parenteral dosage forms of pentamidine isethionate are sterile lyophilized powders that must be made up as sterile aqueous solutions prior to use. Sterile water for injection must be employed for making up the aerosol to avoid precipitation of the pentamidine salt. Adverse reactions to the drug are common. These include cough and bronchospasm (inhalation) and hypertension and hypoglycemia (injection).

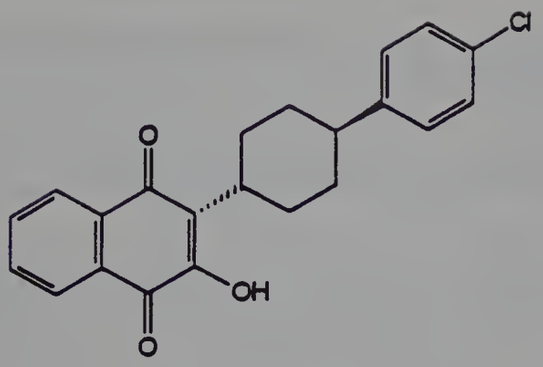
Pentamidine has been used for the prophylaxis and treatment of African trypanosomiasis. It is also of some value for treating visceral leishmaniasis. Pentamidine rapidly disappears from the plasma after intravenous injection and is distributed to the tissues, where it is stored for a long period. This property probably contributes to the usefulness of the drug as a prophylactic agent.

ATOVAQUONE, USP

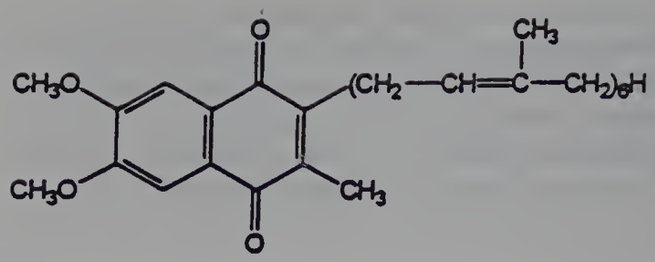
3-[4-(4-Chlorophenyl)-cyclohexyl]-2-hydroxy-1,4-naphthoquinone (Mepron) is a highly lipophilic, water-insoluble analog of ubiquinone 6, an essential component of the mitochondrial electron transport chain in microorganisms. The structural similarity between atovaquone and ubiquinone suggests that the former may act as an antimetabolite for the latter and thereby interfere with the function of electron transport enzymes. Atovaquone was originally developed as an antimalarial drug, but *Plasmodium falciparum* were found to develop a rapid tolerance to its action. More recently, the effectiveness of atovaquone against *Pneumocystis*



Pentamidine Isethionate



Atovaquone



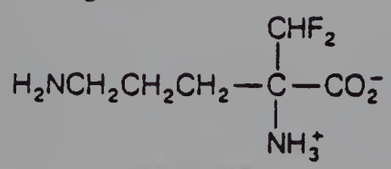
Ubiquinone 6

carinii was discovered. It is currently recommended as an alternative to trimethoprim/sulfamethoxazole (TMP-SMZ) for the treatment and prophylaxis of PCP in patients intolerant to this combination. Atovaquone has also been demonstrated to be effective in eradicating *Toxoplasma gondii* in preclinical animal studies.

The oral absorption of atovaquone is slow and incomplete, in part because of the low water solubility of the drug. Aqueous suspensions provide significantly better absorption than do tablets. Food, especially if it has a high fat content, increases atovaquone absorption. Significant enterohepatic recycling of atovaquone occurs, and the majority (nearly 95%) of the drug is excreted unchanged in the feces. In vivo, atovaquone is largely confined to the plasma, where it is extensively protein-bound (>99.9%). The half-life of the drug ranges from 62 to 80 hr. The primary side effects relate to gastrointestinal intolerance.

EFLORNITHINE, USP

DL-α-Difluoromethylornithine, or DFMO (Ornidyl), an amino acid derivative, is an enzyme-activated inhibitor of ornithine decarboxylase, a pyridoxal phosphate-requiring enzyme responsible for catalyzing the rate-limiting step in the biosynthesis of the diamine putresine and the polyamines spermine and spermidine. Polyamines are essential for the regulation of DNA synthesis and cell proliferation in animal tissues and microorganisms.



Eflornithine

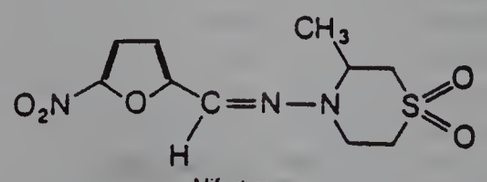
Eflornithine is employed for the treatment of West African sleeping sickness, caused by *Trypanosoma brucii gambiense*. It is specifically indicated for the meningoencephalitic stage of the disease. Eflornithine is a myelosuppressive drug that causes high incidences of anemia, leukopenia, and thrombocytopenia. Blood cell counts should be monitored during the course of therapy.

The irreversible inactivation of ornithine decarboxylase by eflornithine is accompanied by decarboxylation and release of fluoride ion from the inhibitor,[69] suggesting enzyme-catalyzed activation of the inhibitor. Only the (−)-isomer, stereochemically related to L-ornithine, is active.

Eflornithine is supplied as the hydrochloride salt. It may be administered intravenously or orally. Approximately 80% of the unchanged drug is excreted in the urine. Penetration of eflornithine into the CSF is facilitated by inflammation of the meninges.

NIFURTIMOX

Nifurtimox is 4-[(5-nitrofurfurylidene)amino]-3-methylthiomorpholine 1,1-dioxide, or Bayer 2502 (Lampit). The observation that various derivatives of 5-nitrofuraldehyde possessed, in addition to their antibacterial and antifungal properties, significant and potentially useful antiprotozoal activity eventually led to discovery of particular nitrofurans with antitrypanosomal activity. The most important of such compounds is nifurtimox because of its demonstrated effectiveness against *T. cruzi*, the parasite responsible for South American trypanosomiasis. In fact, use of this drug represents the only clinically proven treatment for both acute and chronic forms of the disease. Nifurtimox is available in the United States from the Centers for Disease Control.

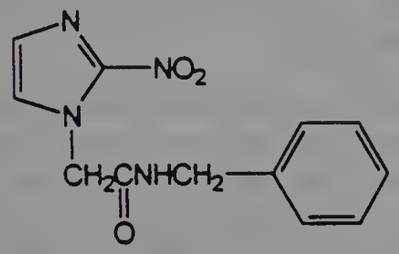


Nifurtimox

Nifurtimox is administered orally. Oral bioavailability is high, but considerable first-pass metabolism occurs. The half-life of nifurtimox is 2 to 4 hr. The drug is poorly tolerated, with a high incidence of nausea, vomiting, abdominal pain, and anorexia reported. Symptoms of central and peripheral nervous system toxicity also frequently occur with nifurtimox.

BENZNIDAZOLE, USP

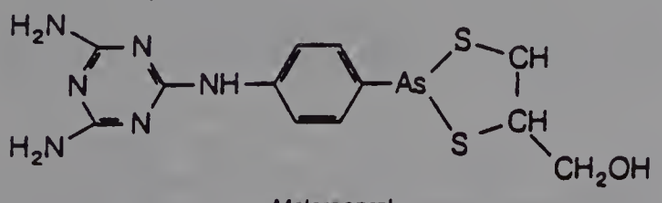
N-Benzyl-2-nitroimidazole-1-acetamide (Radanil, Rochagan) is a nitroimidazole derivative that is employed for the treatment of Chagas' disease. It is not available in the United States, but is used extensively in South America. The effectiveness of benznidazole is similar to that of nifurtimox. Therapy for American trypanosomiasis with oral benznidazole requires several weeks and is frequently accompanied by adverse effects such as peripheral neuropathy, bone marrow depression, and allergic-like reactions.



Benznidazole

MELARSOPROL

2-*p*-(4,6-Diamino-s-triazin-2-yl-amino)phenyl-4-hydroxymethyl-1,3,2-dithiarsoline (Mel B, Arsobal) is prepared by reduction of the corresponding pentavalent arsenilate to the trivalent arsenoxide followed by reaction of the latter with 2,3-dimercaptopropanol, or (British anti-Lewisite) BAL. It has become the drug of choice for the treatment of the latter stages of both forms of African trypanosomiasis. Melarsoprol has the advantage of excellent penetration into the central nervous system and, therefore, is effective against meningoencephalitic forms of *T. gambiense* and *T. rhodesiense*. Trivalent arsenicals tend to be more toxic to the host (as well as the parasites) than the corresponding pentavalent compounds. The bonding of arsenic with sulfur atoms tends to reduce host toxicity, increase chemical stability (to oxidation), and improve distribution of the compound to the arsenoxide. However, melarsoprol shares the toxic properties of other arsenicals, and its use must be monitored for signs of arsenic toxicity.

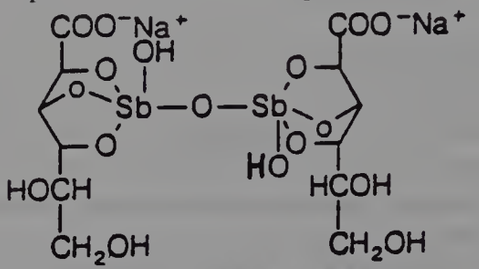


Melarsoprol

SODIUM STIBOGLUCONATE

Sodium antimony gluconate (Pentostam) is a pentavalent antimonial compound intended primarily for the treatment of various forms of leishmaniasis. It is available from the Centers for Disease Control as the disodium salt, which is chemically stable and freely soluble in water. The 10% aqueous solution used for either intramuscular or intravenous injection has a pH of ~5.5. Similar to all antimonial drugs, this drug has a low therapeutic index, and patients undergoing therapy with it should be monitored carefully for signs of heavy metal poisoning. Other organic antimonial compounds are employed primarily for the treatment of schistosomiasis and other flukes.

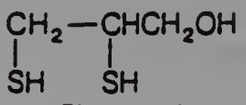
The antileishmanial action of sodium stibogluconate requires reduction to the trivalent form, which is believed to inhibit phosphofructokinase in the parasite.



Sodium Stibogluconate

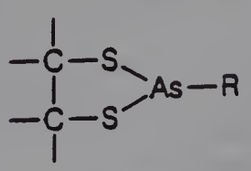
DIMERCAPROL, USP

2,3-Dimercapto-1-propanol, BAL, or dithioglycerol is a foul-smelling, colorless liquid. It is soluble in water (1:20) and alcohol. It was developed by the British during World War II as an antidote for "Lewisite," hence the name British anti-Lewisite or BAL. Dimercaprol is effective topically and systematically as an antidote for poisoning caused by arsenic, antimony, mercury, gold, and lead. It can therefore also be employed to treat arsenic and antimony toxicity associated with overdose or accidental ingestion of organoarsenicals or organoantimonials.



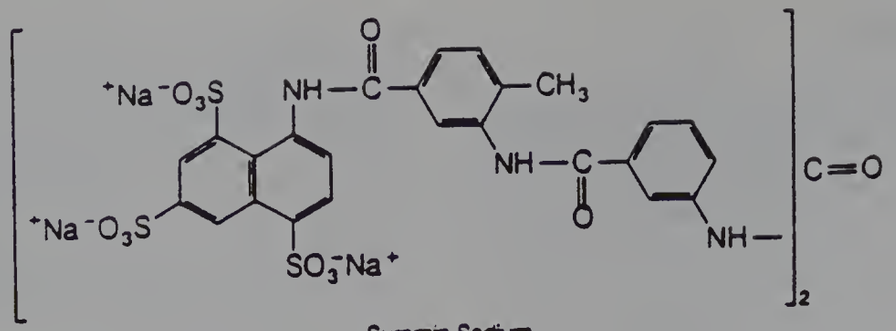
Dimercaprol

The antidotal properties of BAL are associated with the property of heavy metals to react with sulfhydryl (SH) groups in proteins (such as the enzyme pyruvate oxidase) and interfere with their normal function. 1,2-dithiol compounds, such as BAL, compete effectively with such proteins for the metal by reversibly forming metal ring compounds of the following type:



These are relatively nontoxic, metabolically conjugated (as glucuronides), and rapidly excreted.

BAL may be applied topically as an ointment or injected intramuscularly as a 5% or 10% solution in peanut oil.



Suramin Sodium

SURAMIN SODIUM

Suramin sodium is a high-molecular-weight bisurea derivative containing six sulfonic acid groups as their sodium salts. It was developed in Germany shortly after World War I as a by-product of research efforts directed toward the development of potential antiparasitic agents from dyestuffs.

The drug has been used for more than half a century for the treatment of early cases of trypanosomiasis. It was not until several decades later, however, that suramin was discovered to be a long-term prophylactic agent, the effectiveness of which, after a single intravenous injection, is maintained for periods of up to 3 months. The drug is tightly bound to plasma proteins, causing its excretion in the urine to be almost negligible.

Tissue penetration of the drug does not occur, apparently because of its high molecular weight and highly ionic character. Thus, an injected dose remains in the plasma for a very long period. Newer, more effective drugs are now available for short-term treatment and prophylaxis of African sleeping sickness. Suramin is also used for prophylaxis of onchocerciasis. It is available from the Centers for Disease Control.

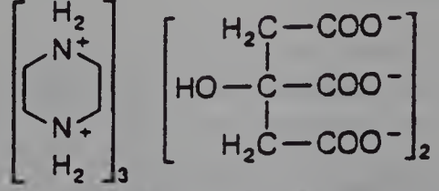
ANTHELMINTICS

Anthelmintics are drugs that have the capability of ridding the body of parasitic worms or helminths. The prevalence of human helminthic infestations is widespread throughout the globe and represents a major world health problem, particularly in Third World countries. Helminths parasitic to humans and other animals are derived from two phyla: Platyhelminthes and Nemathelminthes. Cestodes (tapeworms) and trematodes (flukes) belong to the former, and the nematodes or true roundworms to the latter. The helminth infestations of major concern on the North American continent are caused by roundworms (i.e., hookworm, pinworm, and Ascaris). Human tapeworm and fluke infestations are rarely seen in the United States.

Several classes of chemicals are used as anthelmintics and include phenols and derivatives, piperazine and related compounds, antimalarial compounds (Chap. 9), various heterocyclic compounds, and natural products.

PIPERAZINE, USP

Hexahydropyrazine or diethylenediamine (Arthriticine, Dispermin) occurs as colorless, volatile crystals of the hexahydrate that are freely soluble in water. After the discovery of the anthelmintic properties of a derivative diethylcarbamazine, the activity of piperazine itself was established. Piperazine is still employed as an anthelmintic for the treatment of pinworm [*Enterobius (Oxyuris) vermicularis*] and roundworm (*Ascaris lumbricoides*) infestations. It is available in a variety of salt forms, including the citrate (official in the USP) in syrup and tablet forms.

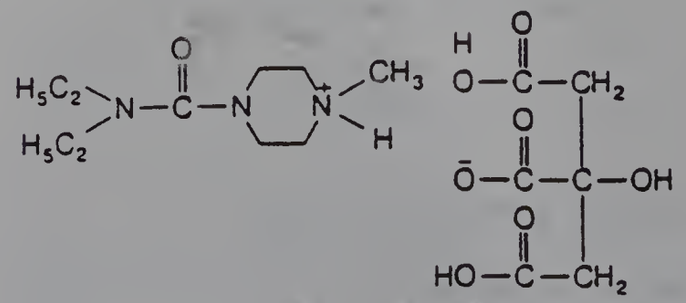


Piperazine Citrate

Piperazine blocks the response of the ascaris muscle to acetylcholine, causing a flaccid paralysis in the worm, which is dislodged from the intestinal wall and expelled in the feces.

DIETHYLCARBAMAZINE CITRATE, USP

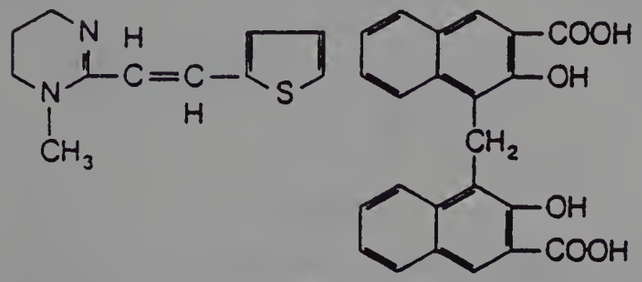
N,N-Diethyl-4-methyl-1-piperazinecarboxamide citrate or 1-diethylcarbamyl-4-methylpiperazine dihydrogen citrate (Hetrazan) is a highly water-soluble crystalline compound that has selective anthelmintic activity. It is effective against various forms of filariasis, including Bancroft's, onchocerciasis, and laviasis. It is also active against ascariasis. Relatively few adverse reactions have been associated with diethylcarbamazine.



Diethylcarbamazine Citrate

PYRANTEL PAMOATE, USP

trans - 1,4,5,6, - Tetrahydro - 1 - methyl - 2 - [2 - (2 -thienyl)vinyl] pyrimidine pamoate (Antiminth) is a depolarizing neuromuscular blocking agent that causes spastic paralysis in susceptible helminths. It is employed in the treatment of infestations caused by pinworms and roundworms (ascariasis). Because its action opposes that of piperazine, the two anthelmintics should not be used together.

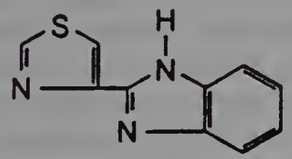


Pyrantel Pamoate

Over half of the oral dose is excreted in the feces unchanged. Adverse effects associated with its use are primarily gastrointestinal.

THIABENDAZOLE, USP

2-(4-Thiazolyl)benzimidazole (Mintezol) occurs as a white crystalline substance that is only slightly soluble in water, but soluble in strong mineral acids. Thiabendazole is a basic compound with a pK_a of 4.7 that forms complexes with metal ions.



Thiabendazole

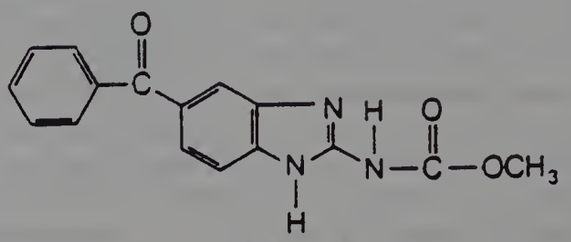
Thiabendazole inhibits the helminth-specific enzyme fumarate reductase.[70] It is not known whether metal ions are involved or if the inhibition of the enzyme is related to thiabendazole's anthelmintic effect. Benzimidazole anthelmintic drugs such as thiabendazole and mebendazole also arrest nemotode cell division in metaphase by interfering with microtubule assembly.[71] They exhibit a high affinity for tubulin, the precursor protein for microtubule synthesis.

Thiabendazole has broad-spectrum anthelmintic activity. It is used to treat enterobiasis, strongyloidiasis (threadworm infection), ascariasis, uncinariasis (hookworm infection), and trichuriasis (whipworm infection). It has also been used to relieve symptoms associated with cutaneous larva migrans (creeping eruption) and the invasive phase of trichinosis.

In addition to its use in human medicine, thiabendazole is widely employed in veterinary practice to control intestinal helminths in livestock.

MEBENDAZOLE, USP

Methyl 5-benzoyl-2-benzimidazolecarbamate (Vermox) is a broad-spectrum anthelmintic that is effective against a variety of nematode infestations, including whipworm, pinworm, roundworm, and hookworm. Mebendazole irreversibly blocks glucose uptake in susceptible helminths, thereby depleting glycogen stored in the parasite. It apparently does not affect glucose metabolism in the host. It also inhibits cell division in nematodes.[71]

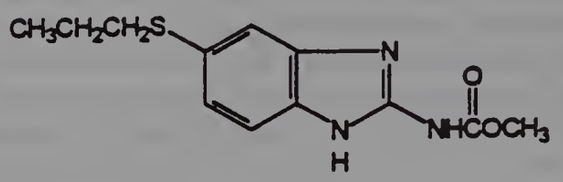


Mebendazole

Mebendazole is poorly absorbed by the oral route. Adverse reactions are uncommon and usually consist of abdominal discomfort. It is teratogenic in laboratory animals and, therefore, should not be given during pregnancy.

ALBENAZOLE, USP

Methyl 5-(Propylthio)-2-benzimidazolecarbamate (Eskazole, Zeutel) is a broad-spectrum anthelmintic that is not currently marketed in North America. It is available from the manufacturer on a compassionate use basis. Albenazole is widely employed throughout the world for the treatment of intestinal nematode infection. It is effective as a single-dose treatment for ascariasis, New and Old World hookworm infections, and trichuriasis. Multiple-dose therapy with albenazole can eradicate pinworm, threadworm, capillariasis, clonorchiasis, and hydatid disease. The effectiveness of albenazole against tapeworms (cestodes) is generally more variable and less impressive.



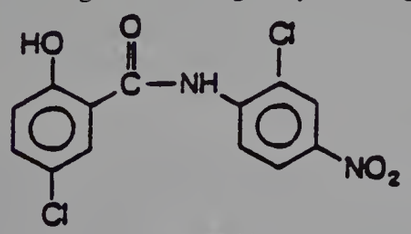
Albenazole

Albenazole occurs as a white crystalline powder that is virtually insoluble in water. The oral absorption of albenazole is enhanced by a fatty meal. The drug undergoes rapid and extensive first-pass metabolism to the sulfoxide, which is the active form in the plasma. The elimination half-life of the sulfoxide ranges from 10 to 15 hr. Considerable biliary excretion and enterohepatic recycling of albenazole sulfoxide occurs. Albenazole is generally well tolerated in single-dose therapy for intestinal nematodes. The high-dose, pro-

longed therapy required for clonorchiasis or echinococcal disease therapy can result in adverse effects, such as bone marrow depression, elevation of hepatic enzymes, and alopecia.

NICLOSAMIDE, USP

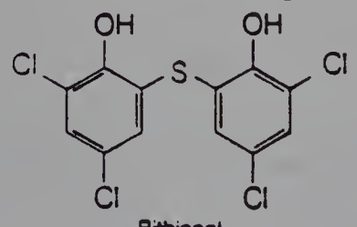
5-Chloro-*N*-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide or 2,5′-dichloro-4′-nitrosalicylanilide (Cestocide, Mansonil, Yomean) occurs as a yellowish-white, water-insoluble powder. It is a potent taeniacide that causes rapid disintegration of worm segments and the scolex. Penetration of the drug into various cestodes appears to be facilitated by the digestive juices of the host because very little of the drug is absorbed by the worms in vitro. Niclosamide is well tolerated following oral administration, and little or no systemic absorption of it occurs. A saline purge 1 to 2 hr after the ingestion of the taeniacide is recommended to remove the damaged scolex and worm segments. This procedure is mandatory in the treatment of pork tapeworm infestation to prevent possible cysticercosis resulting from release of live ova from worm segments damaged by the drug.



Niclosamide

BITHIONOL

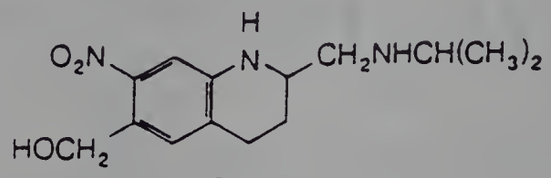
2,2′-Thiobis(4,6-dichlorophenol), or bi(2-hydroxy-3,5-dichlorophenyl) sulfide (Lorothidol, Bitin), a chlorinated bisphenol, was formerly used in soaps and cosmetics for its antimicrobial properties, but was removed from the market for topical use because of reports of contact photodermatitis. Bithionol has useful anthelmintic properties and has been employed as a fasciolicide and taeniacide. It is still considered the agent of choice for the treatment of infestations caused by the liver fluke *Fasciola hepatica* and the lung fluke *Paragonimus westermani*. Niclosamide is believed to be superior to it for the treatment of tapeworm infestations.



Bithionol

OXAMNIQUINE, USP

1,2,3,4 - Tetrahydro - 2 - [(isopropylamino)methyl] - 7 - nitro - 6-quinolinemethanol (Vansil) is an antischistosomal agent that is indicated for the treatment of *S. mansoni* (intestinal schistosomiasis) infection. It has been shown to inhibit DNA, RNA, and protein synthesis in schistosomes.[72] The 6-hydroxymethyl group is critical for activity; metabolic activation of precursor 6-methyl derivatives is critical. The oral bioavailability of oxamniquine is good; effective plasma levels are achieved in 1 to 1.5 hr. The plasma half-life is 1 to 2.5 hr. The drug is extensively metabolized to inactive metabolites, of which the principal one is the 6-carboxy derivative.

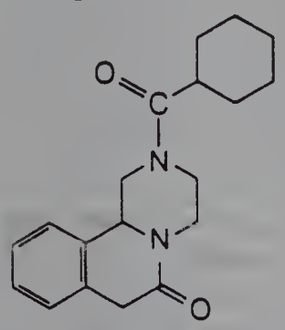


Oxamniquine

The free base occurs as a yellow crystalline solid that is slightly soluble in water, but soluble in dilute aqueous mineral acids and soluble in most organic solvents. It is available in capsules containing 250 mg of the drug. Oxamniquine is generally well tolerated. Dizziness and drowsiness are common, but transitory with its use. Serious reactions, such as epileptiform convulsions, are rare.

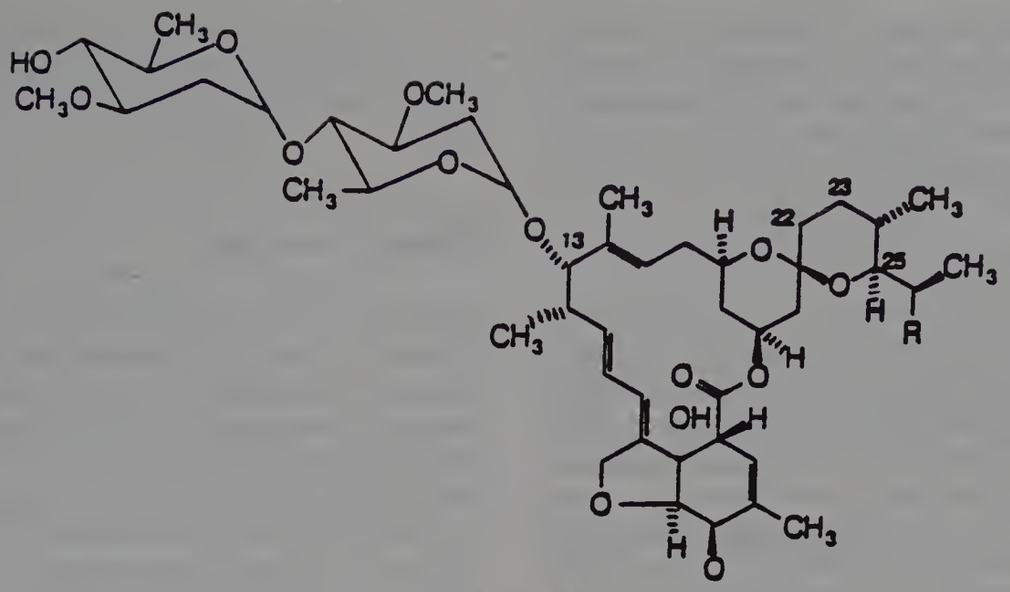
PRAZIQUANTEL, USP

2 - (Cyclohexylcarbonyl) - 1,2,3,6,7,11b - hexahydro - 4H - pyrazino[2,1-α]isoquinolin-4-one (Biltricide) is a broad-spectrum agent that is effective against a variety of trematodes (flukes). It has become the agent of choice for the treatment of infections caused by schistosomes (blood flukes). The drug also provides effective treatment for fasciolopsiasis (intestinal fluke), clonorchiasis (Chinese liver fluke), fascioliasis (sheep liver fluke), opisthorchosis (liver fluke), and paragonimiasis (lung fluke). Praziquantel increases cell membrane permeability of susceptible worms, resulting in the loss of extracellular calcium. Massive contractions and ultimate paralysis of the fluke musculature occurs, followed by phagocytosis of the parasite.



Praziquantel

Following oral administration, ~80% of the dose is absorbed. Maximal plasma concentrations are achieved in 1 to 3 hr. The drug is rapidly metabolized in the liver in the first pass. It is likely that some of the metabolites are also active.



Praziquantel occurs as a white crystalline solid that is insoluble in water. It is available as 600-mg film-coated tablets. The drug is generally well tolerated.

IVERMECTIN, USP

Ivermectin (Cardomec, Eqvalan, Ivomec) is a mixture of 22,23-dihydro derivatives of avermectins B_{1a} and B_{1b} prepared by catalytic reduction. Avermectins are members of a family of structurally complex antibiotics produced by a strain of *Streptomyces avermitilis*. Their discovery is the result of an intensive search for anthelmintic agents from natural sources.[73] Ivermectin is active in low dosage against a wide variety of nematodes and arthropods that parasitize animals.[74]

The structures of the avermectins were established, by a combination of spectroscopic[75] and x-ray crystallographic[76] techniques, to contain pentacyclic 16-membered ring aglycones glycosidically linked at the 3-position to a disaccharide that comprises two oleandrose sugar residues. The side chain at the 25-position of the aglycone is *sec*-butyl in avermectin B_{1a}, whereas in avermectin B_{1b} it is isopropyl. Ivermectin contains at least 80% of 22,23-dihydroavermectin B_{1a} and no more than 20% of 22,23-dihydroavermectin B_{1b}.

Ivermectin has achieved widespread use in veterinary practice in the United States and many countries throughout the world for the control of endoparasites and ectoparasites in domestic animals.[74] It has been found to be effective for the treatment of onchocerciasis (''river blindness'') in humans,[77] an important disease caused by the roundworm *Oncocerca volvulus*, prevalent in West and Central Africa, the Middle East, and South and Central America. Ivermectin destroys the microfilariae, immature forms of the nematode that create the skin and tissue nodules characteristic of the infestation and can lead to blindness. It also inhibits the release of microfilariae by the adult worms living in the host. Studies on the mechanism of action of ivermectin indicate that it blocks interneuron-motor neuron transmission in nematodes by stimulating the release of the inhibitory neurotransmitter γ-amino butyric acid (GABA).[74] The drug has been made available by the manufacturer on a humanitarian basis to qualified treatment programs through the World Health Organization.

ANTISCABIOUS AND ANTIPEDICULAR AGENTS

Scabicides (antiscabious agents) are compounds used to control the mite *Sarcoptes scabei*, an organism that thrives under conditions of poor personal hygiene. The incidence of scabies is believed to be increasing in the United States and worldwide and has, in fact, reached pandemic proportions.[78] Pediculocides (antipedicular agents) are employed to eliminate head, body, and crab lice. Ideal scabicides and pediculocides must kill the adult parasites and destroy their eggs.

BENZYL BENZOATE, USP

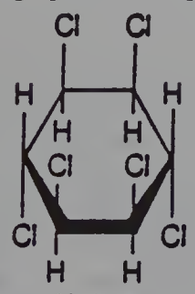
Benzyl benzoate is a naturally occurring ester obtained from Peru balsam and other resins. It is also prepared synthetically from benzyl alcohol and benzoyl chloride. The ester is a clear colorless liquid with a faint aromatic odor. It is insoluble in water, but soluble in organic solvents.

Benzyl benzoate is an effective scabicide when topically applied. Immediate relief from itching probably results from a local anesthetic effect; however, a complete cure is frequently achieved with a single application of a 25% emulsion

of benzyl benzoate in oleic acid, stabilized with triethanolamine. This preparation has the additional advantage of being essentially odorless, nonstaining, and nonirritating to the skin. It is applied topically as a lotion over the entire dampened body, except the face.

LINDANE, USP

Lindane is 1,2,3,4,5,6-hexachlorocyclohexane, γ-benzene hexachloride, or benzene hexachloride (Kwell, Scabene, Kwildane, G-Well). This halogenated hydrocarbon is prepared by the chlorination of benzene. A mixture of isomers is obtained in this process, five of which have been isolated: α, β, γ, δ, and ϵ. The γ-isomer, present to the extent of 10% to 13% in the mixture, is responsible for the insecticidal activity. The γ-isomer may be separated by a variety of extraction and chromatographic techniques.



Lindane

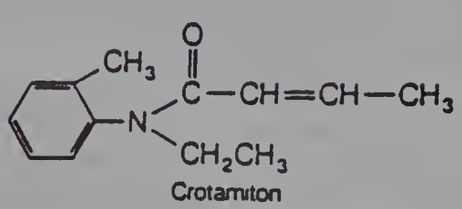
Lindane occurs as light buff to tan powder, with a persistent musty odor, and it is bitter. It is insoluble in water, but soluble in most organic solvents. It is stable under acidic or neutral conditions, but undergoes elimination reactions under alkaline conditions.

The action of lindane against insects is threefold: it is a direct contact poison; it has a fumigant effect; and it acts as a stomach poison. The effect of lindane on insects is similar to that of DDT. Its toxicity in humans is somewhat lower than that of DDT. However, because of its lipid solubility properties, lindane tends to accumulate in the body when ingested.

Lindane is employed locally as a cream, lotion, or shampoo for the treatment of scabies and pediculosis.

CROTAMITON, USP

N-Ethyl-*N*-(2-methylphenyl)-2-butenamide, or N-ethyl-o-crotonotoluidide (Eurax), is a colorless, odorless oily liquid. It is virtually insoluble in water, but soluble in most organic solvents.

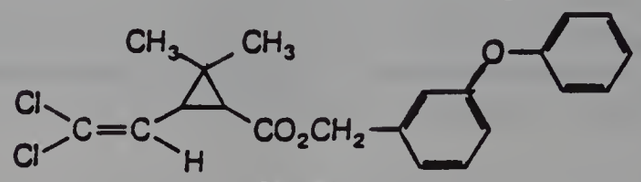


Crotamiton

Crotamiton is available in 10% concentration in a lotion and a cream intended for the topical treatment of scabies. Its antipruritic effect is probably due to a local anesthetic action.

PERMETHRIN, USP

Permethrin is 3-(2,2-Dichloroethenyl)-2,2-dimethylcyclopropanecarboxylic acid (3-phenoxyphenyl)methyl ester or 3-(phenoxyphenyl)methyl (±)-*cis, trans*-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane carboxylate (Nix). This synthetic pyrethrenoid compound is more stable chemically than most natural pyrethrins and is at least as active as an insecticide. Of the four isomers present, the *1R, trans-* and *1R, cis*-isomers are primarily responsible for the insecticidal activity. The commercial product is a mixture consisting of 60% *trans* and 40% *cis* racemic isomers. It occurs as colorless to pale yellow low-melting crystals or as a pale yellow liquid and is insoluble in water, but is soluble in most organic solvents.



Permethrin

Permethrin exerts a lethal action against lice, ticks, mites, and fleas. It acts on the nerve cell membranes of the parasites to disrupt sodium channel conductance. It is employed as a pediculicide for the treatment of head lice. A single application of a 1% solution is known to effect cures in >99% of cases. The most frequent side effect is pruritus, which occurred in ~6% of the patients tested.

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CHAPTER 8

Sulfonamides, Sulfones, and Folate Reductase Inhibitors with Antibacterial Action

Dwight S. Fullerton

SULFONAMIDES AND FOLATE REDUCTASE INHIBITORS

PRONTOSIL AND GERHARD DOMAGK (1895–1964)

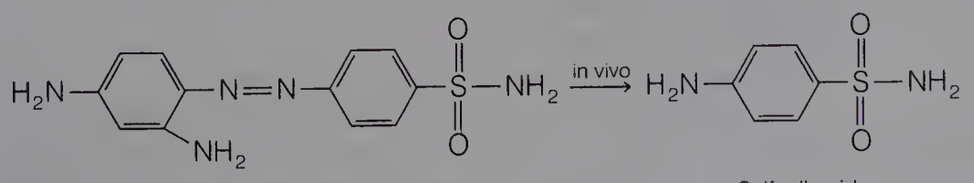
The founding of chemotherapy, drug design, and medicinal chemistry by Paul Ehrlich (1854–1915) and the antisyphilitic drug Salvarsan in 1908 are discussed in Chapter 9. Ehrlich's discovery led to intensive investigations of dyes as antimicrobial agents, especially in Germany. Although Salvarsan and related drugs were revolutionary in treating some protozoan infections and syphilis, they were not useful in treating two major killers of the times, streptococcal and staphylococcal infections.

Fritz Mietzsch and Joseph Klarer of the I.G. Farbenindustrie (Bayer) laboratories began a systematic synthesis of azo dyes as possible antimicrobials. Sulfonamide azo dyes were included because they were relatively easy to synthesize and had improved staining properties. The Bayer pathologist–bacteriologist who evaluated the new Mietzsch-Klarer dyes was Gerhard Domagk, who, like Ehrlich, was a physician by training.[1–3] In 1932, Domagk began a study of a bright red dye, later to be named Prontosil, and found that it caused remarkable cures of streptococcal infections in mice.[1] However, Prontosil was inactive on bacterial cultures. Domagk's studies on Prontosil continued, and in 1933, the first of many human cures of severe staphylococcal septicemias was reported.[4] Domagk even saved the life of his own daughter which was threatened by a severe streptococcal infection. For his pioneering efforts in chemotherapy, Domagk was awarded the Nobel prize for medicine and physiology in 1939. The Gestapo prevented him from actually accepting the award, but he received it in Stockholm in 1947.

Prontosil's inactivity in vitro, but excellent activity in vivo, attracted much attention. In 1935, Trefouel and coworkers[5] reported their conclusion from a structure–activity study of sulfonamide azo dyes, that the azo linkage was metabolically broken to release the active ingredient, sulfanilamide. Their reported finding was confirmed in 1937 when Fuller[6] isolated sulfanilamide from the blood and urine of patients being treated with Prontosil. Modern chemotherapy and the concept of the prodrug (see Chap. 4) were firmly established.

THE MODERN ERA

Following Prontosil's dramatic successes, a cascade of sulfanilamide derivatives began to be synthesized and tested—more than 4,500 by 1948 alone.[7] From these, only about two dozen actually have been used in clinical practice. In the late 1940s, penicillins began to replace the sulfanilamides in chemotherapy. This was largely because of the sulfanilamides' toxicity for some patients and because sulfanilamide-resistant bacterial strains were becoming an increasing problem, the result of indiscriminant use worldwide.



Prontosil Sulfanilamide

Today, a few sulfonamides and, especially, sulfonamide–trimethoprim combinations are used extensively for opportunistic infections in patients with AIDS[8] (*Pneumocystis carinii* pneumonia treatment and prophylaxis, cerebral toxoplasmosis treatment and prophylaxis), urinary tract infections, and burn therapy.[8–12] They are also the drugs of choice or alternates for a few other types of infection (Table 8-1), but their overall use is otherwise quite limited in modern antimicrobial chemotherapy,[8–12] having been largely replaced by antibiotics.

TABLE 8-1

CURRENT THERAPY WITH SULFONAMIDE ANTIBACTERIALS[8,10,11]

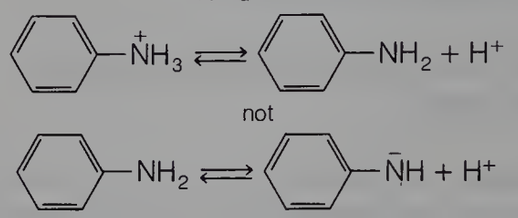
Disease/Infection	*Sulfonamides Commonly Used*
WIDE USE	
Treatment and prophylaxis of *Pneumocystis carinii* pneumonia	Trimethoprim–sulfamethoxazole
Treatment and prophylaxis of cerebral toxoplasmosis	Pyrimethamine–sulfadiazine
First attack of urinary tract infection	Sulfamethoxazole and trimethoprim Sulfisoxazole
Burn therapy: prevention and treatment of bacterial infection	Silver sulfadiazine Mafenide
Conjunctivitis and related superficial ocular infections	Sodium sulfacetamide
Chloroquine-resistant malaria (Chap. 9)	Combinations with quinine, others Sulfadoxine Sulfalene
LESS COMMON INFECTIONS/DISEASES: DRUGS OF CHOICE OR ALTERNATES	
Nocardiosis	Trimethoprim–sulfamethoxazole
Severe traveler's diarrhea[13]	Trimethoprim–sulfamethoxazole
Meningococcal infections	Sulfonamides, only if proved to be sulfonamide-sensitive; otherwise, penicillin G, ampicillin, or (for penicillin-allergic patients) chloramphenicol should be used
GENERALLY NOT USEFUL	
Streptococcal infections	Most are resistant to sulfonamides
Prophylaxis of rheumatic fever recurrences	Most are resistant to sulfonamides
Other bacterial infections	Penicillin's low cost and bacterial resistance to sulfonamides have decreased sulfonamide use worldwide but still used in a few countries
Vaginal infections	FDA Bulletin[12] and USP DI find no evidence of effectiveness
Reduction of bowel flora	Effectiveness not established[9]
Ulcerative colitis	Corticosteroid therapy often preferred Relapses common with sulfanilamides Phthalylsulfathiazole Salicylazosulfapyridine Side effects of the sulfanilamides sometimes seem like ulcerative colitis[14]

CHEMISTRY AND NOMENCLATURE

The term "sulfonamide" is commonly used to refer to antibacterials that are (1) aniline-substituted sulfonamides, the "sulfanilamides" (Fig. 8-1); (2) prodrugs that produce sulfanilamides (e.g., sulfasalazine); and (3) nonaniline sulfonamides (e.g., mafenide). However, several other widely used drugs are also sulfonamides or sulfanilamides. Included among these nonantibacterial sulfonamides are tolbutamide (an oral diabetic drug, see Chap. 19), furosemide (a potent diuretic, see Chap. 18), and chlorthalidone (also a diuretic, see Chap. 18).

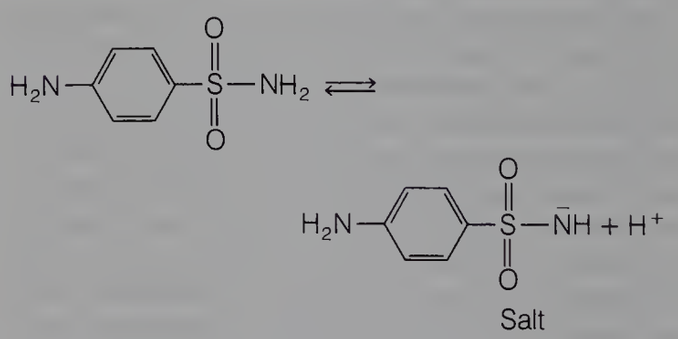
As reviewed in Chapter 4, pK_b values are not used in pharmaceutical chemistry to compare compounds. If a pK_a of an amine is given, it refers to its salt acting as the conjugate acid, for example:

Aniline, pK_a 4.6 refers to:

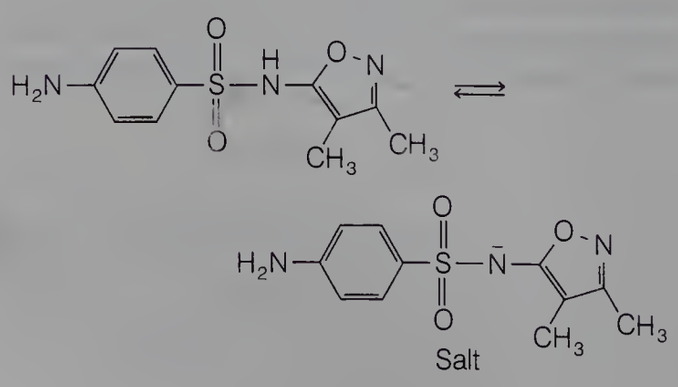


A minus charge on a nitrogen atom is normally not very stable, unless the change can be greatly delocalized by resonance. This is exactly the case with the sulfanilamides. Thus, the single pK_a usually given with sulfanilamides refers to loss of an amide H^+, for example:

Sulfanilamide, pK_a 10.4 refers to:



and Sulfisoxazole, pK_a 5.0 refers to:



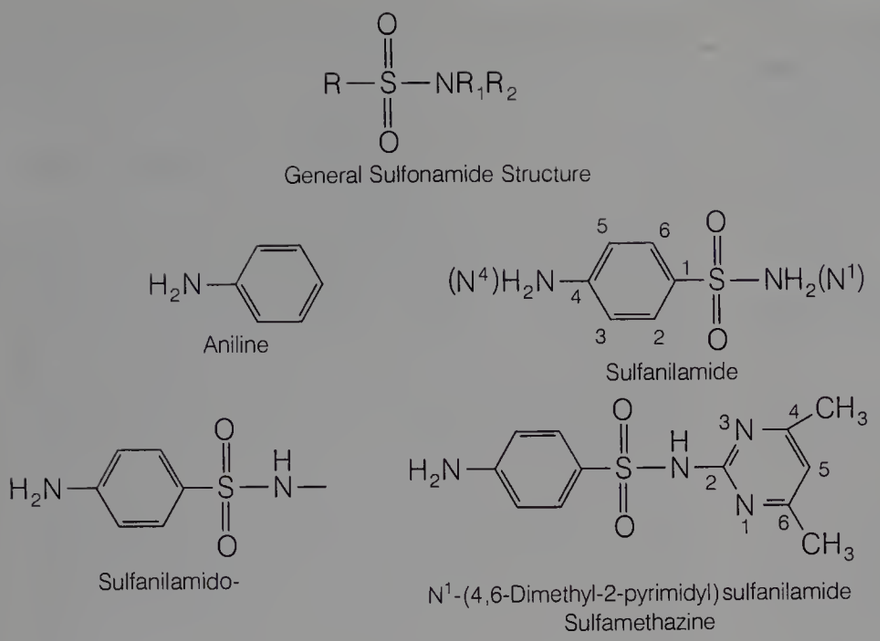
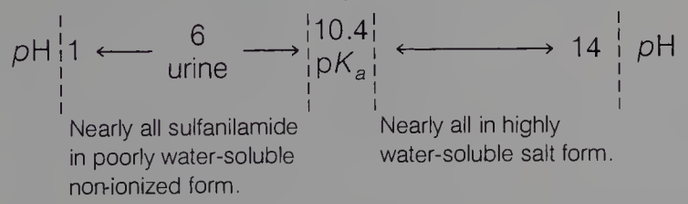


FIG. 8-1.

Thus, sulfisoxazole (pK_a 5.0) is a slightly weaker acid than acetic acid (pK_a 4.8).

REDUCING CRYSTALLURIA BY LOWERING pK_a

Sulfanilamide, although revolutionary in the early 1930s, often caused severe kidney damage by forming crystals in the kidneys. Sulfanilamides and their metabolites (usually acetylated at N^4) are excreted almost entirely in the urine. Unfortunately, sulfanilamide is not very water soluble. Unless the *p*H is above the pK_a (i.e., above *p*H 10.4), little of its water-soluble salt is present. Because urine *p*H is typically about 6, and often slightly lower during bacterial infections, essentially all sulfanilamide is in the relatively insoluble nonionized form in the kidneys.



At *p*H = pK_a, that is, at *p*H 10.4 for sulfanilamide, there will be a 1:1 mixture of nonionized and salt forms.

How can a sulfanilamide be made more soluble in the urine? There are several options:

1. Greatly increase urine flow. Thus, during the early days of sulfanilamide and sulfanilamide derivative use, patients were warned to "force fluids."
2. Raise the *p*H of the urine. The closer the *p*H of the urine gets to 10.4 (for sulfanilamide itself), the more of the highly water-soluble salt form will be present. Thus, sometimes oral sodium bicarbonate was, and occasionally still is, given to raise urine *p*H.
3. Make derivatives of sulfanilamide that have lower pK_a values, closer to the *p*H of urine. This has been the approach taken with virtually all sulfonamides clinically used today, for example:

Sulfon	**pK_a**
Sulfadiazine	6.5
Sulfamerazine	7.1
Sulfamethazine	7.4
Sulfisoxazole	5.0
Sulfamethoxazole	6.1

4. Mix sulfonamides to reach the total dose. Because solubilities of sulfanilamides are independent, more of a mixture of sulfanilamides can stay in water solution at a particular *p*H than a single sulfonamide can. Thus, trisulfapyrimidines, USP ("triple sulfas") contain a mixture of sulfadiazine, sulfamerazine, and sulfamethazine. However, such mixtures are seldom used today because the individual agents have sufficiently low pK_a values to be adequately urine soluble, *providing that at least normal urine flow is maintained.* Patients must still be cautioned to maintain a normal fluid intake, even if they do not feel like drinking during the illness. Forcing fluids, however, is no longer necessary.

It would be reasonable to ask, "Why do the modern sulfonamides have such low pK_a values?" The answer is that the heterocyclic rings attached to N^1 are electron-withdrawing, providing additional stability for the salt form. Therefore, the nonionized forms can more easily give up an H^+, so the pK_a values are lower. Why are simpler electron-withdrawing groups not used, such as *p*-nitrophenyl, the usual example studied in introductory organic chemistry classes? Such compounds, in fact, were investigated extensively, as were thousands of others. However, the other sul-

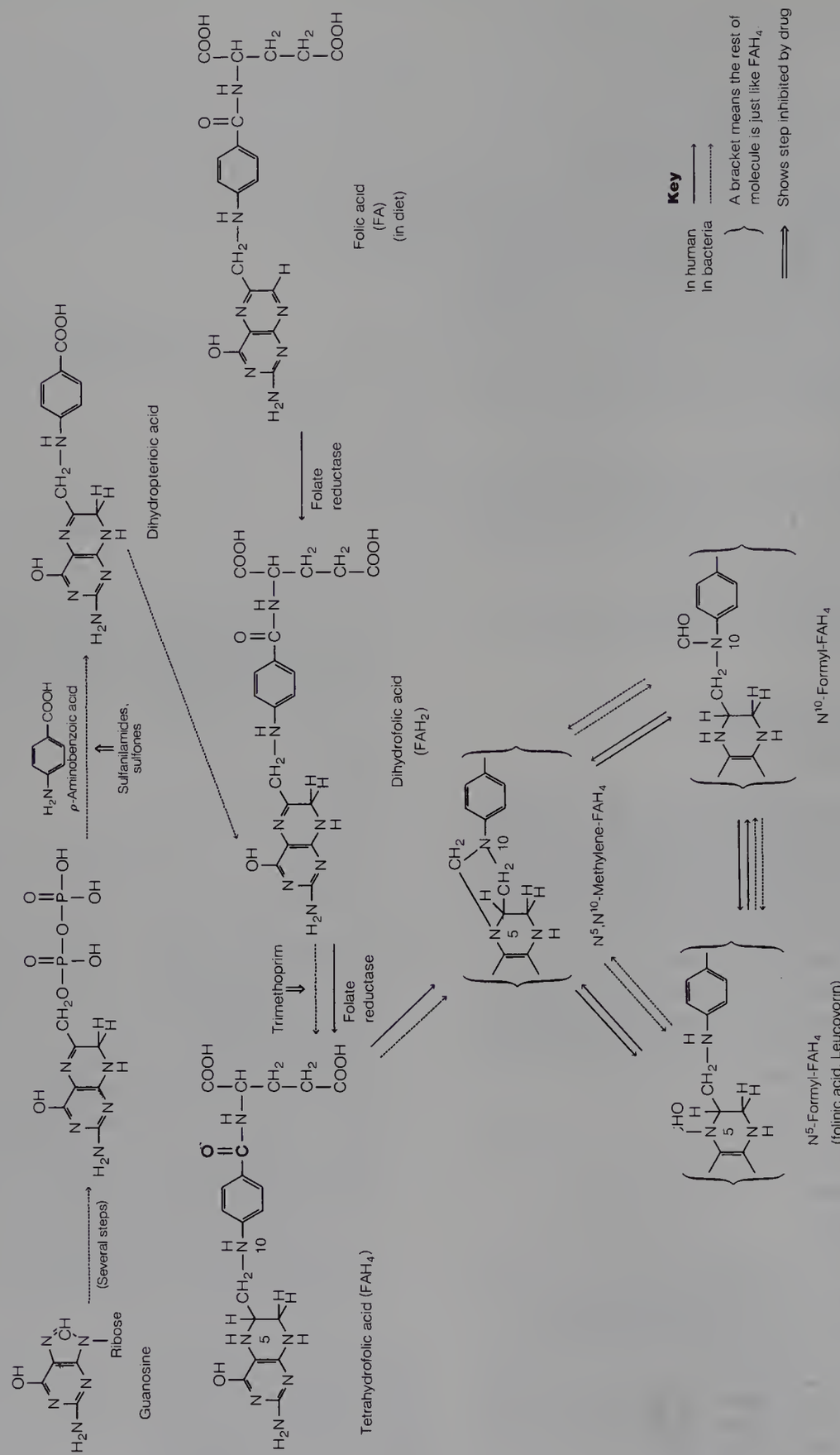

Guanosine
Ribose
(Several steps)
Dihydropterioic acid
p-Aminobenzoic acid
Sulfanilamides, sulfones
Folic acid (FA) (in diet)
Folate reductase
Dihydrofolic acid (FAH_2)
Trimethoprim
Folate reductase
Tetrahydrofolic acid (FAH_4)
N^5,N^{10}-Methylene-FAH_4
N^5-Formyl-FAH_4 (folinic acid, Leucovorin)
N^{10}-Formyl-FAH_4
Key
In human
In bacteria
A bracket means the rest of molecule is just like FAH_4
Shows step inhibited by drug

FIG. 8-2.

fonamides generally were too toxic, not sufficiently active, or both.

MECHANISM OF ACTION

Folinic acid (N^5-formyltetrahydrofolic acid, Fig. 8-2), N^5, N^{10}-methylenetetrahydrofolic acid, and N^{10}-formyltetrahydrofolic acid are indispensable for several biosynthetic pathways in humans, bacteria, animals, and plants (Fig. 8-3). Without these folate coenzymes, for example, thymidine monophosphate will not be available to produce nucleic acids needed for cell division. Other reactions requiring the folate coenzymes are shown in Fig. 8-3. The result of any drug blocking the biosynthesis of folate coenzymes in bacteria, for example, is that growth and cell division are stopped. Such drugs, including the sulfonamides and trimethoprim, are thus *bacteriostatic.*

As shown in the biosynthetic pathway in Fig. 8-2, folate coenzymes are biosynthesized from dietary folic acid in humans (and other animals). However, bacteria (and protozoa, see Chap. 9) must make them from *p*-aminobenzoic acid (PABA). The microbes cannot use dietary folic acid from the host, for reasons not yet completely understood.[3] It may be that folic acid cannot penetrate the cell wall.

The sulfonamide and sulfone antibacterials act as competitive inhibitors for the incorporation of PABA to form dihydropteroic acid (Fig. 8-2). Trimethoprim is an inhibitor of folate reductase, needed to convert dihydrofolic acid (FAH_2) into tetrahydrofolic acid (FAH_4) in bacteria. Evidence for these inhibitions has been summarized in detail by Anand.[3]

Although trimethoprim does not have a very high affinity for malaria protozoa's folate reductase (see Chap. 9), it does have high affinity for the bacterial folate reductase. The reverse situation exists for the antimalarial drug pyrimethamine.[15] Trimethoprim does have some affinity for human folate reductase, the cause of some toxic effects discussed later in this chapter and in Chapter 9.

Drugs with additional or modified mechanisms of action (e.g., silver sulfadiazine) are discussed with individual descriptions, which follow later in the chapter.

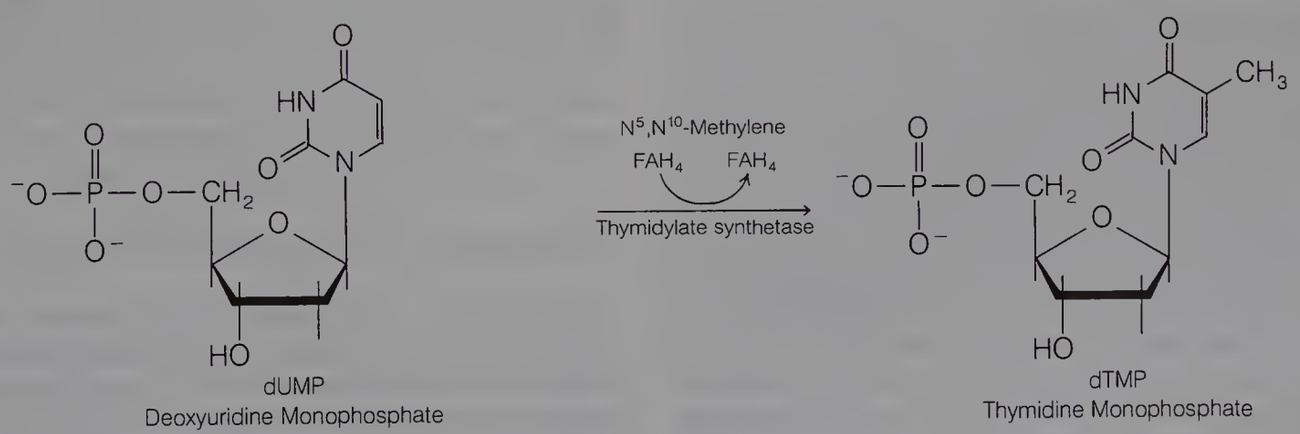
SYNERGISM OF SULFONAMIDES AND FOLATE REDUCTASE INHIBITORS

Blocking the biosynthesis of folate coenzymes at more than one point in the biosynthetic pathway of bacteria (or protozoa, see Chap 9) will result in a synergistic antimicrobial effect. An additional benefit is that the microbe will not be able to develop resistance as quickly as with a single pathway blocker. This synergistic approach is used widely in antibacterial therapy with the combination of sulfamethoxazole and trimethoprim[16,17] and in antimalarial therapy with pyrimethamine plus a sulfanilamide or quinine (Table 8-1). Additional explanations for the synergistic antimicrobial actions of trimethoprim and sulfamethoxazole have at times been debated vigorously.[3,16–18]

Other combinations of trimethoprim have also been investigated (e.g., with rifampin).[19,20]

MECHANISMS OF RESISTANCE

As noted earlier in this chapter, wide and unselective use of sulfonamides led to the emergence of many drug-resistant strains of bacteria. The cause of the resistance is probably increased production of PABA by the resistant bacteria,[21] though other mechanisms may account for resistance in some cases.[3,17] If a microbe is resistant to one sulfonamide,



Other examples

Coenzyme	Reaction
N^{10}-Formyl FAH_4	Met-tRNA ⟶ Formyl Met-tRNA
N^5,N^{10}-Methylene FAH_4	Glycine ⟶ Serine
N^5-Formyl FAH_2	Homocysteine ⟶ Methionine

FIG. 8-3.

it is generally resistant to all. A special concern is the finding that sulfonamide resistance can be transferred from a resistant bacterial species to a previously sulfanilamide-sensitive species. The transferring substances have been called "R-factors."[22]

Several explanations have been presented to account for bacterial resistance to trimethoprim,[17] including natural (intrinsic) resistance, development of an ability by the bacteria to use the host's deoxythymidine monophosphate (Fig. 8-3), and R-factor transmission.

TOXICITY AND SIDE EFFECTS

A variety of serious toxicity and hypersensitivity problems have been reported with sulfonamide and sulfonamide–trimethoprim combinations. Mandell and Petri[9] note that these problems occur in about 5% of all patients. Hypersensitivity reactions include drug fever, Stevens-Johnson syndrome, skin eruptions, allergic myocarditis, photosensitization, and related conditions. Hematologic side effects also sometimes occur, especially hemolytic anemia in individuals with a deficiency of glucose-6-phosphate (discussed further in Chap. 9). Other hematologic side effects that have been reported include agranulocytosis, aplastic anemias, and others. Crystalluria may still occur, even with modern sulfonamides, when the patient does not maintain a normal fluid intake. Nausea and related gastrointestinal side effects are sometimes noted.

Detailed summaries of incidences of side effects with trimethoprim–sulfamethoxazole have been published by Wormser and Deutsch[16] and by Gleckman and associates.[17]

METABOLISM, PROTEIN BINDING, AND DISTRIBUTION

With the exceptions of the poorly absorbed sulfonamides used for ulcerative colitis and reduction of bowel flora and the topical burn preparations (e.g., mafenide), sulfonamides and trimethoprim tend to be absorbed quickly and distributed well. As Mandell and Petri noted, sulfonamides can be found in the urine "within 30 minutes after oral ingestion."[9]

The sulfonamides vary widely in plasma protein binding—for example, sulfisoxazole 76%, sulfamethoxazole 60%, sulfamethoxypyridazine 77%, and sulfadiazine 38%. (An excellent table comparing the percentage of protein binding, lipid solubility, plasma half-life, and N^4-metabolites has been published by Anand.[3]) The fraction that is protein-bound is not active as an antibacterial, but because the binding is reversible, free, and therefore active, sulfonamide eventually becomes available. Generally, the more lipid-soluble a sulfonamide is, at physiologic *p*H, the more it will be protein-bound. Fujita and Hansch[23] have found that among sulfonamides with similar pK_a values, the lipophilicity of the N^1 group has the largest effect on protein binding. N^4-Acetate metabolites of the sulfonamides are more lipid-soluble and, therefore, better protein-bound than the starting drugs themselves (which have a free 4-NH_2 group that decreases lipid solubility). Surprisingly, the N^4-acetylated metabolites, although more strongly protein-bound, are excreted more rapidly than the starting drugs.

Currently, the relationship between plasma protein binding and biologic half-life is not clear. Many competing factors are involved, as reflected in sulfadiazine, with a serum half-life of 17 hr, which is much less protein-bound than sulfamethoxazole, with a serum half-life of 11 hr.[3]

Sulfonamides are excreted primarily as mixtures of unmetabolized drugs, N^4-acetates, and glucuronides.[24] The N^4-acetates and glucuronides are inactive. Sulfisoxazole, for example, is excreted about 80% unchanged and sulfamethoxazole 20% unchanged. Sulfadimethoxine is about 80% excreted as the glucuronide. The correlation between structure and route of metabolism has not yet been delineated, though progress has been made by Fujita.[25] Vree and co-workers, however, have described the excretion kinetics and pK_a values of N^1- and N^4-acetylsulfamethoxazole and other sulfonamides.[24]

Both trimethoprim and sulfamethoxazole are partially plasma protein-bound—about 45% of trimethoprim and about 66% of sulfamethoxazole. Whereas about 80% of excreted trimethoprim and its metabolites are active as antibacterials, only 20% of sulfamethoxazole and its metabolites are active most of the activity comes from mostly unmetabolized sulfamethoxazole. Six metabolites of trimethoprim are known.[25] It is likely, therefore, that sulfonamide–trimethoprim combinations using a sulfonamide with a higher active urine concentration will be developed in the future for urinary tract infections. Sulfamethoxazole and trimethoprim have similar half-lives, about 10 to 12 hours, but the half-life of the active fraction of sulfamethoxazole is less—about 9 hours.[25] (Ranges of half-lives have been summarized by Gleckman and co-workers,[17] and a detailed summary of pharmacokinetics has been made by Hansen.[25]) In patients with impaired renal function, sulfamethoxazole and its metabolites may greatly increase in the plasma. A fixed combination of sulfamethoxazole and trimethoprim should not be used for patients with low creatinine clearances.

STRUCTURE–ACTIVITY RELATIONSHIPS

As noted earlier in this chapter, several thousand sulfonamides have been investigated as antibacterials (and many as antimalarials, see Chapter 9). From these efforts, several structure–activity relationships have been proposed, elegantly summarized by Anand.[3] The aniline (N^4) amino group is very important for activity because any modification of it other than to make prodrugs results in a loss of activity. As noted earlier, for example, all of the N^4-acetylated metabolites of sulfonamide are inactive.

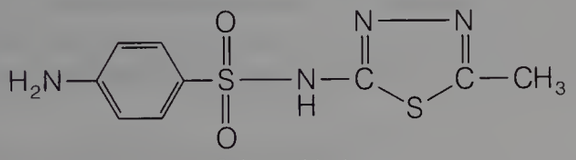
A variety of studies have shown that the active form of sulfonamide is the N^1-ionized salt. Thus, although many

modern sulfonamides are much more active than unsubstituted sulfanilamide, they are only two to six times more active when comparing amounts of N^1-ionized forms.[26] Maximal activity seems to be exhibited by sulfonamides between pK_a 6.6 and 7.4.[26–29] This reflects, in part, the need for enough nonionized (i.e., more lipid-soluble) drug to be present at physiologic *p*H to be able to pass through bacterial cell walls.[30] Fujita and Hansch[23] also related pK_a, partition coefficients, and electronic (Hammett) parameters with sulfonamide activity.

WELL-ABSORBED, SHORT-, AND INTERMEDIATE-ACTING SULFONAMIDES

Sulfamethizole, USP

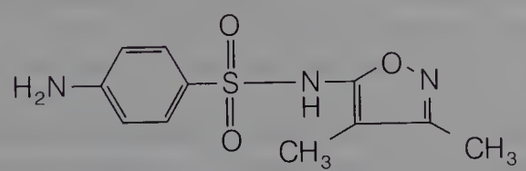
4-Amino-*N*-(5-methyl-1,3,4-thiadiazole-2yl)benzenesulfonamide; N^1-(5-methyl-1,3,4-thiadiazol-2-yl)sulfanilamide; 5-methyl-2-sulfanilamido-1,3,4-thiadiazole. Sulfamethizole's plasma half-life is 2.5 hr. This compound is a white crystalline powder soluble 1:2,000 in water.



Sulfamethizole

Sulfisoxazole, USP

4-Amino-*N*-(3,4-dimethyl-5-isoxazolyl)benzenesulfonamide; N^1-(3,4-dimethyl-5-isoxazolyl)sulfanilamide; 5-sulfanilamido-3,4-dimethylisoxazole. Sulfisoxazole's plasma half-life is 6 hr. This compound is a white, odorless, slightly bitter, crystalline powder. Its pK_a is 5.0. At *p*H 6 this sulfonamide has a water solubility of 350 mg in 100 mL and its acetyl derivative has a solubility of 110 mg in 100 mL of water.

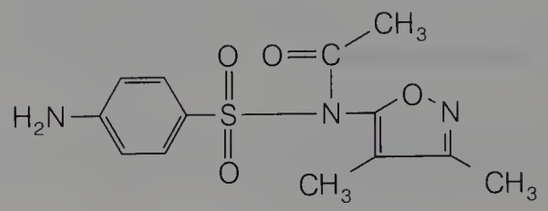


Sulfisoxazole

Sulfisoxazole possesses the action and the uses of other sulfonamides and is used for infections involving sulfonamide-sensitive bacteria. It is claimed to be effective in the treatment of gram-negative urinary infections.

Sulfisoxazole Acetyl, USP

N-[(4-Aminophenyl)sulfonyl]-*N*-(3,4-dimethyl-5-isoxazolyl)acetamide; *N*-(3,4-dimethyl-5-isoxazolyl)-*N*-sulfanilylacetamide; N^1-acetyl-N^1-(3,4-dimethyl-5-isoxazolyl)sulfanilamide. Sulfisoxazole acetyl shares the actions and uses of the parent compound, sulfisoxazole. The acetyl derivative is tasteless and, therefore, suitable for oral administration, especially in liquid preparations. The acetyl compound is split in the intestinal tract and absorbed as sulfisoxazole; that is, it is a *prodrug* for sulfisoxazole.



Sulfisoxazole Acetyl

Sulfisoxazole Diolamine, USP

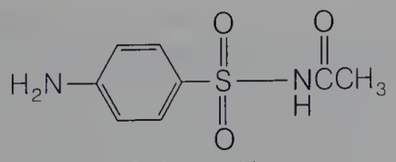
4-Amino-*N*-(3,5-dimethyl-5-isoxazolyl)benzenesulfonamide compound with 2,2′-iminobis[ethanol](1:1); 2,2′-iminodiethanol salt of N^1-(3,4-dimethyl-5-isoxazolyl)sulfanilamide. This salt is prepared by adding enough diethanolamine to a solution of sulfisoxazole to bring the *p*H to about 7.5. It is used as a salt to make the drug more soluble at the physiologic *p*H range of 6.0 to 7.5 and is used in solution for systemic administration of the drug by slow intravenous, intramuscular, or subcutaneous injection when sufficient blood levels cannot be maintained by oral administration alone. It also is used for instillation of drops or ointment in the eye for the local treatment of susceptible infections.

Sulfamethazine, USP

4-Amino-*N*-(4,6-dimethyl-2-pyrimidinyl)benzenesulfonamide; N^1-(4,6-dimethyl-2-pyrimidinyl)sulfanilamide; 2-sulsulfanilamido-4,6-dimethylpyrimidine. Sulfamethazine's plasma half-life is 7 hr. This compound is similar in chemical properties to sulfamerazine and sulfadiazine but does have greater water solubility than either of them. Its pK_a is 7.2. Because it is more soluble in acid urine than sulfamerazine is, the possibility of kidney damage from use of the drug is decreased. The human body appears to handle the drug unpredictably; hence, there is some disfavor to its use in this country except in combination sulfa therapy (in trisulfapyrimidines, USP) and in veterinary medicine. (For the structure of sulfamethazine, see Fig. 8-1.)

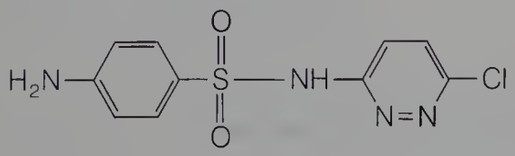
Sulfacetamide

N-[(4-Aminophenyl)sulfonyl]-acetamide; *N*-sulfanilylacetamide; N^1-acetylsulfanilamide. Sulfacetamide's plasma half-life is 7 hr. This compound is a white crystalline powder, soluble in water (1:62.5 at 37°C) and in alcohol. It is very soluble in hot water, and its water solution is acidic. It has a pK_a of 5.4.


Sulfacetamide

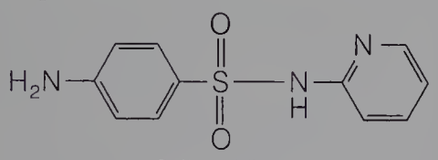
Sulfachloropyridazine

N^1-(6-Chloro-3-pyridazinyl)sulfanilamide. Sulfachloropyridazine's plasma half-life is 8 hr.


Sulfachloropyridazine

Sulfapyridine, USP

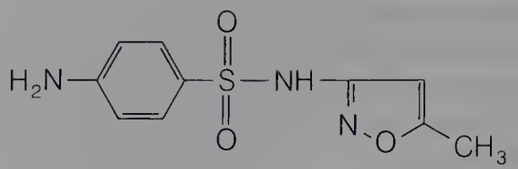
4-Amino-*N*-2-pyridinyl-benzenesulfonamide; N^1-2-pyridylsulfanilamide. Sulfapyridine's plasma half-life is 9 hr. This compound is a white, crystalline, odorless, and tasteless substance. It is stable in air but slowly darkens on exposure to light. It is soluble in water (1:3,500), in alcohol (1:440), and in acetone (1:65) at 25°C. It is freely soluble in dilute mineral acids and aqueous solutions of sodium and potassium hydroxide. The pK_a is 8.4. Its outstanding effect in curing pneumonia was first recognized by Whitby; however, because of its relatively high toxicity, it has been supplanted largely by sulfadiazine and sulfamerazine. Several cases of kidney damage have resulted from acetylsulfapyridine crystals deposited in the kidneys. It also causes severe nausea in most patients. Because of its toxicity, it is used only for dermatitis herpetiformis.


Sulfapyridine

Sulfapyridine was the first drug to have an outstanding curative action on pneumonia. It gave impetus to the study of the whole class of N^1-heterocyclically substituted derivatives of sulfanilamide.

Sulfamethoxazole, USP

4-Amino-*N*-(5-methyl-3-isoxazolyl)benzenesulfonamide; N^1-(5-methyl-3-isoxazolyl)sulfanilamide (Gantanol). Sulfamethoxazole's plasma half-life is 11 hr.

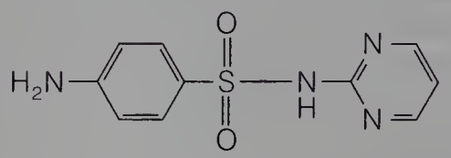

Sulfamethoxazole

Sulfamethoxazole is a sulfonamide drug closely related to sulfisoxazole in chemical structure and antimicrobial activity. It occurs as a tasteless, odorless, almost white crystalline powder. The solubility of sulfamethoxazole at the *p*H range of 5.5 to 7.4 is slightly less than that of sulfisoxazole but greater than that of sulfadiazine, sulfamerazine, or sulfamethazine.

Following oral administration, sulfamethoxazole is not absorbed as completely or as rapidly as sulfisoxazole, and its peak blood level is only about 50% as high.

Sulfadiazine, USP

4-Amino-*N*-2-pyrimidinyl-benzenesulfonamide; N^1-2-pyrimidinylsulfanilamide; 2-sulfanilamidopyrimidine. Sulfadiazine's plasma half-life is 17 hr. It is a white, odorless crystalline powder soluble in water to the extent of 1:8,100


Sulfadiazine

at 37°C and 1:13,000 at 25°C, in human serum to the extent of 1:620 at 37°C, and sparingly soluble in alcohol and acetone. It is readily soluble in dilute mineral acids and bases. Its pK_a is 6.3.

Sulfadiazine Sodium, USP

Soluble sulfadiazine. This compound is an anhydrous, white, colorless, crystalline powder soluble in water (1:2) and slightly soluble in alcohol. Its water solutions are alkaline (*p*H 9 to 10) and absorb carbon dioxide from the air with precipitation of sulfadiazine. It is administered as a 5% solution in sterile water intravenously for patients requiring an immediate high blood level of the sulfonamide.

MIXED SULFONAMIDES

The danger of crystal formation in the kidneys from administration of sulfonamides has been greatly reduced through the use of the more soluble sulfonamides, such as sulfisoxazole. This danger may be diminished still further by administering mixtures of sulfonamides. When several sulfonamides are administered together, the antibacterial action of the mixture

is the summation of the activity of the total sulfonamide concentration present, but the solubilities are independent of the presence of similar compounds. Thus, by giving a mixture of sulfadiazine, sulfamerazine, and sulfacetamide, the same therapeutic level can be maintained with much less danger of crystalluria because only one-third the amount of any one compound is present. Descriptions of some of the mixtures employed follow.

Trisulfapyrimidines, Oral Suspension

This mixture contains equal weights of sulfadiazine, USP; sulfamerazine, USP; and sulfamethazine, USP, either with or without an agent to increase the *p*H of the urine.

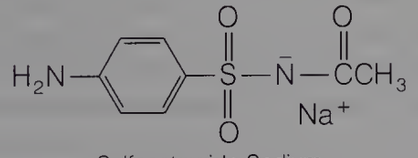
Trisulfapyrimidines, Tablets

These tablets contain essentially equal quantities of sulfadiazine, sulfamerazine, and sulfamethazine.

SULFONAMIDES FOR OPHTHALMIC INFECTIONS

Sulfacetamide Sodium, USP

N-Sulfanilyl-acetamide monosodium salt (Sodium Sulamyd) is obtained as the monohydrate and is a white, odorless, bitter, crystalline powder that is very soluble (1:2.5) in water. Because the sodium salt is highly soluble at the physiologic *p*H of 7.4, it is especially suited, as a solution, for repeated topical applications in the local management of ophthalmic infections susceptible to sulfonamide therapy.



Sulfacetamide Sodium

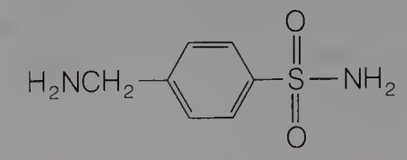
Sulfisoxazole Diolamine, USP

Also used in intravenous and intramuscular preparations, this salt of sulfisoxazole was described along with the short- and intermediate-acting sulfonamides.

SULFONAMIDES FOR BURN THERAPY

Mafenide Acetate

4-(Aminomethyl)benzenesulfonamide acetate (Sulfamylon) is a homologue of the sulfanilamide molecule. It is not a true sulfanilamide-type compound as it is not inhibited by *p*-aminobenzoic acid. Its antibacterial action involves a mechanism that is different from that of true sulfanilamide-type compounds. This compound is particularly effective against *Clostridium welchii* in topical application, and was used during World War II by the German army for prophylaxis of wounds. It is not effective orally. It is currently employed alone or with antibiotics in the treatment of slow-healing, infected wounds.

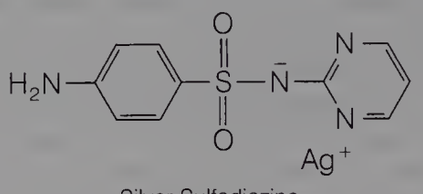


Mafenide

Some patients treated for burns with large quantities of this drug have developed metabolic acidosis. To overcome this side effect, a series of new organic salts was prepared.[16] The acetate in an ointment base proved to be the most efficacious.

Silver Sulfadiazine (Silvadene)

The silver salt of sulfadiazine applied in a water-miscible cream base has proved to be an effective topical antimicrobial agent, especially against *Pseudomonas* species. This is of particular significance in burn therapy because pseudomonad are often responsible for failures in therapy. The salt is only very slightly soluble and does not penetrate the cell wall but acts on the external cell structure. Studies using radioactive silver have shown essentially no absorption into body fluids. Sulfadiazine levels in the serum were about 0.5 to 2 mg/100 mL.



Silver Sulfadiazine

This preparation is reported to be easier to use than other standard burn treatments, such as application of freshly prepared dilute silver nitrate solutions or mafenide ointment.

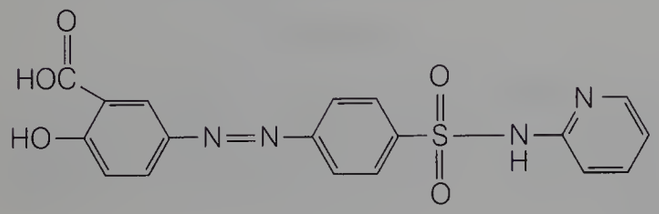
SULFONAMIDES FOR INTESTINAL INFECTIONS, ULCERATIVE COLITIS, OR REDUCTION OF BOWEL FLORA

Each of the sulfonamides in this group is a prodrug, which is designed to be poorly absorbable, though usually, in practice, a little is absorbed. Therefore, usual precautions with sulfonamide therapy should be observed. In the large intestine, the N^4-protecting groups are cleaved, releasing the free

sulfonamide antibacterial agent. Today, only one example is used clinically–sulfasalazine.

Sulfasalazine, USP

2-Hydroxy-5[[4-[(2-pyridinylamino)sulfonyl]phenyl]azo] benzoic acid; 5-[[*p*-(2-pyridylsulfamoyl)phenyl]azo]salicylic acid. This compound is a brownish yellow, odorless powder, slightly soluble in alcohol but practically insoluble in water, ether, and benzene.



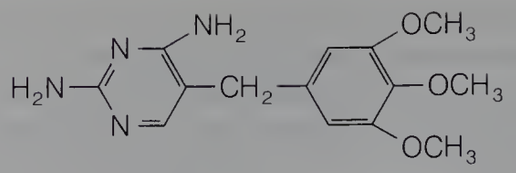
Sulfasalazine

It is broken down in the body to *m*-aminosalicylic acid and sulfapyridine. The drug is excreted through the kidneys and is detectable colorimetrically in the urine, producing an orange-yellow color when the urine is alkaline and no color when the urine is acid.

FOLATE REDUCTASE INHIBITORS

Trimethoprim, USP

5-[(3,4,5-Trimethoxyphenyl)methyl]-2,4-pyrimidinediamine; 2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine. Trimethoprim is closely related to several antimalarials, but it does not have good antimalarial activity by itself; however, it is a potent antibacterial. Originally introduced in combination with sulfamethoxazole, it is now available as a single agent. Approved by the FDA in 1980, trimethoprim as a single agent is used only for the treatment of uncomplicated urinary tract infections. The argument for trimethoprim to be a single agent was summarized in 1979 by Wormser and Deutsch.[16] They point out, for example, that several studies comparing trimethoprim with trimethoprim–sulfamethoxazole for treatment of chronic urinary tract infections found no statistically relevant difference between the two treatments. Furthermore, some patients cannot take sulfonamide products for the reasons discussed previously in this chapter, especially hypersensitivity. In contrast with these and similar arguments, the concern is that, when used as a single agent, bacteria now susceptible to trimethoprim will rapidly develop resistance. However, in combination with a sulfonamide, the bacteria will be less likely to do so. That is, they will not survive long enough to easily develop resistance to both drugs.



Trimethoprim

Sulfamethoxazole and Trimethoprim

The synergistic action of the combination of these two drugs has been discussed previously in this chapter.

SULFONES

The sulfones are primarily of interest as antibacterial agents, though there are some reports of their use in the treatment of malarial and rickettsial infections. They are less effective than the sulfonamides. *p*-Aminobenzoic acid partially antagonizes the action of many of the sulfones, suggesting that the mechanism of action is similar to that of the sulfonamides. It has also been observed that infections that arise in patients being treated with sulfones are cross-resistant to sulfonamides. Several sulfones have proved useful in the treatment of leprosy, but among them only dapsone is generally used today.

It has been estimated that there are about 11 million cases of leprosy in the world, of which about 60% are in Asia (with 3.5 million in India alone). The first reports of dapsone resistance prompted the use of multidrug therapy, with dapsone, rifampin, and clofazimine combinations, in some geographic areas.[31]

The search for antileprotic drugs has been hampered by the inability to cultivate *Mycobacterium leprae* in artificial media and by the lack of experimental animals susceptible to human leprosy. A method of isolating and growing *M. leprae* in the footpads of mice and in armadillos has been reported and has permitted a much wider range of research. Sulfones were introduced into the treatment of leprosy after it was found that sodium glucosulfone was effective in experimental tuberculosis in guinea pigs.

The parent sulfone, dapsone (4,4′-sulfonyldianiline), is the prototype for a variety of analogues that have been studied widely. Four variations on this structure have given active compounds:

1. Substitution on both the 4- and 4′-amino functions
2. Monosubstitution on only one of the amino functions
3. Nuclear substitution on one of the benzenoid rings
4. Replacement of one of the phenyl rings with a heterocyclic ring

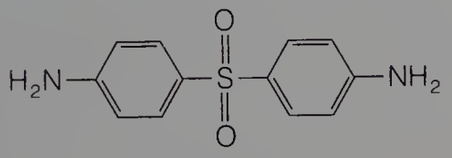
The antibacterial activity and the toxicity of the disubstituted sulfones are thought to be due chiefly to the formation in vivo of dapsone. Hydrolysis of disubstituted derivatives to the parent sulfone apparently occurs readily in the acid

medium of the stomach but only to a very limited extent following parenteral administration. Monosubstituted and nuclear-substituted derivatives are believed to act as entire molecules.

PRODUCTS

Dapsone, USP

4,4′-Sulfonylbisbenzenamine; 4,4′-sulfonyldianiline; *p,p′*-diaminodiphenylsulfone; DDS (Avlosulfone). Dapsone occurs as an odorless, white crystalline powder that is very slightly soluble in water and sparingly soluble in alcohol. The pure compound is light-stable, but the presence of traces of impurities, including water, makes it photosensitive and thus susceptible to discoloration in light. Although no chemical change is detectable following discoloration, the drug should be protected from light.



Dapsone

Dapsone is used in the treatment of both lepromatous and tuberculoid types of leprosy. Dapsone is used widely for all forms of leprosy, often in combination with clofazimine and rifampin. Initial treatment often includes rifampin with dapsone, followed by dapsone alone. It is also used to prevent the occurrence of multibacillary leprosy when given prophylactically.

Dapsone is also the drug of choice for dermatitis herpetiformis, sometimes used with pyrimethamine for treatment of malaria (see Chap. 9) and with trimethoprim for *Pneumocystis carinii* pneumonia.

Serious side effects can include hemolytic anemia, methemoglobinemia, and toxic hepatic effects. Hemolytic effects can be pronounced in patients with glucose-6-phosphate dehydrogenase deficiency. With all patients, blood counts during therapy are important.

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CHAPTER 9

Antimalarials

Dwight S. Fullerton

> Few people realize that there are far more kinds of parasitic than nonparasitic organisms in the world. Even if we exclude viruses, rickettsias . . . and the many kinds of parasitic bacteria and fungi, the parasites are still in the majority. The parasitic way of life . . . is a highly successful one. Humans are hosts to over 100 kinds of parasites, again not counting viruses, bacteria and fungi. . . . At least 45,000 species of protozoa have been described to date, many of which are parasitic. Parasitic protozoa still kill, mutilate, and debilitate more people in the world than any other group of disease organisms. . .
>
> G. D. Schmidt and L. S. Roberts
> *Foundations of Parasitology*[1]

Malaria, African sleeping sickness, leishmaniasis, Chagas' disease, and other protozoan diseases (Table 9-1) of humans and their livestock continue to have a devastating impact worldwide[1–19] More than 12 million South Americans suffer from Chagas' disease alone and millions in Africa and the Mediterranean from African sleeping sickness or the disfigurement of leishmaniasis.

However, none of these protozoan diseases has had the enormous effect upon civilization, either historically or in modern times, as has malaria. Malaria now affects about 250 million people annually.[2] Over one million African children alone still die each year from the disease, largely from the protozoa Plasmodium falciparum. Loss of productivity from the debilitating and cyclic clinical stages of malaria is enormous. It has been noted by Schmidt and Roberts[1] that a single day of malarial fever requires the caloric equivalent of 2 days of hard labor (and thus of food). With malaria protozoa in many areas becoming resistant to commonly used antimalarial drugs[1–6] and with insecticide-related problems increasing (resistance by the mosquito vector, human and environmental harm), the adverse impact of malaria upon the world is likely to continue. Unfortunately, as noted by White:

> There is little pharmaceutical industry interest in developing new antimalarial drugs; the risks are great but returns on investment low. Much of the world's malaria occurs in countries with an annual per capita expenditure on health of less than $10. If drug resistance in *P. falciparum* continues to increase at the current rate, malaria may become untreatable in parts of Southeast Asia by the beginning of the next millennium.[2]

Although some protozoan infections of farm animals (e.g., coccidiosis in chickens) have been at times a serious problem in the United States and trichomonal vaginitis in humans is common, malaria and other life-threatening protozoan infections are not. However, the ease of international travel has caused increased awareness of the prevention and treatment of protozoan infections by American physicians and pharmacists. During the Vietnam War, several thousand cases of malaria were reported in the United States, largely attributed to returning servicemen.

The epidemiology, diagnosis, microbiology, medicinal chemistry, and chemotherapy of malaria and other parasitic diseases have been reviewed.[1–10] Current approaches to the prevention and treatment of parasitic infections including malaria, as well as drugs of choice and recommended doses, have been summarized by White.[2]

ETIOLOGY

Malaria in humans is caused by four species of *Plasmodium* protozoa, which, as shown in Figure 9-1, spend half their life cycle in female *Anopheles* mosquitos. (Male *Anopheles* mosquitos do not feed on vertebrate blood.) Several hundred *Anopheles* species are known, many of which are commonly found in the United States. Resistance to DDT, dieldrin, and other insecticides has been reported for an increasing number of *Anopheles* species, making malaria control more difficult. Once infected, the mosquito carries sporozoites for life.

As can be seen in the simplified life cycle illustrated in Figure 9-1, the malaria protozoa undergo several morphologic changes in the human host.

TABLE 9-1

MALARIA AND OTHER COMMON PROTOZOAN INFECTIONS IN HUMANS AND FARM ANIMALS

Disease	*Protozoa*	*Insect Vector*	*Primary Occurrence*	*Clinical Notes*
Malaria	*Plasmodium vivax*, others	Mosquitos	Tropical	High fever and chills, cyclic
African sleeping sickness	*Trypanosoma*[17] *rhodesiense, T gambiense*, others	Tsetse flies	Tropical Africa	*T. rhodesiense* infection usually causes death before CNS depression, but this is commonly seen with *T. gambiense*
Chagas' disease	*T. cruzi*	Common "bedbug"	South America	Bug usually bites victim close to mouth, so is called the "kissing bug." Protozoa invade many tissues, including the heart; disfiguring edema
Leishmaniasis (kala-azar)	*Leishmania donovani*, others	Sandfly	Middle East, tropical Africa, tropical South America	Progressive wasting and anemia, severely enlarged spleen and liver
Amebiasis (amebic dysentery)	*Entamoeba histolytica, E. vaginalis*	None—transmitted by human and animal wastes	Tropical regions	
Trichomonal vaginitis	*Trichomonas vaginalis*	None—usually transmitted sexually	Worldwide	Can be serious in women and men (see discussion by Kreier)[19]
Coccidiosis in farm animals	*Eimeria* sp.	None—usually by animal wastes	Worldwide	Great economic losses even in United States
Toxoplasmosis	*Toxoplasma* sp.	None—usually by contact with infected cats	Worldwide	
Babesiasis in cattle	*Babesia* sp.	Ticks	Worldwide	

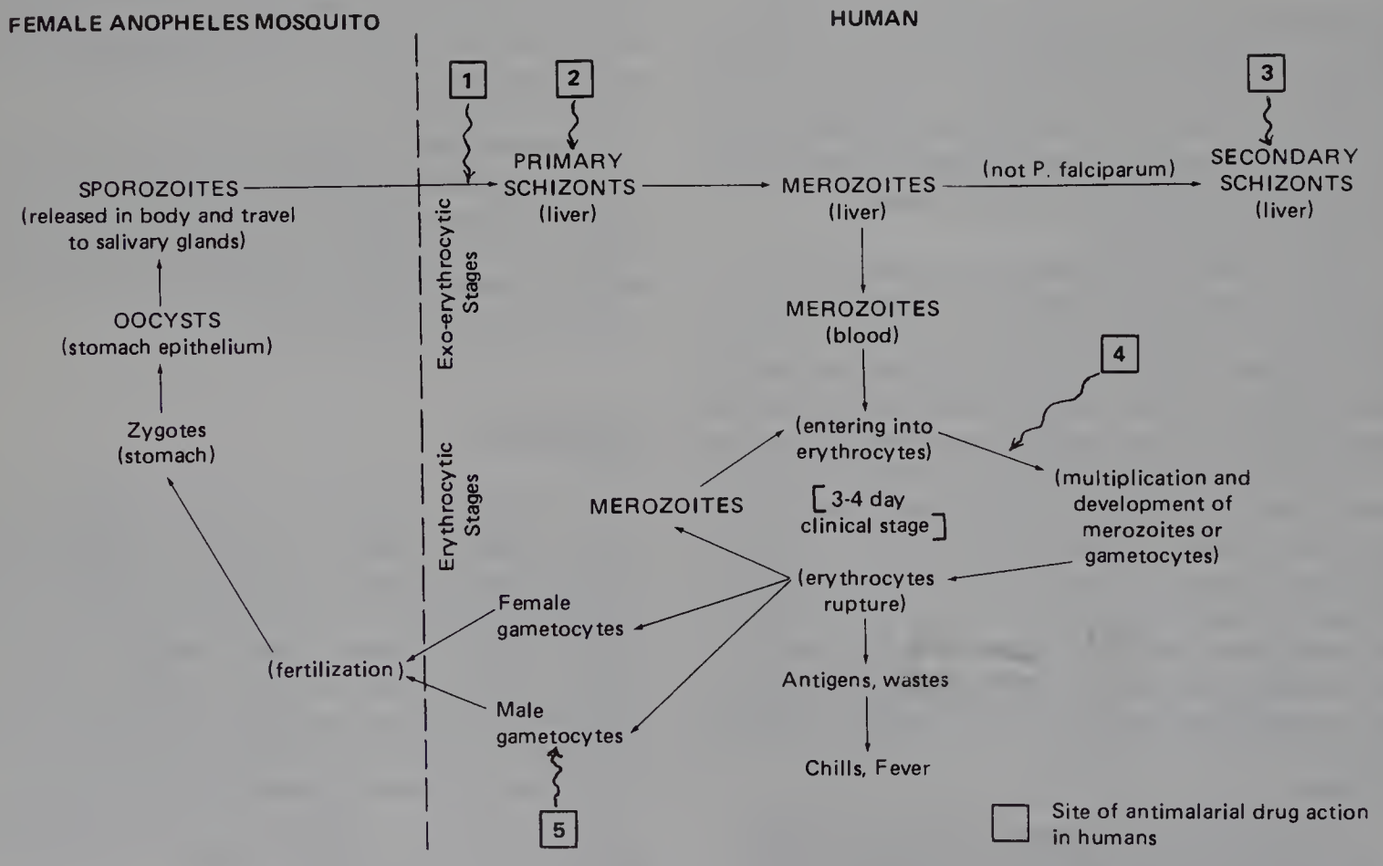
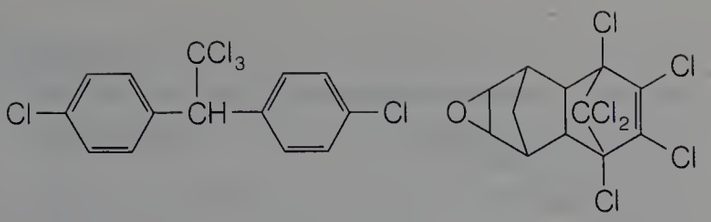


FIG. 9-1.



DDT Dieldrin

The rates at which these changes take place vary among the *Plasmodium* species. The patient generally has no adverse symptoms until erythrocytes rupture (generally 1.5 to 2 weeks after the initial bite), releasing antigenic cell residues and protozoan wastes, which leads to (1) recurring (every 3 to 4 days) attacks of nausea, vomiting, severe chills, delirium, and high fevers (38° to 40°C); (2) severe anemia (and thrombocytopenia) from hemolysis of erythrocytes as more and more merozoites are produced; and (3) jaundice from excess bilirubin (a metabolite of hemoglobin) production.

The four *Plasmodium* species are:

P. falciparum ("Malignant Tertian, Subtertian")

Of the four, only *P. falciparum* does not have a secondary schizont (secondary exoerythrocytic) stage. However, it is the most lethal. Enormously high concentrations of the protozoa in the host's blood are often found, with more than 65% of the erythrocytes affected in some cases. Unlike malaria from the other three species, the patient may feel quite ill between acute attacks. Because all of the merozoites are released from the primary schizont stage at the same time, reinfection from a secondary schizont stage is not a problem. About half of all malaria is caused by this species.

P. vivax ("Benign Tertian Malaria")

This form of malaria is called tertian because clinical symptoms usually recur every 48 hours (i.e., if the patient is sick on day 1, sickness will occur again on day 3, the *tertiary* day). Not as many merozoites are produced as with *P. falciparum*, but many reenter new liver cells to form secondary schizonts, which can cause relapses for several years. Drugs specific for this stage (site 3) must be given for the patient to be truly cured. Only young erythrocytes are attacked, thus limiting the total erythrocytic involvement. About 40% of all malaria is caused by *P. vivax.*

P. malariae ("Quartan Malaria")

This species has a life cycle similar to that of *P. vivax.* Relapses of this "benign" malaria may occur for decades.

P. ovale ("Mild Tertian Malaria")

P. ovale is the least common of the four types of malaria and, similar to *P. vivax* and *P. malariae*, has a long-lasting secondary schizont stage. It also is considered one of the three "benign" forms of malaria.

BIOCHEMICAL DEPENDENCE ON HOST ERYTHROCYTES

Malaria protozoa are very species-specific, some infecting only birds and others only humans. Only *Anopheles* mosquitos harbor the protozoa in half the life cycle; none of the several thousand other mosquitos of other genera do so. This specificity reflects specialized biochemical dependence upon vertebrate host and vector host. The biochemistry of this specialized metabolic and biochemical dependence has been studied intensively, especially with the goal of discovering biochemical (drug-attackable) vulnerability of the malaria protozoa. Nevertheless, many questions remain.

Most research has focused on the biochemical dependence of malaria protozoa on erythrocytes. The reasons are primarily that parasite–erythrocyte biochemistry can be studied with fewer technical problems than with other tissues, and drug treatment and study at this stage are direct (it is easy to get drugs into blood, to obtain blood samples, etc.).

Malaria protozoa need host erythrocytes to make (replicate) their own DNA and RNA, needed for the rapid production of numerous merozoites. The malaria parasites can, and must, synthesize their own pyrimidines (cytosine, uracil, thymine) because they cannot use pyrimidines of the host. However, the protozoa cannot synthesize purines (adenine and guanine) and must obtain them from erythrocytes of the host. Phosphate is also obtained from the host.

Host hemoglobin and plasma are digested by proteolytic enzymes of the malaria protozoa and used as sources of several amino acids. Up to 75% of hemoglobin of infected erythrocytes is digested, with *haemozoin* (an insoluble pigment) formed as a by-product. The protozoa can also synthesize some amino acids of their own.

It appears that the malaria protozoa may be dependent upon the host as a source of pentoses (for DNA and RNA synthesis). There remains disagreement on whether or not these protozoa have an operable pentose phosphate pathway.

Malaria protozoa cannot synthesize their own cholesterol and fatty acids, needed for cell membrane and glyceride biosynthesis. In addition, they require most of the same vitamins and coenzymes needed by their hosts. Similar to many bacteria, they must synthesize their own folic acid (FA). Consequently, drugs such as sulfonamides that block FA biosynthesis, can also block malaria protozoan growth.

Large amounts of glucose are used by these protozoa, particularly during the erythrocytic stage. Hypoglycemia can result in some patients, requiring treatment with parenteral glucose in critical cases.

The roles of sulfonamides and related drugs in blocking FA biosynthesis are discussed in Chapter 8. The 1990 review "Dihydrofolate reductase as a therapeutic target" by Bertino and co-workers[7] is recommended for further reading.

HISTORY

EARLY CINCHONA USE

The general antifebrile properties of the bark of the cinchona undoubtedly were known to the Incas before the arrival of

the Spaniards early in the 16th century. However, it was probably the observations of early Jesuit missionaries that led to the discovery that infusions of cinchona bark were effective for the treatment of the tertian "ague," which was common in tropical Central and South America even then, and to the introduction of the crude drug into Western Europe. The first recorded use in South America was about 1630 and in Europe 1639. Thus began the first era in the chemotherapy of malaria. Cinchona and the purified alkaloids obtained from it were to remain the only drugs of significance in the treatment of malaria for three centuries.

PAUL EHRLICH AND THE FIRST SYNTHETIC ANTIMALARIALS

Much has been written about Paul Ehrlich (1854–1915), who can be considered the founder of modern medicinal chemistry, chemotherapy, and molecular pharmacology.[20–23] (Films about his life are also available for classroom use.[23]) The Nobel prize for medicine in 1908 (with Elie Metchnikoff [Ilya Mechnikov]), however, was for his research on immunology and the development of diphtheria and other antitoxins.

In the late 1800s, the German dye industry was producing hundreds of new dyes. Ehrlich's first work on the biologic properties of these dyes was on blood cells, demonstrating the specific staining of parts of certain leukocytes, the basis of modern hematology. Could pathogenic microorganisms also be stained specifically and perhaps killed without harm to the host (the "magic bullet" concept)? Ehrlich's laboratory set about finding out, focusing first on malaria protozoa using methylene blue and related dyes. (Methylene blue, synthesized by Hoechst in 1885, was known to stain nerve tissues selectively without toxic effect.) For 5 years, Ehrlich and his associates studied hundreds of dyes, many of which they synthesized, leading to the first synthetic antimalarial, trypan red (Fig. 9-2) in 1904. However, trypan red did not have the antimalarial potency needed for an effective human

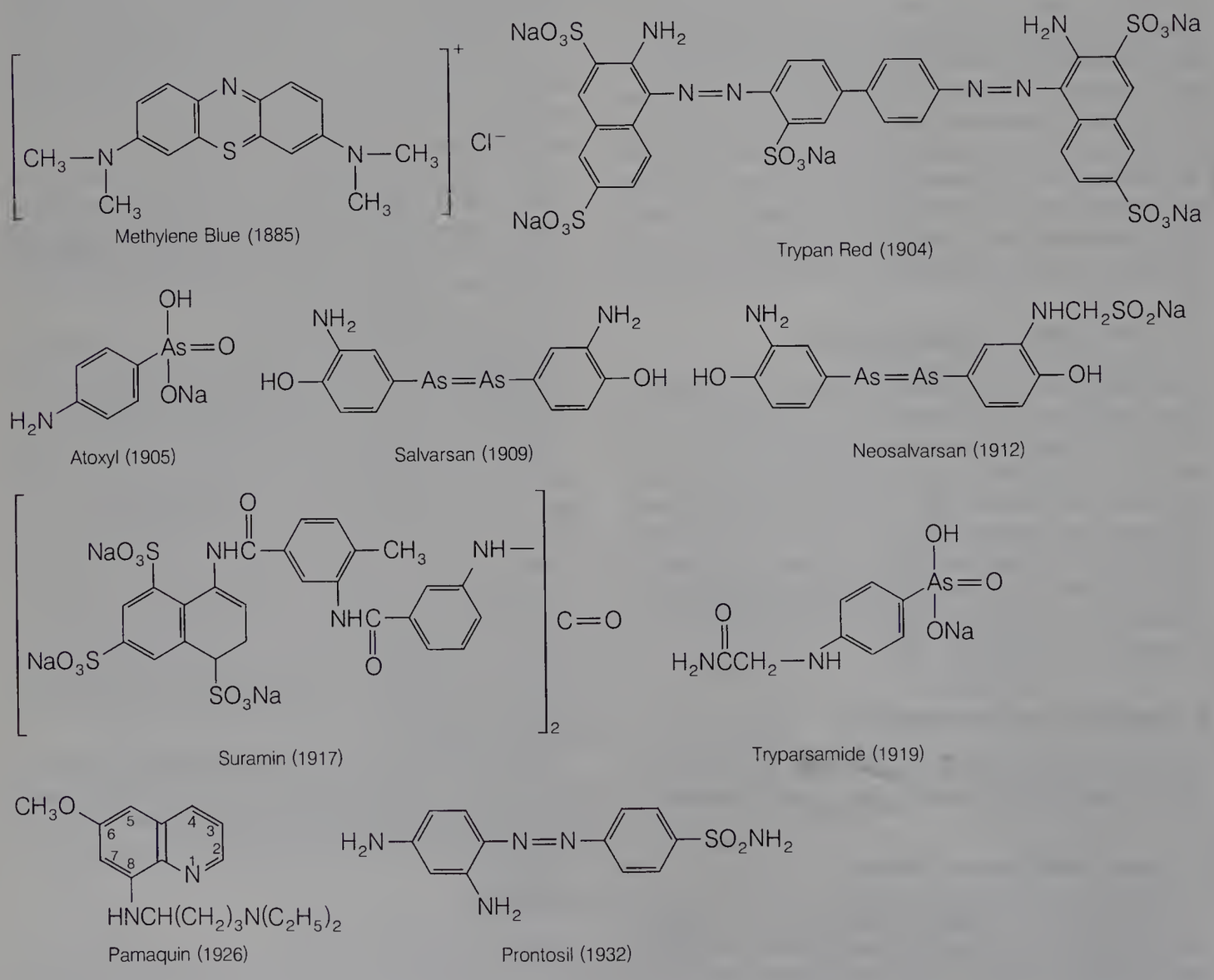


FIG. 9-2.

TABLE 9-2

DRUGS OF CHOICE FOR HUMAN MALARIA*

Malaria Species	*Therapeutic Goal*	*Drug of Choice*	*Alternative*
AREAS NOT KNOWN TO HAVE CHLOROQUINONE-RESISTANT *P. FALCIPARUM*			
All	Suppression while visiting endemic area (begin 1 wk before visit, continue for 4 wk after leaving)†	Chloroquine phosphate once a week	Hydroxychloroquine sulfate
P. vivax, P. ovale, P. malariae†	Prevention of relapses	Primaquine phosphate§	
P. vivax, P. ovale, P. malariae	Elimination of secondary schizonts after leaving endemic area (take for 2 wk—the last 2 wk of chloroquine therapy above)‖	Primaquine phosphate§	
All	Treatment of uncomplicated attack (clinical stage)	Chloroquine phosphate	Hydroxychloroquine sulfate
All	Parenteral treatment of severe attack when patient cannot take an oral dose	Quinine dihydrochloride	Chloroquine hydrochloride
AREAS WITH CHLOROQUINONE-RESISTANT *P. FALCIPARUM*			
P. falciparum	Suppression while visiting endemic area†,**	Mefloquine**	Doxycycline#
P. falciparum	Treatment of uncomplicated attack (clinical stage)	Quinine sulfate and pyrimethamine or sulfadiazine	Mefloquine
P. falciparum	Parenteral treatment of severe illness when patient cannot take an oral dose	Quinine dihydrochloride	

* Modified after references 2 and 6.
† Avoiding mosquito bites is the most important first line of prevention. Insect repellents containing at least 30% diethyltoluamide should be used, as well as mosquito netting at night.
‡ *P. falciparum* does not have secondary schizonts; hence, unless the patient is reinfected by a mosquito bite, relapses do not occur.
§ Primaquine phosphate may cause hemolytic anemias in persons who are glucose-6-phosphate dehydrogenase (G6PD)-deficient. Patients should be tested for this deficiency before primaquine is prescribed.
‖ Because of hemolytic anemia in patients who are G6PD-deficient, some physicians do not recommend primaquine. Rather, they monitor their patients for signs of relapse and then treat accordingly.
Doxycycline should not be taken by pregnant women, or children less than 8 years old.
** See references 4 and 6 for a description of which geographic areas have a risk for chloroquine resistant malaria.
†† Chloroguanide (proguanil) is taken during exposure and 4 weeks afterward.
‡‡ Use of doxycycline is investigational, and, as with all tetracyclines, this drug should not be taken by young children or pregnant women.

cure. Perhaps a potent poison, such as arsenic, could be combined with an effective protozoan stain to carry the poison selectively to the malaria protozoa: one part of the drug for good affinity, one part of the drug for good intrinsic activity. Ehrlich had simultaneously conceived the basis of modern drug design and chemotherapy of the live receptor (see the discussion by Albert[22]).

Atoxyl was Ehrlich's first antimalarial success using the concept, but it was still too neurotoxic to the host. In 1906, he began focusing on spirochetes, specifically on *Treponema pallidum*, identified in 1905 as the cause of syphilis. His organoarsenicals seemed to be as toxic to these spirochetes as to malaria protozoa, and syphilis was a far greater cause of death in Europe than was malaria. In 1909, his #606 (marketed in 1910 as Salvarsan) was the world's first successful antisyphilitic drug. Ehrlich's Neosalvarsan, with greater water solubility (permitting larger intravenous doses) and less toxicity, followed in 1912. In the subsequent 5 years, the incidence of syphilis in Europe dropped by 50% to 80% from its pre-1910 levels.

Erhlich's early success with drug design and chemotherapy with organic dyes and organoarsenicals prompted an explosion of research in drug design and chemotherapy: suramin in 1917 (still a drug of choice[7] for African sleeping sickness), the antimalarial pamaquin in 1926 (Table 9-2), and the first sulfonamide antimalarial–antibacterial, Prontosil (sulfamidochrysoidine), in 1932. The development of other clinically useful organoarsenical and antimonial antiprotozoan agents is also a direct outcome of Ehrlich's drug design success with arsphenamine (Salvarsan).

STIMULATION OF ANTIMALARIAL RESEARCH BY WAR

Following the pioneering work of Ehrlich and his immediate successors in antimalarial drug design, the necessities of war provided the greatest stimulus to the development of new antimalarials. During 1941 to 1946, for example, more than 15,000 substances were synthesized and screened as possible

antimalarial agents by the United States, Australia, and Great Britain. Activity increased again during the Vietnam War, especially because of the increasing problem of resistance to commonly used antimalarials. During the decade 1968 to 1978, more than 250,000 compounds were investigated as part of a U.S. Army search program.[24]

MODERN MALARIA CHEMOTHERAPY: AN OVERVIEW

Most drugs used in modern malaria chemotherapy (Table 9-2)—chloroquine, mefloquine, amodiaquine, pyrimethamine, quinine, and sulfonamides—act primarily at the erythrocytic stage in the malaria life cycle (Fig. 9-1). Because the severe and life-threatening clinical symptoms of malaria occur at this stage, these drugs are very useful in treating and preventing clinical symptoms of all four human malarias. Unfortunately, drug resistant strains have become an extremely serious problem. *P. falciparum* is now virtually untreatable with chlorquine in most parts of the world.[5] Three species, *P. vivax, P. malariae*, and *P. ovale*, have a ''secondary exoerythrocytic'' (secondary schizont) stage that can periodically release new merozoites for years or decades. An additional drug that is effective at this stage—usually primaquine—is prescribed by many physicians when the patient leaves the endemic area, finally resulting in a cure for these three species (Table 9-2). Unfortunately, primaquine can cause hemolytic anemia in patients who are glucose-6-phosphate dehydrogenase (G6PD)–deficient.

It obviously would be desirable to have drugs available that would protect humans from initial infection by the mosquito sporozoites (Fig. 9-1). Unfortunately, no drugs are yet available that are effective at this stage in the life cycle.

Primaquine is quite active at the exoerythrocytic primary schizont stage, so it could be used as a prophylactic against all forms of human malaria. However, its toxicity generally precludes its long-term prophylactic use. Pyrimethamine and chloroguanide are not as effective. Primaquine is also effective as a gametocytocide.

A complicating factor in modern malaria chemotherapy is that drug-resistant strains of *Plasmodium* (especially *P. falciparum*) have been reported in many geographic areas, especially chloroquine-resistant *P. falciparum*. Chloroquine-resistant *P. vivax* has been reported in some areas and quinine-resistant *P. falciparum* in others. Similarly, resistance of certain *Anopheles* species to insecticides is a growing problem.

DEVELOPMENT OF MALARIA VACCINES: PROMISING OR A FAILED PROMISE?

In 1988, results were published on human clinical trials of the first vaccine for protection against the schizont–merozoite stages of malaria.[25] Since the early 1980s, there has been a tremendous effort worldwide to design malaria vaccines, largely based on cell surface proteins of sporozoites, merozoites, or schizonts.[25–38] The use of recombinant DNA techniques to determine the structure of these proteins and to direct their syntheses has been the foundation of most of these studies.

Three primary lines of research include

- Development of sporozoite–merozoite vaccines to block clinical stages of the disease
- Development of sporozoite vaccines to stop infection and spread of the disease
- Development of vaccines that inactivate or block specific metabolic steps in the parasite after infecting humans

Since 1984, the advantages of using vaccines to control diseases of the Third World have received significant attention, though there are major obstacles, as highlighted by Kwiatkowski and Marsh in 1997.[39] In view of the difficulties of improving sanitation on a wide scale, and often of controlling insect vectors, vaccines offer major advantages. As Hoffman and co-workers noted in their 1986 study on the feasibility of developing a sporozoite vaccine, ''There is now little hope that malaria, which affects 100 to 300 million persons . . . per year, can be adequately controlled without vaccines.''[35] Stability of vaccine products in areas without refrigeration is another concern. The World Health Organization has expressed concern about the possibility of clinical trials that might not be well planned or developed. Guidelines for clinical trials, therefore, have been drafted.[37]

Most developmental vaccine work with malaria has centered on *P. falciparum* because it is the primary cause of malaria mortality worldwide. In 1984, the first gene coding the major surface proteins for the *P. falciparum* sporozoite was cloned,[38] opening the possibility for a vaccine that would stop the spread of malaria. The general approach was described by Godson in a well-illustrated *Scientific American* article the following year.[13] Oral vaccines developed from radiation-attenuated sporozoites have also been investigated and may be feasible.[31]

Certa and co-workers[27] have reported that the immunity developed as a result of injection of purified proteins of irradiated merozoites and schizonts may result from antibodies to the malaria parasite's aldolase. (Aldolase catalyzes the reaction of fructose-1,6-diphosphate to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate.) As highlighted by Cox,

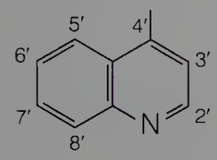
> The importance of this enzyme is that the blood stages of the malaria parasite lack a citric acid cycle, and so use vast amounts of glucose. . . . The inhibition of aldolase activity would therefore totally inhibit the maturation of the parasite and subsequent invasion of fresh blood cells. . . . There is [therefore] a real possibility that aldolase could be a basis for a vaccine against many, if not all types of malaria.[26]

Research on malaria vaccines continues, but as summarized in *Science* in early 1990 (''Malaria Vaccines: The Failed Promise''[29]), the challenges are still considerable:

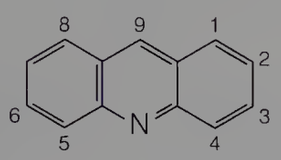
The parasite has shown a surprising immunologic variability, and vaccine strategies that once seemed straightforward have proven frustratingly ineffective in recent years . . . . The sporozoite has acquired the capacity to change its CS (coating) protein in a myriad of ways . . . . The merozoite is [also] extremely variable immunologically . . . . A vaccine remains the likeliest way of preventing entire populations from having a severe attack of malaria. When might such a boon arrive? After the frustrated hopes of the mid-1980's, no one is willing to hazard a guess.

QUINOLINES AND ANALOGUES

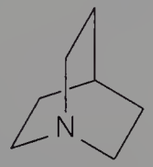
All of the antimalarial agents in Table 9-3 have one common structural feature—a quinoline ring, or "a quinoline with an additional benzene added" (an *acridine ring*). Some, but not all, have quinine's CH_3O group on the quinoline ring. None except the cinchona alkaloids has a quinuclidine ring.



Quinoline

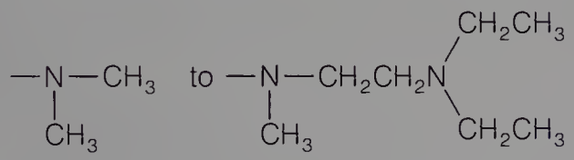


Acridine



Quinuclidine

Quinine's structure (Table 9-3) has been known since 1908[40] (and proved by total synthesis by Woodward and Doering in 1945[41]). It was the structural model for all of the other quinoline antimalarials shown in Table 9-3, along with the methylene blue–trypan red structures. Changing methylene blue's two amino groups from



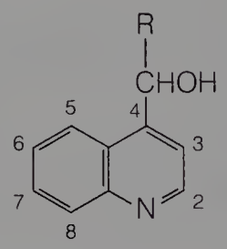
improved antimalarial activity.[16] The approach used by Schulemann and co-workers in developing pamaquine in 1926[42] (see Fig. 9-2 and Table 9-3) was to synthesize combinations of the 6-methoxy quinoline moiety of quinine with variations of the "improved methylene blue side group" shown above. Later followed the development of all of the other 8-aminoquinoline antimalarials in Table 9-3.

The same general approach with the acridine structure (since methylene blue has three rings and acridine is a quinoline analogue) led to the less toxic quinacrine (Table 9-3) in 1932.[43,44] Including a chlorine atom in the structure was entirely fortuitous, but quinacrine's good activity stimulated the synthesis of many chloroquinolines in the decades that followed. Many other 9-aminoacridines were synthesized in later years, but none was superior to quinacrine.

The next logical step in drug design, taken by German chemists, was to "divide" the quinacrine structure into its two 4-aminoquinoline "parts," one of which is chloroquine itself.

Although chloroquine was active, it was considered too toxic, and many other 7-chloro-4-aminoquinolines (Table 9-3), such as chloroquine, were investigated by the Germans in the late 1930s. Sontoquine (Table 9-3) was actually used by German soldiers during World War II. After samples of sontoquine were captured by the Allied forces[45] more than 200 7-chloro-4-aminoquinolines were synthesized in the United States. Hundreds of 9-aminoacridine analogues were also synthesized.

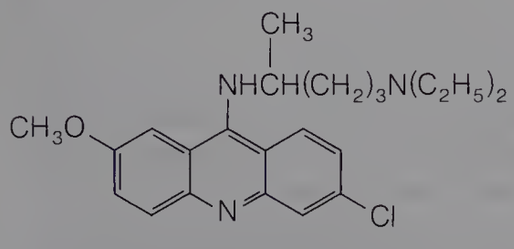
Another drug design approach, begun in the late 1930s, was to make derivatives of 4-quinoline-methanol, the central part of the quinine structure:



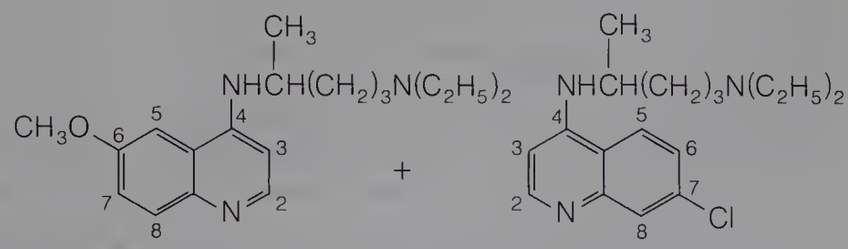
Many antimalarials of this structure have been synthesized, the most promising being mefloquine (Lariam), approved by the FDA in 1989.[2] Mefloquine is discussed later in this chapter.

STRUCTURE–ACTIVITY RELATIONSHIPS

Quinine has four asymmetric centers—C-8, C-9, C-3, and C-4. Quinidine is the diastereomer formed by "inverting" C-8 and C-9. (Inversion is used only in the discussion sense. The biosyntheses of the cinchona alkaloids have been elucidated elegantly by Leete.[46] Cinchonidine is the desmethoxy derivative of quinine and cinchonine of quinidine. All four of these naturally occurring cinchona alkaloids are active



Quinacrine

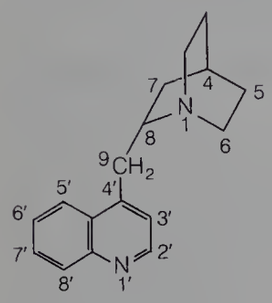


(2)
Chloroquine

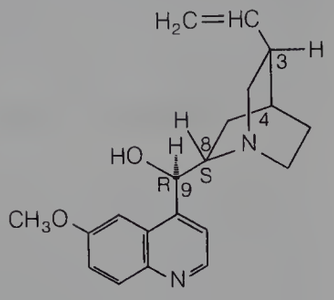
TABLE 9-3

QUINOLINES AND ANALOGUES

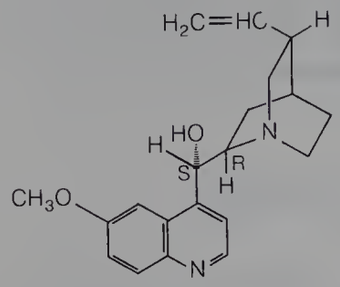
CINCHONA ALKALOIDS AND OTHER 4-QUINOLINEMETHANOLS



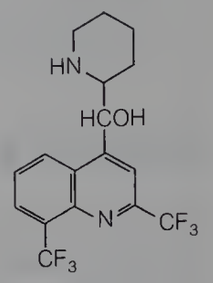
Rubane



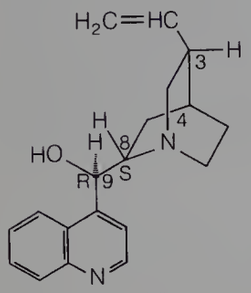
Quinine



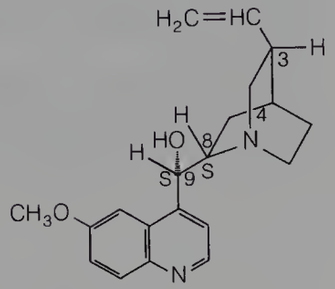
Quinidine



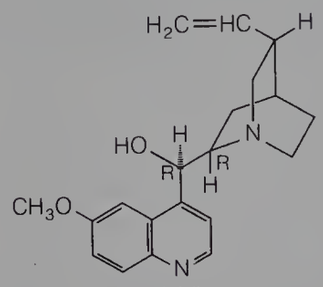
Mefloquine



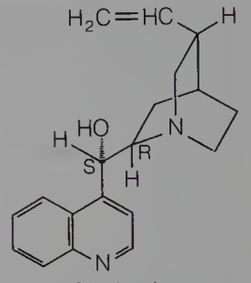
Cinchonidine



Epiquinine

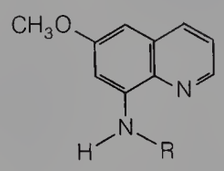


Epiquinidine



Cinchonine

8-AMINOQUINOLINES



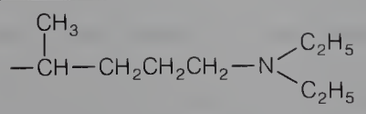
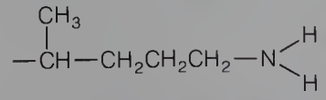
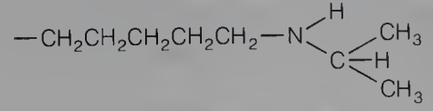
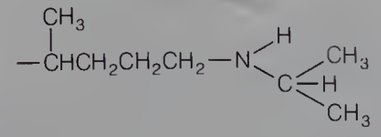
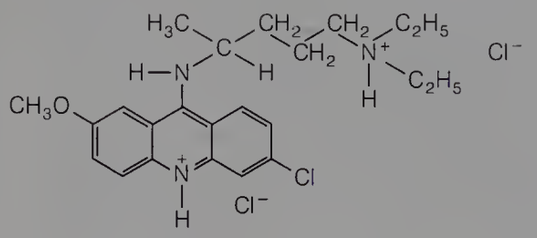
Compound	*R*
Pamaquine	
Primaquine	
Pentaquine	
Isopentaquine	

TABLE 9-3 *Continued*

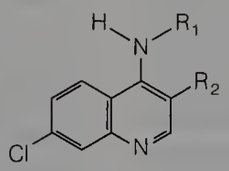
QUINOLNES AND ANALOGUES

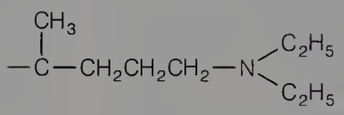
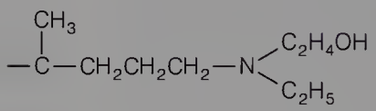
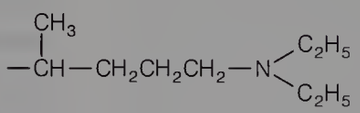
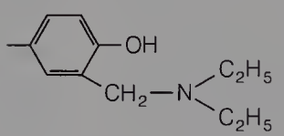
9-AMINOACRIDINES

Quinacrine Hydrochloride



7-CHLORO-4-AMINOQUINOLINES



Compound	R_1	R_2
Chloroquine		H
Hydroxychloroquine		H
Sontoquine		CH_3
Amodiaquine		H

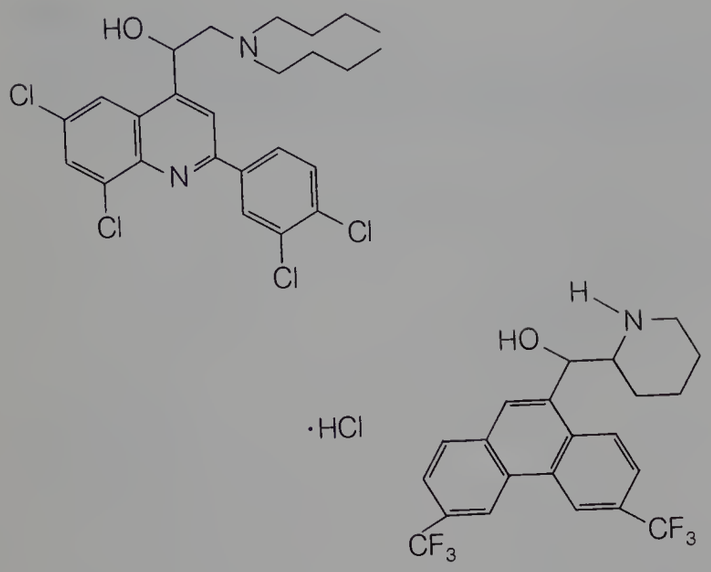
antimalarials (activity varying depending upon the malarial species used in biologic testing). Thus, the 6-CH_3O group is not essential for activity, as illustrated also by mefloquine and the 7-chloro-4-aminoquinolines (Table 9-3).

Quinine is found in highest concentration in cinchona bark (about 5%; quinidine, 0.1%; conchonine, 0.3%; cinchonidine, 0.4%) and is the commercially used antimalarial of the group. Quinidine's cardiac effects (see Chap. 19) also preclude its use as an antimalarial. Inversion of only C-9 gives epiquinine and epiquinidine, both of which are inactive.

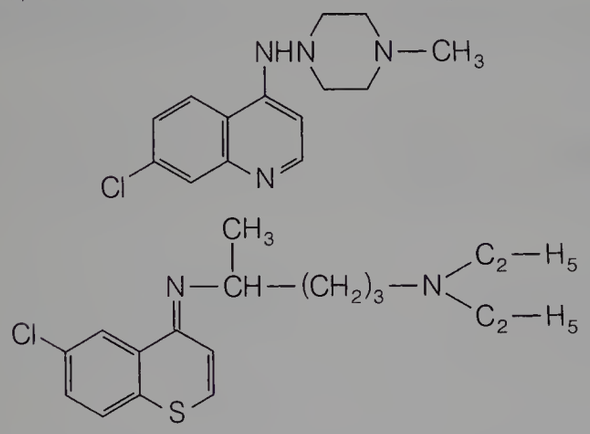
The good antimalarial activity of the 8-aminoquinolines, quinacrine, and the 7-chloro-4-aminoquinolines shows that the CH_2OH (amino alcohol) of the cinchona alkaloids is not essential for activity. Similarly, a chlorine atom on the quinoline ring is not a necessity, but it substantially increases activity for some analogues.

What are the exact structural requirements for good antimalarial activity of the quinoline–acridine analogue group? Although thousands of quinoline analogues have been synthesized in the more than 50 years since the development of pamaquine in 1926, the question cannot yet be answered satisfactorily. Major progress in the development of a quantitative structural model was reported by Kim and co-workers in 1979.[47] The difficulty is compounded because a variety of different malaria protozoan species—many species that do not attack humans—have been used for evaluation of new antimalarials. Furthermore, as discussed in the next section, some quinoline analogues' antimalarial action may not involve a specific receptor.

It is also noteworthy that other aromatic ring systems, besides quinoline and acridine, have been used to make active antimalarials, for example:



Intensive investigations of quinolines and acridines have revealed useful structure–activity guidelines. The quinoline ring system is more active for a given set of substituents than is the acridine ring system. The dialkylaminoalkylamino $-N(CH_2)_nNR_2$ side groups seen throughout compounds in Table 9-3 provide maximal activity, particularly when n = 2 to 5 or 6. However, as illustrated with the active analogues below, considerable structural variation is possible:



A chlorine group at C-7 generally provides maximal activity (and toxicity) with chloro-4-aminoquinolines, but some C-6-chloro analogues are equally active. Other relationships have also been found (e.g., with 8-aminoquinolines); 6-CH_3O analogues are more active (and toxic) than 2- or 4-CH_3O analogues.

MECHANISMS OF ACTION

Interest in delineating the mechanism of action of the quinoline antimalarials has intensified as the problem of chloroquine resistance has increased worldwide.[1–6] Reviews by Ward[8] and Peters[5] are recommended for further reading. A wide variety of possible mechanisms has been explored and many discounted, from intercalation of the aminoquinoline into plasmodial DNA to direct inhibition of protein synthesis.

Two models that have attracted considerable attention are

- **Binding of chloroquine to ferriprotoporphyrin IX (FPIX):** FPIX can cause erythrocytes and malaria protozoan cells to lyse, and it can bind to the aminoquinolines with good affinity. However, even after binding to aminoquinolines in vitro, it can still lyse cells. This finding and related studies suggest that this probably, by itself, is not the operative mechanism of action.
- **Chloroquine (pK_a 8.1, 9.9) gets trapped in the malaria lysosome (*p*H 4.8 to 5.2), raises *p*H, and thereby inactivates hemoglobin-digesting enzymes:** At the acidic *p*H of the lysosome, chloroquine would exist as the salt and, as such, might not pass easily through the lysosomal membrane. The *p*H of the lysozyme would increase, and *p*H-sensitive proteolytic enzymes would not be able to function efficiently. Additionally, it has been suggested that the trapped chloroquine may inactivate the lysosomal proton pump. In fact, about 1,000 times more chloroquine accumulates in lysozymes than could be accounted for by *p*H or pK_a considerations alone; therefore, other chloroquine-concentrating mechanisms must exist. FPIX binding may be an additional component.

Other investigators have proposed that a cytoplasmic proton carrier or permease is inactivated by chloroquine.[6,16]

MECHANISMS OF RESISTANCE

Resistant strains of malaria protozoa accumulate less chloroquine than do chloroquine-sensitive strains, even though the rate of chloroquine uptake is similar.[2,8,9] Work by Van Dyke and Ye[48–50] has shown that drug-resistant malaria protozoa contain increased amounts of a membrane protein that appears to pump drugs out of the protozoa. It seems that the protein may be very similar to the P-glycoprotein that causes multidrug resistance in cancer cells.[51] Verapamil, in addition to being a calcium channel blocker, can reverse drug resistance in malaria, apparently by blocking the P-glycoprotein pump. However, the large doses of verapamil required to reverse the drug resistance of malaria also result in cardiac arrhythmias. A search for selective blockers for the proposed drug efflux pump has led to the intensive study of tetrandrine, a natural product of *Stephania tetrandra*, a plant used for centuries in Chinese herbal medicine in treating a variety of illnesses.

It has also been proposed that resistant strains may metabolize chloroquine at a faster rate, probably related to increased activity of cytochrome P-450.

CINCHONA ALKALOIDS

ABSORPTION, DISTRIBUTION, AND EXCRETION

After oral administration, the cinchona alkaloids are absorbed rapidly and nearly completely, with peak blood level

concentrations occurring in 1 to 4 hr. About 70% is protein-bound. Blood levels fall off very quickly after administration is stopped. A single dose of quinine is disposed of in about 24 hr. Various tissues contain enzymes capable of metabolizing the cinchona alkaloids, but the principal action apparently takes place in the liver, in which an oxidative process results in the addition of a hydroxyl group to the 2′-position of the quinoline ring. The resulting degradation products, called *carbostyrils*, are much less toxic, are eliminated more rapidly, and possess lower antimalarial activity than the parent compounds. The carbostyrils may be oxidized further to dihydroxy compounds. Excretion is mainly in the urine.

TOXICITY

Toxic reactions to the cinchona alkaloids have been studied extensively. These drugs are not as effective as chloroquine and are more toxic; therefore, they are not generally used, except as noted in Table 9-2 (and in some poor countries). Acute poisoning with quinine is not common. In one case, a death was reported after administration of 18 g; in another case, the patient recovered after administration of 19.8 g. A fatality resulted after the intravenous administration of 1 g of quinine. The most common toxic manifestations are due to hypersensitivity to the alkaloids and are referred to collectively as ''cinchonism.'' Frequent effects are allergic skin reactions, tinnitus, slight deafness, vertigo, and slight mental depression. The most serious is amblyopia, which may follow administration of very large doses of quinine but is not common; usual therapeutic regimens do not produce this effect.

OTHER ROUTES OF ADMINISTRATION AND DOSAGE FORMS

In addition to antimalarial action, cinchona alkaloids are antipyretic. The action of quinine on the central temperature-regulating mechanism causes peripheral vasodilation. This effect accounts for the traditional use of quinine in cold remedies and fever treatments. Quinine has been used as a diagnostic agent for myasthenia gravis (by accentuating the symptoms). Also, it has been used for the treatment of night cramps or ''restless legs.'' The antifibrillating effect of quinidine in the treatment of cardiac arrhythmias is discussed in Chapter 19.

The antimalarial action of cinchona alkaloids may be obtained by oral, intravenous, or intramuscular administration. Administration by injection, particularly intravenous injection, is not without hazard and should be used cautiously. For intramuscular injection, quinine dihydrochloride is usually used.

Crude extract preparations containing the alkaloids of cinchona have been used widely as economical antimalarials for oral administration. During World War II, a mixture known as quinetum, containing a large amount of quinine, was used. As the interest in pure quinine increased, another crude mixture (''cinchona fibrifuge''), composed of the alkaloids remaining after quinine removal, was introduced to replace quinetum. Subsequently, the Malaria Commission of the League of Nations introduced totaquine, containing 7% to 12% of anhydrous crystallizable cinchona alkaloids. Totaquine is now the most widely used of inexpensive antimalarial drugs. The usual dose is 600 mg.

Quinine

Quinine is obtained from quinine sulfate prepared by extraction from the crude drug. To obtain it from solutions of quinine sulfate, a solution of the sulfate is alkalinized with ammonia or sodium hydroxide. Another method is to pour an aqueous solution of quinine bisulfate into excess ammonia water, with stirring. In either procedure, the precipitated base is washed and recrystallized. The pure alkaloid crystallizes with three molecules of water. It is efflorescent, losing one molecule of water at 20°C under normal conditions and two molecules in a dry atmosphere. All water is removed at 100°C.

Quinine occurs as a levorotatory, odorless, white crystalline powder, possessing an intensely bitter taste. It is only slightly soluble in water (1 : 1,500), but it is quite soluble in alcohol (1 : 1), chloroform (1 : 1), or ether.

Quinine behaves as a diacidic base and forms salts readily. These may be of two types, the *acid*, or *bi-* salts and the *neutral* salts. Neutral salts are formed by involvement of only the tertiary nitrogen in the quinuclidine nucleus, and acid salts are the result of involvement of both basic nitrogens. Inasmuch as the quinoline nitrogen is very much less basic than the quinuclidine nitrogen, involvement of both nitrogens results in a definitely acidic compound.

PRODUCTS

Quinine Sulfate, USP

6′-Methoxycinchonan-9-ol sulfate dihydrate; quininium sulfate. Quinine sulfate is the usual salt of quinine and is ordinarily the ''quinine'' asked for by the layman.

It is prepared in one of two ways: from the crude bark or from the free base. When prepared from the crude bark, the powdered cinchona is alkalinized and then extracted with a hot, high-boiling petroleum fraction to remove the alkaloids. By carefully adding diluted sulfuric acid to the extract, the alkaloids are converted to sulfates, the sulfate of quinine crystallizing out first. The crude alkaloidal sulfate is decolorized and recrystallized to obtain the article of commerce. Commercial quinine sulfate is not pure but contains from 2% to 3% of impurities, which consist mainly of hydroqui-

nine and cinchonidine. To obtain quinine sulfate from the free base, it is neutralized with dilute sulfuric acid. The resulting sulfate, when recrystallized from hot water, forms masses of crystals with the approximate formula $(C_{20}H_{24}O_2N_2)_2 \cdot H_2SO_4 \cdot 8H_2O$. This compound readily effloresces in dry air to the official dihydrate, which occurs as fine, white needles of a somewhat bulky nature.

Quinine sulfate is often prescribed in liquid mixtures. From a taste standpoint, it is better to suspend the salt rather than to dissolve it. However, in the event that a solution is desired, it may be accomplished by the use of alcohol or, more commonly, by addition of a small amount of sulfuric acid to convert it to the more soluble bisulfate. The capsule form of administration is the most satisfactory for masking the taste of quinine when it is to be administered orally.

The sulfate salts of cinchonidine and cinchonine may be used as antimalarials. The dextrorotatory cinchonine salt is of value in the treatment of patients who display a sensitivity to the levorotatory cinchona alkaloids.

7-CHLORO-4-AMINOQUINOLINES

ABSORPTION, DISTRIBUTION, AND EXCRETION

Chloroquine is absorbed readily from the gastrointestinal tract, but amodiaquin gives lower plasma levels than others in the group. Peak plasma concentrations are reached in 1 to 3 hr, with blood levels falling off rather rapidly after administration is stopped. About half the drug in the plasma is protein-bound. These drugs tend to concentrate in the liver, the spleen, the heart, the kidneys, and the brain. The half-life of chloroquine is about 3 days from a single dose and 1 week or more following daily dosage for 2 weeks. Small amounts of 4-aminoquinolines have been found in the skin but probably not in sufficient quantity to account for their suppressant action on polymorphous light dermatoses. These compounds are excreted rapidly, most of the unmetabolized drug being accounted for in the urine.

TOXICITY

The toxicity of the 4-aminoquinolines is quite low in the usual antimalarial regimen. Side effects can include nausea, vomiting, anorexia, abdominal cramps, diarrhea, headache, dizziness, pruritus, and urticaria. Long-term administration in high doses (for uses other than malaria) may have serious effects on the eyes, and ophthalmologic examinations should be carefully carried out. Also, periodic blood examinations should be taken. Patients with liver diseases should be particularly watched when 4-aminoquinolines are used.

OTHER USES, ROUTES OF ADMINISTRATION, AND DOSAGE FORMS

The 4-aminoquinolines, particularly chloroquine and hydroxychloroquine, have been used in the treatment of extraintestinal amebiasis. They are of value in the treatment of chronic discoid lupus erythematosus but are of questionable value in the treatment of the systemic form of the disease. Symptomatic relief has been secured through the use of 4-aminoquinolines in the treatment of rheumatoid arthritis. Although the mechanism for their effect in collagen diseases has not been established, these drugs appear to suppress the formation of antigens that may be responsible for hypersensitivity reactions that cause the symptoms to develop. Long-term therapy of at least 4 to 5 weeks is usually required before beneficial results are obtained in the treatment of collagen diseases.

For the treatment of malaria, these drugs are usually given orally as salts of the amines in tablet form. If nausea or vomiting occurs after oral administration, intramuscular injection may be used. For prophylactic treatment, the drugs may be incorporated into table salt. To protect the drugs from the high humidity of tropical climates, coating of the granules with a combination of cetyl and stearyl alcohols has been employed. These drugs are sometimes combined with other drugs, such as chloroguanide or pyrimethamine, to obtain a broader spectrum of activity (Table 9-2).

PRODUCTS

Chloroquine, USP

N^4-(7-Chloro-4-quinolinyl)-N^1, N^1-diethyl-1,4-pentanediamine; 7-chloro-4-[[4-(diethylamino)-1-methylbutyl]amino]-quinoline; CQ. Chloroquine occurs as a white or slightly yellow crystalline powder that is odorless and has a bitter taste. It is usually partly hydrated, very slightly soluble in water, and soluble in dilute acids, chloroform, and ether.

Chloroquine Phosphate, USP

N^4-(7-Chloro-4-quinolinyl)-N^1, N^1-diethyl-1,4-pentanediamine phosphate (1:2); 7-chloro-4-[[4-(diethylamino)-1-methylbutyl]-amino]quinoline phosphate (Aralen, Resochin). Chloroquine phosphate occurs as a white crystalline powder that is odorless and bitter and slowly discolors on exposure to light. It is freely soluble in water, and aqueous solutions have a *p*H of about 4.5. It is almost insoluble in alcohol, ether, and chloroform. It exists in two polymorphic forms, either of which (or a mixture of both) may be used medicinally.

Hydroxychloroquine Sulfate, USP

2-[[4-[(7-Chloro-4-quinolyl)amino]pentyl]ethylamino]ethanol sulfate (1:1) (Plaquenil sulfate). Hydroxychloroquine sulfate occurs as a white, or nearly white, crystalline powder that is odorless but bitter. It is freely soluble in water, producing solutions with a *p*H of about 4.5. It is practically insoluble in alcohol, ether, and chloroform.

Although successful as an antimalarial, hydroxychloroquine sulfate has achieved greater use than chloroquine in the control and treatment of collagen diseases because it is somewhat less toxic.

Amodiaquine Hydrochloride, USP

4-[(7-Chloro-4-quinolynyl)amino]-2-[(diethylamino)methyl]-phenol dihydrochloride dihydrate; 4-[(7-chloro-4-quinolyl)-amino]-α-(diethylamino)-*o*-cresol dihydrochloride dihydrate (Camoquin hydrochloride). Amodiaquine hydrochloride occurs as a yellow, odorless, bitter crystalline powder. It is soluble in water, sparingly soluble in alcohol, and very slightly soluble in ether, chloroform, and benzene. The *p*H of a 1% solution is between 4 and 4.8. The synthesis of amodiaquine hydrochloride is more expensive than that of chloroquine.

This compound is an economically important antimalarial. Amodiaquine hydrochloride is highly suppressive in *P. vivax* and *P. falciparum* infections, being three to four times as active as quinine. However, it has no curative activity, except against *P. falciparum*. Amodiaquine hydrochloride is altered rapidly in vivo to yield products that appear to be excreted slowly and to have a prolonged suppressive activity.

8-AMINOQUINOLINES

ABSORPTION, DISTRIBUTION, AND EXCRETION

The 8-aminoquinolines are absorbed rapidly from the gastrointestinal tract, to the extent of 85% to 95% within 2 hr after oral administration. Peak plasma concentration is reached within 2 hr after ingestion, after which the drug rapidly disappears from the blood. The drugs are localized mainly in liver, lung, brain, heart, and muscle tissue. Metabolic changes in the drug are produced very rapidly, and on excretion, metabolic products account for nearly all of the drug. Only about 1% of the drug is eliminated unchanged through the urine. It may be that the antiplasmodial and toxic properties of these drugs are produced by metabolic transformation products. To maintain therapeutic blood level concentrations, frequent administration of 8-aminoquinolines may be necessary.

TOXICITY

The toxic effects of the 8-aminoquinolines are principally in the central nervous system and the hematopoietic system. Occasionally, anorexia, abdominal pain, vomiting, and cyanosis may be produced. The toxic effects related to the blood system are more common; hemolytic anemia (particularly in dark-skinned people), leukopenia, and methemoglobinemia are the usual findings. A genetic deficiency of G6PD—found in up to 100 million people[52] but rarely in Caucasians—weakens erythrocytes and makes them more easily damaged by drugs such as the 8-aminoquinolines. Patients are often tested for G6PD deficiency before primaquine is prescribed. Since primaquine is the drug of choice in preventing relapse of *P. vivax, P. ovale*, and *P. malariae* (Table 9-2), some physicians do not recommend it unless signs of relapse actually occur. For most Caucasians, primaquine is quite nontoxic. Toxicity is increased by quinacrine; therefore, the simultaneous use of quinacrine and 8-aminoquinolines must be avoided.

USES, ROUTES OF ADMINISTRATION, AND DOSAGE FORMS

Primaquine is used mainly to prevent relapses caused by the exoerythrocytic forms of the parasites (Table 9-1). Primaquine is usually administered orally, in tablet form, as salts such as hydrochlorides or phosphates. Pamaquine is used as the methylene-bis-β-hydroxynaphthoate (naphthoate or pamoate) because this salt is of low solubility and is absorbed slowly, and thus, blood levels are maintained for longer periods and are more uniform.

PRODUCTS

Primaquine Phosphate, USP

N^4-(6-Methoxy-8-quinolinyl)pentanediamine phosphate; primaquinium phosphate; 8-[(4-amino-1-methylbutyl)-amino]-6-methoxyquinoline phosphate. Primaquine phosphate is an orange-red crystalline substance with a bitter taste. It is soluble in water and insoluble in chloroform and ether. Its aqueous solutions are acid to litmus. It may be noted that it is the primary amine homologue of pamaquine.

Primaquine is the most effective and the best tolerated of the 8-aminoquinolines. Against *P. vivax,* it is four to six times as active an exoerythrocytic schizontocide as pamaquine and about one-half as toxic. When 15 mg of the base are administered daily for 14 days, radical cure is achieved in most *P. vivax* infections. Success has been achieved against some very resistant strains of *P. vivax* by administering 45 mg of the base once a week for 8 weeks, with simultaneous administration of 300 mg of chloroquine base. This

regimen also tends to lessen the toxic hemolytic effects produced in primaquine-sensitive individuals.

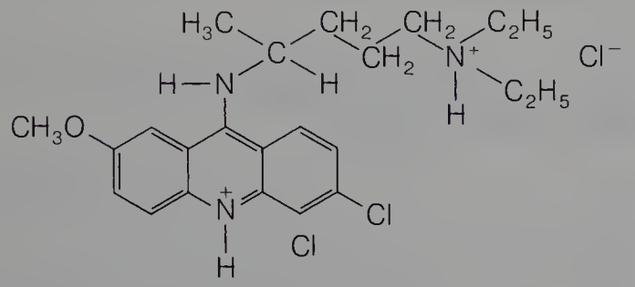
9-AMINOACRIDINES

Quinacrine, the most useful compound in this class, is now rarely used for malaria. A brief description of its use has been consolidated in the following discussion.

PRODUCTS

Quinacrine Hydrochloride, USP

N^4-(6-Chloro-2-methoxy-9-acridinyl)-N^1, N^1-diethylpentanediamine dihydrochloride, dihydrate; 6-chloro-9-[[4(diethylamino)-1-methylbutyl]amino]-2-methoxyacridine dihydrochloride; mepacrine hydrochloride (Atabrine, Atabrin).



Quinacrine Hydrochloride

The wide use of this compound during the early 1940s resulted in many synonyms for quinacrine in various countries throughout the world.

The dihydrochloride salt is a bitter yellow crystalline powder. It is sparingly soluble (1:35) in water and soluble in alcohol. A 1:100 aqueous solution has a *p*H of about 4.5 and shows a fluorescence. Solutions of the dihydrochloride are not stable and should not be stored. A dimethanesulfonate salt produces somewhat more stable solutions, but they too should not be kept for any length of time.

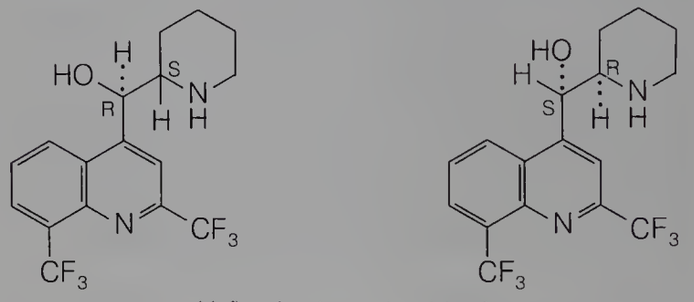
The yellow color that quinacrine imparts to the urine and the skin is temporary and should not be mistaken for jaundice. Quinacrine may produce toxic effects in the central nervous system, such as headaches, epileptiform convulsions, and transient psychoses that may be accompanied by nausea and vomiting. Hematopoietic disturbances, such as aplastic anemia, may occur. Skin reactions and hepatitis are other symptoms of toxicity. Deaths have occurred from exfoliative dermatitis caused by quinacrine.

As an antimalarial, quinacrine acts as an erythrocytic schizontocide in all kinds of human malaria. It has some effectiveness as a gametocytocide in *P. vivax* and *P. malariae* infections. It may be employed in the treatment of blackwater fever when the use of quinine is contraindicated. It is also an effective curative agent for the treatment of giardiasis caused by *Giardia lamblia*, eliminating the parasite from the intestinal tract. It is an important drug for use in the elimination of intestinal cestodes such as *Taenia saginata* (beef tapeworm), *T. solium* (pork tapeworm), and *Hymenolepis nana* (dwarf tapeworm). Like the 4-aminoquinolines, quinacrine may also be used to treat light-sensitive dermatoses such as chronic discoid lupus erythematosus.

MEFLOQUINE

Mefloquine (Lariam),[2,53,54] approved by the FDA in 1989, has become a drug of choice for malaria prophylaxis (250 mg once weekly starting 1 week prior to entry of an endemic area and continuing for 4 weeks after departure), particularly in areas of known chloroquine-resistant strains. It has also been very effective in curing multidrug-resistant *P. falciparum* malaria, apparently at the erythrocytic stage in the life cycle. Single doses (1,250 mg) have cured 90% to 100% of patients. However, resistance to mefloquine has been reported.[2,53,54] Mefloquine is recommended after leaving areas with resistant *P. falciparum* (Table 9-2).

The half-life of mefloquine is 10 to 24 days, which allows for single-dose treatment of malaria. The drug has a high affinity for binding to red cell membranes. It does not intercalate with DNA but does, similar to quinine, interfere with clumping of hemoglobin in the parasite.



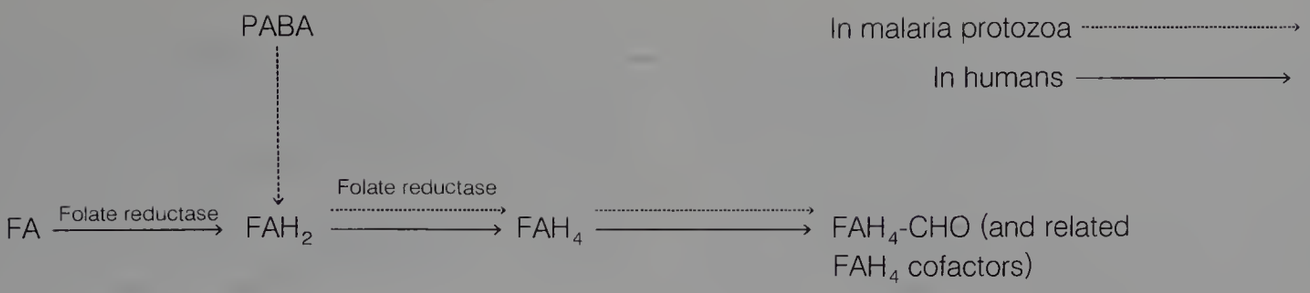
Mefloquine (racemic mixture)

TETRAHYDROFOLATE SYNTHESIS INHIBITORS

USES AND MECHANISMS OF ACTION

The biosyntheses and roles of *p*-aminobenzoic acid (PABA), FA, dihydrofolic acid (FAH_2), tetrahydrofolic acid (FAH_4), and FAH_4 cofactors (such as folinic acid, FAH_4-CHO) have been reviewed by Schweitzer et al.[7] and are described in Chapter 8. The FAH_4 cofactors are essential as one-carbon donors on several biosynthetic pathways, especially the conversion of uridine to thymidine, nucleic acids needed for DNA synthesis. Malaria protozoa cannot convert FA to FAH_4 but can convert FAH_2 to FAH_4.[55]

Ferone and co-workers[55–57] have found that malarial dihydrofolate reductase is structurally different from mammalian dihydrofolate reductase and up to 2,000 times more sensitive



to the antimalarial drugs discussed in this section (much less so with trimethoprim). As discussed earlier in this chapter, the malaria protozoa are unable to use the host's pyrimidine nucleosides (but can use purine nucleosides) and must synthesize their own. Synthesizing thymidine nucleotides requires 5,10-methylenetetrahydrofolate or FAH_4-CHO which may be converted into it. Thus, any drug that can inhibit the malaria protozoa's biosynthesis of FAH_2 or can selectively inhibit the protozoa's dihydrofolate reductase can inhibit the growth of and kill the protozoa.

Several structurally different small molecules have been very effective in competitively inhibiting one of the steps involved in the formation of FAH_4 in malaria protozoa (Fig. 9-3). An examination of the drugs of choice (Table 9-2), however, shows that these drugs are usually reserved for treatment of malaria strains resistant to one or more of the quinoline-type antimalarials. Because the FAH_4 synthesis inhibitors tend to be slow-acting, they are often given in combination with a quinoline antimalarial for treatment of acute clinical attacks. Combinations of FAH_4 synthesis inhibitors are often used, but it should be noted that indiscriminate use may lead to resistant strains (as with any antimalarial) of malaria, as well as resistant bacterial strains.

The biguanides, diaminopyrimidines, and dihydrotriazines are selective inhibitors of malaria protozoa dihydrofolate reductase. The sulfonamides and sulfones apparently competitively block the incorporation of PABA into the malaria protozoans' dihydropteroic acid (FAH_2), the same mechanism as with bacteria.

As shown in the malaria life cycle (Fig. 9-1), pyrimethamine and chloroguanide are effective against both the primary (exoerythrocytic) schizont (site 2) and erythrocytic (site 4) stages. The sulfonamides and sulfones are effective only against the erythrocytic (site 4) stage. Thus, all can cure *P. falciparum* infections but not *P. vivax, P. malariae*, or *P. ovale* infections. They can suppress clinical symptoms of all four species. Both pyrimethamine and chloroguanide could theoretically be effective as prophylactic agents (because they act at the primary schizont stage), but chloroguanide's slow onset of action and short half-life limit its use for this purpose. Pyrimethamine, however, is an effective prophylactic and excellent clinical suppressive.

The number of FAH_4 synthesis inhibitors in actual clinical use is small, but extensive structure–activity studies have been completed.

DIAMINOPYRIMIDINES

After the observations made in the late 1940s that some 2,4-diaminopyrimidines were capable of interfering with the utilization of FA by *Lactobacillus casei*, a property also shown by chloroguanide, these compounds received intensive study as potential antimalarials. It was noted that certain 2,4-diamino-5-phenoxypyrimidines possessed a structural resemblance to chloroguanide, and a series of such compounds was synthesized and found to possess good antimalarial action. Subsequently, a large series of 2,4-diamino-5-phenylpyrimidines was prepared and tested for activity. Maximum activity was obtained when an electron-denoting group was present in the 6-position of the pyrimidine ring and when a chlorine atom was present in the *para* position of the phenyl ring. If the two rings were separated by either an oxygen atom or a carbon atom, antimalarial action decreased. The best in the series of compounds was the one that became known as pyrimethamine.

PRODUCTS

Pyrimethamine, USP

5-(4-Chlorophenyl)-6-ethyl-2,4-pyrimidinediamine; 2,4-diamino-5-(*p*-chlorophenyl)-6-ethylpyrimidine (Daraprim). Pyrimethamine is an effective erythrocytic schizontocide against all human malarias. It also acts as a primary exoerythrocytic schizontocide in most infections.

Pyrimethamine is absorbed slowly but completely from the gastrointestinal tract. It is localized in the liver, lungs, kidneys, and spleen and is excreted slowly through the urine, chiefly in metabolized form. A single weekly dose of 25 mg is sufficient for suppression. It is relatively nontoxic, but overdoses may lead to depression of cell growth by inhibition of FA activity.

It is administered in the form of the free base, a relatively tasteless powder.

Trimethoprim, USP

5-[(3,4,5-Trimethoxyphenyl)methyl]-2,4-pyrimidinediamine; 2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine.

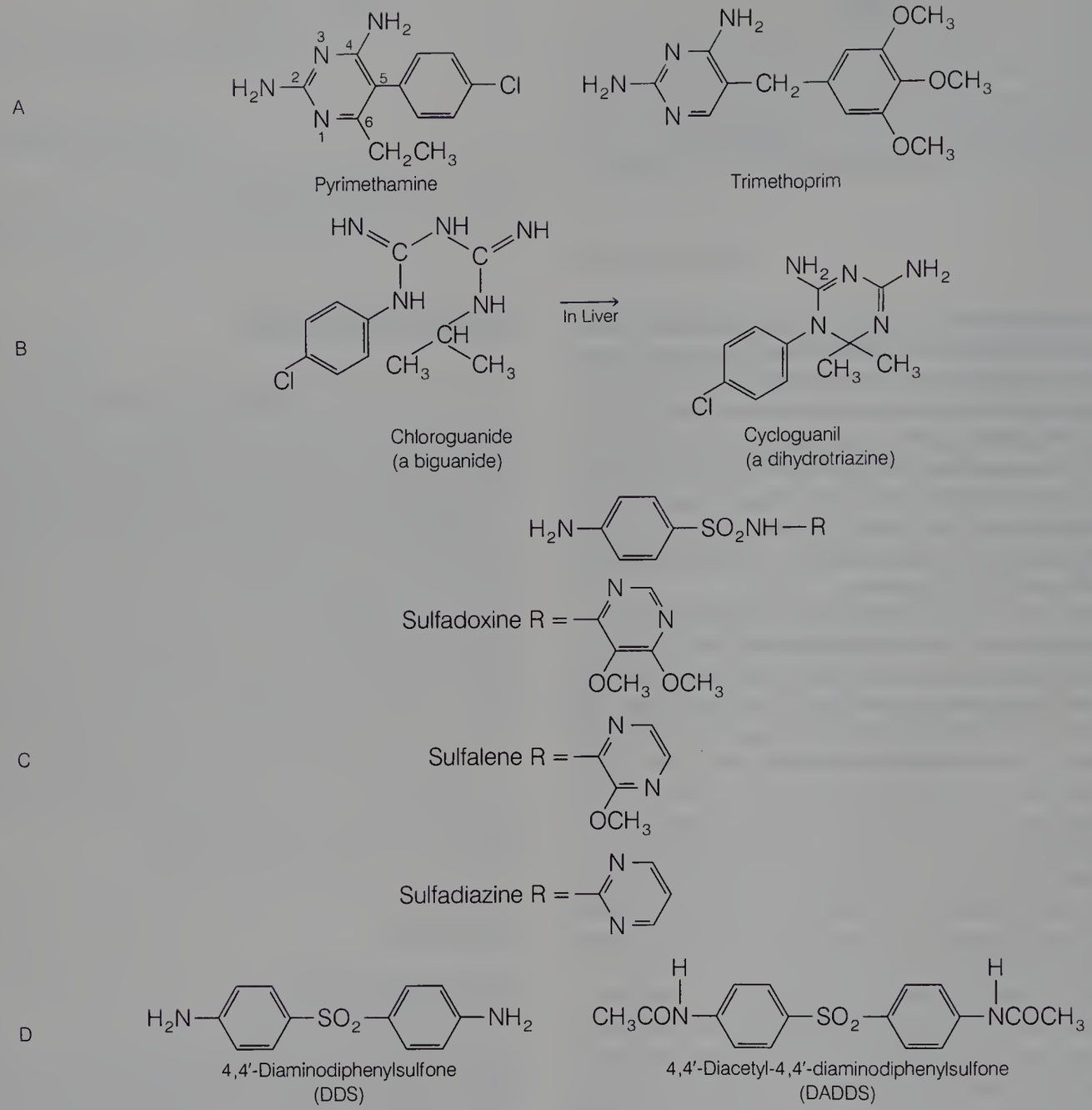


FIG. 9-3.

Trimethoprim is marketed by itself and in combination with sulfonamides (e.g., in the combination products Bactrim, Septra, and others) primarily as antibacterial products (see Chap. 8). It was developed as an antibacterial agent, but subsequent tests showed it to be active as an antimalarial. Studies by Ferone and co-workers[55–57] have shown that trimethoprim is not as selective for protozoan dihydrofolate reductase as is pyrimethamine. As a result, when used as an antimalarial, it is usually combined with another drug. Its antimalarial effectiveness for human malarias can best be described as mixed. (For a review of the early studies, see the discussion of Krishna.[5]) An additional limiting factor in its antimalarial use is trimethoprim's much shorter half-life (about 24 hr) than that of pyrimethamine.

BIGUANIDES AND DIHYDROTRIAZINES

Although in several malaria species the biguanides have some antimalarial activity, they are largely prodrugs for their active metabolites, the dihydrotriazines. For example, as shown in Figure 9-3, chloroguanide is metabolized rapidly to the potent antimalarial dihydrotriazine cycloguanil. The dihydrotriazines such as cycloguanil are metabolized so rapidly that, with one exception (cycloguanil pamoate), they are not used for human infections. Cycloguanil pamoate is an intramuscularly injectable depot preparation that can provide antimalarial protection for several months from a single dose.

Numerous biguanide and dihydrotriazine antimalarial

agents have been synthesized. Useful structure–activity relationships have been found; for example, substitution of a halogen on the *para* position of the phenyl ring significantly increases activity. Chlorine is used in chloroguanide, but the bromine analogue is also very active. Later, it was observed that a second chlorine added to the 3-position of the phenyl ring of chloroguanide further enhanced activity. However, the dichloro compound chlorproguanil is more toxic than chloroguanide itself.

The biguanides are absorbed from the gastrointestinal tract very quickly but not as rapidly as quinine or chloroquine. They concentrate in the liver, lungs, spleen, and kidneys but appear not to cross the blood–brain barrier. Of the amount in plasma, about 75% is protein-bound. They are metabolized mostly in the body and are eliminated very rapidly, principally in the urine. As a result, frequent administration of these drugs is necessary.

The toxic manifestations of biguanides are very mild in humans. Some gastrointestinal disturbances may occur if the drugs are taken on an empty stomach but not if they are taken after meals. With excessive doses (1 g of chloroguanide), some renal disorders, such as hematuria and albuminuria, may develop.

PRODUCTS

Chloroguanide Hydrochloride

1-(*p*-Chlorophenyl)-5-isopropylbiguanide hydrochloride; proguanil hydrochloride (Paludrine) occurs as a white crystalline powder or as colorless crystals that are soluble in water (1:75) and alcohol (1:30). It is odorless, bitter, and stable in air but slowly darkens on exposure to light.

Cycloguanil Pamoate

4,6-Diamino-1-(*p*-chlorophenyl)-1,2-dihydro-2,2-dimethyl-*s*-triazine (2:1) with 4,4′-methylenebis[3-hydroxy-2-naphthoic acid]; CI-501; cycloguanil embonate (Camolar). A single intramuscular injection of cycloguanil pamoate can provide protection against all four human malaria species for several months. As with all antimalarials, effectiveness is dependent upon the malaria strain not being resistant to the drug or its structural analogues (e.g., chloroguanide). Unfortunately, resistance develops quickly to cycloguanil pamoate, and injection sites can be painful. The problems do not seem to be improved significantly by combinations with other antimalarial drugs.[58]

SULFONAMIDES

As can be seen in Table 9-2, sulfonamides, such as sulfadoxine, are used in antimalarial therapy against drug-resistant malaria strains. They are effective against erythrocytic stages of the malaria protozoa (Fig. 9-1, site 4). The azo dye Prontosil (see discussion in Chap. 8) was found to have antimalarial activity in vivo against both *P. falciparum* and *P. vivax* in the late 1930s. Later, when it was discovered that Prontosil was a prodrug for sulfanilamide, many other related sulfonamides were investigated as antibacterials and antimalarials.

Medium- or long-acting sulfonamides have been used clinically as antimalarials, particularly sulfadiazine, sulfadoxine, and sulfalene (see Chap. 8). However, each is much more effective when given in combination with pyrimethamine. Trimethoprim combinations have also been investigated.

SULFONES

It has been known for some time that 4,4′-diaminodiphenylsulfone, dapsone USP (DDS), was active against several of the *Plasmodium* species causing malaria.[59] However, it was considered an inferior antimalarial drug until it was discovered that it served effectively as a chemoprophylactic agent against chloroquine-resistant *P. falciparum* infections in Southeast Asia.

The effectiveness of DDS has prompted the development of programs seeking the synthesis of sulfone compounds of superior activity and with longer duration of action. Among the compounds tested, *N,N*-diacetyl-4,4′-diaminodiphenylsulfone (DADDS) is promising. Its more prolonged activity and lower toxicity compared with DDS are probably related to its slow conversion to either the monoacetyl derivative or DDS itself, both of which act as antimalarial agents. Long-acting depot combinations of DADDS and cycloguanil pamoate are also under clinical investigation.

OTHER ANTIMALARIALS

The emergence of drug-resistant strains of malaria has prompted a reinvestigation of antibiotics and an intensive investigation into new types of antimalarial.[1,2,5,6] Tetracyclines in combination with other antimalarials have been effective against chloroquine-resistant strains of *P. falciparum* (Table 9-2). As noted earlier, tricyclic antidepressants and Ca^{2+} channel blockers may also offer new approaches to the treatment of chloroquine-resistant strains.

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CHAPTER **10**

Antibacterial Antibiotics

Arnold R. Martin

HISTORICAL BACKGROUND

Sir Alexander Fleming's accidental discovery of the antibacterial properties of penicillin in 1929[1] is largely credited with initiating the modern antibiotic era. However, it was not until 1938, when Florey and Chain introduced penicillin into therapy, that practical medical exploitation of this important discovery began to be realized. Centuries earlier, humans had learned to use crude preparations empirically for the topical treatment of infections, which we now assume to be effective because of the antibiotic substances present. As early as 500 to 600 B.C., molded curd of soybean was used in Chinese folk medicine to treat boils and carbuncles. Moldy cheese had also been employed for centuries by Chinese and Ukrainian peasants to treat infected wounds. The discovery by Pasteur and Joubert in 1877, that anthrax bacilli were killed when grown in culture in the presence of certain bacteria, along with similar observations by other microbiologists led Vuillemin[2] to define *antibiosis* (literally "against life") as the biologic concept of survival of the fittest, in which one organism destroys another to preserve itself. It is from this root that the word "*antibiotic*" was derived. The use of the term by the lay public, as well as the medical and scientific communities, has become so widespread that its original meaning has become obscured.

In 1942, Waksman[3] proposed the widely cited definition that "an antibiotic or antibiotic substance is a substance produced by microorganisms, which has the capacity of inhibiting the growth and even of destroying other microorganisms." Later proposals[4–6] have sought both to expand and to restrict the definition to include any substance produced by a living organism that is capable of inhibiting the growth or survival of one or more species of microorganisms in low concentrations. With the advances made by medicinal chemists to modify naturally occurring antibiotics and to prepare synthetic analogues, it has become necessary to permit the inclusion of semisynthetic and synthetic derivatives in the definition. Therefore, a substance is classified as an antibiotic if the following conditions are met:

1. It is a product of metabolism (although it may be duplicated or even have been anticipated by chemical synthesis).
2. It is a synthetic product produced as a structural analogue of a naturally occurring antibiotic.
3. It antagonizes the growth or survival of one or more species of microorganisms.
4. It is effective in low concentrations.

The isolation of the antibacterial antibiotic tyrocidin from the soil bacterium *Bacillus brevis* by Dubois suggested the probable existence of many antibiotic substances in nature and provided the impetus for the search for them. An organized search of the order Actinomycetales led Waksman and associates to isolate streptomycin from *Streptomyces griseus*. The discovery that this antibiotic possessed in vivo activity against *Mycobacterium tuberculosis*, in addition to numerous species of gram-negative bacilli, was electrifying. It was now evident that soil microorganisms would provide a rich source of antibiotics. Broad screening programs were instituted to find antibiotics that might be effective in the treatment of infections hitherto resistant to existing chemotherapeutic agents, as well as to provide safer and more effective chemotherapy. The discoveries of broad-spectrum antibacterial antibiotics, such as chloramphenicol and the tetracyclines; antifungal antibiotics, such as nystatin and griseofulvin (see Chap. 7); and the ever-increasing number of antibiotics that may be used to treat infections that have developed resistance to some of the older antibiotics attest to the spectacular success of this approach as it has been applied in research programs throughout the world.

CURRENT STATUS

Commercial and scientific interest in the antibiotic field has led to the isolation and identification of antibiotic substances

that may be numbered in the thousands. Numerous semisynthetic and synthetic derivatives have been added to the total. Very few such compounds have found application in general medical practice, however, because in addition to an ability to combat infections or neoplastic disease, an antibiotic must possess other attributes. First, it must exhibit sufficient selective toxicity to be decisively effective against pathogenic microorganisms or neoplastic tissue, on the one hand, without causing significant toxic effects, on the other. Second, an antibiotic should possess sufficient chemical stability that it can be isolated, processed, and stored for a reasonable length of time without deterioration of potency. The amenability of an antibiotic, for oral or parenteral administration, to be converted into suitable dosage forms that will provide active drug in vivo is also important. Third, the rates of biotransformation and elimination of the antibiotic should be sufficiently slow to allow a convenient dosing schedule, yet sufficiently rapid and complete to facilitate removal of the drug and its metabolites from the body soon after administration has been discontinued. Some groups of antibiotics, because of certain unique properties, have been designated for specialized uses, such as the treatment of tuberculosis or fungal infections. Others are employed for cancer chemotherapy. These antibiotics are described along with other drugs of the same therapeutic class: antifungal and antitubercular antibiotics are discussed in Chapter 7 and antineoplastic antibiotics in Chapter 12.

The spectacular success of antibiotics in the treatment of human diseases has prompted the expansion of their use into a number of related fields. Extensive use of their antimicrobial power is made in veterinary medicine. The discovery that low-level administration of antibiotics to meat-producing animals resulted in faster growth, lower mortality, and better quality has led to the use of these products as feed supplements. Several antibiotics are used to control bacterial and fungal diseases of plants. Their use in food preservation is being studied carefully. Indeed, such uses of antibiotics have made necessary careful studies of their long-term effects on humans and their effects on various commercial processes. For example, foods that contain low-level amounts of antibiotics may be capable of producing allergic reactions in hypersensitive persons, or the presence of antibiotics in milk may interfere with the manufacture of cheese.

The success of antibiotics in therapy and related fields has made them one of the most important products of the drug industry today. The quantity of antibiotics produced in the United States each year may now be measured in several millions of pounds and valued at billions of dollars. With research activity stimulated to find new substances to treat viral infections that now are combated with only limited success and with the promising discovery that some antibiotics are active against cancers that may be viral in origin, the future development of more antibiotics and the increase in the amounts produced seem to be assured.

COMMERCIAL PRODUCTION

The commercial production of antibiotics for medicinal use follows a general pattern, differing in detail for each antibiotic. The general scheme may be divided into six steps: (1) preparation of a pure culture of the desired organism for use in inoculation of the fermentation medium; (2) fermentation, during which the antibiotic is formed; (3) isolation of the antibiotic from the culture medium; (4) purification; (5) assays for potency, sterility, absence of pyrogens, and other necessary data; and (6) formulation into acceptable and stable dosage forms.

SPECTRUM OF ACTIVITY

The ability of some antibiotics, such as chloramphenicol and the tetracyclines, to antagonize the growth of numerous pathogens has resulted in their being designated "*broad-spectrum*" antibiotics. Designations of spectrum of activity are of somewhat limited use to the physician, unless they are based on clinical effectiveness of the antibiotic against specific microorganisms. Many of the broad-spectrum antibiotics are active only in relatively high concentrations against some of the species of microorganisms often included in the "spectrum."

MECHANISMS OF ACTION

The manner in which antibiotics exert their actions against susceptible organisms is varied. The mechanisms of action of some of the more common antibiotics are summarized in Table 10-1. In many instances, the mechanism of action is not fully known; for a few (e.g., penicillins), the site of action is known, but precise details of the mechanism are still under investigation. The biochemical processes of microorganisms comprise a lively subject for research, for an understanding of those mechanisms that are peculiar to the metabolic systems of infectious organisms is the basis for the future development of modern chemotherapeutic agents. Antibiotics that interfere with those metabolic systems found in microorganisms and not in mammalian cells are the most successful anti-infective agents. For example, antibiotics that interfere with the synthesis of bacterial cell walls have a high potential for selective toxicity. The fact that some antibiotics structurally resemble some essential metabolites of microorganisms has suggested that competitive antagonism may be the mechanism by which they exert their effects. Thus, cycloserine is believed to be an antimetabolite for D-alanine, a constituent of bacterial cell walls. Many antibiotics selectively interfere with microbial protein synthesis (e.g., the aminoglycosides, the tetracyclines, the macrolides, chloramphenicol, and lincomycin) or nucleic acid synthesis (e.g., rifampin). Others, such as the polymyxins and the polyenes, are believed to interfere with the integrity and function of cell membranes of microorganisms. The mechanism of action of

TABLE 10-1

MECHANISMS OF ANTIBIOTIC ACTION

Site of Action	*Antibiotic*	*Process Interrupted*	*Type of Activity*
Cell wall	Bacitracin	Mucopeptide synthesis	Bactericidal
	Cephalosporins	Cell wall cross-linking	Bactericidal
	Cycloserine	Synthesis of cell wall peptides	Bactericidal
	Penicillin	Cell wall cross-linking	Bactericidal
	Vancomycin	Mucopeptide synthesis	Bactericidal
Cell membrane	Amphotericin B	Membrane function	Fungicidal
	Nystatin	Membrane function	Fungicidal
	Polymyxins	Membrane integrity	Bactericidal
Ribosomes	Chloramphenicol	Protein synthesis	Bacteriostatic
50S subunit	Erythromycin	Protein synthesis	Bacteriostatic
	Lincomycins	Protein synthesis	Bacteriostatic
30S subunit	Aminoglycosides	Protein synthesis and fidelity	Bactericidal
	Tetracyclines	Protein synthesis	Bacteriostatic
Nucleic acids	Actinomycin	DNA and mRNA synthesis	Pancidal
	Griseofulvin	Cell division, microtubule assembly	Fungistatic
DNA and/or RNA	Mitomycin C	DNA synthesis	Pancidal
	Rifampin	mRNA synthesis	Bactericidal

an antibiotic determines, in general, whether the agent exerts a *-cidal* or a *-static* action. The distinction may be important for the treatment of serious, life-threatening infections, particularly if the natural defense mechanisms of the host are either deficient or overwhelmed by the infection. In such situations, a *-cidal* agent is obviously indicated. Much work remains to be done in this area, and as mechanisms of action are revealed, the development of improved structural analogues of effective antibiotics probably will continue to increase.

CHEMICAL CLASSIFICATION

The chemistry of antibiotics is so varied that a chemical classification is of limited value. However, some similarities can be found, indicating that some antibiotics may be the products of similar mechanisms in different organisms and that these structurally similar products may exert their activities in a similar manner. For example, several important antibiotics have in common a macrolide structure, that is, a large lactone ring. This group includes erythromycin and oleandomycin. The tetracycline family comprises a group of compounds very closely related chemically. Several compounds contain closely related amino sugar moieties, such as those found in streptomycins, kanamycins, neomycins, paromomycins, and gentamicins. The antifungal antibiotics nystatin and the amphotericins (see Chap. 7) are examples of a group of conjugated polyene compounds. The bacitracins, tyrothricin, and polymyxin are among a large group of polypeptides that exhibit antibiotic action. The penicillins and cephalosporins are β-lactam ring-containing antibiotics derived from amino acids.

MICROBIAL RESISTANCE

The normal biologic processes of microbial pathogens are varied and complex. Thus, it seems reasonable to assume that there are many ways in which they may be inhibited and that different microorganisms that elaborate antibiotics antagonistic to a common "foe" produce compounds that are chemically dissimilar and that act on different processes. In fact, nature has produced many chemically different antibiotics that are capable of attacking the same microorganism by different pathways. The diversity of structure in antibiotics has proved to be of real clinical value. As the pathogenic cell is called on to combat the effect of one antibiotic and, thus, develops drug resistance, another antibiotic, attacking another metabolic process of the resisting cell, will deal it a crippling blow. The development of new and different antibiotics has been a very important step in providing the means for treating resistant strains of organisms that previously had been susceptible to an older antibiotic. More recently, the elucidation of biochemical mechanisms of microbial resistance to antibiotics, such as the inactivation of penicillins and cephalosporins by β-lactamase–producing bacteria, has stimulated research in the development of semisynthetic analogues that resist microbial biotransformation. The evolution of *nosocomial* (hospital-acquired) strains of staphylococci resistant to penicillin and of gram-negative bacilli (e.g., *Pseudomonas* and *Klebsiella* spp., *Escherichia coli*, and others) often resistant to several antibiotics has become a serious medical problem. No doubt, the promiscuous and improper use of antibiotics has contributed to the emergence of resistant bacterial strains. The successful control of diseases caused by resistant strains of bacteria will require not only the development of new and improved antibiotics but also the rational use of available agents.

β-LACTAM ANTIBIOTICS

Antibiotics that contain the β-lactam (a four-membered cyclic amide) ring structure constitute the dominant class of

agents currently employed for the chemotherapy of bacterial infections. The first antibiotic to be used in therapy, penicillin (penicillin G or benzyl penicillin), and a close biosynthetic relative, phenoxymethyl penicillin (penicillin V), remain the agents of choice for the treatment of infections caused by most species of gram-positive bacteria. The discovery of a second major group of β-lactam antibiotics, the cephalosporins, and chemical modifications of naturally occurring penicillins and cephalosporins have provided semisynthetic derivatives that are variously effective against bacterial species known to be resistant to penicillin, in particular, penicillinase-producing staphylococci and gram-negative bacilli. Thus, apart from a few strains that have either inherent or acquired resistance, almost all bacterial species are sensitive to one or more of the available β-lactam antibiotics.

MECHANISM OF ACTION

In addition to a broad spectrum of antibacterial action, two properties contribute to the unequaled importance of β-lactam antibiotics in chemotherapy: a potent and rapid *-cidal* action against bacteria in the growth phase and a very low frequency of toxic and other adverse reactions in the host. The uniquely lethal antibacterial action of these agents has been attributed to a selective inhibition of bacterial cell wall synthesis.[7] Specifically, inhibition of the biosynthesis of the dipeptidoglycan that is needed to provide strength and rigidity to the cell wall is the basic mechanism involved. Penicillins and cephalosporins acylate a specific bacterial D-transpeptidase,[8] thereby rendering it inactive for its role in forming peptide cross-links of two linear peptidoglycan strands by transpeptidation and loss of D-alanine. Bacterial D-alanine carboxypeptidases are also inhibited by β-lactam antibiotics.

Binding studies with tritiated benzyl penicillin have shown that the mechanisms of action of various β-lactam antibiotics are much more complex than previously assumed. Studies in *E. coli* have revealed the existence of as many as seven different functional proteins, each having an important role in cell wall biosynthesis.[9] These penicillin-binding proteins (PBPs) have the following functional properties:

- PBPs 1_a and 1_b are transpeptidases involved in peptidoglycan synthesis associated with cell elongation. Inhibition results in spheroplast formation and rapid cell lysis,[9,10] caused by autolysins (bacterial enzymes that create nicks in the cell wall for attachment of new peptidoglycan units or for separation of daughter cells during cell division[10]).
- PBP 2 is a transpeptidase involved in maintaining the rod shape of bacilli.[11] Inhibition results in ovoid or round forms that undergo delayed lysis.
- PBP 3 is a transpeptidase required for septum formation during cell division.[12] Inhibition results in the formation of filamentous forms containing rod-shaped units that cannot separate. It is not yet clear if inhibition of PBP 3 is lethal to the bacterium.
- PBPs 4 through 6 are carboxypeptidases responsible for the hydrolysis of D-alanine-D-alanine terminal peptide bonds of the cross-linking peptides. Inhibition of these enzymes is apparently not lethal to the bacterium,[13] despite the fact that cleavage of the terminal D-alanine bond is required before peptide cross-linkage.

The various β-lactam antibiotics differ in their affinities for PBPs. Penicillin G binds preferentially to PBP 3, whereas the first-generation cephalosporins bind with higher affinity to PBP 1_a. In contrast to other penicillins and to cephalosporins, which can bind to PBPs 1, 2, and 3, amdinocillin binds only to PBP 2.

THE PENICILLINS

COMMERCIAL PRODUCTION AND UNITAGE

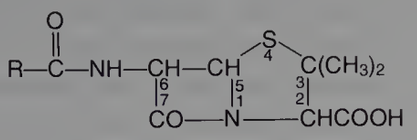
Until 1944, it was assumed that the active principle in penicillin was a single substance and that variation in activity of different products was due to the amount of inert materials in the samples. Now it is known that during the biologic elaboration of the antibiotic several closely related compounds may be produced. These compounds differ chemically in the acid moiety of the amide side chain. Variations in this moiety produce differences in antibiotic effect and in physicochemical properties, including stability. Thus, it has become proper to speak of penicillins as a group of compounds and to identify each of the penicillins specifically. As each of the different penicillins was first isolated, letter designations were used in the United States; the British used Roman numerals.

Over 30 penicillins have been isolated from fermentation mixtures. Some of these occur naturally; others have been biosynthesized by altering the culture medium to provide certain precursors that may be incorporated as acyl groups. Commercial production of biosynthetic penicillins today depends chiefly on various strains of *Penicillium notatum* and *P. chrysogenum.* In recent years, many more penicillins have been prepared semisynthetically, and undoubtedly, many more will be added to the list in attempts to find superior products.

Because penicillin, when first used in chemotherapy, was not a pure compound and exhibited varying activity among samples, it was necessary to evaluate it by microbiologic assay. The procedure for assay was developed at Oxford, England, and the value became known as the *Oxford unit:* 1 Oxford unit is defined as the smallest amount of penicillin that will inhibit, in vitro, the growth of a strain of *Staphylococcus* in 50 mL of culture medium under specified conditions. Now that pure crystalline penicillin is available, the *United States Pharmacopoeia* (USP) defines *unit* as the antibiotic activity of 0.6 μg of USP penicillin G sodium reference standard. The weight–unit relationship of the penicillins will vary with the nature of the acyl substituent and

TABLE 10-2

STRUCTURE OF PENICILLINS



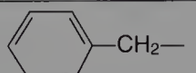
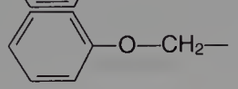
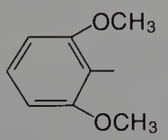
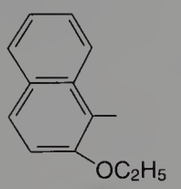
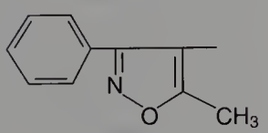
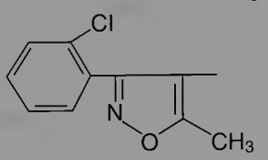
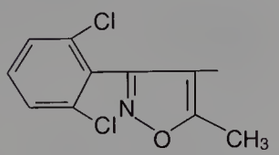
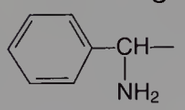
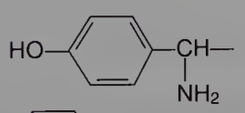
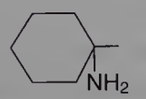
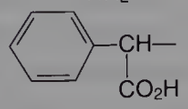
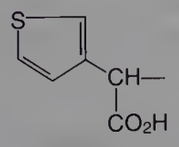
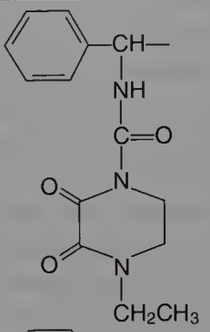
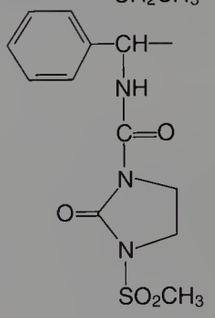
Generic Name	Chemical Name	R Group
Penicillin G	Benzylpenicillin	
Penicillin V	Phenoxymethylpenicillin	
Methicillin	2,6-Dimethoxyphenyl-penicillin	
Nafcillin	2-Ethoxy-1-naphthyl-penicillin	
Oxacillin	5-Methyl-3-phenyl-4-isoxazolylpenicillin	
Cloxacillin	5-Methyl-3-(2-chlorophenyl)-4-isoxazolylpenicillin	
Dicloxacillin	5-Methyl-3-(2,6-dichlorophenyl)-4-isoxazolylpenicillin	
Ampicillin	D-α-Aminobenzyl-penicillin	
Amoxicillin	D-α-Amino-p-hydroxybenzyl-penicillin	
Cyclacillin	1-Aminocyclohexyl-penicillin	
Carbenicillin	α-Carboxybenzyl-penicillin	
Ticarcillin	α-Carboxy-3-thienyl-penicillin	

TABLE 10-2 *Continued*

Generic Name	Chemical Name	R Group
Piperacillin	α-(4-Ethyl-2,3-dioxo-1-piperazinylcarbonyl-amino)benzylpenicillin	
Mezlocillin	α-(1-methanesulfonyl-2-oxoimidazolidino-carbonylamino)benzyl penicillin	

with the salt formed of the free acid: 1 mg of penicillin G sodium is equivalent to 1,667 units; 1 mg of penicillin G procaine is equivalent to 1,009 units; 1 mg of penicillin G potassium is equivalent to 1,530 units.

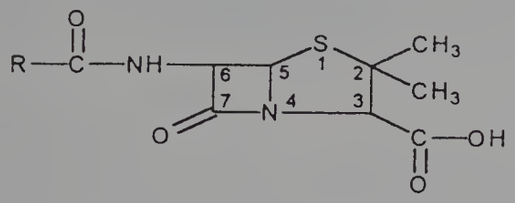
The commercial production of penicillin has increased markedly since its introduction. As production increased, the cost dropped correspondingly. When penicillin was first available, 100,000 units sold for 20 dollars. Currently, the same quantity costs less than a penny. Fluctuations in the production of penicillins through the years have reflected changes in the popularity of broad-spectrum antibiotics compared with penicillins, the development of penicillin-resistant strains of several pathogens, the more recent introduction of semisynthetic penicillins, the use of penicillins in animal feeds and for veterinary purposes, and the increase in marketing problems in a highly competitive sales area.

Table 10-2 shows the general structure of the penicillins and relates the structure of the more familiar ones to their various designations.

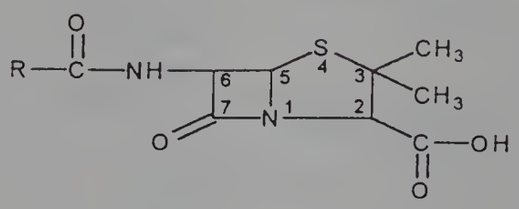
NOMENCLATURE

The nomenclature of penicillins is somewhat complex and very cumbersome. Two numbering systems for the fused bicyclic heterocyclic system are in existence. The *Chemical Abstracts* system initiates the numbering with the sulfur atom and assigns the ring nitrogen as the 4-position. Thus, penicillins are named as 4-thia-l-azabicyclo[3.2.0]heptanes, according to this system. The numbering system adopted by the USP is the reverse of the *Chemical Abstracts* procedure,

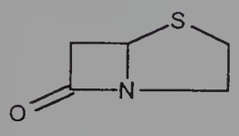
assigning the nitrogen atom as atom number 1 and the sulfur atom as number 4. Three simplified forms of penicillin nomenclature have been adopted for general use. One utilizes the name "penam" for the unsubstituted bicyclic system, including the amide carbonyl group, with one of the foregoing numbering systems as just described. Thus, penicillins generally are designated according to the *Chemical Abstracts* system as 5-acylamino-2,2-dimethylpenam-3-carboxylic acids. The second, seen more frequently in the medical literature, uses the name "penicillanic acid" to describe the ring system with substituents that are generally present (i.e., 2,2-dimethyl and 3-carboxyl). A third form, followed in this chapter, uses trivial nomenclature to name the entire 6-carbonylamino-penicillanic acid portion of the molecule as penicillin and then distinguishes compounds on the basis of the R group of the acyl portion of the molecule. Thus, Penicillin G is named benzylpenicillin, penicillin V is phenoxymethylpenicillin, methicillin is 2,6-dimethoxyphenylpenicillin, and so on. For the most part, the latter two systems serve well for naming and comparing closely similar penicillin structures, but they are too restrictive to be applied to compounds with unusual substituents or to ring-modified derivatives.



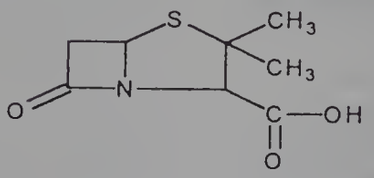
Chemical Abstracts



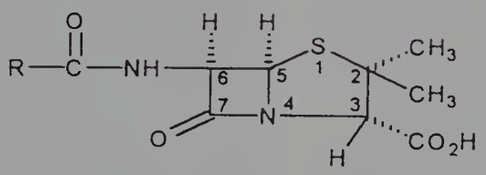
USP



Penam



Penicillanic Acid



Chemical Abstracts

STEREOCHEMISTRY

The penicillin molecule contains three asymmetric carbon atoms (C-3, C-5, and C-6). All naturally occurring and microbiologically active synthetic and semisynthetic penicillins have the same absolute configuration about these three centers. The carbon atom bearing the acylamino group (C-6) has the L-configuration, whereas the carbon to which the carboxyl group is attached has the D-configuration. Thus, the acylamino and carboxyl groups are *trans* to each other, with the former in the α- and the latter in the β-orientation relative to the penam ring system. The atoms comprising the 6-aminopenicillanic acid portion of the structure are derived biosynthetically from two amino acids, L-cysteine (S-1, C-5, C-6, C-7, and 6-amino) and L-valine (2,2-dimethyl, C-2, C-3, N-4, and 3-carboxyl). The absolute stereochemistry of the penicillins is designated as 3S:5R:6R, as shown below.

SYNTHESIS

Examination of the structure of the penicillin molecule shows it to contain a fused ring system of unusual design, the β-lactam thiazolidine structure. The nature of the β-lactam ring delayed elucidation of the structure of penicillin, but its determination was reached as the result of a collaborative research program involving groups in Great Britain and the United States during the years 1943 to 1945.[14] Attempts to synthesize these compounds resulted, at best, in only trace amounts until Sheehan and Henery-Logan[15] adapted techniques developed in peptide synthesis to the synthesis of penicillin V. This procedure is not likely to replace the established fermentation processes because the last step in the reaction series develops only 10% to 12% penicillin. It is of advantage in research because it provides a means of obtain-

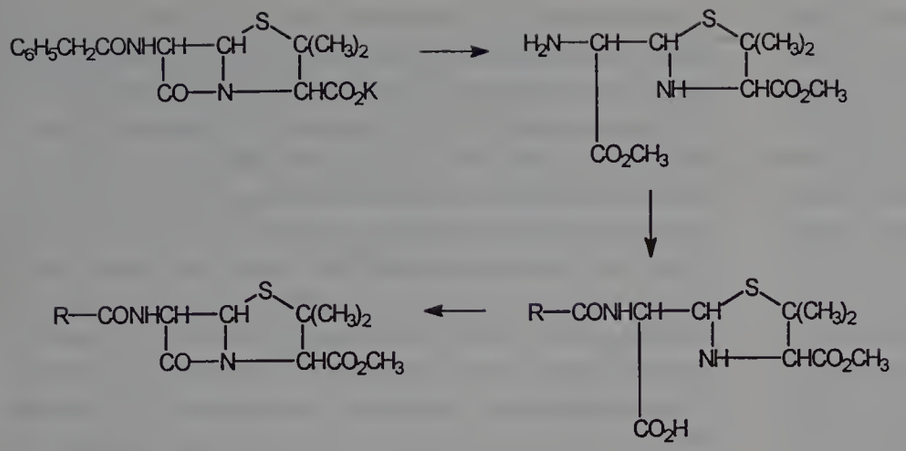


FIG. 10-1. *Conversion of natural penicillin to synthetic penicillin.*

ing many new amide chains hitherto not possible to achieve by biosynthetic procedures.

Two other developments have provided additional means for making new penicillins. A group of British scientists, Batchelor et al.,[16] have reported the isolation of 6-aminopenicillanic acid from a culture of *P. chrysogenum*. This compound can be converted to penicillins by acylation of the 6-amino group. Sheehan and Ferris[17] provided another route to synthetic penicillins by converting a natural penicillin, such as penicillin G potassium, to an intermediate (Fig. 10-1), from which the acyl side chain has been cleaved and which then can be treated to form biologically active penicillins with a variety of new side chains. By these procedures, new penicillins, superior in activity and stability to those formerly in wide use, have been found, and no doubt, others will be produced. The first commercial products of these research activities were phenoxyethylpenicillin (phenethicillin) (Fig. 10-2) and dimethoxyphenylpenicillin (methicillin).

CHEMICAL DEGRADATION

The early commercial penicillin was a yellow to brown amorphous powder that was so unstable that refrigeration was required to maintain a reasonable level of activity for a short time. Improved procedures for purification provided the white crystalline material in use today. Crystalline penicillin must be protected from moisture, but when kept dry, the salts will remain stable for years without refrigeration. Many penicillins have an unpleasant taste, which must be overcome in the formation of pediatric dosage forms. All of the natural penicillins are strongly dextrorotatory. The solubility and other physicochemical properties of the penicillins are affected by the nature of the acyl side chain and by the cations used to make salts of the acid. Most penicillins are acids with pK_a values in the range of 2.5 to 3.0, but some are amphoteric. The free acids are not suitable for oral or parenteral administration. However, the sodium and potas-

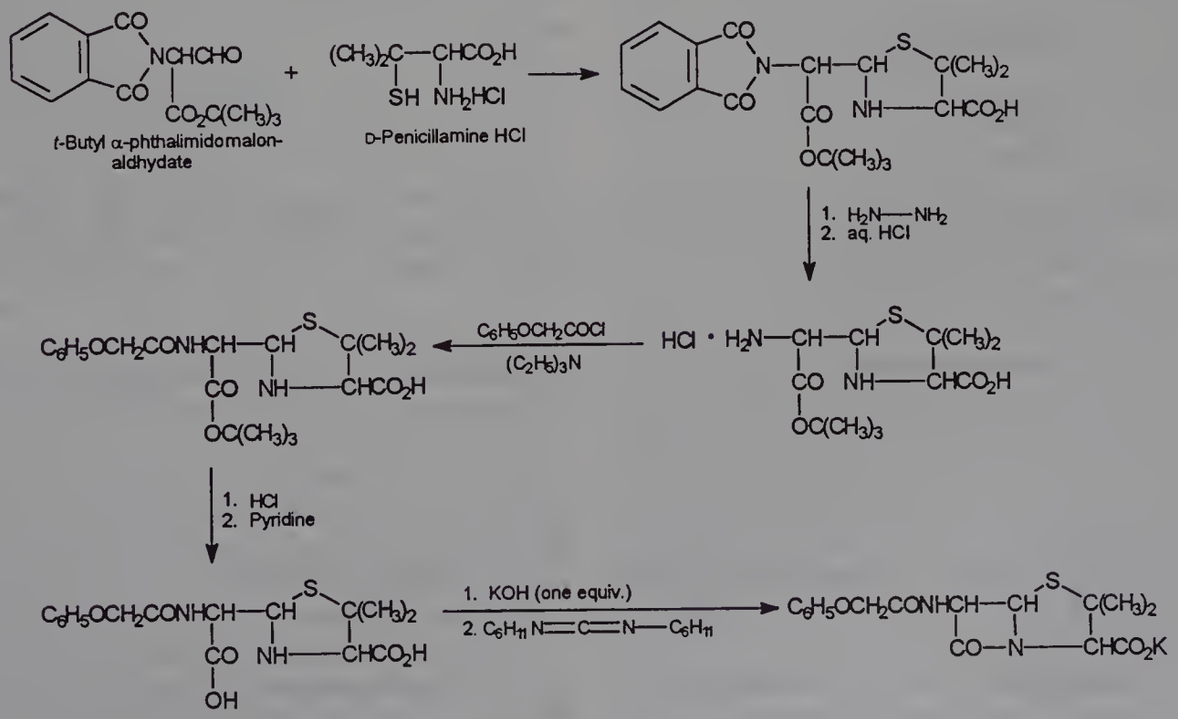


FIG. 10-2. *Synthesis of phenoxymethylpenicillin.*

sium salts of most penicillins are soluble in water and readily absorbed orally or parenterally. Salts of penicillins with organic bases, such as benzathine, procaine, and hydrabamine, have limited water solubility and are, therefore, useful as depot forms to provide effective blood levels over a long period in the treatment of chronic infections. Some of the crystalline salts of the penicillins are hygroscopic, making it necessary to store them in sealed containers.

The main cause of deterioration of penicillin is the reactivity of the strained lactam ring, particularly to hydrolysis. The course of the hydrolysis and the nature of the degradation products are influenced by the *p*H of the solution.[18,19] Thus, the β-lactam carbonyl group of penicillin readily undergoes nucleophilic attack by water or (especially) hydroxide ion to form the inactive penicilloic acid, which is reasonably stable in neutral to alkaline solutions but readily undergoes decarboxylation and further hydrolytic reactions in acidic solutions. Other nucleophiles, such as hydroxylamines, alkylamines, and alcohols, open the β-lactam ring to form the corresponding hydroxyamic acids, amides, and esters. It has been speculated[20] that one of the causes of penicillin allergy may be the formation of antigenic penicilloyl proteins formed in vivo by the reaction of nucleophilic groups (e.g., ε-amino) on specific body proteins with the β-lactam carbonyl group. In strongly acidic solutions (*p*H < 3), penicillin undergoes a complex series of reactions leading to a variety of inactive degradation products (Fig. 10-3).[19] The first step appears to involve rearrangement to the penicillenic acid. This process is initiated by protonation of the β-lactam nitrogen, followed by nucleophilic attack of the acyl oxygen atom on the β-lactam carbonyl carbon. The subsequent opening of the β-lactam ring destabilizes the thiazoline ring, which then also suffers acid-catalyzed ring opening to form the penicillenic acid. The latter is very unstable and experiences two major degradation pathways. The most easily understood path involves hydrolysis of the oxazolone ring to form the unstable penamaldic acid. Because it is an enamine, penamaldic acid easily hydrolyzes to penicillamine (a major degradation product) and penaldic acid. The second path involves a complex rearrangement of penicillenic acid to a

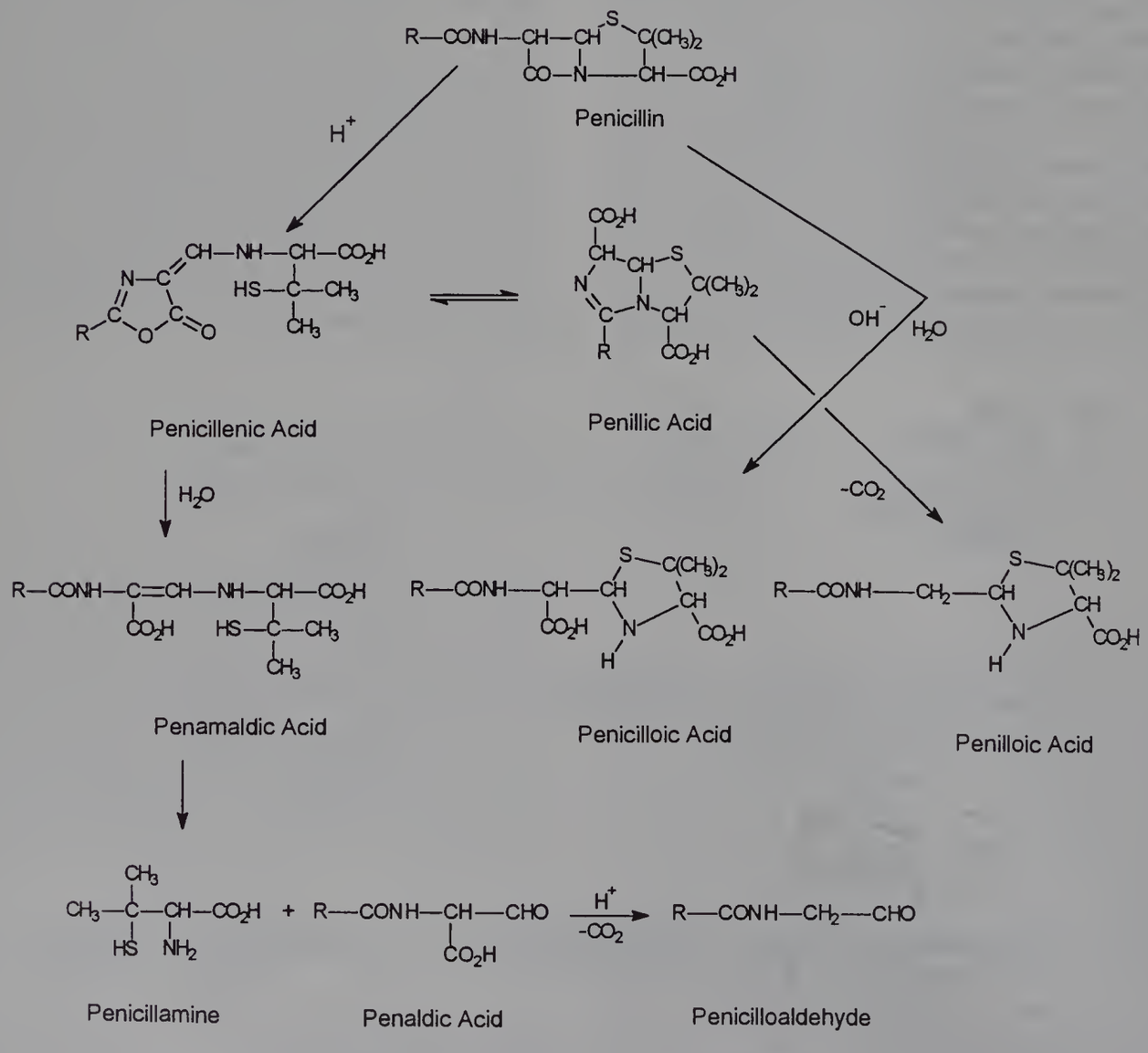


FIG. 10-3. *Degradation of penicillins.*

penillic acid through a series of intramolecular processes that remain to be elucidated completely. Penillic acid (an imidazoline-2-carboxylic acid) readily decarboxylates and suffers hydrolytic ring opening under acidic conditions to form a second major end product of acid-catalyzed penicillin degradation, penilloic acid. Penicilloic acid, the major product formed under weakly acidic to alkaline (as well as enzymatic) hydrolytic conditions, cannot be detected as an intermediate under strongly acidic conditions. However, it is known to exist in equilibrium with penamaldic acid and to undergo decarboxylation in acid to form penilloic acid. The third major product of the degradation is penicilloaldehyde, formed by decarboxylation of penaldic acid (a derivative of malonaldehyde).

By controlling the *p*H of aqueous solutions within a range of 6.0 to 6.8 and by refrigeration of the solutions, aqueous preparation of the soluble penicillins may be stored for periods of up to several weeks. The relationship of these properties to the pharmaceutics of penicillins has been reviewed by Schwartz and Buckwalter.[21] It has been noted that some buffer systems, particularly phosphates and citrates, exert a favorable effect on penicillin stability, independent of the *p*H effect. However, Finholt et al.[22] have shown that these buffers may catalyze penicillin degradation if the *p*H is adjusted to obtain the requisite ions. Hydroalcoholic solutions of penicillin G potassium show about the same degree of instability as aqueous solutions.[23] Because penicillins are inactivated by metal ions, such as zinc and copper, it has been suggested that the phosphates and the citrates combine with these metals to prevent their existing as free ions in solution.

Oxidizing agents also inactivate penicillins, but reducing agents have little effect on them. Temperature affects the rate of deterioration: although the dry salts are stable at room temperature and do not require refrigeration, prolonged heating will inactivate the penicillins.

Acid-catalyzed degradation in the stomach contributes in a major way to the poor oral absorption of penicillin. Thus, efforts to obtain penicillins with improved pharmacokinetic and microbiologic properties have sought to find acyl functionalities that would minimize sensitivity of the β-lactam ring to acid hydrolysis and, at the same time, maintain antibacterial activity.

Substitution of an electron-withdrawing group in the α-position of benzylpenicillin markedly stabilizes the penicillin to acid-catalyzed hydrolysis. Thus, the phenoxymethyl-,α-aminobenzyl-, and α-halobenzylpenicillins are significantly more stable than benzylpenicillin in acid solutions. The increased stability imparted by such electron withdrawing groups has been attributed to a decrease in reactivity (nucleophilicity) of the side chain amide carbonyl oxygen atom toward participation in β-lactam ring opening to form penicillenic acid. Obviously, α-aminobenzylpenicillin (ampicillin) exists as the protonated form in acidic (as well as neutral) solutions, and the ammonium group is known to be powerfully electron-withdrawing.

BACTERIAL RESISTANCE

Some bacteria, in particular most species of gram-negative bacilli, are naturally resistant to the action of penicillins. Other normally sensitive species are capable of developing penicillin resistance (either through natural selection of resistant individuals or through mutation). The best understood, and probably the most important, biochemical mechanism of penicillin resistance is the bacterial elaboration of enzymes that inactivate penicillins. Such enzymes, which have been given the nonspecific name "penicillinases," are of two general types: β-lactamases and acylases. By far the most important of these are the β-lactamases, enzymes that catalyze the hydrolytic opening of the β-lactam ring of penicillins to produce inactive penicilloic acids. Synthesis of bacterial β-lactamases may be under chromosomal or plasmid R-factor control and may be either constitutive or inducible (stimulated by the presence of the substrate), depending on the bacterial species. The well-known resistance among strains of *Staphylococcus aureus* is apparently entirely due to the production of an inducible β-lactamase. Resistance among gram-negative bacilli, however, may be a result of other, poorly characterized "resistance factors" or of constitutive β-lactamase elaboration. β-Lactamases produced by gram-negative bacilli appear to be cytoplasmic enzymes that remain in the bacterial cell, whereas those elaborated by *Staph. aureus* are synthesized in the cell wall and released extracellularly. β-Lactamases from different bacterial species may be classified[24–26] by their structure, their substrate and inhibitor specificities, their physical properties (*p*H optimum, isoelectric point, molecular weight, etc.), and their immunologic properties.

Specific acylases, enzymes that are capable of hydrolyzing the acylamino side chain of penicillins, have been obtained from several species of gram-negative bacteria, but their possible role in bacterial resistance has not been well defined. These enzymes find some commercial use in the preparation of 6-aminopenicillanic acid (6-APA) for the preparation of semisynthetic penicillins. 6-APA is less active and hydrolyzed more rapidly (enzymatically and nonenzymatically) than penicillin.

Another important resistance mechanism, especially in gram-negative bacteria, is decreased permeability to penicillins. The cell envelope in most gram-negative bacteria is more complex than it is in gram-positive bacteria. It contains an outer membrane (linked by lipoprotein bridges to the peptidoglycan cell wall), not present in gram-positive bacteria, which creates a physical barrier to the penetration of antibiotics, especially those that are hydrophobic.[27] Small hydrophilic molecules, however, can traverse the outer membrane through pores formed by proteins called "porins."[28] Alteration of the number or nature of porins in the cell envelope[28] also could be an important mechanism of antibiotic resistance. Bacterial resistance can result from changes in the affinity of PBPs for penicillins.[29] Altered PBP binding has been demonstrated in non–β-lactamase-producing

strains of penicillin-resistant *Neisseria gonorrhoeae*[30] and methicillin-resistant *Staph. aureus* (MRSA).[31]

Certain strains of bacteria are resistant to the lytic properties of penicillins but remain susceptible to their growth-inhibiting effects. Thus, the action of the antibiotic has been converted from bactericidal to bacteriostatic. This mechanism of resistance has been termed "tolerance" and apparently results from impaired autolysin activity in the bacterium.

PENICILLINASE-RESISTANT PENICILLINS

The availability of 6-APA on a commercial scale made possible the synthesis of numerous semisynthetic penicillins modified at the acyl amino side chain. Much of the early work done in the 1960s was directed toward the preparation of derivatives that would resist destruction by β-lactamases, particularly those produced by penicillin-resistant strains of *Staph. aureus*, which constituted a very serious health problem at that time. In general, it was found that increasing the steric hindrance at the α-carbon of the acyl group increased resistance to staphylococcal β-lactamase, with maximal resistance being observed with quaternary substitution.[32] More fruitful from the standpoint of antibacterial potency, however, was the observation that the α-acyl carbon could be part of an aromatic (e.g., phenyl or naphythyl) or heteroaromatic (e.g., 4-isoxazoyl) system.[33] Substitutions at the *ortho* positions of a phenyl ring (e.g., 2,6-dimethoxyl [methicillin]) or the 2-position of a 1-naphthyl system (e.g., 2-ethoxyl [nafcillin]) increase the steric hindrance of the acyl group and confer increased β-lactamase resistance over the unsubstituted compounds or those substituted at positions more distant from the α-carbon. Bulkier substituents are required to confer effective β-lactamase resistance among five-membered ring heterocyclic derivatives.[34] Thus, members of the 4-isoxazoyl penicillin family (e.g., oxacillin, cloxacillin, and dicloxacillin) require both the 3-aryl and 5-methyl (3-methyl and 5-aryl) substituents for effectiveness against β-lactamase–producing *Staph. aureus*.

Increasing the bulkiness of the acyl group is not without its price, however, because all of the clinically available penicillinase-resistant penicillins are significantly less active than either penicillin G or penicillin V against most non–β-lactamase-producing bacteria normally sensitive to the penicillins. The β-lactamase–resistant penicillins tend to be comparatively lipophilic molecules that do not penetrate well into gram-negative bacteria. The isoxazoyl penicillins, particularly those with an electronegative substituent in the 3-phenyl group (cloxacillin, dicloxacillin, and fluoxicillin), are also resistant to an acid-catalyzed hydrolysis of the β-lactam, for the reasons described earlier. However, steric factors that confer β-lactamase resistance do not necessarily also confer stability to acid. Accordingly, methicillin, which has electron-donating groups (by resonance) *ortho* to the carbonyl carbon, is even more labile to acid-catalyzed hydrolysis than is penicillin G because of the more rapid formation of the penicillenic acid derivative.

EXTENDED-SPECTRUM PENICILLINS

Another highly significant advance arising from the preparation of semisynthetic penicillins was the discovery that the introduction of an ionized or polar group into the α-position of the side chain benzyl carbon atom of penicillin G confers activity against gram-negative bacilli. Hence, derivatives with an ionized α-amino group, such as ampicillin and amoxicillin, are generally effective against such gram-negative genera as *Esclierichia, Klebsiella, Haemophilus, Salmonella, Shigella*, and non–indole-producing *Proteus*. Furthermore, activity against penicillin G-sensitive, gram-positive species is largely retained. The introduction of an α-amino group in ampicillin (or amoxicillin) creates an additional asymmetric center. Extension of the antibacterial spectrum brought about by the substituent applies only to the D-isomer, which is two to eight times more active than either the L-isomer or benzylpenicillin (which are equiactive) against various species of the aforementioned genera of gram-negative bacilli.

The basis for the expanded spectrum of activity associated with the ampicillin group is not related to β-lactamase inhibition as ampicillin and amoxicillin are even more labile than penicillin G to the action of β-lactamases elaborated by both *Staph. aureus* and various species of gram-negative bacilli, including strains among the ampicillin-sensitive group. Hydrophilic penicillins, such as ampicillin, penetrate gram-negative bacteria with greater facility than do penicillin G, penicillin V, or methicillin. This selective penetration is believed to take place through the porin channels of the cell membrane.[35]

α-Hydroxy substitution also yields "expanded-spectrum" penicillins with similar activity and stereoselectivity to that of the ampicillin group. However, the α-hydroxybenzylpenicillins are about two to five times less active than their corresponding α-aminobenzyl counterparts and, unlike the latter, not very stable under acidic conditions.

Incorporation of an acidic substituent at the α-benzyl carbon atom of penicillin G also imparts clinical effectiveness against gram-negative bacilli and, furthermore, extends the spectrum of activity to include organisms resistant to ampicillin. Thus, α-carboxybenzylpenicillin (carbenicillin) is active against ampicillin-sensitive, gram-negative species and additional gram-negative bacilli of the genera *Pseudomonas, Klebsiella, Enterobacter*, indole-producing *Proteus, Serratia*, and *Providencia*. The potency of carbenicillin against most species of penicillin G-sensitive, gram-positive bacteria is several orders of magnitude lower than that of either penicillin G or ampicillin, presumably because of poorer penetration of a more highly ionized molecule into these bacteria. (Note that α-aminobenzylpenicillins exist as zwitterions over a broad *p*H range and, as such, are considerably less polar than carbenicillin.) This increased polarity is ap-

parently an advantage of the penetration of carbenicillin through the cell envelope of gram-negative bacteria via porin channels.[35]

Carbenicillin is active against both β-lactamase–producing and non-β-lactamase–producing strains of gram-negative bacteria. It is known to be somewhat resistant to a few of the β-lactamases produced by gram-negative bacteria, especially members of the Enterobacteriaceae family.[36] Resistance to β-lactamases elaborated by gram-negative bacteria, therefore, may be an important component of carbenicillin's activity against some ampicillin-resistant organisms. However, β-lactamases produced by *Pseudomonas* species readily hydrolyzes carbenicillin. Although carbenicillin is also somewhat resistant to staphylococcal β-lactamase, it is considerably less so than methicillin or the isoxazoyl penicillins, and its inherent antistaphylococcal activity is less impressive compared with that of the penicillinase-resistant penicillins. However, the penicillinase-resistant penicillins, despite their resistance to most β-lactamases, share penicillin G's lack of activity against gram-negative bacilli, primarily because of an inability to penetrate the bacterial cell envelope.

When compared with the aminoglycoside antibiotics, the potency of carbenicillin against such gram-negative bacilli as *Ps. aeruginosa, Proteus vulgaris*, and *K. pneumoniae* is much less impressive. Large parenteral doses thus are required to achieve bactericidal concentrations in plasma and tissues. However, the low toxicity of carbenicillin (and the penicillins in general) usually permits (in the absence of allergy) the use of such high doses without untoward effects. Furthermore, carbenicillin (and other penicillins), when combined with aminoglycosides, exerts a synergistic *-cidal* action against bacterial species sensitive to both agents, frequently allowing the use of a lower dose of the more toxic aminoglycoside than normally required for treatment of a life-threatening infection. The chemical incomparability of penicillins and aminoglycosides requires that the two antibiotics be administered separately; otherwise, both are inactivated. Iyengar et al.[37] have shown that acylation of amino groups in the aminoglycoside by the β-lactam of the penicillin occurs.

Unlike the situation with ampicillin, the introduction of asymmetry at the α-benzyl carbon in carbenicillin imparts little or no stereoselectivity of antibacterial action; the individual enantiomers are nearly equally active and readily epimerized to the racemate in aqueous solution. Because it is a derivative of phenylmalonic acid, carbenicillin readily decarboxylates to benzylpenicillin in the presence of acid; therefore, it is not active (as carbenicillin) orally and must be administered parenterally. Esterification of the α-carboxyl group (e.g., as the 5-indanyl ester) partially protects the compound from acid-catalyzed destruction and provides an orally active derivative that is hydrolyzed to carbenicillin in the plasma. However, the plasma levels of free carbenicillin achievable with oral administration of such esters may not be sufficiently high to treat effectively serious infections caused by some species of gram-negative bacilli, such as *Ps. aeruginosa*.

A series of α-acylureido-substituted penicillins, exemplified by azlocillin, mezlocillin, and piperacillin, exhibit enhanced activity against certain gram-negative bacilli compared with carbenicillin. Although the acylureidopenicillins are acylated derivatives of ampicillin, the antibacterial spectrum of activity of the group is more like that of carbenicillin. The acylureidopenicillins are, however, superior to carbenicillin against *Klebsiella* sp., *Enterobacter* sp., and *Ps. aeruginosa*. This enhanced activity is apparently not due to β-lactamase resistance because both inducible and plasmid-mediated β-lactamases hydrolyze these penicillins. More facile penetration through the cell envelope of these particular bacterial species is the most likely explanation for the greater potency. The acylureidopenicillins, unlike ampicillin, are unstable under acidic conditions; therefore, they are not available for oral administration.

PROTEIN BINDING

The nature of the acylamino side chain also determines the extent to which penicillins are plasma protein-bound. Quantitative structure–activity relationship (QSAR) studies of the binding of penicillins to human serum[38,39] indicate that hydrophobic groups (positive π dependence) in the side chain appear to be largely responsible for increased binding to serum proteins. Penicillins with polar or ionized substituents in the side chain exhibit low to intermediate fractions of protein binding. Accordingly, ampicillin, amoxicillin, and cyclacillin experience 25% to 30% protein binding; carbenicillin and ticarcillin show 45% to 55%. Those with nonpolar, lipophilic substituents (nafcillin and isoxazoylpenicillins) are highly protein-bound, with fractions exceeding 90%. The penicillins with less complex acyl groups (benzylpenicillin, phenoxymethylpenicillin, and methicillin) fall in the range of 35% to 80%. Protein binding is thought to restrict the tissue availability of drugs if the fraction of binding is sufficiently high; thus, the tissue distribution of the penicillins in the highly bound group may be inferior to that of other penicillins. The similarity of biologic half-lives for various penicillins, however, indicates that plasma protein binding has little effect on duration of action. All of the commercially available penicillins are secreted actively by the renal active-transport system for anions. The reversible nature of protein binding does not compete effectively with the active tubular secretion process.

ALLERGY TO PENICILLINS

Allergic reactions to various penicillins, ranging in severity from a variety of skin and mucous membrane rashes to drug fever and anaphylaxis, constitute the major problem associated with the use of this class of antibiotics. Estimates place the prevalence of hypersensitivity to penicillin G throughout the world at between 1% and 10% of the population. In the United States and other industrialized countries, it is nearer

to the higher figure, ranking penicillin as the most common cause of drug-induced allergy. The penicillins most frequently implicated as causes of allergic reactions are penicillin G and ampicillin. However, virtually all commercially available penicillins have been reported to cause such reactions and, in fact, cross-sensitivity among most chemical classes of 6-acylaminopenicillanic acid derivatives has been demonstrated.[40]

The chemical mechanisms by which penicillin preparations become antigenic have been studied extensively.[20] Evidence suggests that penicillins, or their rearrangement products formed in vivo (e.g., penicillenic acids),[41] react with lysine-ϵ-amino groups of proteins to form penicilloyl proteins, which are major antigenic determinants.[42,43] Early clinical observations with the biosynthetic penicillins G and V indicated a higher incidence of allergic reactions with unpurified, amorphous preparations compared with highly purified, crystalline forms, suggesting that small amounts of highly antigenic penicilloyl proteins present in unpurified samples were a cause. Polymeric impurities in ampicillin dosage forms have been implicated as possible antigenic determinants and as a possible explanation for the high frequency of allergic reactions with this particular semisynthetic penicillin. Ampicillin is known to undergo *p*H-dependent polymerization reactions (especially in concentrated solutions) that involve nucleophilic attack of the side chain amino group of one molecule on the β-lactam carbonyl carbon atom of a second molecule, and so on.[44] The high frequency of antigenicity shown by ampicillin polymers together with their isolation and characterization in some ampicillin preparations support the theory that they can contribute to ampicillin-induced allergy.[45]

CLASSIFICATION

A variety of designations has been used for classifying penicillins, based on their sources, chemistry, pharmacokinetic properties, resistance to enzymatic spectrum of activity, and clinical uses (Table 10-3). Thus, penicillins may be biosynthetic, semisynthetic, or (potentially) synthetic; acid-resistant or not; orally or (only) parenterally active; and resistant to β-lactamases (penicillinases) or not. They may have a narrow, intermediate, broad, or extended spectrum of antibacterial activity and may be intended for multipurpose or limited clinical use. In the latter two connections, it is important to emphasize that designations of the activity spectrum as narrow, intermediate, broad, or extended are relative and do not necessarily imply the breadth of therapeutic application. Indeed, the classification of penicillin G as a "narrow-spectrum" antibiotic has meaning only relative to other penicillins. Although the β-lactamase–resistant penicillins have a spectrum of activity similar to that of penicillin G, they generally are reserved for the treatment of infections caused by penicillin G-resistant, β-lactamase–producing *Staph. aureus* because their activity against most penicillin G-sensitive bacteria is significantly inferior. Similarly, carbenicillin and ticarcillin usually are reserved for the treatment of infections caused by ampicillin-resistant, gram-negative bacilli because they offer no advantage (and have some disadvantages) to ampicillin or penicillin G in infections sensitive to them.

PRODUCTS

Penicillin G, Benzylpenicillin

For years, the most popular penicillin has been benzylpenicillin. In fact, with the exception of patients allergic to it, penicillin G remains the agent of choice for the treatment of more different kinds of bacterial infection than any other antibiotic. It was first made available in the form of the water-soluble salts of potassium, sodium, and calcium. These salts of penicillin are inactivated by the gastric juice and are not effective when administered orally unless antacids, such as calcium

TABLE 10-3.

CLASSIFICATION AND PROPERTIES OF PENICILLINS

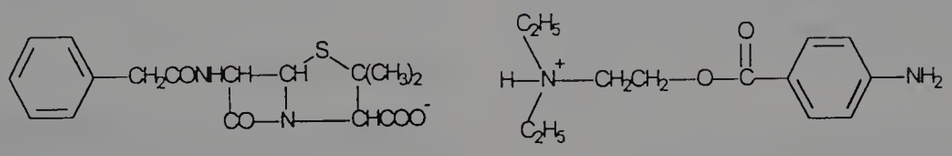
Penicillin	*Source*	*Acid Resistance*	*Oral Absorption (%)*	*Plasma Protein Binding (%)*	*β-Lactamase-Resistant (S. aureus)*	*Spectrum of Activity*	*Clinical Use*
Benzylpenicillin	Biosynthetic	Poor	Poor (20)	50–60	No	Intermediate	Multipurpose
Phenoxymethylpenicillin	Biosynthetic	Good	Good (60)	55–80	No	Intermediate	Multipurpose
Methicillin	Semisynthetic	Poor	Nil	30–40	Yes	Narrow	Limited use
Nafcillin	Semisynthetic	Fair	Variable	90	Yes	Narrow	Limited use
Oxacillin	Semisynthetic	Good	Fair (30)	85–94	Yes	Narrow	Limited use
Cloxacillin	Semisynthetic	Good	Good (50)	88–96	Yes	Narrow	Limited use
Dicloxacillin	Semisynthetic	Good	Good (50)	95–98	Yes	Narrow	Limited use
Ampicillin	Semisynthetic	Good	Fair (40)	20–25	No	Broad	Multipurpose
Amoxicillin	Semisynthetic	Good	Good (75)	20–25	No	Broad	Multipurpose
Carbenicillin	Semisynthetic	Poor	Nil	50–60	No	Extended	Limited use
Ticarcillin	Semisynthetic	Poor	Nil	45	No	Extended	Limited use
Mezlocillin	Semisynthetic	Poor	Nil	50	No	Extended	Limited use
Piperacillin	Semisynthetic	Poor	Nil	50	No	Extended	Limited use

carbonate, aluminum hydroxide, and magnesium trisilicate, or a strong buffer, such as sodium citrate, are added. Also, because penicillin is absorbed poorly from the intestinal tract, oral doses must be very large, about five times the amount necessary with parenteral administration. Only after the production of penicillin had increased sufficiently that low-priced penicillin was available did the oral dosage forms become popular. The water-soluble potassium and sodium salts are used orally and parenterally to rapidly achieve high plasma concentrations of penicillin G. The more water-soluble potassium salt usually is preferred when large doses are required. However, situations in which hyperkalemia is a danger, as in renal failure, require use of the sodium salt; the potassium salt is preferred for patients on salt-free diets or with congestive heart conditions.

The rapid elimination of penicillin from the bloodstream through the kidneys by active tubular secretion and the need for maintaining an effective blood level concentration have led to the development of "repository" forms of this drug. Suspensions of penicillin in peanut oil or sesame oil with white beeswax added were first employed for prolonging the duration of injected forms of penicillin. This dosage form was replaced by a suspension in vegetable oil, to which aluminum monostearate or aluminum distearate was added. Today, most repository forms are suspensions of high-molecular-weight amine salts of penicillin in a similar base.

Penicillin G Procaine, USP (Crysticillin, Duracillin, Wycillin)

The first widely used amine salt of penicillin G was made with procaine. It can be made readily from penicillin G sodium by treatment with procaine hydrochloride. This salt is considerably less soluble in water than are the alkali metal salts, requiring about 250 mL to dissolve 1 g. Free penicillin is released only as the compound dissolves and dissociates. It has an activity of 1,009 units/mg. A large number of preparations for injection of penicillin G procaine are commercially available. Most of these are either suspensions in water to which a suitable dispersing or suspending agent, a buffer, and a preservative have been added or suspensions in peanut oil or sesame oil that have been gelled by the addition of 2% aluminum monostearate. Some commercial products are mixtures of penicillin G potassium or sodium with penicillin G procaine to provide rapid development of a high plasma concentration of penicillin through use of the water-soluble salt plus the prolonged duration of effect obtained from the insoluble salt.

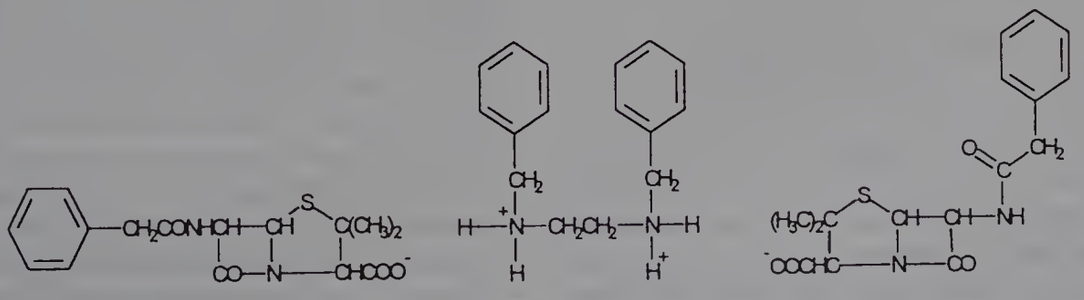


Penicillin G Procaine

Penicillin G Benzathine, USP

N,N'-Dibenzylethylenediamine dipenicillin G (Bicillin, Permapen). Since it is the salt of a diamine, 2 moles of penicillin are available from each molecule. It is very insoluble in water, requiring about 3,000 mL to dissolve 1 g. This property gives the compound great stability and prolonged duration of effect. At the *p*H of gastric juice it is quite stable, and food intake does not interfere with its absorption. It is available in tablet form and in a number of parenteral preparations. The activity of penicillin G benzathine is equivalent to 1,211 units/mg.

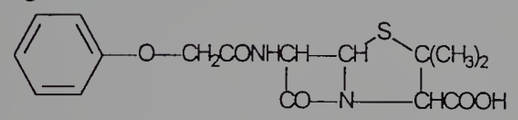
Several other amines have been used to make penicillin salts, and research is continuing on this subject. Other amines that have been used include 2-chloroprocaine; L-*N*-methyl-1,2-diphenyl-2-hydroxyethylamine (L-ephenamine); dibenzylamine; tripelennamine (Pyribenzamine); and *N,N'*-bis-(dehydroabietyl)ethylenediamine (hydrabamine).



Penicillin G Benzathine

Penicillin V, USP

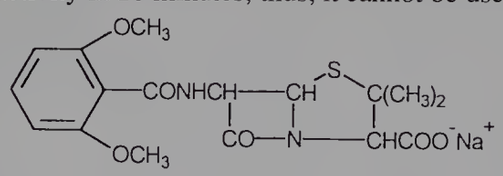
Phenoxymethylpenicillin (Pen Vee, V-Cillin) was reported by Behrens et al.[46] in 1948 as a biosynthetic product. However, it was not until 1953 that its clinical value was recognized by some European scientists. Since then, it has enjoyed wide use because of its resistance to hydrolysis by gastric juice and its ability to produce uniform concentrations in blood (when administered orally). The free acid requires about 1,200 mL of water to dissolve 1 g, and it has an activity of 1,695 units/mg. For parenteral solutions, the potassium salt is usually employed. This salt is very soluble in water. Solutions of it are made from the dry salt at the time of administration. Oral dosage forms of the potassium salt are also available, providing rapid, effective plasma concentrations of this penicillin. The salt of phenoxymethylpenicillin with *N,N'*-bis(dehydroabietyl)ethylenediamine (hydrabamine, Compocillin-V) provides a very long-acting form of this compound. Its high degree of water insolubility makes it a desirable compound for aqueous suspensions used as liquid oral dosage forms.



Penicillin V

Methicillin Sodium, USP

2,6-Dimethoxyphenylpenicillin sodium (Staphcillin). During 1960, the second penicillin produced as a result of the research that developed synthetic analogues was introduced for medicinal use. By reacting 2,6-dimethoxybenzoyl chloride with 6-APA, 6-(2,6-dimethoxybenzamido)penicillanic acid forms. The sodium salt is a white, crystalline solid that is extremely soluble in water, forming clear, neutral solutions. As with other penicillins, it is very sensitive to moisture, losing about half of its activity in 5 days at room temperature. Refrigeration at 5°C reduces the loss in activity to about 20% in the same period. Solutions prepared for parenteral use may be kept as long as 24 hr if refrigerated. It is extremely sensitive to acid, a *p*H of 2 causing a 50% loss of activity in 20 minutes; thus, it cannot be used orally.



Methicillin Sodium

Methicillin sodium is particularly resistant to inactivation by the penicillinase found in staphylococcal organisms and somewhat more resistant than penicillin G to penicillinase from *Bacillus cereus.* Methicillin and many other penicillinase-resistant penicillins are inducers of penicillinase, an observation that has implications against the use of these agents in the treatment of penicillin G-sensitive infections. Clearly, the use of a penicillinase-resistant penicillin should not be followed by penicillin G.

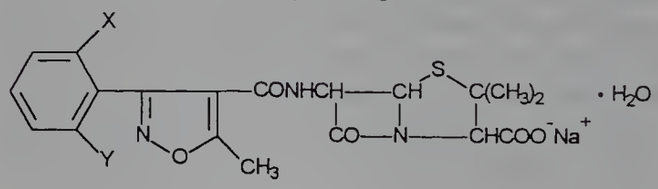
It may be assumed that the absence of the benzylmethylene group of penicillin G and the steric protection afforded by the 2- and 6-methoxy groups make this compound particularly resistant to enzyme hydrolysis.

Methicillin sodium has been introduced for use in the treatment of staphylococcal infections caused by strains found resistant to other penicillins. It is recommended that it not be used in general therapy to avoid the possible widespread development of organisms resistant to it.

The incidence of interstitial nephritis, a probable hypersensitivity reaction, is reportedly higher with methicillin than with other penicillins.

Oxacillin Sodium, USP

(5-Methyl-3-phenyl-4-isoxazolyl)penicillin sodium monohydrate (Prostaphlin). Oxacillin sodium is the salt of a semisynthetic penicillin that is highly resistant to inactivation by penicillinase. Apparently, the steric effects of the 3-phenyl and 5-methyl groups of the isoxazolyl ring prevent the binding of this penicillin to the β-lactamase active site and, thereby, protect the lactam ring from degradation in much the same way as has been suggested for methicillin. It is also relatively resistant to acid hydrolysis and, therefore, may be administered orally with good effect.



Oxacillins

X; Y = H: Sodium Oxacillin
X = Cl; Y = H: Sodium Cloxacillin
X; Y = Cl: Sodium Dicloxacillin
X = Cl; Y = F: Sodium Floxicillin

Oxacillin sodium, which is available in capsule form, is reasonably well absorbed from the gastrointestinal tract, particularly in fasting patients. Effective plasma levels of oxacillin are obtained in about 1 hr, but despite extensive plasma protein binding, it is excreted rapidly through the kidneys. Oxacillin experiences some first-pass metabolism in the liver to the 5-hydroxymethyl derivative. This metabolite has comparable antibacterial activity to oxacillin but is less avidly protein-bound and more rapidly excreted. The halogenated analogues cloxacillin, dicloxacillin, and floxicillin experience less 5-methyl hydroxylation.

The use of oxacillin and other isoxazolylpenicillins should be restricted to the treatment of infections caused by staphy-

lococci resistant to penicillin G. Although their spectrum of activity is similar to that of penicillin G, the isoxazolylpenicillins are, in general, inferior to it and the phenoxymethylpenicillins for the treatment of infections caused by penicillin G-sensitive bacteria. Because they cause allergic reactions similar to those produced by other penicillins, the isoxazolylpenicillins should be used with great caution in patients who are penicillin-sensitive.

Cloxacillin Sodium, USP

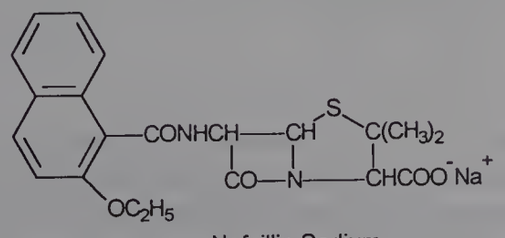
[3-(*o*-Chlorophenyl)-5-methyl-4-isoxazolyl]penicillin sodium monohydrate (Tegopen). The chlorine atom *ortho* to the position of attachment of the phenyl ring to the isoxazole ring enhances the activity of this compound over that of oxacillin, not by an increase in intrinsic antibacterial activity but by enhancing its oral absorption, leading to higher plasma levels. In almost all other respects, it resembles oxacillin.

Dicloxacillin Sodium, USP

[3-(2,6-Dichlorophenyl)-5-methyl-4-isoxazolyl]penicillin sodium monohydrate (Dynapen, Pathocil, Veracillin). The substitution of chlorine atoms on both carbons *ortho* to the position of attachment of the phenyl ring to the isoxazole ring is presumed to enhance further the stability of this oxacillin congener and to produce high plasma concentrations of it. Its medicinal properties and use are similar to those of cloxacillin sodium. However, progressive halogen substitution also increases the fraction of protein binding in the plasma, potentially reducing the concentration of free antibiotic in plasma and tissues. Its medicinal properties and use are the same as those of cloxacillin sodium.

Nafcillin Sodium, USP

6-(2-Ethoxy-1-naphthyl)penicillin sodium (Unipen). Nafcillin sodium is another semisynthetic penicillin produced as a result of the search for penicillinase-resistant compounds. Similar to methicillin, nafcillin has substituents in positions *ortho* to the point of attachment of the aromatic ring to the carboxamide group of penicillin. No doubt, the ethoxy group and the second ring of the naphthalene group play steric roles in stabilizing nafcillin against penicillinase. Very similar structures have been reported to produce similar results in some substituted 2-biphenyl-penicillins.[33]



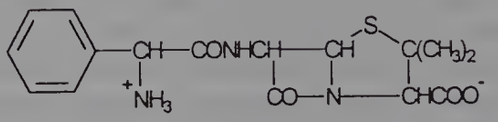
Nafcillin Sodium

Unlike methicillin, nafcillin is sufficiently stable in acid to permit its use by oral administration. When given orally, its absorption is somewhat slow and incomplete, but satisfactory plasma levels may be achieved in about 1 hr. Relatively small amounts are excreted through the kidneys, with the major portion excreted in the bile. Even though some cyclic reabsorption from the gut may occur, nafcillin should be readministered every 4 to 6 hr when given orally. This salt is readily soluble in water and may be administered intramuscularly or intravenously to quickly obtain high plasma concentrations for the treatment of serious infections.

Nafcillin sodium may be used in infections caused solely by penicillin G-resistant staphylococci or when streptococci are present also. Although it is recommended that it be used exclusively for such resistant infections, it is also effective against pneumococci and group A β-hemolytic streptococci. Because, as with other penicillins, it may cause allergic side effects, it should be administered with care.

Ampicillin, USP

6-[D-α-Aminophenylacetamido]penicillanic acid, D-α-aminobenzylpenicillin (Penbritin, Polycillin, Omnipen, Amcil, Principen). With ampicillin, another goal in the research on semisynthetic penicillins—an antibacterial spectrum broader than that of penicillin G—has been attained. This product is active against the same gram-positive organisms that are susceptible to other penicillins, and it is more active against some gram-negative bacteria and enterococcal infections than are other penicillins. Obviously, the α-amino group plays an important role in the broader activity, but the mechanism for its action is unknown. It has been suggested that the amino group confers an ability to cross cell wall barriers that are impenetrable to other penicillins. It is noteworthy that D-(−)-ampicillin, prepared from D-(−)-α-aminophenylacetic acid, is significantly more active than L-(+)-ampicillin.



Ampicillin

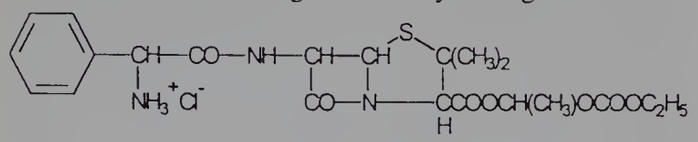
Ampicillin is not resistant to penicillinase, and it produces the allergic reactions and other untoward effects that are found in penicillin-sensitive patients. However, because such reactions are relatively few, it may be used to treat those infections caused by gram-negative bacilli for which a broad-spectrum antibiotic, such as a tetracycline or chloramphenicol, may be indicated but not preferred because of undesirable reactions or lack of bactericidal effect. However, ampicillin is not so widely active that it should be used as a broad-spectrum antibiotic in the same manner as the tetracyclines. It is particularly useful for the treatment of acute urinary tract infections caused by *E. coli* or *Proteus mirabilis* and is the agent of choice against *Haemophilus influenzae*

infections. Ampicillin together with probenecid, to inhibit its active tubular excretion, has become a treatment of choice for gonorrhea in recent years. However, β-lactamase–producing strains of gram-negative bacteria that are highly resistant to ampicillin appear to be increasing in the world population. The threat from such resistant strains is particularly great with *H. influenzae* and *N. gonorrhoeae* because few alternative therapies for infections caused by these organisms are available. Incomplete absorption together with excretion of effective concentrations in the bile may contribute to the effectiveness of ampicillin in the treatment of salmonellosis and shigellosis.

Ampicillin is water-soluble and stable in acid. The protonated α-amino group of ampicillin has a pK_a of 7.3,[46] and, thus, it is protonated extensively in acidic media, which explains ampicillin's stability toward acid hydrolysis and instability toward alkaline hydrolysis. It is administered orally and is absorbed from the intestinal tract to produce peak plasma concentrations in about 2 hr. Oral doses must be repeated about every 6 hr because it is excreted rapidly and unchanged through the kidneys. It is available as a white, crystalline, anhydrous powder that is sparingly soluble in water or as the colorless or slightly buff-colored crystalline trihydrate that is soluble in water. Either form may be used for oral administration, in capsules or as a suspension. Earlier claims of higher plasma levels for the anhydrous form compared with the trihydrate following oral administration have been disputed.[47,48] The white, crystalline sodium salt is very soluble in water, and solutions for injections should be administered within 1 hr after being made.

Bacampicillin Hydrochloride, USP (Spectrobid)

Bacampicillin hydrochloride is the hydrochloride salt of the 1-ethoxycarbonyloxyethyl ester of ampicillin. It is a prodrug of ampicillin with no antibacterial activity. After oral absorption, bacampicillin is hydrolyzed rapidly by esterases in the plasma to form ampicillin. Oral absorption of bacampicillin is more rapid and more complete than that of ampicillin and less affected by food. Plasma levels of ampicillin from oral bacampicillin exceed those of oral ampicillin or amoxicillin for the first 2.5 hr but, thereafter, are the same as for ampicillin and amoxicillin.[49] Effective plasma levels are sustained for 12 hr, allowing twice-a-day dosing.

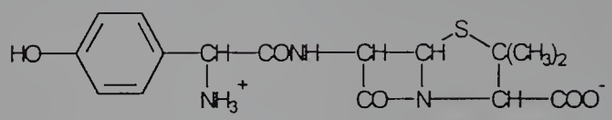


Bacampicillin Hydrochloride

Amoxicillin, USP

6-[D-(−)-α-Amino-*p*-hydroxyphenylacetamido]penicillanic acid (Amoxil, Larotid, Polymox). Amoxicillin, a semisynthetic penicillin introduced in 1974, is simply the *p*-hydroxy analogue of ampicillin prepared by the acylation of 6-APA with *p*-hydroxyphenylglycine. Its antibacterial spectrum is nearly identical to that of ampicillin, and, like ampicillin, it is resistant to acid, susceptible to alkaline and β-lactamase hydrolysis, and weakly protein-bound. Early clinical reports indicated that orally administered amoxicillin possesses significant advantages over ampicillin, including more complete gastrointestinal absorption to give higher plasma and urine levels, less diarrhea, and little or no effect of food on absorption.[50] Thus, amoxicillin has largely replaced ampicillin for the treatment of certain systemic and urinary tract infections for which oral administration is desirable. Amoxicillin is reported to be less effective than ampicillin in the treatment of bacillary dysentery, presumably because of its greater gastrointestinal absorption. Considerable evidence suggests that oral absorption of α-aminobenzyl–substituted penicillins (e.g., ampicillin and amoxicillin) and cephalosporins is, at least in part, carrier-mediated,[51] thus explaining their generally superior oral activity.

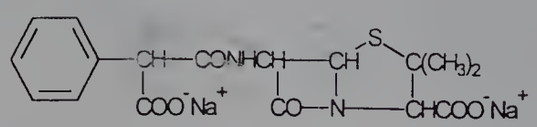
Amoxicillin is a fine, white to off-white, crystalline powder that is sparingly soluble in water. It is available in a variety of oral dosage forms. Aqueous suspensions are stable for 1 week at room temperature.



Amoxicillin

Carbenicillin Disodium, Sterile, USP

Disodium α-carboxybenzylpenicillin (Geopen, Pyopen) is a semisynthetic penicillin released in the United States in 1970, which was introduced in England and first reported by Ancred et al.[52] in 1967. Examination of its structure shows that it differs from ampicillin by having an ionizable carboxyl group, rather than an amino group, substituted on the α-carbon atom of the benzyl side chain. Carbenicillin has a broad range of antimicrobial activity, broader than any other known penicillins, a property attributed to the unique carboxyl group. It has been proposed that the carboxyl group confers improved penetration of the molecule through cell wall barriers of gram-negative bacilli, compared with other penicillins.



Carbenicillin Disodium

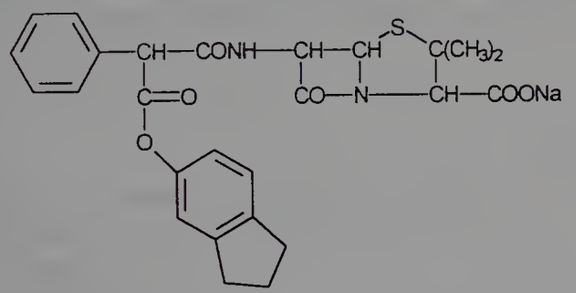
Carbenicillin is not stable in acids and is inactivated by penicillinase. It is a malonic acid derivative and, as such, decarboxylates readily to penicillin G, which is acid-labile. Solutions of the disodium salt should be freshly prepared

but may be kept for 2 weeks when refrigerated. It must be administered by injection and is usually given intravenously.

Carbenicillin has been effective in the treatment of systemic and urinary tract infections caused by *Ps. aeruginosa,* indole-producing *Proteus,* and *Providencia* species, all of which are resistant to ampicillin. The low toxicity of carbenicillin, with the exception of allergic sensitivity, permits the use of large dosages in serious infections. Most clinicians prefer to use a combination of carbenicillin and gentamicin for serious pseudomonad and mixed coliform infections. However, the two antibiotics are chemically incompatible and should never be combined in the same intravenous solution.

Carbenicillin Indanyl Sodium, USP

6-[2-Phenyl-2-(5-indanyloxycarbonyl)acetamido]penicillanic acid (Geocillin). Efforts to obtain orally active forms of carbenicillin led to the eventual release of the 5-indanylester in 1972. Approximately 40% of the usual oral dose of indanyl carbenicillin is absorbed. After absorption, the ester is hydrolyzed rapidly by plasma and tissue esterases to yield carbenicillin. Thus, although the highly lipophilic and highly protein-bound ester has in vitro activity comparable with carbenicillin, its activity in vivo is due to



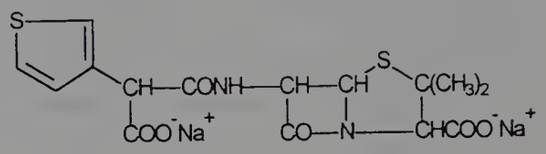
Carbenicillin Indanyl Sodium

carbenicillin. Indanyl carbenicillin thus provides an orally active alternative for the treatment of carbenicillin-sensitive systemic and urinary tract infections caused by *Pseudomonas,* indole-positive *Proteus* species, and selected species of gram-negative bacilli.

In clinical trials with indanyl carbenicillin, a relatively high frequency of gastrointestinal symptoms (nausea, occasional vomiting, and diarrhea) was reported. It seems doubtful that the high doses required for the treatment of serious systemic infections could be tolerated by most patients. Indanyl carbenicillin occurs as the sodium salt, an off-white, bitter powder that is freely soluble in water. It is stable in acid. It should be protected from moisture to prevent hydrolysis of the ester.

Ticarcillin Disodium, Sterile, USP

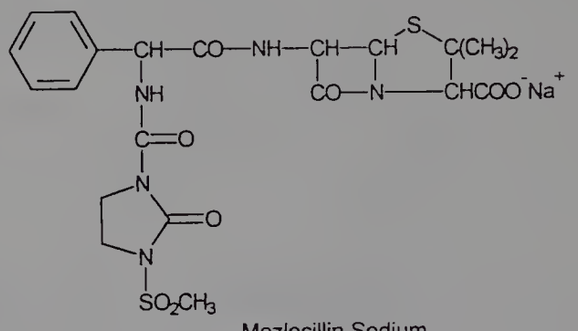
α-Carboxy-3-thienylpenicillin (Ticar) is an isostere of carbenicillin, wherein the phenyl group is replaced by a thienyl group. This semisynthetic penicillin derivative, like carbenicillin, is unstable in acid and, therefore, must be administered parenterally. It is similar to carbenicillin in antibacterial spectrum and pharmacokinetic properties. Two advantages for ticarcillin are claimed: (1) slightly better pharmacokinetic properties, including higher serum levels and a longer duration of action, and (2) greater in vitro potencies against several species of gram-negative bacilli, most notably *Ps. aeruginosa* and *Bacteroides fragilis.* These advantages can be crucial in the treatment of serious infections requiring high-dose therapy.



Ticarcillin Disodium

Mezlocillin Sodium, Sterile, USP (Mezlin)

Mezlocillin is an acylureidopenicillin with an antibacterial spectrum similar to that of carbenicillin and ticarcillin; however, there are some major differences. It is much more active against most *Klebsiella* species, *Ps. aeruginosa*, anaerobic bacteria (such as *Streptococcus faecalis* and *B. fragilis*), and *H. influenzae*. It is recommended for the treatment of serious infections caused by these organisms.



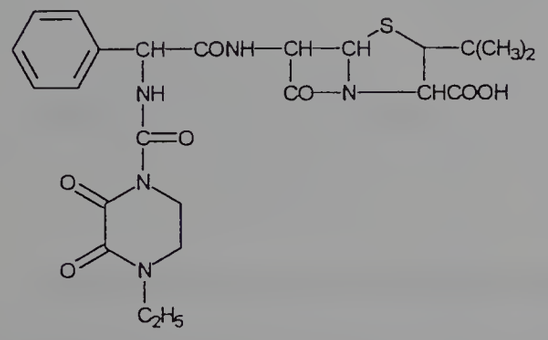
Mezlocillin Sodium

Mezlocillin is not generally effective against β-lactamase–producing bacteria, nor is it active orally. It is available as a white, crystalline, water-soluble sodium salt for injection. Solutions should be prepared freshly and refrigerated if not used within 24 hr. Mezlocillin and other acylureidopenicillins, unlike carbenicillin, exhibit nonlinear pharmacokinetics. Peak plasma levels, half-life, and area under the time curve increase with increased dosage. The effect of mezlocillin on bleeding time is less than that of carbenicillin, and it is less likely to cause hypokalemia.

Piperacillin Sodium, Sterile, USP (Pipracil)

Piperacillin is the more generally useful of the extended-spectrum acylureidopenicillins. It is more active than mezlocillin against susceptible strains of gram-negative aerobic

bacilli, such as *Serratia marcescens, Proteus, Enterobacter, Citrobacter,* and *Ps. aeruginosa.* However, mezlocillin appears to be more active against *Providencia* species and *K. pneumoniae.* Piperacillin is also active against anaerobic bacteria, especially *B. fragilis* and *Streptococcus faecalis* (enterococcus). β-Lactamase–producing strains of these organisms are, however, resistant to piperacillin, which is hydrolyzed by *Staph. aureus* β-lactamase. β-Lactamase susceptibility of piperacillin is not absolute because β-lactamase–producing, ampicillin-resistant strains of *N. gonorrhoeae* and *H. influenzae* are susceptible to piperacillin.



Piperacillin

Piperacillin is destroyed rapidly by stomach acid; therefore, it is active only by intramuscular or intravenous administration. The injectable form is provided as the white, crystalline, water-soluble sodium salt. Its pharmacokinetic properties are very similar to those of the other acylureidopenicillins.

β-LACTAMASE INHIBITORS

The strategy of using a β-lactamase inhibitor in combination with a β-lactamase–sensitive penicillin in the therapy of infections caused by β-lactamase–producing bacterial strains, has, until relatively recently, failed to live up to its obvious promise. Early attempts to obtain synergy against such resistant strains, utilizing combinations consisting of a β-lactamase–resistant penicillin (such as methicillin or oxacillin) as a competitive inhibitor and a β-lactamase–sensitive penicillin (such as ampicillin or carbenicillin) to kill the organisms, met with limited success. Factors that may contribute to the failure of such combinations to achieve synergy include (1) the failure of most lipophilic penicillinase-resistant penicillins to penetrate the cell envelope of gram-negative bacilli in effective concentrations; (2) the reversible binding of penicillinase–resistant penicillins to β-lactamase, requiring high concentrations to prevent substrate binding and hydrolysis; and (3) the induction of β-lactamases by some penicillinase-resistant penicillins.

The discovery of the naturally occurring, mechanism-based inhibitor clavulanic acid, which causes a potent and

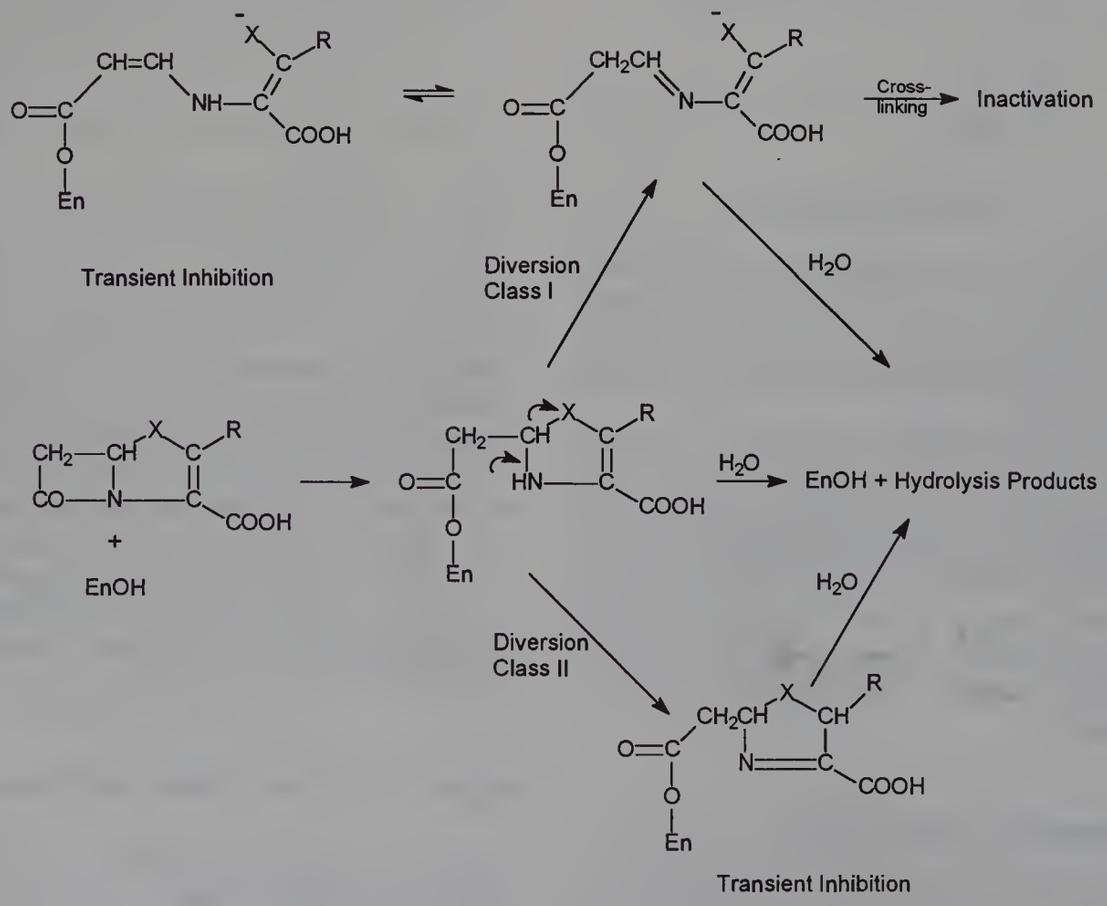


FIG. 10-4. Mechanism-based inhibition of β-lactamases.

progressive inactivation of β-lactamases (Fig. 10-4), has created renewed interest in β-lactam combination therapy. This interest has led to the design and synthesis of additional mechanism-based β-lactamase inhibitors, such as sulbactam and tazobactam, and the isolation of naturally occurring β-lactams, such as the thienamycins, which both inhibit β-lactamases and interact with PBPs.

The chemical events leading to the inactivation of β-lactamases by mechanism-based inhibitors are very complex. In a review of the chemistry of β-lactamase inhibition, Knowles[53] has described two classes of β-lactamase inhibitors: class I inhibitors, such as clavulanic acid and sulbactam, that have a hetero atom leaving group at position 1 and class II inhibitors, such as the carbapenems, that do not. Unlike competitive inhibitors, which bind reversibly with the enzyme they inhibit, mechanism-based inhibitors react with the enzyme in much the same way that the substrate does. With the β-lactamases, an acylenzyme intermediate is formed by reaction of the β-lactam with an active-site serine hydroxyl group of the enzyme. For normal substrates, the acylenzyme intermediate readily undergoes hydrolysis, destroying the substrate and freeing the enzyme to attack more substrate. The acylenzyme intermediate formed when a mechanism-based inhibitor is attacked by the enzyme is diverted by tautomerism to a more stable imine form that hydrolyzes more slowly to eventually free the enzyme (transient inhibition), or, for a class I inhibitor, a second group on the enzyme may be attacked to inactivate it. Because these inhibitors are also substrates for the enzymes that they inactivate, they are sometimes referred to as "suicide substrates."

Because they cause prolonged inactivation of certain β-lactamases, class I inhibitors are particularly useful in combination with extended-spectrum, β-lactamase–sensitive penicillins to treat infections caused by β-lactamase–producing bacteria. Three such inhibitors, clavulanic acid, sulbactam, and tazobactam, are currently marketed in the United States for this purpose. A class II inhibitor, the carbapenem derivative imipenem, has potent antibacterial activity in addition to its ability to cause transient inhibition of some β-lactamases. Certain antibacterial cephalosporins, with a leaving group at the C-3 position, can cause transient inhibition of β-lactamases by forming stabilized acylenzyme intermediates. These are discussed more fully later in this chapter.

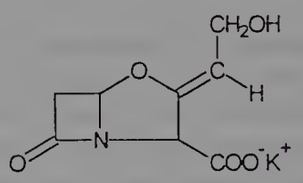
The relative susceptibilities of various β-lactamases to inactivation by class I inhibitors appear to be related to the molecular properties of the enzymes.[25,54,55] β-Lactamases belonging to group A, a large and somewhat heterogenous group of serine enzymes, some having narrow (e.g., penicillinases or cephalosporinases) and some broad (i.e., general β-lactamases) specificities, are generally inactivated by class I inhibitors. However, a large group of chromosomally encoded serine β-lactamases belonging to group C with specificity for cephalosporins are resistant to inactivation by class I inhibitors. A small group of Zn^{2+}-requiring metallo-β-lactamases (group B) with broad substrate specificities[56] are also not inactivated by class I inhibitors.

PRODUCTS

Clavulanate Potassium, USP

Clavulanic acid is an antibiotic isolated from *Streptomyces clavuligeris*. Structurally it is a 1-oxopenam lacking the 6-acylamino side chain of penicillins but possessing a 2-hydroxyethylidene moiety at C-2. Clavulanic acid exhibits very weak antibacterial activity, comparable with 6-APA and, therefore, is not useful as an antibiotic. It is, however, a potent inhibitor of *Staph. aureus* β-lactamase and plasmid-mediated β-lactamases elaborated by gram-negative bacilli.

Combinations of amoxicillin and the potassium salt of clavulanic acid are available (Augmentin) in a variety of fixed-dose, oral dosage forms intended for the treatment of skin, respiratory, ear, and urinary tract infections caused by β-lactamase–producing bacterial strains. These combinations are effective against β-lactamase–producing strains of *Staph. aureus, E. coli, K. pneumoniae, Enterobacter, H. influenzae, Moraxella catarrhalis*, and *H. ducreyi*, which are resistant to amoxicillin alone. The oral bioavailability of amoxicillin and potassium clavulanate is similar. Clavulanic acid is acid-stable. It cannot undergo penicillanic acid formation because it lacks an amide side chain.



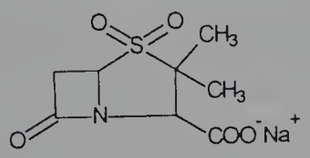
Clavulanate Potassium

Potassium clavulanate and the extended-spectrum penicillin ticarcillin have been combined in a fixed-dose, injectable form for the control of serious infections caused by β-lactamase–producing bacterial strains. This combination has been recommended for septicemia, lower respiratory tract infections, and urinary tract infections caused by β-lactamase–producing *Klebsiella, E. coli, Ps. aeruginosa* and other *Pseudomonas* species, *Citrobacter, Enterobacter, S. marcescens*, and *Staph. aureus*. It also is used in bone and joint infections caused by these organisms. The combination contains 3 g of ticarcillin disodium and 100 mg of potassium clavulanate in a sterile powder for injection (Timentin).

Sulbactam, USP

Sulbactam is penicillanic acid sulfone or 1,1-dioxopenicillanic acid. This synthetic penicillin derivative is a potent inhibitor of *Staph. aureus* β-lactamase, as well as many β-

lactamases elaborated by gram-negative bacilli. Sulbactam has weak intrinsic antibacterial activity but potentiates the activity of ampicillin and carbenicillin against β-lactamase–producing *Staph. aureus* and members of the *Enterobacteriaceae* family. However, it does not synergize with either carbenicillin or ticarcillin against *Ps. aeruginosa* strains resistant to these agents. Failure of sulbactam to penetrate the cell envelope is a possible explanation for the lack of synergy.



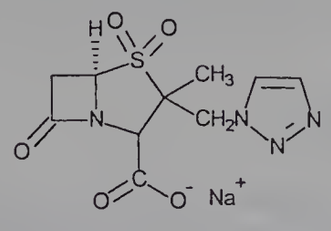
Sulbactam Sodium

Fixed-dose combinations of ampicillin sodium and sulbactam sodium, marketed under the trade name Unasyn as sterile powders for injection, have been approved for use in the United States. These combinations are recommended for the treatment of skin, tissue, intra-abdominal, and gynecologic infections caused by β-lactamase–producing strains of *Staph. aureus, E. coli, Klebsiella, Pr. mirabilis, B. fragilis, Enterobacter*, and *Acinetobacter.*

Tazobactam, USP

Tazobactam is a penicillanic acid sulfone that is similar in structure to sulbactam. As a β-lactamase inhibitor, it is more potent than sulbactam[57] and has a slightly broader spectrum of activity than clavulanic acid. It has very weak antibacterial activity. Tazobactam is available in fixed-dose, injectable combinations with piperacillin, a broad-spectrum penicillin consisting of an 8:1 ratio of piperacillin sodium to tazobactam sodium by weight and marketed under the trade name Zosyn. The pharmacokinetics of the two drugs are very similar. Both have short half-lives (t ½ ~ 1 hr), are minimally protein-bound, experience very little metabolism, and are excreted in active forms in the urine in high concentrations.

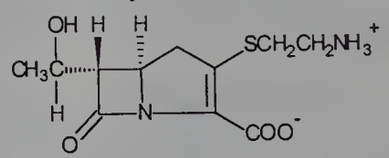
Approved indications for the piperacillin–tazobactam combination include the treatment of appendicitis, postpartum endometritis and pelvic inflammatory disease caused by β-lactamase–producing *E. coli* and *Bacteroides* species, skin and skin structure infections caused by β-lactamase–producing *Staph. aureus*, and pneumonia caused by β-lactamase–producing strains of *H. influenzae.*



Tazobactam Sodium

Carbapenems

Thienamycin. Thienamycin is a novel β-lactam antibiotic first isolated and identified by researchers at Merck[58] from fermentation of cultures of *Streptomyces cattleya*. Its structure and absolute configuration were established by both spectroscopic and total synthesis procedures.[59,60] Two structural features of thienamycin are shared with the penicillins and cephalosporins: a fused bicyclic ring system containing a β-lactam and an equivalently attached 3-carboxyl group. In other respects, the thienamycins represent a significant departure from the established β-lactam antibiotics. The bicyclic system consists of a carbapenem containing a double bond between C-2 and C-3 (i.e., it is a 2-carbapenem, or Δ^2-carbapenem, system). The double bond in the bicyclic structure creates considerable ring strain and increases the reactivity of the β-lactam to ring-opening reactions. The side chain is unique in two respects: it is a simple 1-hydroxyethyl group, instead of the familiar acylamino side chain, and it is oriented to the bicyclic ring system rather than having the usual β-orientation of the penicillins and cephalosporins. The remaining feature is a 2-aminoethylthioether function at C-2. The absolute stereochemistry of thienamycin has been determined to be 5R:6S:8S. Several additional structurally related antibiotics have been isolated from various *Streptomyces* species, including the four epithienamycins, which are isomeric to thienamycin at C-5, C-6, or C-8, and derivatives wherein the 2-aminoethylthio side chain is modified.



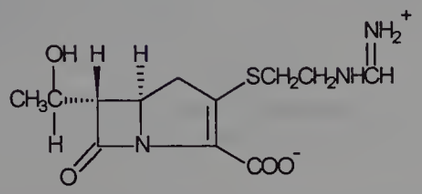
Thienamycin

Thienamycin displays outstanding broad-spectrum antibacterial properties in vitro.[61] It is highly active against most aerobic and anaerobic gram-positive and gram-negative bacteria, including *Staph. aureus, Ps. aeruginosa*, and *B. fragilis*. Furthermore, it is resistant to inactivation by most β-lactamases elaborated by gram-negative and gram-positive bacteria and, therefore, is effective against many strains resistant to penicillins and cephalosporins. Resistance to lactamases appears to be a function of the α-1-hydroxyethyl side chain because this property is lost in the 6-nor derivative and epithienamycins having S-stereochemistry show variable resistance to the different β-lactamases.

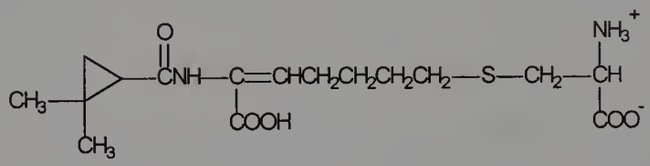
An unfortunate property of thienamycin is its chemical instability in solution. It is more susceptible to hydrolysis in both acidic and alkaline solutions than most β-lactam antibiotics, owing to the strained nature of its fused ring system containing an endocyclic double bond. Furthermore, at its optimally stable *p*H of between 6 and 7, thienamycin undergoes a concentration-dependent inactivation. This inactivation is believed to result from intermolecular aminolysis of the β-lactam by the cysteamine side chain of

a second molecule. Another shortcoming is its susceptibility to hydrolytic inactivation by renal dehydropeptidase-I (DHP-I),[62] which causes it to have an unacceptably short half-life in vivo.

Imipenem–Cilastatin, USP (Primaxin). Imipenem is *N*-formimidoylthienamycin, the most successful of a series of chemically stable derivatives of thienamycin in which the primary amino group is converted to a non-nucleophilic basic function.[63] Cilastatin is an inhibitor of DHP-I. The combination provides a chemically and enzymatically stable form of thienamycin that has clinically useful pharmacokinetic properties. The half-life of the drug is nonetheless short ($t_{1/2}$ ~ 1 hr) due to renal tubular secretion of imipenem.



Imipenem



Cilastatin

Imipenem retains the extraordinary broad-spectrum antibacterial properties of thienamycin. Its bactericidal activity results from the inhibition of cell wall synthesis associated with bonding to PBPs 1_b and 2. Imipenem is very stable to most β-lactamases. It is an inhibitor of β-lactamases from certain gram-negative bacteria resistant to other β-lactam antibiotics, for example, *Ps. aeruginosa, S. marcescens*, and *Enterobacter* sp.

Imipenem is indicated for the treatment of a wide variety of bacterial infections of the skin and tissues, lower respiratory tract, bones and joints, and genitourinary tract, as well as of septicemia and endocarditis caused by β-lactamase–producing strains of susceptible bacteria. These include aerobic gram-positive organisms such as *Staph. aureus, Staph. epidermidis*, enterococci, and *Streptococcus viridans*; aerobic gram-negative bacteria such as *E. coli, Klebsiella, Serratia, Providencia, Haemophilus, Citrobacter*, indole-positive *Proteus, Morganella morgani, Acinetobacter, Enterobacter*, and *Ps. aeroginosa*; and anaerobes such as *B. fragilis, Clostridium, Peptococcus, Peptidostreptococcus, Eubacterium*, and *Fusobacterium*. Some *Pseudomonas* species are resistant, such as *Ps. maltophilia* and *Ps. cepacia*, as are some methicillin-resistant staphylococci. Imipenem is effective against non–β-lactamase-producing strains of these and additional bacterial species, but other less expensive and equally effective antibiotics are preferred for the treatment of infections caused by these organisms.

The imipenem–cilastatin combination is marketed as a sterile powder intended for the preparation of solutions for intravenous infusion. Such solutions are stable for 4 hr at 25°C and up to 24 hr when refrigerated. The concomitant administration of imipenem with an aminoglycoside antibiotic results in synergistic antibacterial activity in vivo. The two types of antibiotics are, however, chemically incompatible and should never be combined in the same intravenous bottle.

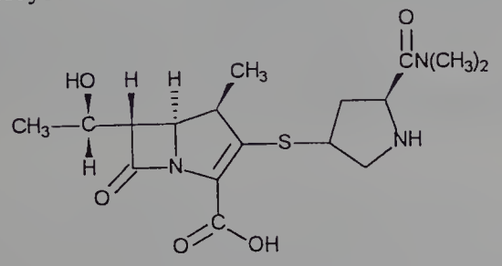
Investigational Carbopenems. The extended spectrum of antibacterial activity associated with the carbopenems together with their resistance to inactivation by most β-lactamases make this class of β-lactams an attractive target for drug development. In the design of new carbopenems, structural variations are being investigated with the objective of developing analogues with advantages over imipenem. Improvements that are particularly desired include stability to hydrolysis catalyzed by DHP-I,[62] stability to bacterial metallo-β-lactamases (''carbopenemases'')[56] that hydrolyze imipenem, activity against MRSA,[31] and increased potency against *Ps. aeruginosa*, especially imipenem-resistant strains. Enhanced pharmacokinetic properties, such as oral bioavailability and a longer duration of action, have heretofore received little emphasis in carbopenem analogue design.

Early structure–activity studies established the critical importance of the Δ^2 position of the double bond, the 3-carboxyl group, and the 6α-hydroxyethyl side chain for both broad-spectrum antibacterial activity and β-lactamase stability in carbopenems. Modifications, therefore, have concentrated on variations at positions 1 and 2 of the carbopenem nucleus. The incorporation of a β-methyl group at the 1-position confers stability of the carbopenems to hydrolysis by renal DHP-I.[64,65] However, substituents at the 2-position appear to affect primarily the spectrum of antibacterial activity of the carbopenems by influencing their penetration into bacteria. The capability of carbopenems to exist as zwitterionic structures (as exemplified by imipenem and biapenem), resulting from the combined features of a basic amine function attached to the 2-position and the 3-carboxyl group, may enable these molecules to enter bacteria via their charged porin channels.

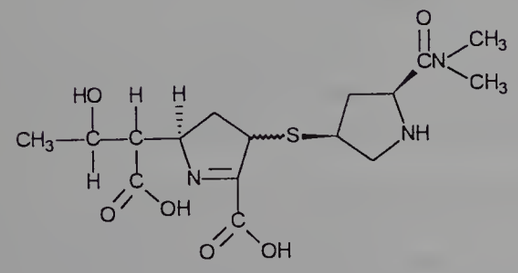
Meropenem. Meropenem is the second-generation carbopenem that has undergone the most extensive clinical evaluation to date.[66] It has recently been approved as Merrem for the treatment of infections caused by multiply resistant bacteria and for empiric therapy for serious infections, such as bacterial meningitis, septicemia, pneumonia, and peritonitis. Meropenem exhibits greater potency against gram-negative and anaerobic bacteria than does imipenem, but it is slightly less active against most gram-positive species. It is not effective against MRSA. Meropenem is not hydrolyzed by DHP-I and is resistant to most β-lactamases, including a few carbopenemases that hydrolyze carbopenem.

Like imipenem, meropenem is not active orally. It is provided in the form of a sterile lyophilized powder to be made up in normal saline or 5% dextrose solution for parenteral

administration. Approximately 70% to 80% of unchanged meropenem is excreted in the urine following intravenous or intramuscular administration. The remainder is the inactive metabolite formed by hydrolytic cleavage of the β-lactam ring. A lower incidence of nephrotoxicity attributed to meropenem compared with imipenem has been correlated with its greater stability to DHP-I and the absence of the DHP-I inhibitor cilastatin in the preparation. Meropenem appears to be less epileptogenic than imipenem when the two agents are employed in the treatment of bacterial meningitis.

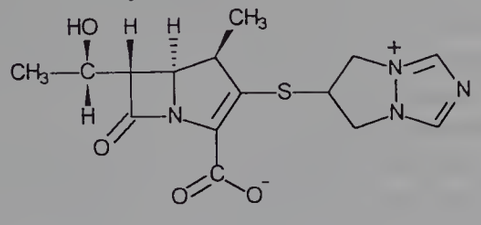


Meropenem



Meropenem metabolite

Biapenem. Biapenem is an investigational second-generation carbopenem with chemical and microbiologic properties similar to those of meropenem.[67] Thus, it has broad-spectrum antibacterial activity that includes most aerobic gram-negative and gram-positive bacteria and anaerobes. Biapenem is stable to DHP-I[67] and resistant to most β-lactamases.[68] It is claimed to be less susceptible to metallo-β-lactamases than either imipenem or meropenem. It is not active orally.

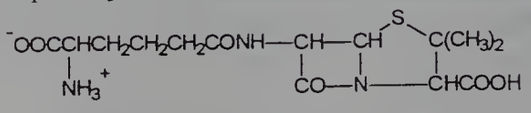


Biapenem

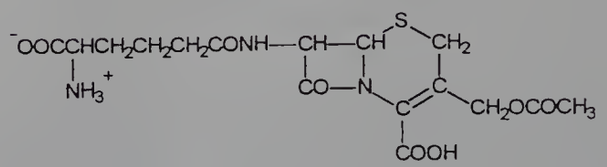
CEPHALOSPORINS

HISTORICAL BACKGROUND

The cephalosporins are β-lactam antibiotics isolated from *Cephalosporium* species or prepared semisynthetically. Most of the antibiotics introduced since 1965 have been semisynthetic cephalosporins. Interest in *Cephalosporium* fungi began in 1945 with Giuseppe Brotzu's discovery that cultures of *C. acremonium* inhibited the growth of a wide variety of gram-positive and gram-negative bacteria. Abraham and Newton[67a] in Oxford, having been supplied cultures of the fungus in 1948, isolated three principal antibiotic components: cephalosporin Pl, a steroid with minimal antibacterial activity; cephalosporin N, later discovered to be identical to synnematin N (a penicillin derivative now called penicillin N that had earlier been isolated from *C. salmosynnematum*); and cephalosporin C.



Penicillin N
(Cephalosporin N, Synnematin B)



Cephalosporin C

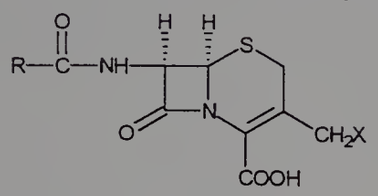
The structure of penicillin N was discovered to be D-(4-amino-4-carboxybutyl)penicillanic acid. The amino acid side chain confers increased activity against gram-negative bacteria, particularly *Salmonella* species, but reduced activity against gram-positive organisms compared with penicillin G. It has been used successfully in clinical trials for the treatment of typhoid fever but was never released as an approved drug.

Cephalosporin C turned out to be a close congener of penicillin N, containing a dihydrothiazine ring instead of the thiazolidine ring of the penicillins. Despite the observation that cephalosporin C was resistant to *Staph. aureus* β-lactamase, early interest in it was not great because its antibacterial potency was inferior to that of penicillin N and other penicillins. However, the discovery that the α-aminoadipoyl side chain could be removed to efficiently produce 7-aminocephalosporanic acid (7-ACA)[69,70] prompted investigations that led to semisynthetic cephalosporins of medicinal value. The relationship of 7-ACA and its acyl derivatives to 6-APA and the semisynthetic penicillins is obvious. Woodward et al.[71] have prepared both cephalosporin C and the clinically useful cephalothin by an elegant synthetic procedure, but the commercially available drugs are obtained from 7-ACA as semisynthetic products.

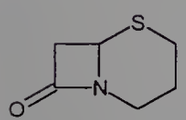
NOMENCLATURE

The chemical nomenclature of the cephalosporins is slightly more complex than even that of the penicillins because of

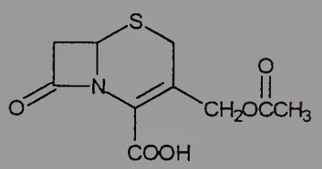
the presence of a double bond in the dihydrothiazine ring. The fused ring system is designated by *Chemical Abstracts* as 5-thia-1-azabicyclo[4.2.0]oct-2-ene. With this system, cephalothin is 3-(acetoxymethyl)-7-[2-(thienylacetyl)amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. A simplification that retains some of the systematic nature of the *Chemical Abstracts* procedure is to name the saturated bicyclic ring system with the lactam carbonyl oxygen as cepham (cf., penam for penicillins). According to this system, all of the commercially available cephalosporins and cephamycins are named as 3-cephems (or Δ^3-cephems) to designate the position of the double bond. (Interestingly, all known 2-cephems are inactive, presumably because the β-lactam lacks the necessary ring strain to be sufficiently reactive.) The trivialized forms of nomenclature of the type that have been applied to the penicillins are not consistently applicable to the naming of cephalosporins because of variations in the substituent at the 3-position. Thus, although some cephalosporins have been named as derivatives of cephalosporanic acids, this practice applies only to the derivatives that have a 3-acetoxymethyl group.



Cephalosporins



Cepham



Cephalosporanic Acid

SEMISYNTHETIC DERIVATIVES

To date, the more useful semisynthetic modifications of the basic 7-ACA nucleus have resulted from acylations of the 7-amino group with different acids or nucleophilic substitution or reduction of the acetoxyl group. Structure–activity relationships (SARs) among the cephalosporins appear to parallel those among the penicillins insofar as the acyl group is concerned. However, the presence of an allylic acetoxyl function in the 3-position provides a reactive site at which various 7-acylaminocephalosporanic acid structures can easily be varied by nucleophilic displacement reactions. Reduction of the 3-acetoxymethyl to a 3-methyl substituent to prepare 7-aminodesacetylcephalosporanic acid (7-ADCA) derivatives can be accomplished by catalytic hydrogenation, but the process currently employed for the commercial synthesis of 7-ADCA derivatives involves the rearrangement of the corresponding penicillin sulfoxide.[72] Perhaps the most noteworthy development thus far is the discovery that 7-phenylglycyl derivatives of 7-ACA and especially 7-ADCA are active orally.

In the preparation of semisynthetic cephalosporins, the following improvements are sought: (1) increased acid stability; (2) improved pharmacokinetic properties, particularly better oral absorption; (3) broadened antimicrobial spectrum; (4) increased activity against resistant microorganisms (as a result of resistance to enzymatic destruction, improved penetration, increased receptor affinity, etc.); (5) decreased allergenicity; and (6) increased tolerance after parenteral administration.

Structures of cephalosporins currently marketed in the United States are represented in Table 10-4.

CHEMICAL DEGRADATION

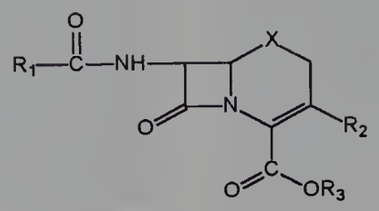
Cephalosporins experience a variety of hydrolytic degradation reactions, the specific nature of which depends on the individual structure (Table 10-4).[73] Among 7-acylaminocephalosporanic acid derivatives, the 3-acetoxylmethyl group is the most reactive site. In addition to its reactivity to nucleophilic displacement reactions, the acetoxyl function of this group readily undergoes solvolysis in strongly acidic solutions to form the desacetylcephalosporin derivatives. The latter lactonize to form the desacetylcephalosporin lactones, which are virtually inactive. The 7-acylamino group of some cephalosporins can also be hydrolyzed under enzymatic (acylases) and possibly nonenzymatic conditions to give 7-ACA (or 7-ADCA) derivatives. Following hydrolysis or solvolysis of the 3-acetoxymethyl group, 7-ACA also lactonizes under acidic conditions (Fig. 10-5).

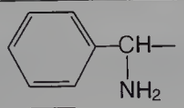
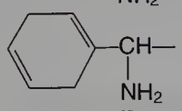
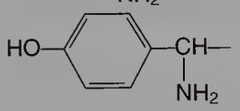
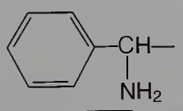
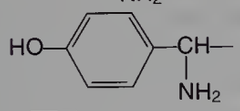
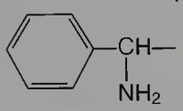
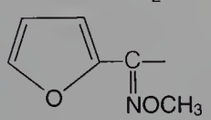
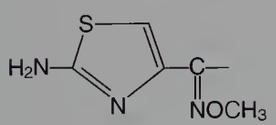
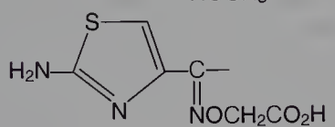
The reactive functionality common to all cephalosporins is the β-lactam. Hydrolysis of the β-lactam of cephalosporins is believed to give initially cephalosporoic acids (in which the R′ group is stable, e.g., R′ = H or S heterocycle) or possibly anhydrodesacetylcephalosporoic acids (for the 7-acylaminocephalosporanic acids). It has not been possible to isolate either of these initial hydrolysis products in aqueous systems. Apparently, both types of cephalosporanic acid undergo fragmentation reactions that have not been characterized fully. However, studies of the in vivo metabolism[74] of orally administered cephalosporins have demonstrated the formation of arylacetylglycines and arylacetamidoethanols, which are believed to be formed from the corresponding arylacetylaminoacetaldehydes by metabolic oxidation and reduction, respectively. The aldehydes, no doubt, arise from nonenzymatic hydrolysis of the corresponding cephalosporoic acids. No evidence for the intramolecular opening of the β-lactam ring by the 7-acylamino oxygen to form oxazolones of the penicillanic acid type has been found in the cephalosporins. However, at neutral to alkaline *p*H intramolecular aminolysis of the β-lactam ring by the α-amino group in the 7-ADCA derivatives cephaloglycin, cephradine, and cefadroxil occurs, forming diketopiperazine derivatives.[75,76]

TABLE 10-4

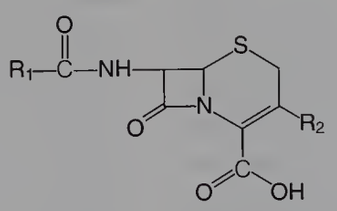
STRUCTURE OF CEPHALOSPORINS

ORAL CEPHALOSPORINS



Generic Name	R_1	R_2	R_3	X
Cephalexin		—CH_3	—H	—S—
Cephradine		—CH_3	—H	—S—
Cefadroxil		—CH_3	—H	—S—
Cefachlor		—Cl	—H	—S—
Cefprozil		—$CH{=}CHCH_3$	—H	—S—
Loracarbef		—Cl	—H	—CH_2—
Cefuroxime axetil		—CH_2OCNH_2 (O)	—$CHOCCH_3$ (O), CH_3	—S—
Cefpodoxime proxetil		—CH_2OCH_3	—$CHOCOCH(CH_3)_2$ (O), CH_3	—S—
Cefixime		—$C{=}CH_2$	—H	—S—

PARENTERAL CEPHALOSPORINS



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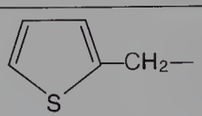
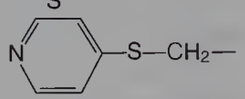
Cephalothin		—CH_2OCCH_3 (O)
Cephapirin		—CH_2OCCH_3 (O)

TABLE 10-4 *Continued*

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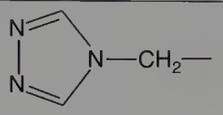
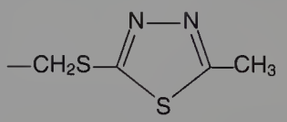
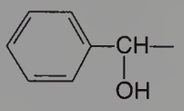
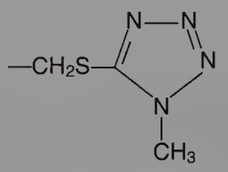
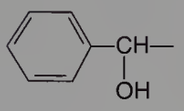
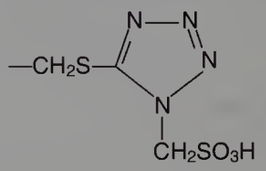
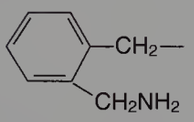
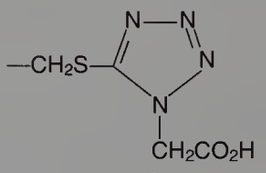
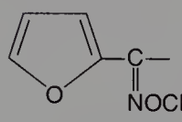
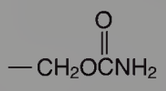
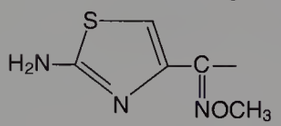
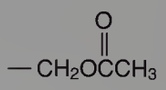
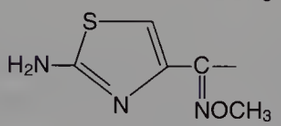
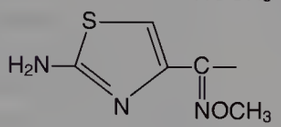
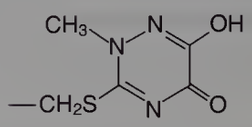
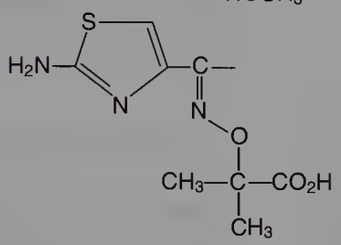
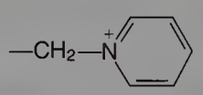
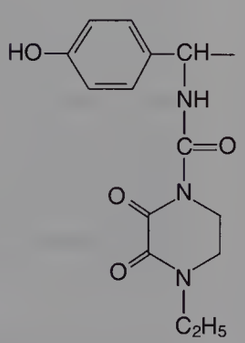
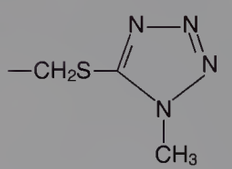
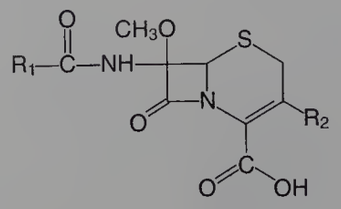
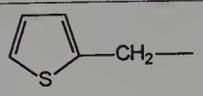
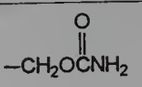
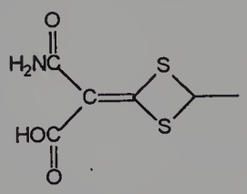
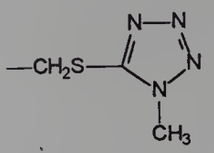
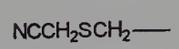
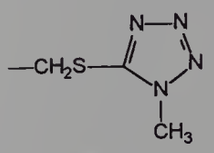
Cefazolin		
Cefamandole		
Cefonacid		
Ceforanide		
Cefuroxime		
Cefotaxime		
Ceftizoxime		—H
Ceftriaxone		
Ceftazidime		
Cefoperazone		

(Continued)

TABLE 10-4 *Continued*

PARENTERAL CEPHAMYCINS



Generic Name	R_1	R_2
Cefoxitin		
Cefotetan		
Cefmetazole		

The formation of dimers, and possibly polymers, from 7-ADCA derivatives containing an α-amino group in the acylamino side chain may also occur, especially in concentrated solutions and at alkaline *p*H values.

ORAL CEPHALOSPORINS

The oral activity conferred by the phenylglycyl substituent is attributed to increased acid stability of the lactam ring, resulting from the presence of a protonated amino group on the 7-acylamino portion of the molecule. Carrier-mediated transport of these dipeptide-like, zwitterionic cephalosporins[51] is also an important factor in their excellent oral activity. The situation, then, is analogous to that of the α-aminobenzylpenicillins (e.g., ampicillin). Also important for high acid stability (and, therefore, good oral activity) of the cephalosporins is the absence of the leaving group at the 3-position. Thus, despite the presence of the phenylglycyl side chain in its structure, the cephalosporanic acid derivative cephaloglycin is poorly absorbed orally, presumably because of solvolysis of the 3-acetoxyl group in the low *p*H of the stomach. The resulting 3-hydroxyl derivative undergoes lactonization under acidic conditions. The 3-hydroxyl derivatives, and especially the corresponding lactones, are considerably less active in vitro than the parent cephalosporins. Generally, acyl derivatives of 7-ADCA show lower in vitro antibacterial potencies than the corresponding 7-ACA analogues.

Oral activity can also be conferred in certain cephalosporins by esterification of the 3-carboxylic acid group to form acid-stable, lipophilic esters that undergo hydrolysis in the plasma. Cefuroxime axetil and cefpodoxime proxetil are two β-lactamase–resistant alkoximino-cephalosporins that are orally active ester prodrug derivatives of cefuroxime and cefprodoxime, respectively, based on this concept.

PARENTERAL CEPHALOSPORINS

Hydrolysis of the ester function, catalyzed by hepatic and renal esterases, is responsible for some in vivo inactivation of parenteral cephalosporins containing a 3-acetoxymethyl substituent (e.g., cephalothin, cephapirin, and cefotaxime). The extent of such inactivation (20% to 35%) is not sufficiently great to compromise seriously the in vivo effectiveness of acetoxyl cephalosporins. Parenteral cephalosporins lacking a hydrolyzable group at the 3-position are not subject to hydrolysis by esterases. Cephradine is the only cephalosporin that is used both orally and parenterally.

SPECTRUM OF ACTIVITY

The cephalosporins are considered broad-spectrum antibiotics with patterns of antibacterial effectiveness comparable to that of ampicillin. Several significant differences exist, however. Cephalosporins are much more resistant to inacti-

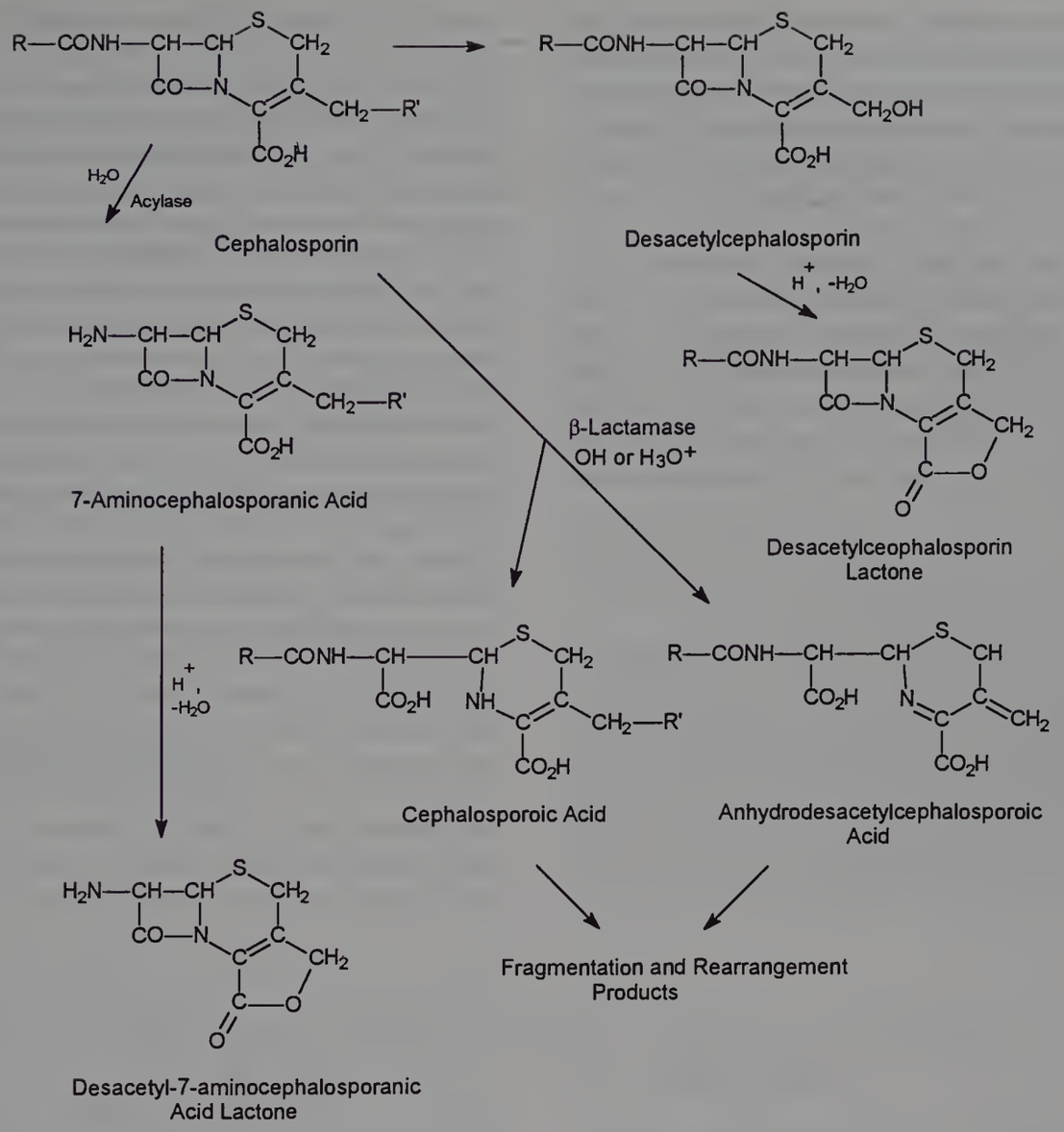


FIG. 10-5. *Degradation of cephalosporins.*

vation by β-lactamases, particularly those produced by gram-positive bacteria, than is ampicillin. However, ampicillin is generally more active against non–β-lactamase-producing strains of gram-positive and gram-negative bacteria sensitive to both it and the cephalosporins. Cephalosporins, among β-lactam antibiotics, exhibit uniquely potent activity against most species of *Klebsiella*. Differential potencies of cephalosporins, compared with penicillins, against different species of bacteria have been attributed to several variable characteristics of individual bacterial species and strains, the most important of which probably are (1) resistance to inactivation by β-lactamases, (2) permeability of bacterial cells, and (3) intrinsic activity against bacterial enzymes involved in cell wall synthesis and cross-linking.

B-LACTAMASE RESISTANCE

The susceptibility of cephalosporins to various lactamases varies considerably with the source and properties of these enzymes. Cephalosporins are significantly less sensitive than all but the β-lactamase–resistant penicillins to hydrolysis by the enzymes from *Staph. aureus* and *Bacillus subtilis*. The "penicillinase" resistance of cephalosporins appears to be a property of the bicyclic cephem ring system, rather than of the acyl group. Despite natural resistance to staphylococcal β-lactamase, the different cephalosporins exhibit considerable variation in rates of hydrolysis by the enzyme.[77] Thus, cephalothin and cefoxitin are the most resistant and cephaloridine and cefazolin are the least resistant of several cephalosporins tested in vitro. The same acyl functionalities that impart β-lactamase resistance in the penicillins unfortunately render cephalosporins virtually inactive against *Staph. aureus* and other gram-positive bacteria.

β-Lactamases elaborated by gram-negative bacteria present an exceedingly complex picture. Well over 100 different enzymes from various species of gram-negative bacilli have been identified and characterized,[25] differing widely in specificity for various β-lactam antibiotics. Most of these en-

zymes hydrolyze penicillin G and ampicillin at faster rates than they do the cephalosporins. However, some inducible β-lactamases, belonging to group C, are "cephalosporinases," which hydrolyze cephalosporins more rapidly. Inactivation by β-lactamases is an important factor in determining resistance to cephalosporins in many strains of gram-negative bacilli.

The introduction of polar substituents in the aminoacyl moiety of cephalosporins appears to confer stability to some β-lactamases.[78] Thus, cefamandole and cefonacid, which contain an α-hydroxyphenylacetyl (or mandoyl) group, and ceforanide, which has an *o*-aminophenyl acetyl group, are resistant to a few β-lactamases. Steric factors also may be important because cefoperazone, an acylureidocephalosporin that contains the same 4-ethyl-2,3-dioxo-1-piperazinylcarbonyl group present in piperacillin, is resistant to many β-lactamases. Oddly enough, piperacillin is hydrolyzed by most of these enzymes.

Two structural features confer broadly based resistance to β-lactamases among the cephalosporins: (1) an alkoximino function in the aminoacyl group and (2) a methoxyl substituent at the 7-position of the cephem nucleus having α-stereochemistry. The structures of several β-lactamase–resistant cephalosporins feature a methoximino acyl group, including cefuroxime, cefotaxime, ceftizoxime, and ceftriaxone. β-Lactamase resistance is enhanced modestly if the oximino substituent also features a polar function, as in ceftazidime, which has a 2-methylpropionic acid substituent on the oximino group. Both steric and electronic properties of the alkoximino group may contribute to the β-lactamase resistance conferred by this functionality since *syn*-isomers are more potent than *anti*-isomers.[78] β-Lactamase–resistant 7α-methoxylcephalosporins, also called cephamycins because they are derived from cephamycin C (an antibiotic isolated from *Streptomyces*), are represented by cefoxitin, cefotetan, cefmetazole, and the 1-oxocephalosporin moxalactam, which is prepared by total synthesis.

Base- or β-lactamase–catalyzed hydrolysis of cephalosporins containing a good leaving group at the 3′-position is accompanied by elimination of the leaving group. The enzymatic process occurs in a stepwise fashion, beginning with the formation of a tetrahedral transition state, which quickly collapses into an acylenzyme intermediate (Fig. 10-6). This intermediate can then either undergo hydrolysis to free the enzyme (path 1) or suffer elimination of the leaving group to form a relatively stable acylenzyme having a conjugated imine structure (path 2). Because of the stability of the acylenzyme intermediate, path 2 leads to transient inhibition of the enzyme. Faraci and Pratt[79] have shown that cephalothin and cefoxitin inhibit certain β-lactamases by this mechanism, whereas analogues lacking a 3′-leaving group do not.

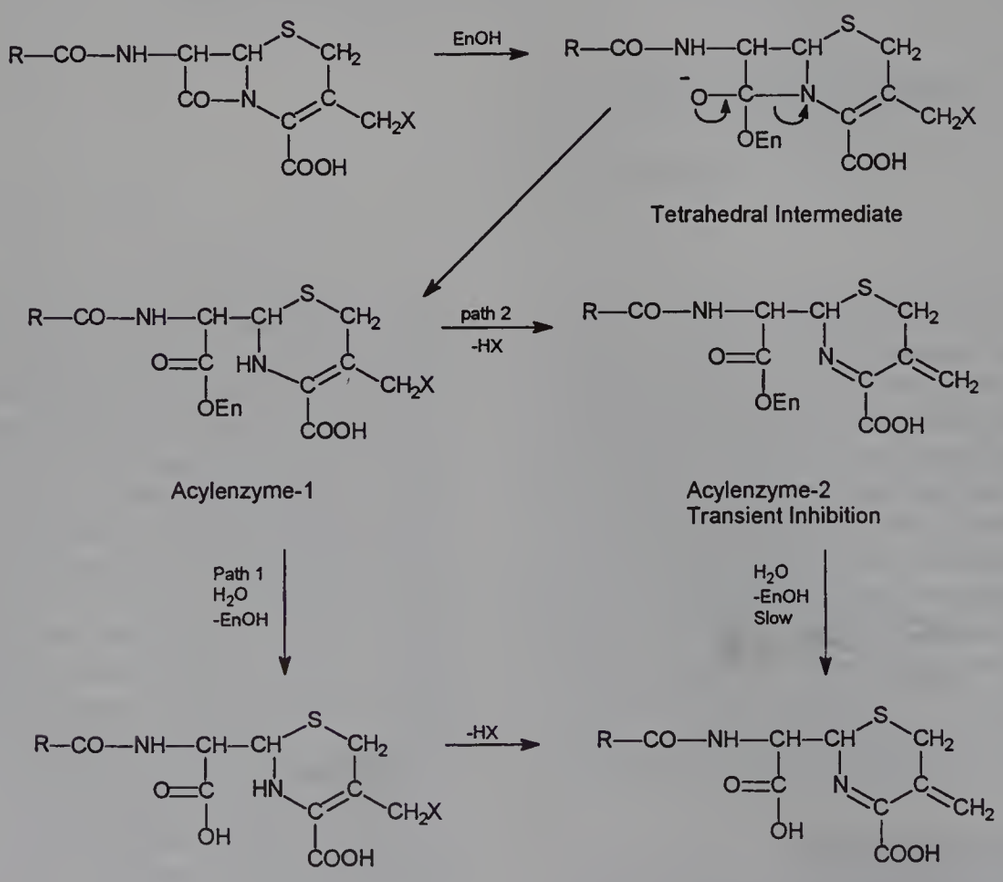


FIG. 10-6. Inhibition of β-lactamases by cephalosporins.

ANTIPSEUDOMONAL CEPHALOSPORINS

Species of *Pseudomonas*, especially *Ps. aeruginosa*, represent a special public health problem because of their ubiquity in the environment and their propensity to develop resistance to antibiotics, including the β-lactams. The primary mechanisms of β-lactam resistance appear to involve destruction of the antibiotics by β-lactamases and/or interference with their penetration through the cell envelope. Apparently, not all β-lactamase–resistant cephalosporins penetrate the cell envelope of *Ps. aeruginosa* as only cefoperazone, moxalactam, cefotaxime, ceftizoxime, ceftriaxone, and ceftazidime have useful antipseudomonal activity. Two cephalosporins, moxalactam and cefoperazone, contain the same polar functionalities, such as carboxy and *N*-acylureido, which facilitate penetration into *Pseudomonas* species in the penicillins (see carbenicillin, ticarcillin, and piperacillin). Unfortunately, strains of *Ps. aeruginosa* resistant to cefoperazone and cefotaxime have been found in clinical isolates. The war between humans and microbes continues.

ADVERSE REACTIONS AND DRUG INTERACTIONS

Like their close relatives the penicillins, the cephalosporin antibiotics are comparatively nontoxic compounds that exhibit a high degree of selective toxicity toward bacteria due to their selective actions on cell wall cross-linking enzymes. The most common adverse reactions to the cephalosporins are allergic and hypersensitivity reactions. These vary from mild skin rashes to life-threatening anaphylactic reactions. Allergic reactions are believed to occur less frequently with cephalosporins than with penicillins. The issue of cross-sensitivity between the two classes of β-lactams is very complex, but the incidence is considered to be very low (estimated at 3% to 7%). The physician faced with the decision of whether or not to administer a cephalosporin to a patient with a history of penicillin allergy must weigh several factors including the severity of the illness being treated, the effectiveness and safety of alternative therapies, and the severity of previous allergic responses to penicillins.

Cephalosporins containing an *N*-methyl-5-thiotetrazole (MTT) moiety at the 3-position (e.g., cefamandole, cefotetan, cefmetazole, moxalactam, and cefoperazone) have been implicated in an increased incidence of hypoprothrombinemia compared with cephalosporins lacking the MTT group. This effect, which is enhanced and can lead to severe bleeding in patients with poor nutritional status, debilitation, recent gastrointestinal surgery, hepatic disease, or renal failure, is apparently due to inhibition of vitamin K-requiring enzymes involved in the carboxylation of glutamic acid residues in clotting factors II, VII, IX, and X to the MTT group.[80] Treatment with vitamin K restores prothrombin time to normal in patients treated with MTT-containing cephalosporins. Weekly vitamin K prophylaxis has been recommended for high-risk patients undergoing therapy with such agents. Cephalosporins containing the MTT group should not be administered to patients receiving oral anticoagulant or heparin therapy because of possible synergism with these drugs.

The MTT group has also been implicated in the intolerance to alcohol associated with certain injectable cephalosporins: cefamandole, cefotetan, cefmetazole, and cefoperazone. Thus, disulfiram-like reactions, attributed to the accumulation of acetaldehyde and resulting from the inhibition of aldehyde dehydrogenase-catalyzed oxidation of ethanol by MTT-containing cephalosporins,[81] may occur in patients who have consumed alcohol before, during, or shortly following the course of therapy.

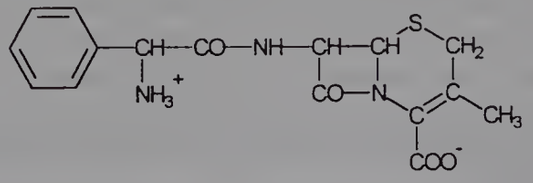
CLASSIFICATION

Cephalosporins are divided into first-, second-, and third-generation agents, based roughly on their time of discovery and their antimicrobial properties (Table 10-5). In general, progression from first to third generation is associated with a broadening of the gram-negative antibacterial spectrum, some reduction in activity against gram-positive organisms, and enhanced resistance to β-lactamases. Individual cephalosporins differ in their pharmacokinetic properties, especially plasma protein binding and half-life, but the structural bases for these differences are not obvious.

PRODUCTS

Cephalexin, USP

7α-(D-Amino-α-phenylacetamido)-3-methylcephemcarboxylic acid (Keflex, Keforal). Cephalexin was designed purposely as an orally active, semisynthetic cephalosporin. The oral inactivation of cephalosporins has been attributed to two causes: instability of the β-lactam ring to acid hydrolysis (cephalothin and cephaloridine) and solvolysis or microbial transformation of the 3-methylacetoxy group (cephalothin, cephaloglycin). The α-amino group of cephalexin renders it acid-stable, and reduction of the 3-acetoxymethyl to a methyl group circumvents reaction at that site.



Cephalexin

Cephalexin occurs as a white crystalline monohydrate. It is freely soluble in water, resistant to acid, and absorbed well orally. Food does not interfere with its absorption. Because of minimal protein binding and nearly exclusive renal excretion, cephalexin is recommended particularly for the

TABLE 10-5

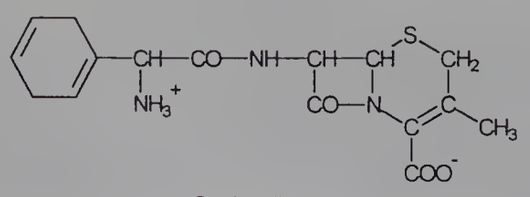
CLASSIFICATION AND PROPERTIES OF CEPHALOSPORINS

Cephalosporin	Generation	Route of Administration	Acid-Resistant	Plasma Protein Binding (%)	β-Lactamase Resistance	Spectrum of Activity	Antipseudomonal Activity
Cephalexin	First	Oral	Yes	5–15	Poor	Broad	No
Cephradine	First	Oral, parenteral	Yes	8–17	Poor	Broad	No
Cefadroxil	First	Oral	Yes	20	Poor	Broad	No
Cephalothin	First	Parenteral	No	65–80	Poor	Broad	No
Cephapirin	First	Parenteral	No	40–54	Poor	Broad	No
Cefazolin	First	Parenteral	No	70–86	Poor	Broad	No
Cefaclor	Second	Oral	Yes	22–25	Poor	Broad	No
Loracarbef	Second	Oral	Yes	25	Poor	Broad	No
Cefprozil	Second	Oral	Yes	36	Poor	Broad	No
Cefamandole	Second	Parenteral	No	56–78	Poor to average	Extended	No
Cefonacid	Second	Parenteral	No	98	Poor to average	Extended	No
Ceforanide	Second	Parenteral	No	80	Average	Extended	No
Cefoxitin	Second	Parenteral	No	13–22	Good	Extended	No
Cefotetan	Second	Parenteral	No	78–91	Good	Extended	No
Cefmetazole	Second	Parenteral	No	65	Good	Extended	No
Cefuroxime	Second	Oral, parenteral	Yes/no	33–50	Good	Extended	No
Cefpodoxime	Second	Oral	Yes	25	Good	Extended	No
Cefixime	Third	Oral	Yes	65	Good	Extended	No
Cefoperazone	Third	Parenteral	No	82–93	Average to good	Extended	Yes
Cefotaxime	Third	Parenteral	No	30–51	Good	Extended	Yes
Ceftizoxime	Third	Parenteral	No	30	Good	Extended	Yes
Ceftriaxone	Third	Parenteral	No	85–95	Good	Extended	Yes
Ceftazidime	Third	Parenteral	No	80–90	Good	Extended	Yes

treatment of urinary tract infections. It is also sometimes employed for upper respiratory tract infections. Its spectrum of activity is very similar to those of cephalothin and cephaloridine. Cephalexin is somewhat less potent than these two agents after parenteral administration and, therefore, is inferior to them for the treatment of serious systemic infections.

Cephradine, USP (Anspor, Velosef)

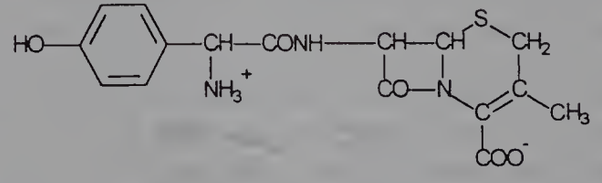
Cephradine is the only cephalosporin derivative available in both oral and parenteral dosage forms. It closely resembles cephalexin chemically (it may be regarded as a partially hydrogenated derivative of cephalexin) and has very similar antibacterial and pharmacokinetic properties. It occurs as a crystalline hydrate, which is readily soluble in water. Cephradine is stable to acid and absorbed almost completely after oral administration. It is minimally protein-bound and excreted almost exclusively through the kidneys. It is recommended for the treatment of uncomplicated urinary tract and upper respiratory tract infections caused by susceptible organisms. Cephradine is available in both oral and parenteral dosage forms.



Cephradine

Cefadroxil, USP (Duricef)

Cefadroxil is an orally active semisynthetic derivative of 7-ADCA, wherein the 7-acyl group is the D-hydroxylphenylglycyl moiety. This compound is absorbed well after oral administration to give plasma levels that reach 75% to 80% of those of an equal dose of its close structural analogue cephalexin. The main advantage claimed for cefadroxil results from its somewhat prolonged duration of action, which permits once-a-day dosing. The prolonged duration of action of this compound is related to relatively slow urinary excretion of the drug compared with other cephalosporins, but the basis for this remains to be explained completely. The antibacterial spectrum of action and therapeutic indications of cefadroxil are very similar to those of cephalexin and cephradine. The D-*p*-hydroxyphenylglycyl isomer is much more active than the L-isomer.

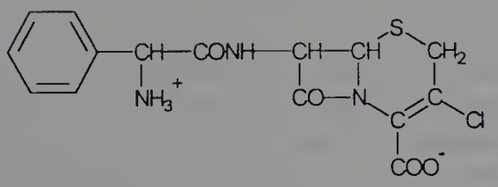


Cefadroxil

Cefaclor, USP (Ceclor)

Cefaclor is an orally active semisynthetic cephalosporin that was introduced in the American market in 1979. It differs

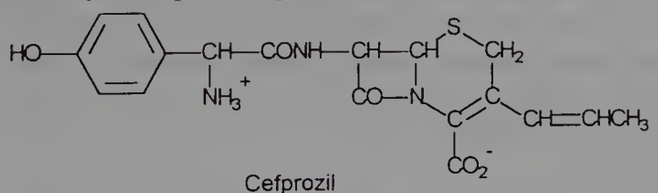
structurally from cephalexin in that the 3-methyl group has been replaced by a chlorine atom. It is synthesized from the corresponding 3-methylenecepham sulfoxide ester by ozonolysis, followed by halogenation of the resulting β-ketoester.[82] The 3-methylenecepham sulfoxide esters are prepared by rearrangement of the corresponding 6-acylaminopenicillanic acid derivative. Cefaclor is moderately stable in acid and achieves sufficient oral absorption to provide effective plasma levels (equal to about two-thirds of those obtained with cephalexin). The compound is apparently unstable in solution because about 50% of its antimicrobial activity is lost in 2 hr in serum at 37°C.[83] The antibacterial spectrum of activity is similar to that of cephalexin, but it is claimed to be more potent against some species sensitive to both agents. Currently, the drug is recommended for the treatment of non–life-threatening infections caused by *H. influenzae*, particularly strains resistant to ampicillin.



Cefaclor

Cefprozil, USP (Cefzil)

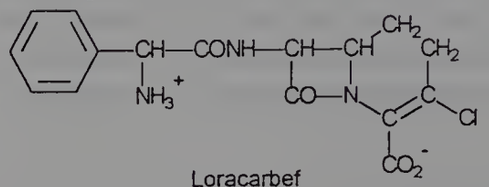
Cefprozil is an orally active second-generation cephalosporin that is similar in structure and antibacterial spectrum to cefadroxil. Oral absorption is excellent (oral bioavailability is around 95%) and is not affected by antacids or histamine-H_2 antagonists. Cefprozil exhibits greater in vitro activity against streptococci, *Neisseria* spp., and *Staph. aureus* than does cefadroxil. It is also more active than the first-generation cephalosporins against members of the *Enterobacteriaceae* family, such as *E. coli, Klebsiella* spp., *Pr. mirabilis*, and *Citrobacter* spp. The plasma half-life of 1.2 to 1.4 hr permits twice-a-day dosing for the treatment of most community-acquired respiratory and urinary tract infections caused by susceptible organisms.



Cefprozil

Loracarbef, USP (Lorabid)

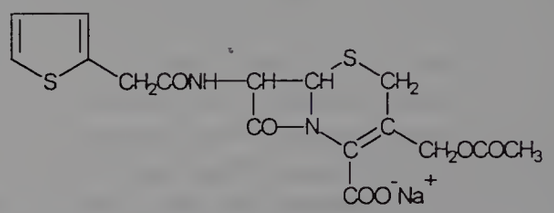
Loracarbef is the first of a series of carbocephems prepared by total synthesis to be introduced.[84] Carbocephems are isosteres of the cephalosporin (or Δ^3-cephem) antibiotics, wherein the 1-sulfur atom has been replaced by a methylene (CH_2) group. Loracarbef is isosteric with cefaclor and has similar pharmacokinetic and microbiologic properties. Thus, the antibacterial spectrum of activity resembles that of cefaclor, but it has somewhat greater potency against *H. influenzae* and *Mor. catarrhalis*, including β-lactamase–producing strains. Unlike cefaclor, which undergoes degradation in human serum, loracarbef is chemically stable in plasma. It is absorbed well orally. Oral absorption is delayed by food. The half-life in plasma is about 1 hr.



Loracarbef

Cephalothin Sodium, USP (Keflin)

Cephalothin sodium occurs as a white to off-white, crystalline powder that is practically odorless. It is freely soluble in water and insoluble in most organic solvents. Although it has been described as a broad-spectrum antibacterial compound, it is not in the same class as the tetracyclines. Its spectrum of activity is broader than that of penicillin G and more similar to that of ampicillin. Unlike ampicillin, cephalothin is resistant to penicillinase produced by *Staph. aureus* and provides an alternative to the use of penicillinase-resistant penicillins for the treatment of infections caused by such strains.



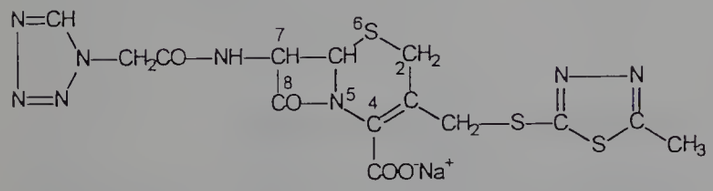
Cephalothin Sodium

Cephalothin is absorbed poorly from the gastrointestinal tract and must be administered parenterally for systemic infections. It is relatively nontoxic and acid-stable. It is excreted rapidly through the kidneys, about 60% being lost within 6 hr of administration. Pain at the site of intramuscular injection and thrombophlebitis following intravenous injection have been reported. Hypersensitivity reactions have been observed, and there is some evidence of cross-sensitivity in patients noted previously to be penicillin-sensitive.

Cefazolin Sodium, Sterile, USP (Ancef, Kefzol)

Cefazolin is one of a series of semisynthetic cephalosporins in which the C-3 acetoxy function has been replaced by a thiol-containing heterocycle, here, 5-methyl-2-thio-1,3,4-thiadiazole. It also contains the somewhat unusual tetrazolylacetyl acylating group. Cefazolin was released in 1973 as a water-soluble sodium salt. It is active only by parenteral administration.

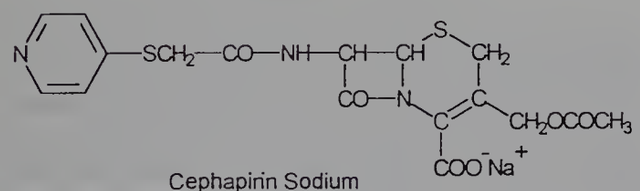
In comparison with other first-generation cephalosporins, cefazolin provides higher serum levels, slower renal clearance, and a longer half-life. It is approximately 75% protein-bound in plasma, a higher value than for most other cephalosporins. Early in vitro and clinical studies suggest that cefazolin is more active against gram-negative bacilli but less active against gram-positive cocci than either cephalothin or cephaloridine. Occurrence rates of thrombophlebitis following intravenous injection and pain at the site of intramuscular injection appear to be the lowest of the parenteral cephalosporins.



Cefazolin Sodium

Cephapirin Sodium, Sterile, USP (Cefadyl)

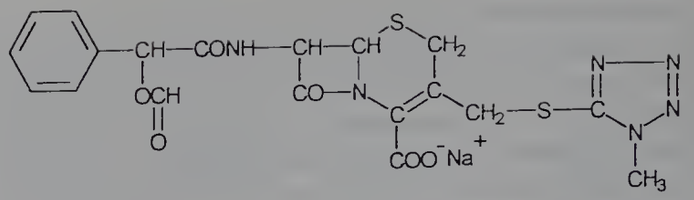
Cephapirin is a semisynthetic 7-ACA derivative released in the United States in 1974. It closely resembles cephalothin in chemical and pharmacokinetic properties. Like cephalothin, cephapirin is unstable in acid and must be administered parenterally in the form of an aqueous solution of the sodium salt. It is moderately protein-bound (45% to 50%) in plasma and cleared rapidly by the kidneys. Cephapirin and cephalothin are very similar in antimicrobial spectrum and potency. Conflicting reports concerning the relative occurrence of pain at the site of injection and thrombophlebitis after intravenous injection of cephapirin and cephalothin are difficult to assess on the basis of available clinical data.



Cephapirin Sodium

Cefamandole Nafate, USP (Mandol)

Cefamandole nafate is the formate ester of cefamandole, a semisynthetic cephalosporin that incorporates D-mandelic acids as the acyl portion and a thiol-containing heterocycle (5-thio-1,2,3,4-tetrazole) in place of the acetoxyl function on the C-3 methylene carbon atom. Esterification of the α-hydroxyl group of the D-mandeloyl function overcomes the instability of cefamandole in solid-state dosage forms[85] and provides satisfactory concentrations of the parent antibiotic in vivo through spontaneous hydrolysis of the ester at neutral to alkaline *p*H. Cefamandole is the first second-generation cephalosporin to be marketed in the United States.



Cefamandole Nafate

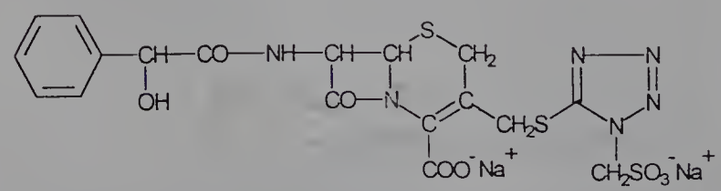
The D-mandeloyl moiety of cefamandole appears to confer resistance to a few β-lactamases since some β-lactamase–producing, gram-negative bacteria (particularly *Enterobacteriaceae*) that show resistance to cefazolin and other first-generation cephalosporins are sensitive to cefamandole. Additionally, it is active against some ampicillin-resistant strains of *Neisseria* and *Haemophilus*. Although resistance to β-lactamases may be a factor in determining the sensitivity of individual bacterial strains to cefamandole, an early study[86] indicated that other factors, such as permeability and intrinsic activity, are frequently more important. It should be noted that the L-mandeloyl isomer is significantly less active than the D-isomer.

Cefamandole nafate is very unstable in solution, hydrolyzing rapidly to release cefamandole and formate. However, there is no loss of potency when such solutions are stored for 24 hr at room temperature or for up to 96 hr when refrigerated. Air oxidation of the released formate to carbon dioxide can cause pressure to build up in the injection vial.

Cefonacid Sodium, Sterile, USP (Monicid)

Cefonacid is a second-generation cephalosporin that is structurally similar to cefamandole, except that it contains a methane sulfonic acid group attached to the N-1 position of the tetrazole ring. The antimicrobial spectrum and limited β-lactamase stability of cefonacid are essentially identical to those of cefamandole.

Cefonacid is unique among the second-generation cephalosporins in that it has an unusually long serum half-life of approximately 4.5 hr. A high fraction of plasma protein binding coupled with slow renal tubular secretion are apparently responsible for the long duration of action. Despite the high fraction of drug bound in plasma, cefonacid is distributed throughout body fluids and tissues, with the exception of the cerebrospinal fluid.

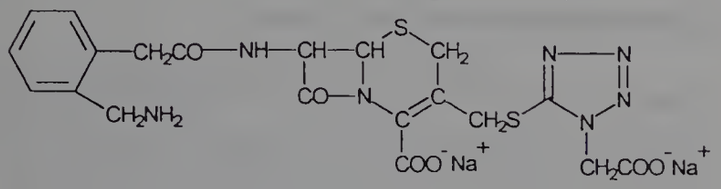


Cefonacid Sodium

Cefonacid is supplied as a highly water-soluble disodium salt, in the form of a sterile powder to be reconstituted for injection. Solutions are stable for 24 hr at 25°C and for 72 hr when refrigerated.

Ceforanide, Sterile, USP (Precef)

Ceforanide was approved for clinical use in the United States in 1984. Despite this recent introduction, however, ceforanide is classified as a second-generation cephalosporin because its antimicrobial properties are similar to those of cefamandole. It exhibits excellent potency against most members of the *Enterobacteriaceae* family, especially *K. pneumoniae, E. coli, Pr. mirabilis*, and *Enterobacter cloacae*. However, it is less active than cefamandole against *H. influenzae*.

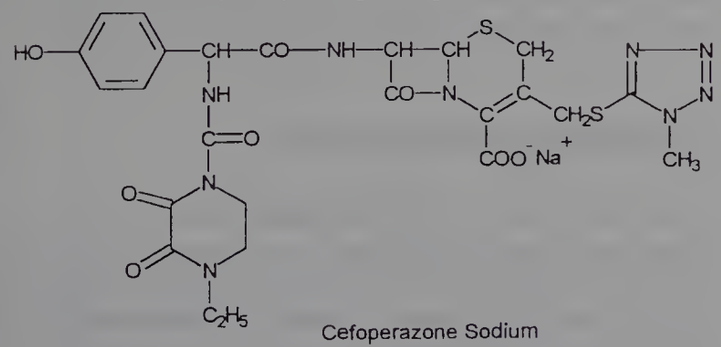


Ceforanide Disodium

The duration of action of ceforanide lies between that of cefamandole and cefonacid. It has a serum half-life of around 3 hr, permitting twice-a-day dosing for most indications. Ceforanide is supplied as the sterile, crystalline disodium salt. Parenteral solutions are stable for 4 hr at 25°C and for up to 5 days when refrigerated.

Cefoperazone Sodium, Sterile, USP (Cefobid)

Cefoperazone is a third-generation, antipseudomonal cephalosporin that resembles piperacillin chemically and microbiologically. It is active against many strains of *Ps. aeruginosa,* indole-positive *Proteus, Enterobacter*, and *S. marcescens* that are resistant to cefamandole. It is less active than cephalothin against gram-positive bacteria and less active than cefamandole against most of the *Enterobacteriaceae.* Similar to piperacillin, cefoperazone is hydrolyzed by many of the β-lactamases that hydrolyze penicillins. Unlike piperacillin, however, it is resistant to some (but not all) of the β-lactamases that hydrolyze cephalosporins.

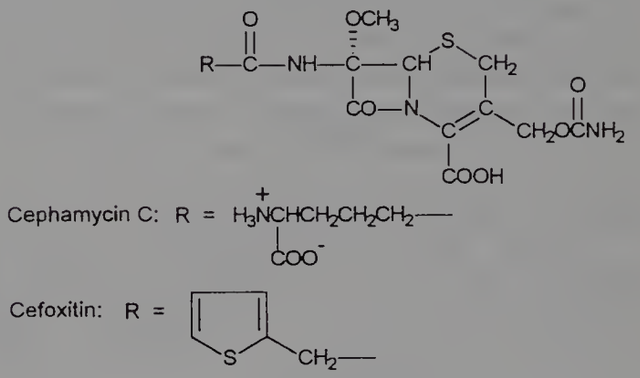


Cefoperazone Sodium

Cefoperazone is excreted primarily in the bile. Hepatic dysfunction can affect its clearance from the body. Although only 25% of the free antibiotic is recovered in the urine, urinary concentrations are high enough to be effective in the management of urinary tract infections caused by susceptible organisms. The relatively long half-life (2 hr) allows for dosing twice a day. Solutions prepared from the crystalline sodium salt are stable for up to 4 hr at room temperature. If refrigerated, they will last 5 days without appreciable loss of potency.

Cefoxitin Sodium, Sterile, USP (Mefoxin)

Cefoxitin is a semisynthetic derivative obtained by modification of cephamycin C, a 7α-methoxy-substituted cephalosporin isolated independently from various *Streptomyces* by research groups in Japan[87] and the United States. Although it is less potent than cephalothin against gram-positive bacteria and cefamandole against most of the *Enterobacteriaceae,* cefoxitin is effective against certain strains of gram-negative bacilli (e.g., *E. coli, K. pneumoniae, Providencia, S. marcescens,* indole-positive *Proteus,* and *Bacteroides*) that are resistant to these cephalosporins. It is also effective against penicillin-resistant *Staph. aureus* and *N. gonorrhoeae.*



Cephamycin C: R =

Cefoxitin: R =

The activity of cefoxitin and cephamycins, in general, against resistant bacterial strains is due to their resistance to hydrolysis by β-lactamases conferred by the 7α-methoxyl substituent.[88] Cefoxitin is a potent competitive inhibitor of many β-lactamases. It is also a potent inducer of chromosomally mediated β-lactamases. The temptation to exploit the β-lactamase–inhibiting properties of cefoxitin by combining it with β-lactamase–labile β-lactam antibiotics should be tempered by the possibility that antagonism may occur. In fact, cefoxitin antagonizes the action of cefamandole against *Ent. cloacae* and that of carbenicillin against *Ps. aeruginosa.*[89] Cefoxitin alone is essentially ineffective against these organisms.

The pharmacokinetic properties of cefoxitin resemble those of cefamandole. Because its half-life is relatively short, cefoxitin must be administered three or four times daily. Solutions of the sodium salt intended for parenteral administration are stable for 24 hr at room temperature and for 1 week if refrigerated. 7α-Methoxyl substitution stabilizes the β-lactam to alkaline hydrolysis to some extent.

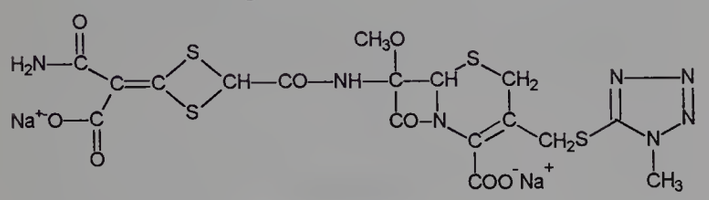
The principal role of cefoxitin in therapy seems to be for the treatment of certain anaerobic and mixed aerobic–anaerobic infections. It is also used to treat gonorrhea caused by β-lactamase–producing strains. It is classified as a second-generation agent because of its spectrum of activity.

Cefotetan Disodium (Cefotan)

Cefotetan is a third-generation cephalosporin that is structurally similar to cefoxitin. Like cefoxitin, cefotetan is resistant to destruction by β-lactamases. It is also a competitive inhibitor of many β-lactamases and causes transient inactivation of some of these enzymes. Cefotetan is reported to synergize with β-lactamase–sensitive β-lactams but, unlike cefoxitin, does not appear to cause antagonism.[90]

The antibacterial spectrum of cefotetan closely resembles that of cefoxitin. It is, however, generally more active against *Staph. aureus* and members of the *Enterobacteriaceae* family sensitive to both agents. It also exhibits excellent potency against *H. influenzae* and *N. gonorrhoeae*, including β-lactamase–producing strains. Cefotetan is slightly less active than cefoxitin against *B. fragilis* and other anaerobes. *Enterobacter* species are generally resistant to cefotetan, and the drug is without effect against *Pseudomonas*.

Cefotetan has a relatively long half-life of around 3.5 hr. It is administered on a twice-daily dosing schedule. It is excreted largely unchanged in the urine. Aqueous solutions for parenteral administration maintain potency for 24 hr at 25°C. Refrigerated solutions are stable for 4 days.

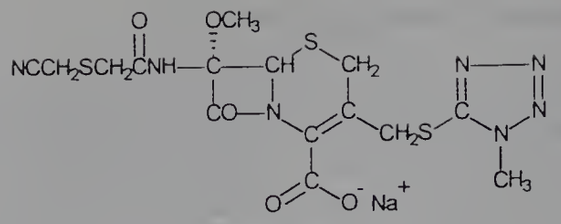


Cefotetan Disodium

Cefotetan contains the MTT group that has been associated with hypoprothrombinemia and alcohol intolerance. Another cephalosporin that lacks these properties should be selected for patients at risk for severe bleeding or alcoholism.

Cefmetazole Sodium, USP (Zefazone)

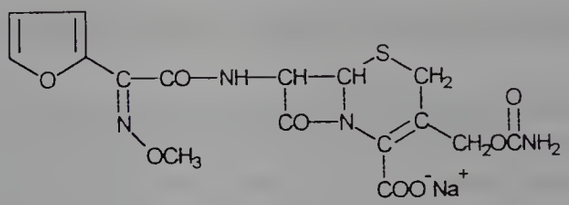
Cefmetazole is a semisynthetic, third-generation, parenteral cephalosporin of the cephamycin group. Like other cephamycins, the presence of the 7α-methoxyl group confers resistance to many β-lactamases. Compared with cefoxitin, cefmetazole exhibits significantly higher potency against members of the *Enterobacteriaceae* family but lower activity against *Bacteroides* spp. It is highly active against *N. gonorrhoeae*, including β-lactamase–producing strains. In common with other cephamycins, cefmetazole is ineffective against indole-positive *Proteus, Enterobacter, Providencia, Serratia,* and *Pseudomonas*. Cefmetazole has the MTT moiety associated with increased bleeding in certain high-risk patients. The plasma half-life is 1.1 hr.



Cefmetazole Sodium

Cefuroxime Sodium, USP (Zinacef)

Cefuroxime is the first of a series of α-methoximinoacyl-substituted cephalosporins that constitute most of the third-generation agents available for clinical use. A *syn*-alkoximino substituent is associated with β-lactamase stability in these cephalosporins.[78] Cefuroxime is classified as a second-generation cephalosporin because its spectrum of antibacterial activity more closely resembles that of cefamandole. It is, however, active against β-lactamase–producing strains that are resistant to cefamandole, such as *E. coli, K. pneumoniae, N. gonorrhoeae,* and *H. influenzae*. Other important gram-negative pathogens, such as *Serratia*, indole-positive *Proteus, Ps. aeruginosa,* and *B. fragilis,* are resistant.



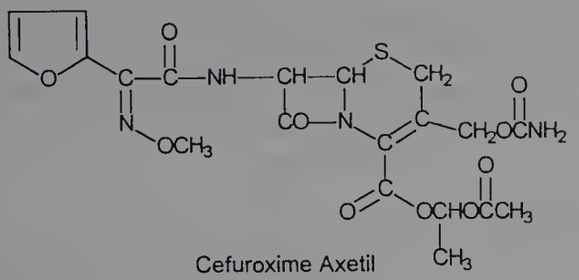
Cefuroxime Sodium

Cefuroxime is distributed throughout the body. It penetrates inflamed meninges in sufficiently high concentrations to be effective in meningitis caused by susceptible organisms. Thrice-daily dosing is required to maintain effective plasma levels for most sensitive organisms, such as *Neisseria meningitidis, Streptococcus pneumoniae,* and *H. influenzae*. It has a plasma half-life of 1.4 hr.

Cefuroxime Axetil, USP (Ceftin)

Cefuroxime axetil is the 1-acetyoxyethyl ester of cefuroxime. This acid-stable, lipophilic, oral prodrug derivative of cefuroxime is hydrolyzed to cefuroxime during absorption by intestinal and/or plasma enzymes. The axetil ester provides an oral bioavailability of 35% to 50% of cefuroxime, depending on conditions. Oral absorption of the ester is increased by food but decreased by antacids and H_2-histamine antagonists. The latter effect may be due to spontaneous hydrolysis of the ester in the intestine due to the higher *p*H created by these drugs. Axetil is employed for the oral treatment of non–life-threatening infections caused by bacteria

that are susceptible to cefuroxime. The prodrug form permits twice-a-day dosing for such infections.

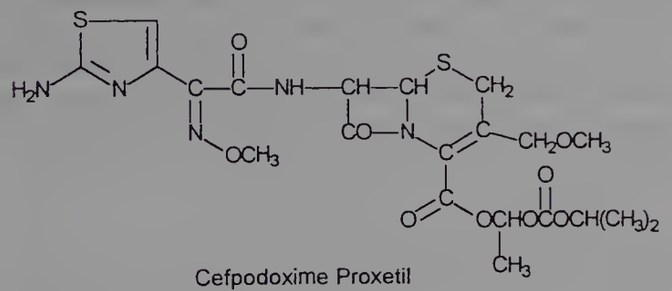


Cefuroxime Axetil

Cefpodoxime Proxetil, USP (Vantin)

Cefpodoxime proxetil is the isopropyloxycarbonylethyl ester of the third-generation cephalosporin cefpodoxime. This orally active prodrug derivative is hydrolyzed by esterases in the intestinal wall and in the plasma to provide cefpodoxime. Tablets and a powder for the preparation of an aqueous suspension for oral pediatric administration are available. The oral bioavailability of cefpodoxime from the proxetil is estimated to be around 50%. Administration of the prodrug with food enhances its absorption. The plasma half-life is 2.2 hr, which permits the proxetil to be administered on a twice-daily schedule.

Cefpodoxime is a broad-spectrum cephalosporin with useful activity against a relatively wide range of gram-positive and gram-negative bacteria. It is also resistant to many β-lactamases. Its spectrum of activity includes *Streptococcus pneumoniae, Streptococcus pyogenes, Staph. aureus, H. influenzae, Mor. catarrhalis,* and *Neisseria* spp. Cefpodoxime is also active against members of the *Enterobacteriaceae* family, including *E. coli, K. pneumoniae*, and *Pr. mirabilis*. It thus finds use in the treatment of upper and lower respiratory infections, such as pharyngitis, bronchitis otitis media, and community-acquired pneumonia. It is also useful for the treatment of uncomplicated gonorrhea.

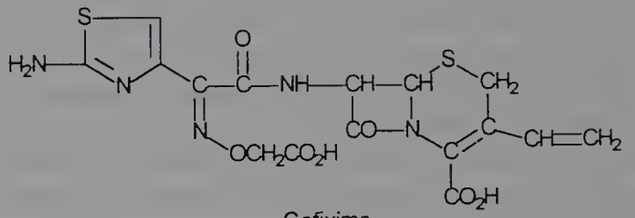


Cefpodoxime Proxetil

Cefixime, USP (Suprax)

Cefixime is the first orally active, third-generation cephalosporin that is not an ester prodrug to be approved for therapy in the United States. Oral bioavailability is surprisingly high, ranging from 40% to 50%. Facilitated transport of cefixime across intestinal brush border membranes involving the carrier system for dipeptides may explain its surprisingly good oral absorption.[91] This result was not expected because cefixime lacks the ionizable α-amino group present in dipeptides and β-lactams previously known to be transported by the carrier system.[51,91]

Cefixime is a broad-spectrum cephalosporin that is resistant to many β-lactamases. It is particularly effective against gram-negative bacilli, including *E. coli, Klebsiellia, Pr. mirabilis,* indole-positive *Proteus, Providencia*, and some *Citrobacter* spp. Most *Pseudomonas, Enterobacter*, and *Bacteroides* species are resistant. It also has useful activity against streptococci, gonococci, *H. influenzae*, and *Mor. catarrhalis*. It is much less active against *Staph. aureus*. Cefixime is employed for the treatment of a variety of respiratory tract infections (e.g., acute bronchitis, pharyngitis, and tonsillitis) and otitis media. It is also used to treat uncomplicated urinary tract infections and gonorrhea caused by β-lactamase–producing bacterial strains.

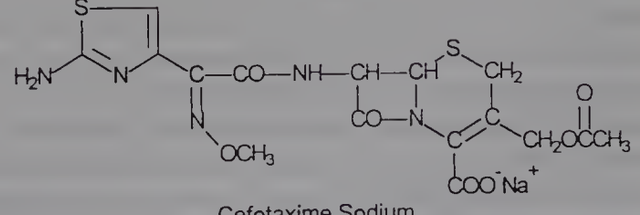


Cefixime

The comparatively long half-life of cefixime (t ½ is 3 to 4 hr) allows it to be administered on a twice-a-day schedule. Renal tubular reabsorption and a relatively high fraction of plasma protein binding (about 65%) contribute to the long half-life. It is provided in two oral dosage forms: 200- or 400-mg tablets and a powder for the preparation of an aqueous suspension.

Cefotaxime Sodium, Sterile, USP (Claforan)

Cefotaxime was the first of the third-generation cephalosporins to be introduced. It possesses excellent broad-spectrum activity against gram-positive and gram-negative aerobic and anaerobic bacteria. It is more active than moxalactam against gram-positive organisms. Many β-lactamase–producing bacterial strains are sensitive to cefotaxime, including *N. gonorrhoeae, Klebsiella, H. influenzae, Staph. aureus*, and *Ent. cloacae*. Some, but not all, *Pseudomonas* strains are sensitive. Enterococci and *Listeria monocytogenes* are resistant.



Cefotaxime Sodium

The *syn*-isomer of cefotaxime is significantly more active than the *anti*-isomer against β-lactamase–producing bacteria. This potency difference is due in part to greater resis-

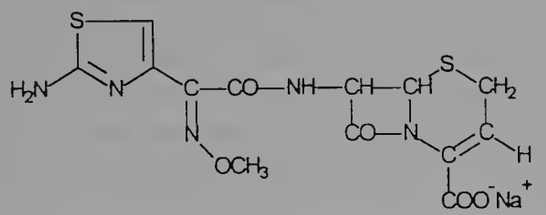
tance of the *syn*-isomer to the action of β-lactamases.[78] However, the higher affinity of the *syn*-isomer for PBPs may also be a factor.[92]

Cefotaxime is metabolized in part to the less active desacetyl metabolite. Approximately 20% of the metabolite and 25% of the parent drug are excreted in the urine. The parent drug reaches the cerebrospinal fluid in sufficient concentrations to be effective in the treatment of meningitis.

Solutions of cefotaxime sodium should be used within 24 hr. If stored, they should be refrigerated. Refrigerated solutions maintain potency for up to 10 days.

Ceftizoxime Sodium, Sterile, USP (Ceftizox)

Ceftizoxime is a third-generation cephalosporin that was introduced in 1984. This β-lactamase–resistant agent exhibits excellent activity against the *Enterobacteriaceae*, especially *E. coli, K. pneumoniae, Ent. cloacae, Ent. aerogenes*, indole-positive and indole-negative *Proteus*, and *S. marcescens*. Ceftizoxime is claimed to be more active than cefoxitin against *B. fragilis*. It is also very active against gram-positive bacteria. Its activity against *Ps. aeruginosa* is somewhat variable and less than that of either cefotaxime or cefoperazone.

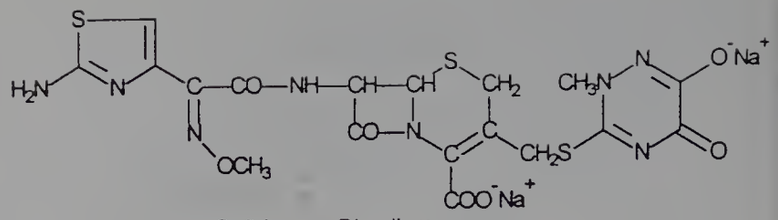


Ceftizoxime Sodium

Ceftizoxime is not metabolized in vivo. It is excreted largely unchanged in the urine. Adequate levels of the drug are achieved in the cerebrospinal fluid for the treatment of gram-negative or gram-positive bacterial meningitis. It must be administered on a three-times-daily dosing schedule because of its relatively short half-life. Ceftizoxime sodium is very stable in the dry state. Solutions maintain potency for up to 24 hr at room temperature and for 10 days when refrigerated.

Ceftriaxone Disodium, Sterile, USP (Rocephin)

Ceftriaxone is a β-lactamase–resistant cephalosporin with an extremely long serum half-life. Once-daily dosing suffices for most indications. Two factors contribute to the prolonged duration of action of ceftriaxone: a high fraction of protein binding in the plasma and a slow urinary excretion. Ceftriaxone is excreted both in the bile and in the urine. Its urinary excretion is not affected by probenecid. Despite its comparatively low volume of distribution, it reaches the cerebrospinal fluid in concentrations that are effective in meningitis. Nonlinear pharmacokinetics are observed.



Ceftriaxone Disodium

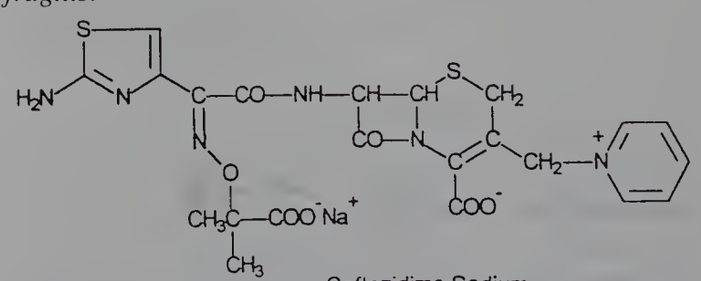
Ceftriaxone contains a highly acidic heterocyclic system on the 3-thiomethyl group. This unusual dioxotriazine ring system is believed to confer the unique pharmacokinetic properties of this agent. Ceftriaxone has been associated with sonographically detected "sludge," or pseudolithiasis, in the gall bladder and common bile duct.[93] Symptoms of cholecystitis may occur in susceptible patients, especially on prolonged or high-dose ceftriaxone therapy. The culprit has been identified as the calcium chelate.

Ceftriaxone exhibits excellent broad-spectrum antibacterial activity against both gram-positive and gram-negative organisms. It is highly resistant to most chromosomally and plasmid-mediated β-lactamases. The activity of ceftriaxone against *Enterobacter, Citrobacter, Serratia*, indole-positive *Proteus*, and *Pseudomonas* is particularly impressive. It is also effective in the treatment of ampicillin-resistant gonorrhea and *H. influenzae* infections but generally less active than cefotaxime against gram-positive bacteria and *B. fragilis*.

Solutions of ceftriaxone sodium should be used within 24 hr. They may be stored up to 10 days if refrigerated.

Ceftazidime Sodium, Sterile, USP (Fortax, Taxidime)

Ceftazidime is a new β-lactamase–resistant third-generation cephalosporin, which is noted for its antipseudomonal activity. It is active against some strains of *Ps. aeruginosa* that are resistant to cefoperazone and ceftriaxone. Ceftazidime is also highly effective against β-lactamase–producing strains of the *Enterobacteriaceae* family. It is generally less active than cefotaxime against gram-positive bacteria and *B. fragilis*.



Ceftazidime Sodium

The structure of ceftazidime contains two noteworthy features: (1) a 2-methylpropionicoxaminoacyl group that confers β-lactamase resistance and possibly increased permeability through the porin channels of the cell envelope and

(2) a pyridinium group at the 3′-position that confers zwitterionic properties to the molecule.

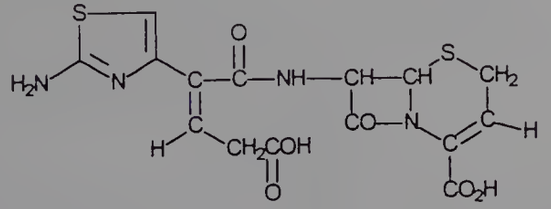
Ceftazidime is administered parenterally two or three times daily, depending on the severity of the infection. Its serum half-life is about 1.8 hr. It has been used effectively for the treatment of meningitis caused by *H. influenzae* and *N. meningitidis*.

Investigational Cephalosporins

Cephalosporins currently undergoing clinical trials and having the highest probability of being marketed in the United States fall into two categories: (1) orally active β-lactamase–resistant cephalosporins and (2) parenteral β-lactamase–resistant antipseudomonal cephalosporins. The status of these investigational compounds awaits more extensive clinical evaluation. Nonetheless, it appears that any advances they represent will be relatively modest.

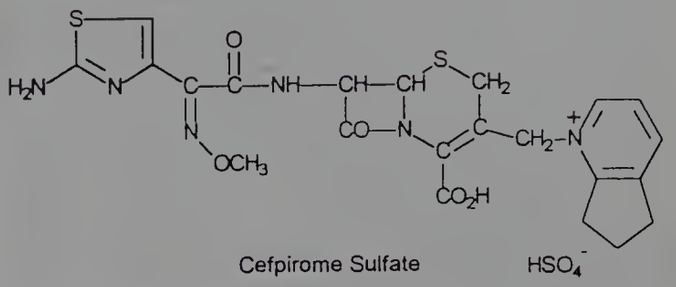
Ceftibuten (Cedax) Ceftibuten is a recently introduced, chemically novel analogue of the oximinocephalosporins, wherein an olefinic methylene group (C═CHCH$_2$—) having *Z*-stereochemistry has replaced the *syn*-oximino (C═NO—) group. This isosteric replacement results in a compound that retains resistance to hydrolysis catalyzed by many β-lactamases, has enhanced chemical stability, and is orally active. Oral absorption is rapid and nearly complete. It has the highest oral bioavailability of the third-generation cephalosporins.[94] Ceftibuten is excreted largely unchanged in the urine and has a half-life of around 2.5 hr. Plasma protein binding of this cephalosporin is estimated to be 63%.

Ceftibuten possesses excellent potency against most members of the *Enterobacteriaceae* family, *H. influenzae, Neisseria* spp., and *Mor. catarrhalis*. It is not active against *Staph. aureus* or *Ps. aeruginosa* and exhibits modest antistreptococcal activity. Ceftibuten is recommended in the management of community-acquired respiratory tract, urinary tract, and gynecologic infections.



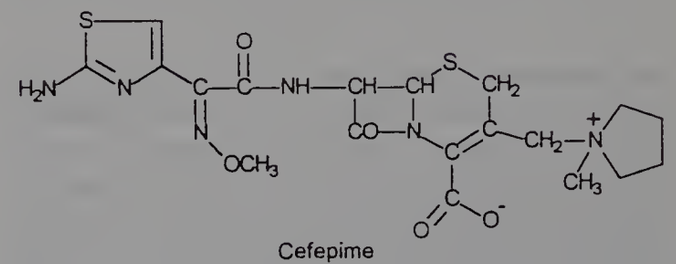
Ceftibuten

Cefpirome (Cefrom). Cefpirome is a new parenteral, β-lactamase–resistant cephalosporin with a quaternary ammonium group at the 3-position of the cephem nucleus. Because its potency against gram-positive and gram-negative bacteria rivals that of the first-generation and third-generation cephalosporins, respectively, cefpirome is being touted as the first fourth-generation cephalosporin.[95] Its broad spectrum includes methacillin-sensitive staphylococci; penicillin-resistant pneumococci; and β-lactamase–producing strains of *E. coli, Enterobacter, Citrobacter*, and *Serratia*. Its efficacy against *Ps. aeruginosa* is comparable to that of ceftazidime. Cefpirome is excreted largely unchanged in the urine with a half-life of 2 hr.



Cefpirome Sulfate HSO_4^-

Cefepime (Maxipime, Axepin). Cefepime is a parenteral, β-lactamase–resistant cephalosporin that is chemically and microbiologically similar to cefpirome. It also has a broad antibacterial spectrum that encompasses significant activity against both gram-positive and gram-negative bacteria, including streptococci, staphylococci, *Pseudomonas* spp., and the *Enterobacteriaceae*. It is active against some bacterial isolates that are resistant to ceftazidime.[96] The efficacy of cefepime has been demonstrated in the treatment of urinary tract infections, lower respiratory tract infections, skin and soft tissue infections, chronic osteomyelitis, and intra-abdominal and biliary infections. It is excreted in the urine with a half-life of 2.1 hr. It is bound minimally to plasma proteins.



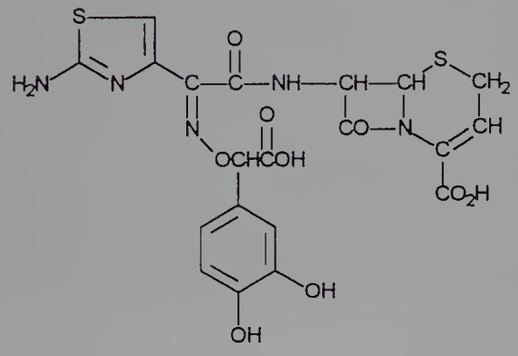
Cefepime

Future Developments in Cephalosporin Design

Recent research efforts in the cephalosporin field have focused primarily on two desired antibiotic properties: (1) increased permeability into gram-negative bacilli, leading to enhanced efficacy against permeability-resistant strains of *Enterobacteriaceae* and *Ps. aeruginosa*, and (2) increased affinity for altered PBPs, in particular the PBP 2a (or PBP 2′) of MRSA.[31]

The observation that certain catechol-substituted cephalosporins exhibit marked broad-spectrum antibacterial activity led to the discovery that such compounds and other analogues capable of chelating iron could mimic natural siderophores (iron-chelating peptides) and thereby be actively transported into bacterial cells via the *tonB*-dependent iron-transport system.[97,98] A means of attacking bacterial strains that resist cellular penetration of cephalosporins is thus provided.

A catechol-containing cephalosporin that exhibits excellent in vitro antibacterial activity against clinical isolates and promising pharmacokinetic properties is GR-69153. GR-69153 is a parenteral β-lactamase–resistant cephalosporin with a broad spectrum of activity against gram-positive and gram-negative bacteria. The antibacterial spectrum of GR-69153 includes most members of the *Enterobacteriaceae* family, *Ps. aeruginosa, H. influenzae, N. gonorrhoeae, Mor. catarrhalis,* staphylocci, steptococci, and *Acinetobacter.* It was not active against enterococci, *B. fragilis*, or MRSA. The half-life of GR-69153 in human volunteers was determined to be 3.5 hr, suggesting that metabolism by catechol-*O*-methyltransferase may not be an important factor. The relatively long half-life would permit once-a-day parenteral dosing for the treatment of many serious bacterial infections.

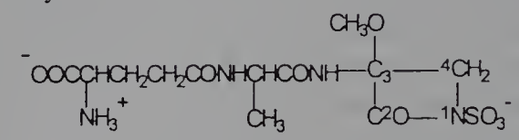


GR 69153

An experimental cephalosporin that has exhibited considerable promise against MRSA in preclinical evaluations is TOC-039. TOC-039 is a parenteral, β-lactamase–resistant, hydroxyiminocephalosporin with a vinylthiopyridyl side chain attached to the 3-position of the cephem nucleus. It is a broad-spectrum agent that exhibits good activity against most aerobic gram-positive and gram-negative bacteria, including staphylococci, streptococci, enterococci, *H. influenzae, Mor. catarrhalis,* and most of the *Enterobacteriaceae* family.[99] A few strains of *Pr. vulgaris, S. marcescens* and *Citrobacter freundii* are resistant and TOC-039 is inactive against *Ps. aeruginosa*. Although the minimum inhibiting concentration (MIC) of TOC-039 against MRSA is slightly less than that of vancomycin, it is more rapidly bacteriocidal. Future clinical evaluations will determine if TOC-039 has the appropriate pharmacokinetic and antibacterial properties in vivo to be approved for the treatment of bacterial infections in humans.

MONOBACTAMS

The development of useful monobactam antibiotics began with the independent isolation of sulfazecin (SQ 26,445) and other monocyclic β-lactam antibiotics from saprophytic soil bacteria in Japan[100] and the United States.[101] Sulfazecin was found to be weakly active as an antibacterial agent but highly resistant to β-lactamases. Extensive SAR studies[102] eventually led to the development of aztreonam, which has useful properties as an antibacterial agent. Early work established that the 3-methoxy group, which was in part responsible for β-lactamase stability in the series, contributed to the low antibacterial potency and poor chemical stability of these antibiotics. A 4-methyl group, however, increases stability to β-lactamases and activity against gram-negative bacteria at the same time. Unfortunately, potency against gram-positive bacteria decreases. 4,4-Gem-dimethyl substitution confers to oral administration a slightly decreased antibacterial potency.

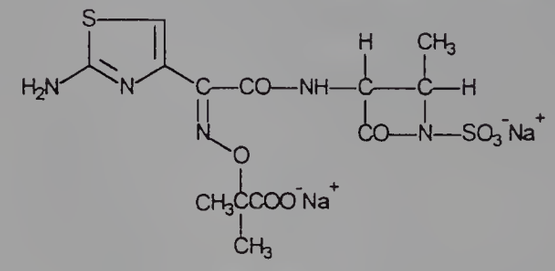


Sulfazecin (SQ 26,445)

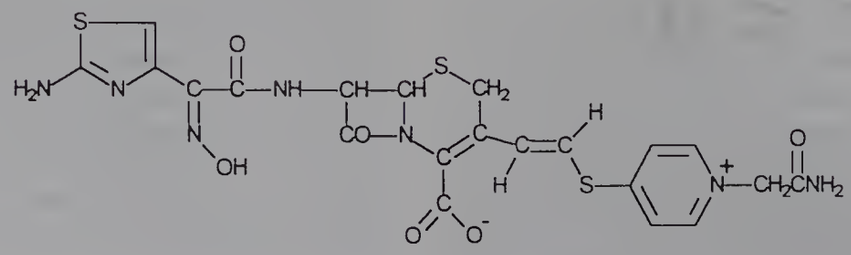
PRODUCTS

Aztreonam Disodium, USP (Azactam)

Aztreonam is a monobactam prepared by total synthesis. It binds with high affinity to PBP 3 in gram-negative bacteria only. It is inactive against gram-positive bacteria and anaerobes. β-Lactamase resistance is similar to that of ceftazidime, which has the same isobutyric acid oximinoacyl group. Aztreonam is not an inducer of chromosomally mediated β-lactamases.



Aztreonam Disodium



TOC-039

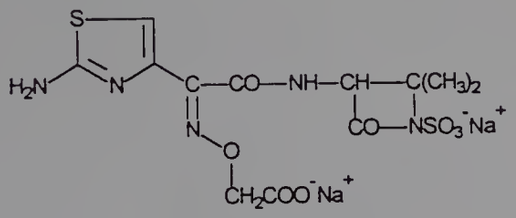
Aztreonam is particularly active against aerobic gram-negative bacilli, including *E. coli, K. pneumoniae, K. oxytoca, Pr. mirabilis, Sr. marcescens, Citrobacter* spp. and *Ps. aeruginosa*. It is used to treat urinary and lower respiratory tract infections, intra-abdominal infections, and gynecologic infections, as well as septicemias caused by these organisms. Aztreonam is also effective against, but is not currently used to treat, infections caused by *Haemophilus, Neisseria, Salmonella*, indole-positive *Proteus*, and *Yersinia*. It is not active against gram-positive bacteria, anaerobic bacteria, or other species of *Pseudomonas*.

Urinary excretion is about 70% of the administered dose. Some is excreted through the bile. Serum half-life is 1.7 hr, thereby allowing aztreonam to be administered two or three times daily, depending on the severity of the infection. Less than 1% of an orally administered dose of aztreonam is absorbed, prompting the suggestion that this β-lactam could be used to treat intestinal infections.

The disodium salt of aztreonam is very soluble in water. Solutions for parenteral administration containing 2% or less are stable for 48 hr at room temperature. Refrigerated solutions retain full potency for 1 week.

Tigemonam

Tigemonam is an investigational monobactam that is orally active.[103] It is highly resistant to β-lactamases. The antibacterial spectrum of activity resembles that of aztreonam. It is very active against the *Enterobacteriaceae*, including *E. coli, Klebsiella, Proteus, Citrobacter, Serratia*, and *Enterobacter* species. It also exhibits good potency against *H. influenzae* and *N. gonorrhoeae*. Tigemonam is not particularly active against gram-positive or anaerobic bacteria and is inactive against *Ps. aeruginosa*.



Tigemonam Disodium

In contrast to the poor oral bioavailability of aztreonam, the oral absorption of tigemonam is excellent. It could become a valuable agent for the oral treatment of urinary tract infections and other non–life-threatening infections caused by β-lactamase–producing gram-negative bacteria.

THE AMINOGLYCOSIDES

The discovery of streptomycin, the first aminoglycoside antibiotic to be used in chemotherapy, was the result of a planned and deliberate search begun in 1939 and brought to fruition in 1944 by Schatz and associates.[104] This success stimulated worldwide searches for antibiotics from the actinomycetes and, particularly, from the genus *Streptomyces*. Among the many antibiotics isolated from that genus, several are compounds closely related in structure to streptomycin. Six of them–kanamycin, neomycin, paromomycin, gentamicin, tobramycin, and netilmicin–currently are marketed in the United States. Amikacin, a semisynthetic derivative of kanamycin A, has been added, and it is possible that additional aminoglycosides will be introduced in the future.

All aminoglycoside antibiotics are absorbed very poorly (less than 1% under normal circumstances) following oral administration, and some of them (kanamycin, neomycin, and paromomycin) are administered by that route for the treatment of gastrointestinal infections. Because of the potent broad-spectrum nature of their antimicrobial activity, they are also used for the treatment of systemic infections. However, their undesirable side effects, particularly oto- and nephrotoxicity, have led to restrictions in their systemic use for serious infections or infections caused by bacterial strains resistant to other agents. When administered for systemic infections, aminoglycosides must be given parenterally, usually by intramuscular injection. An additional antibiotic obtained from *Streptomyces*, spectinomycin, is also an aminoglycoside but differs chemically and microbiologically from other members of the group. It is employed exclusively for the treatment of uncomplicated gonorrhea.

CHEMISTRY

Aminoglycosides are so named because their structures consist of amino sugars linked glycosidically. All have at least one aminohexose and some have a pentose lacking an amino group (e.g., streptomycin, neomycin, and paromomycin). Additionally, each of the clinically useful aminoglycosides contains a highly substituted 1,3-diaminocyclohexane central ring: in kanamycin, neomycin, gentamicin, and tobramycin, it is deoxystreptamine; and in streptomycin it is streptadine. The aminoglycosides are thus strongly basic compounds that exist as polycations at physiologic *p*H. Their inorganic acid salts are very soluble in water. All are available as sulfates. Solutions of the aminoglycoside salts are stable to autoclaving. The high water solubility of the aminoglycosides no doubt contributes to their pharmacokinetic properties. They distribute well into most body fluids but not into the central nervous system, bone, or fatty or connective tissues. They tend to concentrate in the kidneys and are excreted by glomerular filtration. Metabolism of aminoglycosides in vivo apparently does not occur.

SPECTRUM OF ACTIVITY

Although the aminoglycosides are classified as broad-spectrum antibiotics, their greatest usefulness lies in the

treatment of serious systemic infections caused by aerobic gram-negative bacilli. The choice of agent is generally between kanamycin, gentamicin, tobramycin, netilmicin, and amikacin. Aerobic gram-negative and gram-positive cocci (with the exception of staphylococci) tend to be less sensitive; thus, the β-lactam and other antibiotics tend to be preferred for the treatment of infections caused by these organisms. Anaerobic bacteria are invariably resistant to the aminoglycosides. Streptomycin is the most effective of the group for the chemotherapy of tuberculosis, brucellosis, tularemia, and yersina infections. Paromomycin is used primarily in the chemotherapy of amebic dysentery. Under certain circumstances, aminoglycoside and β-lactam antibiotics exert a synergistic action in vivo against certain bacterial strains when the two are administered jointly. Thus, for example, carbenicillin and gentamicin are synergistic against gentamicin-sensitive strains of *Ps. aeruginosa* and several other species of gram-negative bacilli, and penicillin G and streptomycin (or gentamicin or kanamycin) tend to be more effective than either agent alone in the treatment of enterococcal endocarditis. The two antibiotic types should not be combined in the same solution because they are chemically incompatible. Damage to the cell wall caused by the β-lactam is believed to increase penetration of the aminoglycoside into the bacterial cell.

MECHANISM OF ACTION

Most studies concerning the mechanism of antibacterial action of the aminoglycosides have been carried out with streptomycin. However, the specific actions of other aminoglycosides are thought to be qualitatively similar. The aminoglycosides act directly on the bacterial ribosome to inhibit the initiation of protein synthesis and to interfere with the fidelity of translation of the genetic message. They bind to the 30S ribosomal subunit to form a complex that is unable to initiate proper amino acid polymerization.[105] The binding of streptomycin and other aminoglycosides to ribosomes also causes misreading mutations of the genetic code, apparently resulting from failure of specific aminoacyl RNAs to recognize the proper codons on mRNA and the incorporation of improper amino acids into the peptide chain.[106] Evidence suggests that the deoxystreptamine-containing aminoglycosides differ quantitatively from streptomycin in causing misreading at lower concentrations than are required to prevent initiation of protein synthesis, whereas streptomycin inhibits initiation and causes misreading equally effectively.[107] Spectinomycin, however, prevents the initiation of protein synthesis but apparently does not cause misreading. All of the commercially available aminoglycoside antibiotics are bactericidal, except spectinomycin. The mechanistic basis for the bactericidal action of the aminoglycosides has not been elucidated.

MICROBIAL RESISTANCE

The development of strains of *Enterobacteriaceae* resistant to antibiotics has become well recognized as a serious medical problem. Nosocomial (hospital acquired) infections caused by these organisms are often resistant to antibiotic therapy. Research has established clearly that multiple resistance among gram-negative bacilli to a variety of antibiotics occurs and can be transmitted to previously nonresistant strains of the same species and, indeed, to different species of bacteria. The mechanism of transfer of resistance from one bacterium to another has been attributed directly to extrachromosomal R-factors (DNA), which are self-replicative and transferable by conjugation (direct contact). The aminoglycoside antibiotics, because of their potent bactericidal action against gram-negative bacilli, are now preferred for the treatment of many serious infections caused by coliform bacteria. However, a pattern of bacterial resistance to each of the aminoglycoside antibiotics has developed as their clinical use has become more widespread. Consequently, there are bacterial strains resistant to streptomycin, kanamycin, and gentamicin. Strains carrying R-factors for resistance to these antibiotics synthesize enzymes capable of acetylating, phosphorylating, or adenylylating key amino or hydroxyl groups of the aminoglycosides. Much of the recent effort in aminoglycoside research is directed toward identification of new, or modification of existing, antibiotics that are resistant to inactivation by bacterial enzymes.

Resistance of individual aminoglycosides to specific inactivating enzymes can be understood, in large measure, by using chemical principles. As a first principle, it can be assumed that if the target functional group is absent in a position of the structure normally attacked by an inactivating enzyme, then the antibiotic will be resistant to the enzyme. Second, steric factors may confer resistance to attack at functionalities otherwise susceptible to enzymatic attack. For example, conversion of a primary amino group to a secondary amine has been shown to inhibit *N*-acetylation by certain aminoglycoside acetyl transferases. At least nine different types of aminoglycoside-inactivating enzyme have been identified and partially characterized.[108] The sites of attack of these enzymes and the biochemistry of the inactivation reactions is described briefly, using the kanamycin B structure (which holds the dubious distinction of being a substrate for all of the enzymes described) for illustrative purposes (Fig. 10-7). Aminoglycoside-inactivating enzymes include (1) aminoacetyltransferases (designated AAC), which acetylate the 6′-NH_2 of ring I, the 3-NH_2 of ring II, or the 2′-NH_2 of ring I; (2) phosphotransferases (designated APH), which phosphorylate the 3′-OH of ring 1 or the 2″-OH of ring III; and nucleotidyltransferases, which adenylate the 2″-OH of ring III, the 4′-OH of ring I, or the 4″-OH of ring III.

The gentamicins and tobramycin lack a 3′-hydroxyl group in ring I (see the section on the individual products for structures) and, consequently, are not inactivated by the phospho-

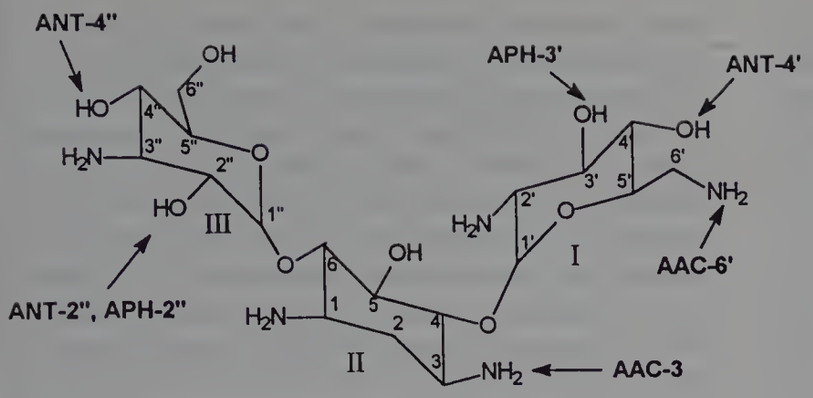


FIG. 10-7. Inactivation of kanamycin B by bacterial enzymes.

transferase enzymes that phosphorylate that group in the kanamycins. Gentamicin C_1 (but not gentamicins C_{1a} or C_2 or tobramycin) is resistant to the acetyltransferase that acetylates the 6′-amino group in ring I of kanamycin B. All gentamicins are resistant to the nucleotidyltransferase enzyme that adenylylates the secondary equatorial 4″-hydroxyl group of kanamycin B because the 4″-hydroxyl group in the gentamicins is *tertiary* and has an *axial* orientation. Removal of functional groups susceptible to attacking an aminoglycoside occasionally can lead to derivatives that resist enzymatic inactivation and retain activity. For example, the 3′-deoxy-, 4′-deoxy-, and 3′,4′-dideoxykanamycins are more similar to the gentamicins and tobramycin in their patterns of activity against clinical isolates that resist one or more of the aminoglycoside-inactivating enzymes.

The most significant breakthrough yet achieved in the search for aminoglycosides resistant to bacterial enzymes has been amikacin, the 1-*N*-L(−)-amino-α-hydroxybutyric acid (L-AHBA) derivative of kanamycin A. This remarkable compound retains most of the intrinsic potency of kanamycin A and is resistant to virtually all aminoglycoside-inactivating enzymes known, except the aminoacetyltransferase that acetylates the 6′-amino group and the nucleotidyltransferase that adenylylates the 4′-hydroxyl group of ring I.[108,109] The cause of amikacin's resistance to enzymatic inactivation is not known, but it has been suggested that introduction of the L-AHBA group into kanamycin A markedly decreases its affinity for the inactivating enzymes. The importance of amikacin's resistance to enzymatic inactivation is reflected in the results of an investigation on the comparative effectiveness of amikacin and other aminoglycosides against clinical isolates of bacterial strains known to be resistant to one or more of the aminoglycosides.[110] In this study, amikacin was effective against 91% of the isolates (with a range of 87% to 100%, depending on the species). The strains found susceptible to other systemically useful aminoglycosides were kanamycin, 18%; gentamicin, 36%; and tobramycin, 41%.

Low-level resistance associated with diminished aminoglycoside uptake has been observed in certain strains of *Ps. aeruginosa* isolated from nosocomial infections.[111] Bacterial susceptibility to aminoglycosides requires uptake of the drug by an energy-dependent active process.[112] Uptake is initiated by the binding of the cationic aminoglycoside to anionic phospholipids of the cell membrane. Electron transport-linked transfer of the aminoglycoside through the cell membrane then occurs. Divalent cations, such as Ca^{2+} and Mg^{2+}, antagonize the transport of aminoglycosides into bacterial cells by interfering with their binding to cell membrane phospholipids. The resistance of anaerobic bacteria to the lethal action of the aminoglycosides is apparently due to the absence of the respiration-driven active-transport process for transporting the antibiotics.

STRUCTURE–ACTIVITY RELATIONSHIPS

Despite the complexity inherent in various aminoglycoside structures, some conclusions on SARs in this antibiotic class have been made.[113] Such conclusions have been formulated on the basis of comparisons of naturally occurring aminoglycoside structures, the results of selective semisynthetic modifications, and the elucidation of sites of inactivation by bacterial enzymes. It is convenient to discuss sequentially aminoglycoside SARs in terms of substituents in rings I, II, and III.

Ring I is crucially important for characteristic broad-spectrum antibacterial activity, and it is the primary target for bacteria-inactivating enzymes. Amino functions at 6′ and 2′ are particularly important as kanamycin B (6′-amino, 2′-amino) is more active than kanamycin A (6′-amino, 2′-hydroxyl), which in turn is more active than kanamycin C (6′-hydroxyl, 2′-amino). Methylation at either the 6′-carbon or the 6′-amino positions does not lower appreciably antibacterial activity and confers resistance to enzymatic acetylation of the 6′-amino group. Removal of the 3′-hydroxyl or the 4′-hydroxyl group or both in the kanamycins (e.g., 3′,4′-dideoxykanamycin B or dibekacin) does not reduce antibacterial potency. The gentamicins also lack oxygen functions at these positions, as do sisomicin and netilmicin, which also have a 4′,5′-double bond. None of these derivatives is inactivated by phosphotransferase enzymes that phosphorylate the 3′-hydroxyl group. Evidently the 3′-phosphorylated derivatives have very low affinity for aminoglycoside-binding sites in bacterial ribosomes.

Few modifications of ring II (deoxystreptamine) functional groups are possible without appreciable loss of activity in most of the aminoglycosides. However, the 1-amino group of kanamycin A can be acylated (e.g., amikacin), and activity is largely retained. Netilmicin (1-*N*-ethylsisomicin) retains the antibacterial potency of sisomicin and is resistant to several additional bacteria-inactivating enzymes. 2″-Hydroxysisomicin is claimed to be resistant to bacterial strains that adenylate the 2″-hydroxyl group of ring III, whereas 3-deaminosisomicin exhibits good activity against bacterial strains that elaborate 3-acetylating enzymes.

Ring III functional groups appear to be somewhat less sensitive to structural changes than those of either ring I or ring II. Although the 2″-deoxygentamicins are significantly less active than their 2″-hydroxyl counterparts, the 2″-amino derivatives (seldomycins) are highly active. The 3″-amino group of gentamicins may be primary or secondary with high antibacterial potency. Furthermore, the 4″-hydroxyl group may be *axial* or *equatorial* with little change in potency.

Despite improvements in antibacterial potency and spectrum among newer naturally occurring and semisynthetic aminoglycoside antibiotics, efforts to find agents with improved margins of safety have been disappointing. The potential for toxicity of these important chemotherapeutic agents continues to restrict their use largely to the hospital environment.

The discovery of agents with higher potency/toxicity ratios remains an important goal of aminoglycoside research. In a now somewhat dated review, however, Price[114] expressed doubt that many significant clinical breakthroughs in aminoglycoside research would occur in the future.

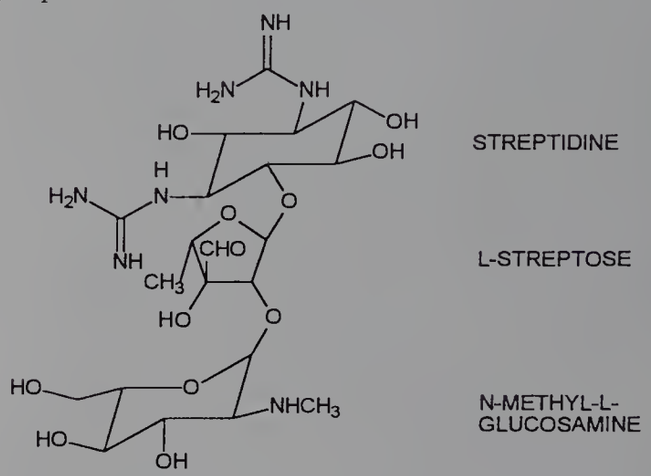
PRODUCTS

Streptomycin Sulfate, Sterile, USP

Streptomycin sulfate is a white, odorless powder that is hygroscopic but stable toward light and air. It is freely soluble in water, forming solutions that are slightly acidic or nearly neutral. It is very slightly soluble in alcohol and is insoluble in most other organic solvents. Acid hydrolysis yields streptidine and streptobiosamine, the compound that is a combination of L-streptose and *N*-methyl-L-glucosamine.

Streptomycin acts as a triacidic base through the effect of its two strongly basic guanidino groups and the more weakly basic methylamino group. Aqueous solutions may be stored at room temperature for 1 week without any loss of potency, but they are most stable if the *p*H is between 4.5 and 7.0. The solutions decompose if sterilized by heating, so sterile solutions are prepared by adding sterile distilled water to the sterile powder. The early salts of streptomycin contained impurities that were difficult to remove and caused a histamine-like reaction. By forming a complex with calcium chloride, it was possible to free the streptomycin from these impurities and to obtain a product that was generally well tolerated.

The organism that produces streptomycin, *Streptomyces griseus*, also produces several other antibiotic compounds: hydroxystreptomycin, mannisidostreptomycin, and cycloheximide (*q.v.*). None of these has achieved importance as a medicinally useful substance. The term "streptomycin A" has been used to refer to what is commonly called streptomycin, and mannisidostreptomycin has been called "streptomycin B." Hydroxystreptomycin differs from streptomycin in having a hydroxyl group in place of one of the hydrogens of the streptose methyl group. Mannisidostreptomycin has a mannose residue attached by glycosidic linkage through the hydroxyl group at C-4 of the *N*-methyl-L-glucosamine moiety. The work of Dyer and colleagues[115,116] to establish the stereostructure of streptomycin has been completed, with the total synthesis of streptomycin and dihydrostreptomycin by Japanese scientists.[117]



Streptomycin

A clinical problem that sometimes develops with the use of streptomycin is the early development of resistant strains of bacteria, making necessary a change in therapy. Another factor that limits its therapeutic efficacy is its chronic toxicity. Certain neurotoxic reactions have been observed after the use of streptomycin. They are characterized by vertigo, disturbance of equilibrium, and diminished auditory acuity. Minor toxic effects include skin rashes, mild malaise, muscular pains, and drug fever.

As a chemotherapeutic agent, the drug is active against numerous gram-negative and gram-positive bacteria. One of the greatest virtues of streptomycin is its effectiveness against the tubercle bacillus. In itself, it is not a cure, but it is a valuable adjunct to the standard treatment of tuberculosis. The greatest drawback to the use of this antibiotic is the rather rapid development of resistant strains of microorganisms. In infections that may be due to both streptomycin- and penicillin-sensitive bacteria, the combined administration of the two antibiotics has been advocated. The possible development of damage to the otic nerve by the continued use of streptomycin-containing preparations has led to dis-

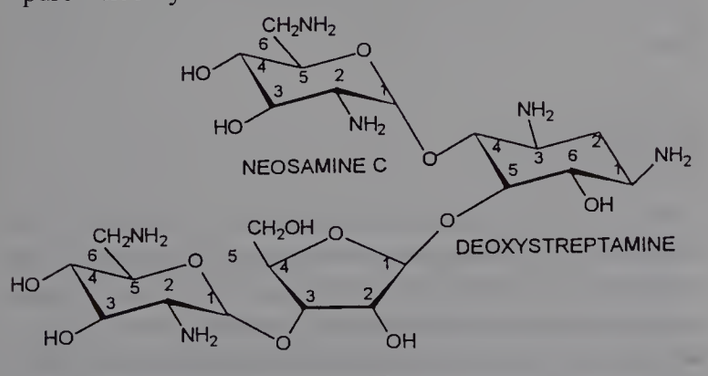
couragement of the use of such products. There is an increasing tendency to reserve the use of streptomycin products for the treatment of tuberculosis. However, it remains one of the agents of choice for the treatment of certain "occupational" bacterial infections, such as brucellosis, tularemia, bubonic plague, and glanders. Because streptomycin is not absorbed when given orally or destroyed significantly in the gastrointestinal tract, at one time, it was used rather widely in the treatment of infections of the intestinal tract. For systemic action, streptomycin usually is given by intramuscular injection.

Neomycin Sulfate, USP (Mycifradin, Neobiotic)

In a search for less toxic antibiotics than streptomycin, Waksman and Lechevalier[118] obtained neomycin in 1949 from *Streptomyces fradiae*. Since then, neomycin has increased steadily in importance, and today, it is considered to be one of the most useful antibiotics in the treatment of gastrointestinal infections, dermatologic infections, and acute bacterial peritonitis. Also, it is employed in abdominal surgery to reduce or avoid complications caused by infections from bacterial flora of the bowel. It has a broad-spectrum activity against a variety of organisms. It shows a low incidence of toxic and hypersensitivity reactions. It is absorbed very slightly from the digestive tract, so its oral use ordinarily does not produce any systemic effect. Neomycin-resistant strains of pathogens seldom have been reported to develop from those organisms against which neomycin is effective.

Neomycin as the sulfate salt is a white to slightly yellow, crystalline powder that is very soluble in water. It is hygroscopic and photosensitive (but stable over a wide *p*H range and to autoclaving). Neomycin sulfate contains the equivalent of 60% of the free base.

Neomycin, as produced by *S. fradiae*, is a mixture of closely related substances. Included in the "neomycin complex" is neamine (originally designated neomycin A) and neomycins B and C. *S. fradiae* also elaborates another antibiotic, fradicin, which has some antifungal properties but no antibacterial activity. This substance is not present in "pure" neomycin.



Neomycin C

The structures of neamine and neomycin B and C are known, and the absolute configurational structures of neamine and neomycin have been reported by Hichens and Rinehart.[119] Neamine may be obtained by methanolysis of neomycins B and C, during which the glycosidic link between deoxystreptamine and D-ribose is broken. Therefore, neamine is a combination of deoxystreptamine and neosamine C, linked glycosidically (α) at the 4-position of deoxystreptamine. According to Hichens and Rinehart, neomycin B differs from neomycin C by the nature of the sugar attached terminally to D-ribose. That sugar, called neosamine B, differs from neosamine C in its stereochemistry. It has been suggested by Rinehart et al.[120] that in neosamine the configuration is that of 2,6-diamino-2,6-dideoxy-L-idose, in which the orientation of the 6-aminomethyl group is inverted to that of the 6-amino-6-deoxy-D-glucosamine in neosamine C. In both instances, the glycosidic links were assumed to be α. However, Huettenrauch[121] later suggested that both of the diamino sugars in neomycin C have the D-glucose configuration and that the glycosidic link is β in the one attached to D-ribose. The latter stereochemistry has been confirmed by the total synthesis of neomycin C.[122]

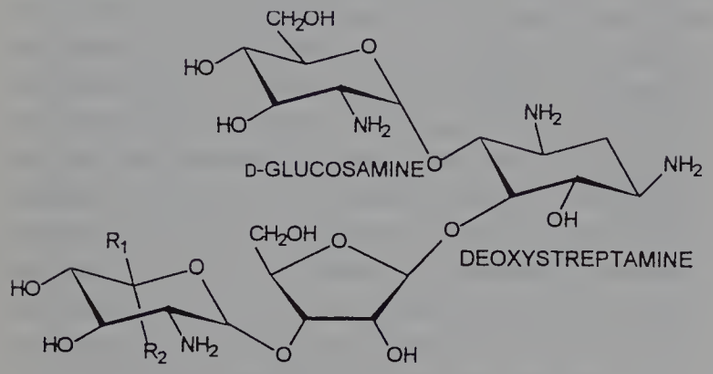
Paromomycin Sulfate, USP (Humatin)

The isolation of paromomycin was reported in 1956 as an antibiotic obtained from a *Streptomyces* species (PD 04998) said to resemble closely *S. rimosus*. The parent organism had been obtained from soil samples collected in Colombia. However, paromomycin more closely resembles neomycin and streptomycin in antibiotic activity than it does oxytetracycline, the antibiotic obtained from *S. rimosus*.

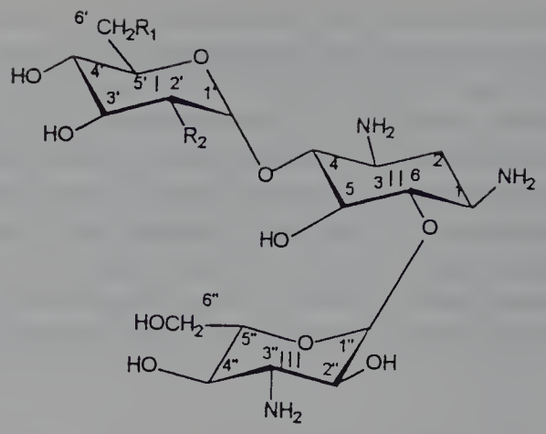
The general structure of paromomycin was reported by Haskell et al.[123] as one compound. Subsequently, chromatographic determinations have shown paromomycin to consist of two fractions, paromomycin I and paromomycin II. The absolute configurational structures for the paromomycins, as shown in the structural formula, were suggested by Hichens and Rinehart[119] and confirmed by DeJongh et al.[124] by mass spectrometric studies. The structure of paromomycin is the same as that of neomycin B, except that paromomycin contains D-glucosamine instead of the 6-amino-6-deoxy-D-glucosamine found in neomycin B. The same structural relationship is found between paromomycin II and neomycin C. The combination of D-glucosamine and deoxystreptamine is obtained by partial hydrolysis of both paromomycins and is called paromamine [4-(2-amino-2-deoxy-α-4-glucosyl)-deoxystreptamine].

Paromomycin has broad-spectrum antibacterial activity and has been employed for the treatment of gastrointestinal infections caused by *Salmonella, Shigella*, and enteropathogenic *E. coli*. However, currently, its use is restricted largely

to the treatment of intestinal amebiasis. Paromomycin is soluble in water and stable to heat over a wide *p*H range.



Paromomycin I: R_1 = H; R_2 = CH_2NH_2
Paromomycin II: R_1 = CH_2NH_2; R_2 = H



Kanamycin A: R_1 = NH_2; R_2 = OH
Kanamycin B: R_1 = NH_2; R_2 = NH_2
Kanamycin C: R_1 = OH; R_2 = NH_2

Kanamycin Sulfate, USP (Kantrex)

Kanamycin was isolated in 1957 by Umezawa and co-workers[125] from *Streptomyces kanamyceticus*. Its activity against mycobacteria and many intestinal bacteria, as well as a number of pathogens that show resistance to other antibiotics, brought a great deal of attention to this antibiotic. As a result, kanamycin was tested and released for medical use in a very short time.

Research activity has been focused intensively on determining the structures of the kanamycins. It has been determined by chromatography that *S. kanamyceticus* elaborates three closely related structures: kanamycins A, B, and C. Commercially available kanamycin is almost pure kanamycin A, the least toxic of the three forms. The kanamycins differ only by the nature of the sugar moieties attached to the glycosidic oxygen on the 4-position of the central deoxystreptamine. The absolute configuration of the deoxystreptamine in kanamycins has been reported by Tatsuoka et al.,[126] as represented in the following diagram. The chemical relationships among the kanamycins, the neomycins, and the paromomycins have been reported by Hichens and Rinehart.[119] The kanamycins do not have the D-ribose molecule that is present in neomycins and paromomycins. Perhaps this structural difference is significant in the lower toxicity observed with kanamycins. The kanosamine fragment linked glycosidically to the 6-position of deoxystreptamine is 3-amino-3-deoxy-D-glucose (3-D-glucosamine) in all three kanamycins. The structures of the kanamycins have been proved by total synthesis.[127,128] They differ in the nature of the substituted D-glucoses attached glycosidically to the 4-position of the deoxystreptamine ring. Kanamycin A contains 6-amino-6-deoxy-D-glucose; kanamycin B contains 2,6-diamino-2,6-dideoxy-D-glucose; and kanamycin C contains 2-amino-2-deoxy-D-glucose (see diagram above).

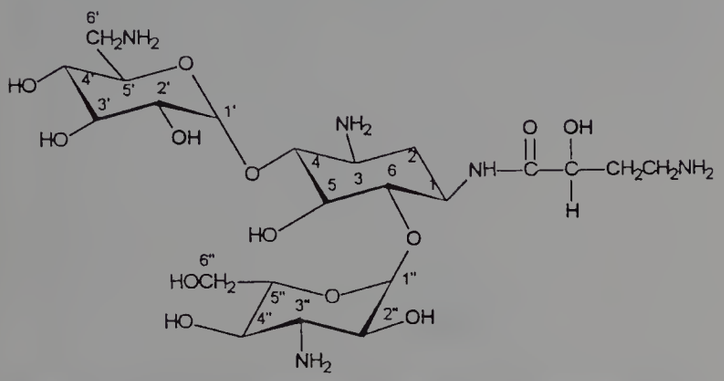
Kanamycin is basic and forms salts of acids through its amino groups. It is water-soluble as the free base, but it is used in therapy as the sulfate salt, which is very soluble. It is stable to both heat and chemicals. Solutions resist both acids and alkali within the *p*H range of 2.0 to 11.0. Because of possible inactivation of either agent, kanamycin and penicillin salts should not be combined in the same solution.

The use of kanamycin in the United States usually is restricted to infections of the intestinal tract (such as bacillary dysentery) and to systemic infections arising from gram-negative bacilli (e.g., *Klebsiella, Proteus, Enterobacter*, and *Serratia*) that have developed resistance to other antibiotics. It has also been recommended for preoperative antisepsis of the bowel. It is absorbed poorly from the intestinal tract; consequently, systemic infections must be treated by intramuscular or, for serious infections, intravenous injections. Injections of it are rather painful, and the concomitant use of a local anesthetic is indicated. The use of kanamycin in the treatment of tuberculosis has not been widely advocated since the discovery that mycobacteria develop resistance to it very rapidly. In fact, clinical experience as well as experimental work[129] indicate that kanamycin does develop cross-resistance in the tubercle bacilli with dihydrostreptomycin, viomycin, and other antitubercular drugs. Similar to streptomycin, kanamycin may cause a decrease in, or complete loss of, hearing. Upon development of such symptoms, its use should be stopped immediately.

Amikacin, USP

1-*N*-Amino-α-hydroxybutyrylkanamycin A (Amikin) is a semisynthetic aminoglycoside first prepared in Japan. The synthesis formally involves simple acylation of the 1-amino group of the deoxystreptamine ring of kanamycin A with L-AHBA. This particular acyl derivative retains about 50% of the original activity of kanamycin A against sensitive strains

of gram-negative bacilli. The L-AHBA derivative is much more active than the D-isomer.[130] The remarkable feature of amikacin is that it resists attack by most bacteria-inactivating enzymes and, therefore, is effective against strains of bacteria that are resistant to other aminoglycosides,[110] including gentamicin and tobramycin. In fact, it is resistant to all known aminoglycoside-inactivating enzymes, except the aminotransferase that acetylates the 6′-amino group[109] and the 4′-nucleotidyl transferase that adenylylates the 4′-hydroxyl group of aminoglycosides.[108]



Amikacin

Preliminary studies indicate that amikacin may be less ototoxic than either kanamycin or gentamicin.[131] However, higher dosages of amikacin generally are required for the treatment of most gram-negative bacillary infections. For this reason, and to discourage the proliferation of bacterial strains resistant to it, amikacin currently is recommended for the treatment of serious infections caused by bacterial strains resistant to other aminoglycosides.

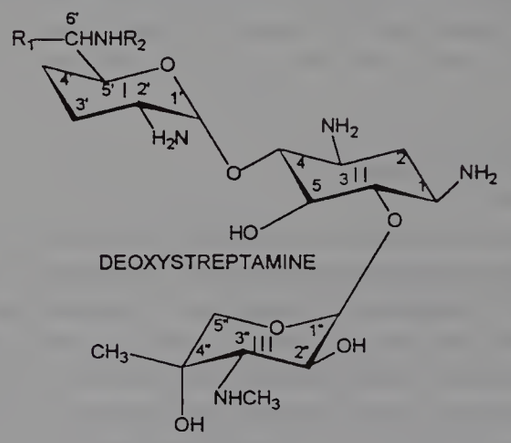
Gentamicin Sulfate, USP (Garamycin)

Gentamicin was isolated in 1958 and reported in 1963 by Weinstein et al.[132] to belong to the streptomycinoid (aminocyclitol) group of antibiotics. It is obtained commercially from *Micromonospora purpurea*. Similar to the other members of its group, it has a broad spectrum of activity against many common pathogens of both gram-positive and gram-negative types. Of particular interest is its high degree of activity against *Ps. aeruginosa* and other gram-negative enteric bacilli.

Gentamicin is effective in the treatment of a variety of skin infections for which a topical cream or ointment may be used. However, because it offers no real advantage over topical neomycin in the treatment of all but pseudomonal infections, it is recommended that topical gentamicin be reserved for use in such infections and in the treatment of burns complicated by pseudomonemia. An injectable solution containing 40 mg of gentamicin sulfate per milliliter may be used for serious systemic and genitourinary tract infections caused by gram-negative bacteria, particularly *Pseudomonas, Enterobacter*, and *Serratia* species. Because of the development of strains of these bacterial species resistant to previously effective broad-spectrum antibiotics, gentamicin has been employed for the treatment of hospital-acquired infections caused by such organisms. However, resistant bacterial strains that inactivate gentamicin by adenylylation and acetylation appear to be emerging with increasing frequency.

Gentamicin sulfate is a mixture of the salts of compounds identified as gentamicins C_1, C_2, and C_{1a}. These gentamicins have been reported by Cooper et al.[133] to have the structures shown in the following diagram. Furthermore, the absolute stereochemistries of the sugar components and the geometries of the glycosidic linkages have been established.[134]

Coproduced, but not a part of the commercial product, are gentamicins A and B. Their structures have been reported by Maehr and Schaffner[135] and are closely related to the gentamicins C. Although gentamicin molecules are similar in many ways to other aminocyclitols, such as streptomycins, they are sufficiently different that their medical effectiveness is significantly greater. Gentamicin sulfate is a white to buff substance that is soluble in water and insoluble in alcohol, acetone, and benzene. Its solutions are stable over a wide *p*H range and may be autoclaved. It is chemically incompatible with carbenicillin, and the two should not be combined in the same intravenous solution.



DEOXYSTREPTAMINE

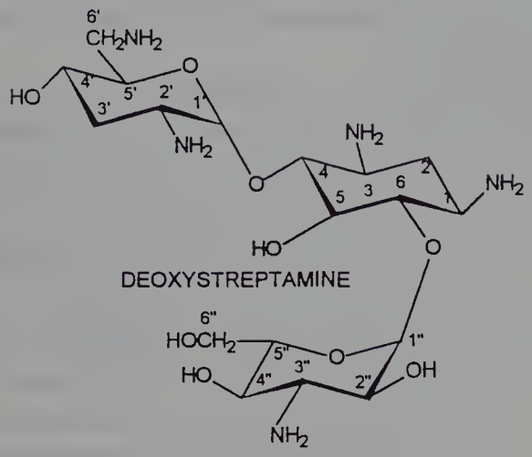
GAROSAMINE

Gentamicin C_1: $R_1 = R_2 = CH_3$
Gentamicin C_2: $R_1 = CH_3$; $R_2 = H$
Gentamicin C_{1a}: $R_1 = R_2 = H$

Tobramycin Sulfate, USP (Nebcin)

Introduced in 1976, tobramycin sulfate is the most active of the chemically related aminoglycosides called nebramycins, obtained from a strain of *Streptomyces tenebrarius*. Five members of the nebramycin complex have been identified chemically.[136] Factors 4 and 4′ are 6″-*O*-carbamoylkanamycin B and kanamycin B, respectively; factors 5′ and 6 are

6″-*O*-carbamoyltobramycin and tobramycin; and factor 2 is apramycin, a tetracyclic aminoglycoside with an unusual bicyclic central ring structure. Kanamycin B and tobramycin probably do not occur in fermentation broths per se but are formed by hydrolysis of the 6-*O*″-carbamoyl derivatives in the isolation procedure.



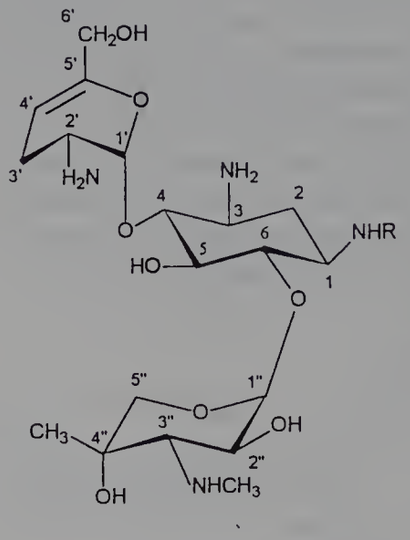
Tobramycin

The most important property of tobramycin is its activity against most strains of *Ps. aeruginosa*, exceeding that of gentamicin by two- to fourfold. Some gentamicin-resistant strains of this troublesome organism are sensitive to tobramycin, but others are resistant to both antibiotics.[137] Other gram-negative bacilli and staphylococci are generally more sensitive to gentamicin. Tobramycin more closely resembles kanamycin B in structure (it is 3′-deoxykanamycin B).

Netilmicin Sulfate, USP

1-*N*-Ethylsisomicin (Netromycin) is a semisynthetic derivative prepared by reductive ethylation[138] of sisomicin, an aminoglycoside antibiotic obtained from *Micromonospora inyoensis*.[139] Structurally, sisomicin and netilmicin resemble gentamicin C_{1a}, a component of the gentamicin complex.

Against most strains of *Enterobacteriaceae, Ps. aeruginosa*, and *Staph. aureus*, sisomicin and netilmicin are comparable with gentamicin in potency.[140] However, netilmicin is active against many gentamicin-resistant strains, in particular among *E. coli, Enterobacter, Klebsiella*, and *Citrobacter*. A few strains of gentamicin-resistant *Ps. aeruginosa, S. marcescens*, and indole-positive *Proteus* are also sensitive to netilmicin. However, very few gentamicin-resistant bacterial strains are sensitive to sisomicin. The potency of netilmicin against certain gentamicin-resistant bacteria is attributed to its resistance to inactivation by bacterial enzymes that adenylylate or phosphorylate gentamicin and sisomicin. Evidently, the introduction of a 1-ethyl group in sisomicin markedly decreases the affinity of these enzymes for the molecule in a manner similar to that observed in the 1-*N*-γ-amino-α-hydroxybutyryl amide of kanamycin A (amikacin). Netilmicin, however, is inactivated by most of the bacterial enzymes that acetylate aminoglycosides, whereas amikacin is resistant to most of these enzymes.



Sisomicin: R = H
Netilmicin: R = C_2H_5

The pharmacokinetic and toxicologic properties of netilmicin and gentamicin appear to be similar clinically, though animal studies have indicated greater nephrotoxicity for gentamicin.

Sisomicin Sulfate, USP

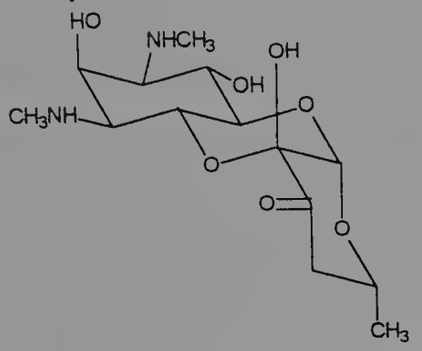
Although it has been approved for human use in the United States, sisomicin has not been marketed in this country. Its antibacterial potency and effectiveness against aminoglycoside-inactivating enzymes resemble those of gentamicin. Sisomicin also exhibits pharmacokinetics and pharmacologic properties similar to those of gentamicin.

Spectinomycin Hydrochloride, Sterile, USP (Trobicin)

This aminocyclitol antibiotic, isolated from *Streptomyces spectabilis* and once called actinospectocin, was first described by Lewis and Clapp.[141] Its structure and absolute stereochemistry have been confirmed by x-ray crystallography.[142] It occurs as the white, crystalline dihydrochloride pentahydrate, which is stable in the dry form and very soluble in water. Solutions of spectinomycin, a hemiacetal, slowly hydrolyze on standing and should be prepared freshly and used within 24 hr. It is administered by deep intramuscular injection.

Spectinomycin is a broad-spectrum antibiotic with moderate activity against many gram-positive and gram-negative bacteria. It differs from streptomycin and the streptamine-containing aminoglycosides in chemical and antibacterial properties. Similar to streptomycin, spectinomycin interferes with the binding of tRNA to the ribosomes and, thereby,

with the initiation of protein synthesis. Unlike streptomycin or the streptamine-containing antibiotics, however, it does not cause misreading of the messenger. Spectinomycin exerts a bacteriostatic action and is inferior to other aminoglycosides for most systemic infections. Currently, it is recommended as an alternative to penicillin G salts for the treatment of uncomplicated gonorrhea. A cure rate of greater than 90% has been observed in clinical studies for this indication. Many physicians prefer to use a tetracycline or erythromycin for prevention or treatment of suspected gonorrhea in penicillin-sensitive patients because, unlike these agents, spectinomycin is ineffective against syphilis. Furthermore, it is considerably more expensive than erythromycin and most of the tetracyclines.



Spectinomycin

THE TETRACYCLINES

CHEMISTRY

Among the most important broad-spectrum antibiotics are members of the tetracycline family. Nine such compounds—tetracycline, rolitetracycline, oxytetracycline, chlortetracycline, demeclocycline, meclocycline, methacycline, doxycycline, and minocycline—have been introduced into medical use. Several others possess antibiotic activity. The tetracyclines are obtained by fermentation procedures from *Streptomyces* species or by chemical transformations of the natural products. Their chemical identities have been established by degradation studies and confirmed by the synthesis of three members of the group, oxytetracycline,[143,144] 6-demethyl-6-deoxytetracycline,[145] and anhydrochlortetracycline,[146] in their (±) forms. The important members of the group are derivatives of an octahydronaphthacene, a hydrocarbon system that comprises four annelated six-membered rings. It is from this tetracyclic system that the group name is derived. The antibiotic spectra and chemical properties of these compounds are very similar but not identical.

The stereochemistry of the tetracyclines is very complex. Carbon atoms 4, 4a, 5, 5a, 6, and 12a are potentially asymmetric, depending on substitution. Oxytetracycline and doxycycline, each with a 5α-hydroxyl substituent, have six asymmetric centers, whereas the others, lacking asymmetry at C-5, have only five. Determination of the complete absolute stereochemistry of the tetracyclines was a difficult problem. Detailed x-ray diffraction analysis[147–149] established

TABLE 10-6

STRUCTURE OF TETRACYCLINES

	R_1	R_2	R_3	R_4
Tetracycline	H	CH_3	OH	H
Chlortetracycline	Cl	CH_3	OH	H
Oxytetracycline	H	CH_3	OH	OH
Demeclocycline	Cl	H	OH	H
Methacycline	H	CH_2		OH
Doxycycline	H	H	CH_3	OH
Minocycline	$N(CH_3)_2$	H	H	H

that the stereochemical formula shown in Table 10-6 represents the orientations found in the natural and semisynthetic tetracyclines. These studies also confirmed that conjugated systems exist in the structure from C-10 through C-12 and from C-1 through C-3 and that the formula represents only one of several canonical forms existing in those portions of the molecule.

The tetracyclines are amphoteric compounds, forming salts with either acids or bases. In neutral solutions, these substances exist mainly as zwitterions. The acid salts, which are formed through protonation of the enol group on C-2, exist as crystalline compounds that are very soluble in water. However, these amphoteric antibiotics will crystallize out of aqueous solutions of their salts unless stabilized by an excess of acid. The hydrochloride salts are used most commonly for oral administration and usually are encapsulated because they are bitter. Water-soluble salts may be obtained also from bases, such as sodium or potassium hydroxides, but they are not stable in aqueous solutions. Water-insoluble salts are formed with divalent and polyvalent metals.

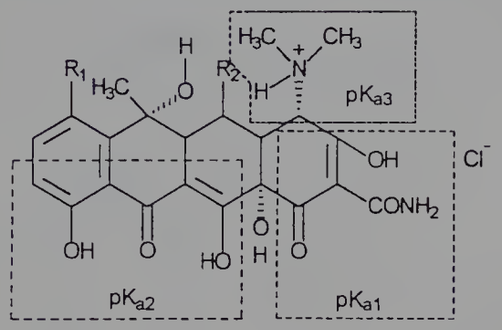
The unusual structural groupings in the tetracyclines produce three acidity constants in aqueous solutions of the acid salts (Table 10-7). The particular functional groups responsible for each of the thermodynamic pK_a values have been determined by Leeson et al.,[150] as shown in the following diagram. These groupings had been identified previously by Stephens et al.[151] as the sites for protonation, but their earlier

TABLE 10-7

pK_a VALUES (OF HYDROCHLORIDES) IN AQUEOUS SOLUTION AT 25%

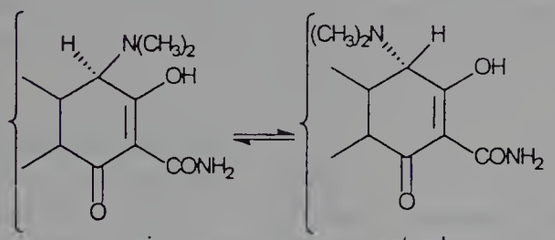
	pK_{a1}	pK_{a2}	pK_{a3}
Tetracycline	3.3	7.7	9.5
Chlortetracycline	3.3	7.4	9.3
Demeclocycline	3.3	7.2	9.3
Oxytetracycline	3.3	7.3	9.1
Doxycycline	3.4	7.7	9.7
Minocycline	2.8	7.8	9.3

assignments, which produced the values responsible for pK_{a2} and pK_{a3}, were opposite those of Leeson et al.[150] This latter assignment has been substantiated by Rigler et al.[152]



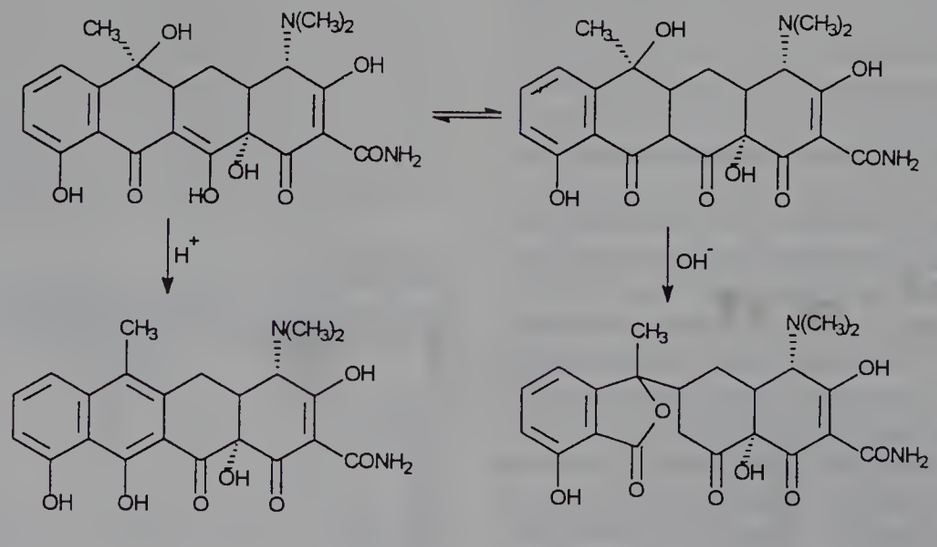
The approximate pK_a values for each of these groups in the six tetracycline salts in common use are shown (Table 10-7). The values are taken from Stephens et al.,[151] Benet and Goyan,[153] and Barringer et al.[154] The pK_a of the 7-dimethylamino group of minocycline (not listed) is 5.0.

An interesting property of the tetracyclines is their ability to undergo epimerization at C-4 in solutions of intermediate *p*H range. These isomers are called *epitetracyclines*. Under acidic conditions, an equilibrium is established in about 1 day and consists of approximately equal amounts of the isomers. The partial structures below indicate the two forms of the epimeric pair. The 4-epitetracyclines have been isolated and characterized. They exhibit much less activity than the "natural" isomers, thus accounting for a decrease in therapeutic value of aged solutions.



Strong acids and strong bases attack the tetracyclines with a hydroxyl group on C-6, causing a loss in activity through modification of the C ring. Strong acids produce a dehydration through a reaction involving the 6-hydroxyl group and the 5a-hydrogen. The double bond thus formed between positions 5a and 6 induces a shift in the position of the double bond between C-11a and C-12 to a position between C-11 and C-11a, forming the more energetically favored resonant system of the naphthalene group found in the inactive anhydrotetracyclines. Bases promote a reaction between the 6-hydroxyl group and the ketone group at the 11-position, causing the bond between the 11 and 11a atoms to cleave and to form the lactone ring found in the inactive isotetracycline. These two unfavorable reactions stimulated research that led to the development of the more stable and longer-acting compounds 6-deoxytetracycline, methacycline, doxycycline, and minocycline.

Stable chelate complexes are formed by the tetracyclines with many metals, including calcium, magnesium, and iron. Such chelates are usually very insoluble in water, accounting for the impairment in absorption of most (if not all) tetracyclines in the presence of milk; calcium-, magnesium-, and aluminum-containing antacids; and iron salts. Soluble alkalinizers, such as sodium bicarbonate, also decrease the gastrointestinal absorption of the tetracyclines.[155] Deprotonation of tetracyclines to more ionic species and their observed instability in alkaline solutions may account for this observation. The affinity of tetracyclines for calcium causes them to be laid down in newly formed bones and teeth as tetracycline–calcium orthophosphate complexes. Deposits of these antibiotics in teeth cause a yellow discoloration that darkens (a photochemical reaction) over time. Tetracyclines are distributed into the milk of lactating mothers and cross the placental barrier into the fetus. The possible effects of these agents on the bones and teeth of the child should be taken into consideration before their use during pregnancy or in children under 8 years of age.



MECHANISM OF ACTION AND RESISTANCE

The strong binding properties of the tetracyclines with metal ions caused Albert[156] to suggest that their antibacterial properties may be due to an ability to remove essential metal ions as chelated compounds. Elucidation of details of the mechanism of action of the tetracyclines,[157] however, has defined more clearly the specific roles of magnesium ions in molecular processes affected by these antibiotics in bacteria. Tetracyclines are specific inhibitors of bacterial protein synthesis. They bind to the 30S ribosomal subunit and, thereby, prevent the binding of aminoacyl tRNA to the mRNA–ribosome complex. Both the binding of aminoacyl tRNA and the binding of tetracyclines at the ribosomal-binding site require magnesium ions.[158] Tetracyclines also bind to mammalian ribosomes but with lower affinities, and they apparently do not achieve sufficient intracellular concentrations to interfere with protein synthesis. The selective toxicity of the tetracyclines against bacteria depends strongly on the self-destructive capacity of bacterial cells to concentrate these agents in the cell. Tetracyclines enter bacterial cells by two processes: passive diffusion and active transport. The active uptake of tetracyclines by bacterial cells is an energy-dependent process that requires adenosine triphosphate (ATP) and magnesium ions.[159]

Three biochemically distinct mechanisms of resistance to tetracyclines have been described in bacteria:[160] (1) efflux, mediated by transmembrane spanning, active-transport proteins, which results in reduction of the intracellular tetracycline concentration; (2) ribosomal protection, in which the bacterial protein synthesis apparatus is rendered resistant to the action of tetracyclines by an inducible cytoplasmic protein; and (3) enzymatic oxidation. Efflux mediated by plasmid or the chromosomal protein determinants tetA-E, -G, -H, -K, and -L, and ribosomal protection mediated by the chromosomal protein determinants tetM, -O, and -S are the more frequently encountered and more clinically significant resistance mcchanisms for tetracyclines.

SPECTRUM OF ACTIVITY

The tetracyclines have the broadest spectrum of any known antibacterial agents. They are active against a wide range of gram-positive and gram-negative bacteria, spirochetes, mycoplasmas, rickettsiae, and chlamydiae. Their potential indications are, therefore, numerous. However, their bacteriostatic action is a disadvantage in the treatment of life-threatening infections, such as septicemia, endocarditis, and meningitis, wherein the aminoglycosides and/or cephalosporins usually are preferred for gram-negative and the penicillins for gram-positive infections. Because of incomplete absorption and effectiveness against the natural bacterial flora of the intestine, tetracyclines may induce superinfections caused by the pathogenic yeast *Candida albicans*. Resistance to tetracyclines among both gram-positive and gram-negative bacteria is relatively common. Superinfections caused by resistant *Staph. aureus* and *Ps. aeruginosa* have resulted from the use of these agents over time. Parenteral tetracyclines may cause severe liver damage, especially when given in excessive dosage to pregnant women or to patients with impaired renal function.

STRUCTURE–ACTIVITY RELATIONSHIPS

As a result of the large amount of research carried out to prepare semisynthetic modifications of the tetracyclines and to obtain individual compounds by total synthesis, several interesting SARs have emerged. Reviews are available that discuss SARs among the tetracyclines in detail[161–163] and their molecular and clinical properties,[164] as well as their synthesis and chemical properties.[162,163,165,166] Only a brief review of the salient structure–activity features is presented here. All derivatives containing fewer than four rings are inactive or nearly inactive. The simplest tetracycline derivative that retains the characteristic broad-spectrum activity associated with this antibiotic class is 6-demethyl-6-deoxytetracycline. It has become evident that many of the precise structural features present in this molecule must remain unmodified for derivatives to retain activity. Consequently, the integrity of substituents at carbon atoms 1, 2, 3, 4, 10, 11, 11a, and 12, representing the hydrophilic "southern and eastern" faces of the molecule, cannot be violated drastically without deleterious effects on the antimicrobial properties of the resulting derivatives.

Only very slight modifications of A-ring substituents can be made without dramatic loss of antibacterial potency. The enolized tricarbonylmethane system at C-1 to C-3 must be intact for good activity. Replacement of the amide at C-2 with other functions, such as aldehyde or nitrile, reduces or abolishes activity. Monoalkylation of the amide nitrogen reduces activity proportionately to the size of the alkyl group. Aminoalkylation of the amide nitrogen, accomplished by the Mannich reaction, yields derivatives that are substantially more water-soluble than the parent tetracycline and are hydrolyzed to it in vivo (e.g., rolitetracycline). The dimethylamino group at the 4-position must have the α-orientation: 4-epitetracyclines are very much less active than the natural isomers. Removal of the 4-dimethylamino group reduces activity even further. Activity is largely retained in the primary and *N*-methyl secondary amines but rapidly diminishes in the higher alkylamines. A *cis*-A/B-ring fusion with a β-hydroxyl group at C-12a is apparently also essential. Esters of the C-12a hydroxyl group are inactive, with the exception of the formyl ester, which readily hydrolyzes in aqueous solutions. Alkylation at C-11a also leads to inactive compounds, demonstrating the importance of an enolizable β-diketone functionality at C-11 and C-12. The importance of the shape of the tetracyclic ring system is illustrated further by a substantial loss in antibacterial potency resulting from epimerization at C-5a. Dehydrogenation to form a double bond between C-5a and C-11a markedly decreases activity, as does aromatization of ring C to form anhydrotetracyclines.

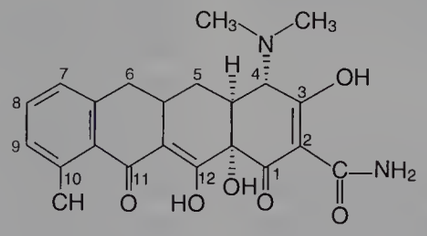
In contrast, substituents at positions 5, 5a, 6, 7, 8, and 9, representing the largely hydrophobic "northern and western" faces of the molecule, can be modified with varying degrees of impunity, resulting in retention and, sometimes, improvement of antibiotic activity. A 5-hydroxyl group, as in oxytetracycline and doxycycline, may influence pharmacokinetic properties but does not change antimicrobial activity. 5a-Epitetracyclines (prepared by total synthesis), although highly active in vitro, are unfortunately much less impressive in vivo. Acid-stable 6-deoxytetracyclines and 6-demethyl-6-deoxytetracyclines have been used to prepare a variety of mono- and disubstituted derivatives by electrophilic substitution reactions at C-7 and C-9 of the D ring. The more useful results have been achieved with the introduction of substituents at C-7. Oddly, both strongly electron-withdrawing groups (e.g., chloro [chlortetracycline] and nitro) and strongly electron-donating groups (e.g., dimethylamino [minocycline]) enhance activity. This unusual circumstance is reflected in QSAR studies of 7- and 9-substituted tetracyclines,[162,167] which indicated a squared (parabolic) dependence on σ, Hammet's electronic substituent constant, and in vitro inhibition of an *E. coli* strain. The effect of introducing substituents at C-8 has not been studied because this position cannot be substituted directly by classic electrophilic aromatic substitution reactions; thus, 8-substituted derivatives are available only through total synthesis.[168]

The most fruitful site for semisynthetic modification of the tetracyclines has been the 6-position. Neither the 6α-methyl nor the 6β-hydroxyl group is essential for antibacterial activity. In fact, doxycycline and methacycline are more active in vitro than their parent oxytetracycline against most bacterial strains. The conversion of oxytetracycline to doxycycline, which can be accomplished by reduction of methacycline,[169] gives a 1 : 1 mixture of doxycycline and epidoxycycline (which has a β-oriented methyl group), whereas if the C-11a α-fluoro derivative of methacycline is employed, the β-methyl epimer is formed exclusively.[170] 6-Epidoxycycline is much less active than doxycycline. 6-Demethyl-6-deoxytetracycline, synthesized commercially by catalytic hydrogenolysis of the 7-chloro and 6-hydroxyl groups of 7-chloro-6-demethyltetracycline, obtained by fermentation of a mutant strain of *Streptomyces aureofaciens*,[171] is slightly more potent than tetracycline. More successful from a clinical standpoint, however, is 6-demethyl-6-deoxy-7-dimethylaminotetracycline (minocycline)[172] because of its activity against tetracycline-resistant bacterial strains.

6-Deoxytetracyclines also possess important chemical and pharmacokinetic advantages over their 6-oxy counterparts. Unlike the latter, they are incapable of forming anhydrotetracyclines under acidic conditions because they cannot dehydrate at C-5a and C-6. They are also more stable in base because they do not readily undergo β-ketone cleavage, followed by lactonization, to form isotetracyclines. Despite the fact that it lacks a 6-hydroxyl group, methacycline shares the instability of the 6-oxytetracyclines in strongly acetic conditions. It suffers prototropic rearrangement to the anhydrotetracycline in acid but is stable to β-ketone cleavage followed by lactonization to the isotetracycline in base. Reduction of the 6-hydroxyl group also brings about a dramatic change in the solubility properties of tetracyclines. This effect is reflected in the significantly higher oil/water partition coefficients of the 6-deoxytetracyclines compared with the tetracyclines (Table 10-8).[173,174] The greater lipid solubility of the 6-deoxy compounds has important pharmacokinetic consequences.[162,164] Hence, doxycycline and minocycline are absorbed more completely following oral administration,

TABLE 10-8

PHARMACOKINETIC PROPERTIES* OF TETRACYCLINES



Tetracycline	Substituents C-5α	C-6α	C-6β	C-7	$K_{pc}^{\dagger}$ Octanol/ Water pH 5.6	% Absorbed Orally	% Excreted Feces	% Excreted Urine	% Protein Bound	Volume of Distribution (% body weight)	Renal Clearance (ml/min/ 1.73 m²)	Half-Life (hr)
Tetracycline	H	CH_6	OH	H	0.056	58	20–50	60	24–65	156–306	50–80	10
Oxytetracycline	OH	CH_6	OH	H	0.075	77–80	≈50	70	20–35	189–305	99–102	9
Chlortetracycline	H	CH_6	OH	Cl	0.41	25–30	>50	18	42–54	149	32	7
Demeclocycline	H	H	OH	Cl	0.25	66	23–72	42	68–77	179	35	15
Doxycycline	OH	CH_6	H	H	0.95	93	20–40	27–39	60–91	63	18–28	15
Minocycline	H	H	H	$N(CH_3)_2$	1.10	≈100	40	5–11	55–76	74	5–15	19

* Values taken from Brown and Ireland[146] and references cited therein.
† Values taken from Colazzi and Klink.[156]

exhibit higher fractions of plasma protein binding, and have higher volumes of distribution and lower renal clearance rates than the corresponding 6-oxytetracyclines.

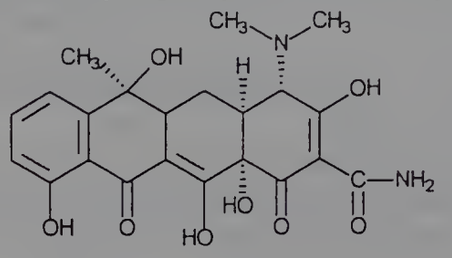
Polar substituents (i.e., hydroxyl groups) at C-5 and C-6 contribute decreased lipid versus water solubility to the tetracyclines. However, the 6-position is considerably more sensitive than the 5-position to this effect. Thus, doxycycline (6-deoxy-5-oxytetracycline) has a much higher partition coefficient than either tetracycline or oxytetracycline. Nonpolar substituents (those with positive π values; see Chap. 2), for example, 7-dimethylamino, 7-chloro, and 6-methyl, have the opposite effect. Accordingly, the partition coefficient of chlortetracycline is substantially greater than that of tetracycline and slightly greater than that of demeclocycline. Interestingly, minocycline (5-demethyl-6-deoxy-7-dimethylaminotetracycline) has the highest partition coefficient of the commonly used tetracyclines.

The poorer oral absorption of the more water-soluble compounds, tetracycline and oxytetracycline, can be attributed to several factors. In addition to their comparative difficulty in penetrating lipid membranes, the polar tetracyclines probably experience more complexation with metal ions in the gut and undergo some acid-catalyzed destruction in the stomach. Poorer oral absorption coupled with biliary excretion of some tetracyclines is also thought to cause a higher incidence of superinfections from resistant microbial strains. However, the more polar tetracyclines are excreted in higher concentrations in the urine (e.g., 60% for tetracycline and 70% for oxytetracycline) than the more lipid-soluble compounds (e.g., 33% for doxycycline and only 11% for minocycline). Significant passive renal tubular reabsorption coupled with higher fractions of protein binding contribute to the lower renal clearance and more prolonged durations of action of doxycycline and minocycline compared with the other tetracyclines, especially tetracycline and oxytetracycline. Minocycline also experiences a significant amount of *N*-dealkylation catalyzed by cytochrome P450 oxygenases in the liver, which contributes to its comparatively low renal clearance. Although all tetracyclines are distributed widely into tissues, the more polar ones have larger volumes of distribution than the nonpolar compounds. The more lipid-soluble tetracyclines, however, distribute better to poorly vascularized tissue. It is also claimed that the distribution of doxycycline and minocycline into bone is less than that of other tetracyclines.[175]

PRODUCTS

Tetracycline, USP (Achromycin, Cyclopar, Panmycin, Tetracyn)

During chemical studies on chlortetracycline, it was discovered that controlled catalytic hydrogenolysis would selectively remove the 7-chloro atom and thereby produce tetracycline. This process was patented by Conover[176] in 1955. Later, tetracycline was obtained from fermentations of *Streptomyces* species, but the commercial supply is still chiefly dependent on the hydrogenolysis of chlortetracycline.



Tetracycline

Tetracycline is 4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6 -methyl-1,11-dioxo-2-naphthacenecarboxamide. It is a bright yellow, crystalline salt that is stable in air but darkens in color upon exposure to strong sunlight. Tetracycline is stable in acid solutions having a *p*H higher than 2. It is somewhat more stable in alkaline solutions than chlortetracycline, but, like those of the other tetracyclines, such solutions rapidly lose potency. One gram of the base requires 2,500 mL of water and 50 mL of alcohol to dissolve it. The hydrochloride salt is used most commonly in medicine, though the free base is absorbed from the gastrointestinal tract about equally well. One gram of the hydrochloride salt dissolves in about 10 mL of water and in 100 mL of alcohol. Tetracycline has become the most popular antibiotic of its group, largely because its plasma concentration appears to be higher and more enduring than that of either oxytetracycline or chlortetracycline. Also, it is found in higher concentration in the spinal fluid than the other two compounds.

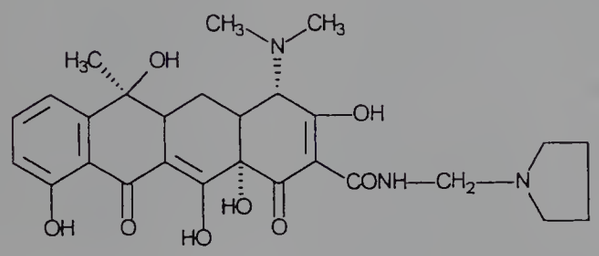
A number of combinations of tetracycline with agents that increase the rate and the height of plasma concentrations are on the market. One such adjuvant is magnesium chloride hexahydrate (Panmycin). Also, an insoluble tetracycline phosphate complex (Tetrex) is made by mixing a solution of tetracycline, usually as the hydrochloride, with a solution of sodium metaphosphate. A variety of claims concerning the efficacy of these adjuvants has been made. The mechanisms of their actions are not clear, but it has been reported[177,178] that these agents enhance plasma concentrations over those obtained when tetracycline hydrochloride alone is administered orally. Remmers et al.[179,180] have reported on the effects that selected aluminum–calcium gluconates complexed with some tetracyclines have on plasma concentrations when administered orally, intramuscularly, or intravenously. Such complexes enhanced plasma levels in dogs when injected but not when given orally. They also observed enhanced plasma levels in experimental animals when complexes of tetracyclines with aluminum metaphosphate, aluminum pyrophosphate, or aluminum–calcium phosphinicodilactates were administered orally. As noted previously, the tetracyclines are capable of forming stable chelate complexes with metal ions, such as calcium and magnesium, that would retard absorption from the gastrointestinal tract. The complexity of the systems involved has not permitted unequivocal substantiation of the idea that these

adjuvants compete with the tetracyclines for substances in the alimentary tract that would otherwise be free to complex with these antibiotics and thereby retard their absorption. Certainly, there is no evidence that the metal ions act, by any virtue they possess, as buffers, an idea alluded to sometimes in the literature.

Tetracycline hydrochloride is also available in ointments for topical and ophthalmic administration. A topical solution is used for the management of acne vulgaris.

Rolitetracycline, USP

N-(Pyrrolidinomethyl)-tetracycline (Syntetrin) was introduced for use by intramuscular or intravenous injection. This derivative is made by condensing tetracycline with pyrrolidine and formaldehyde in the presence of t-butyl alcohol. It is very soluble in water, 1 g dissolving in about 1 mL, and provides a means of injecting the antibiotic in a small volume of solution. It has been recommended in cases for which the oral dosage forms are not suitable, but it is no longer widely used.

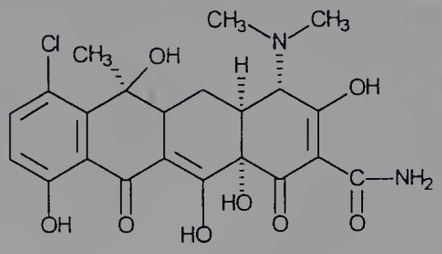


N-(pyrrolidinomethyl)tetracycline

Chlortetracycline Hydrochloride, USP (Aureomycin Hydrochloride)

Chlortetracycline was isolated by Duggar[181] in 1948 from *S. aureofaciens*. This compound, which was produced in an extensive search for new antibiotics, was the first of the group of highly successful tetracyclines. It soon became established as a valuable antibiotic with broad-spectrum activities. It is used in medicine chiefly as the acid salt of the compound whose systematic chemical designation is 7-chloro - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro - 3,6,10,12,12a - pentahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naph thacenecarboxamide. The hydrochloride salt is a crystalline powder with a bright yellow color, which suggested its brand name, Aureomycin. It is stable in air but slightly photosensitive and should be protected from light. It is odorless and bitter. One gram of the hydrochloride salt will dissolve in about 75 mL of water, producing a *p*H of about 3. It is only slightly soluble in alcohol and practically insoluble in other organic solvents.

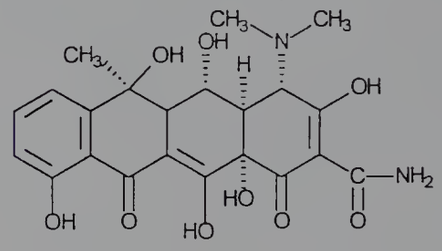
Oral and parenteral forms of chlortetracycline are no longer used because of the poor bioavailability and inferior pharmacokinetic properties of the drug. It is still marketed in ointment forms for topical and ophthalmic use.



Chlortetracycline

Oxytetracycline Hydrochloride, USP (Terramycin)

Early in 1950, Finlay et al.[182] reported the isolation of oxytetracycline from *Streptomyces rimosus*. It was soon established that this compound was a chemical analogue of chlortetracycline and showed similar antibiotic properties. The structure of oxytetracycline was elucidated by Hochstein et al.,[183] and this work provided the basis for the confirmation of the structure of the other tetracyclines.



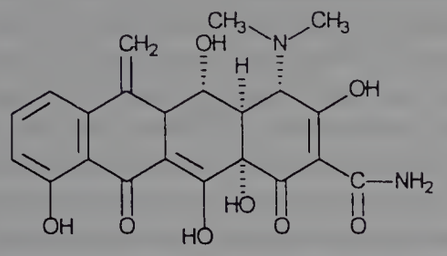
Oxytetracycline

Oxytetracycline hydrochloride is a pale yellow, bitter, crystalline compound. The amphoteric base is only slightly soluble in water and slightly soluble in alcohol. It is odorless and stable in air but darkens upon exposure to strong sunlight. The hydrochloride salt is a stable yellow powder that is more bitter than the free base. It is much more soluble in water, 1 g dissolving in 2 mL, and more soluble in alcohol than the free base. Both compounds are inactivated rapidly by alkali hydroxides and by acid solutions below *p*H 2. Both forms of oxytetracycline are absorbed rapidly and equally well from the digestive tract, so the only real advantage the free base offers over the hydrochloride salt is that it is less bitter. Oxytetracycline hydrochloride is also used for parenteral administration (intravenously and intramuscularly).

Methacycline Hydrochloride, USP

6-Deoxy-6-demethyl-6-methylene-5-oxytetracycline hydrochloride (Rondomycin). The synthesis of methacycline, reported by Blackwood et al.[184] in 1961, was accomplished by chemical modification of oxytetracycline. It has an antibiotic spectrum similar to that of the other tetracyclines but a greater potency: about 600 mg of methacycline is equivalent to 1 g of tetracycline. Its particular value lies in its longer

serum half-life: doses of 300 mg produce continuous serum antibacterial activity for 12 hr. Its toxic manifestations and contraindications are similar to those of the other tetracyclines.

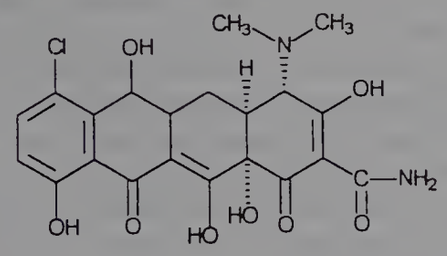


Methacycline

The greater stability of methacycline, both in vivo and in vitro, is a result of the modification at C-6. Removal of the 6-hydroxy group markedly increases the stability of ring C to both acids and bases, preventing the formation of isotetracyclines by bases. However, anhydrotetracyclines still can form by acid-catalyzed isomerization under strongly acidic conditions. Methacycline hydrochloride is a yellow to dark yellow, crystalline powder that is slightly soluble in water and insoluble in nonpolar solvents. It should be stored in tight, light-resistant containers in a cool place.

Demeclocycline, USP

7-Chloro-6-demethyltetracycline (Declomycin) was isolated in 1957 by McCormick et al.[171] from a mutant strain of *S. aureofaciens*. Chemically, it is 7-chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a - octahydro - 3,6,10,12,12a - pentahydroxy-1,11-dioxo-2-naphthacenecarboxamide. Thus, it differs from chlortetracycline only by the absence of the methyl group on C-6.

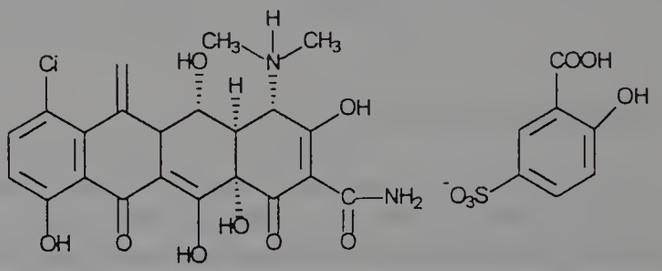


Demeclocycline

Demeclocycline is a yellow, crystalline powder that is odorless and bitter. It is sparingly soluble in water. A 1% solution has a *p*H of about 4.8. It has an antibiotic spectrum similar to that of other tetracyclines, but it is slightly more active than the others against most of the microorganisms for which they are used. This, together with its slower rate of elimination through the kidneys, gives demeclocycline an effectiveness comparable to that of the other tetracyclines, at about three-fifths of the dose. Similar to the other tetracyclines, it may cause infrequent photosensitivity reactions that produce erythema after exposure to sunlight. Demeclocycline may produce the reaction somewhat more frequently than the other tetracyclines. The incidence of discoloration and mottling of the teeth in youths from demeclocycline appears to be as low as with the other tetracyclines.

Meclocycline Sulfosalicylate, USP

7 - Chloro - 6 - deoxy - 6 - demethyl - 6 - methylene - 5 -oxytetracycline sulfosalicylate (Meclan) is a semisynthetic derivative prepared from oxytetracycline.[184] Although meclocycline has been used in Europe for many years, it became available only relatively recently in the United States for a single therapeutic indication, the treatment of acne. It is available as the sulfosalicylate salt in a 1% cream.



Meclocycline Sulfosalicylate

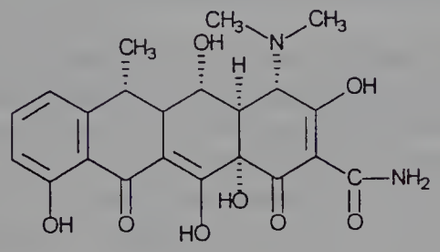
Meclocycline sulfosalicylate is a bright yellow, crystalline powder that is slightly soluble in water and insoluble in organic solvents. It is light-sensitive and should be stored in light-resistant containers.

Doxycycline, USP

α-6-Deoxy-5-oxytetracycline (Vibramycin). A more recent addition to the tetracycline group of antibiotics available for antibacterial therapy is doxycycline, first reported by Stephens et al.[185] in 1958. It was obtained first in small yields by a chemical transformation of oxytetracycline, but it is now produced by catalytic hydrogenation of methacycline or by reduction of a benzylmercaptan derivative of methacycline with Raney nickel. In the latter process, a nearly pure form of the 6α-methyl epimer is produced. It is noteworthy that the 6α-methyl epimer is more than three times as active as its β-epimer.[169] Apparently, the difference in orientation of the methyl groups, slightly affecting the shapes of the molecules, causes a substantial difference in biologic effect. Also, absence of the 6-hydroxyl group produces a compound that is very stable in acids and bases and that has a long biologic half-life. In addition, it is absorbed very well from the gastrointestinal tract, thus allowing a smaller dose to be administered. High tissue levels are obtained with it, and unlike other tetracyclines, doxycycline apparently does not accumulate in patients with impaired renal function. Therefore, it is preferred for uremic patients with infections outside the urinary tract. However, its low renal clearance may limit its effectiveness in urinary tract infections.

Doxycycline is available as a hydrate salt, a hydrochloride

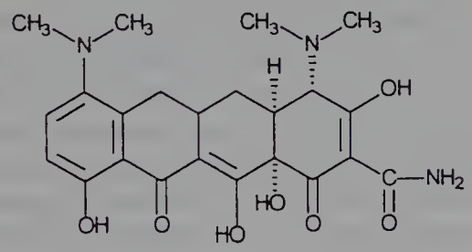
salt solvated as the hemiethanolate hemihydrate, and a monohydrate. The hydrate form is sparingly soluble in water and is used in the capsule dosage form; the monohydrate is water-insoluble and is used for aqueous suspensions, which are stable for up to 2 weeks when kept in a cool place.



Doxycycline

Minocycline Hydrochloride, USP

7 - Dimethylamino - 6 - demethyl - 6 - deoxytetracycline (Minocin, Vectrin). Minocycline, the most potent tetracycline currently employed in therapy, is obtained by reductive methylation of 7-nitro-6-demethyl-6-deoxytetracycline.[172] It was released for use in the United States in 1971. Because minocycline, like doxycycline, lacks the 6-hydroxyl group, it is stable in acids and does not dehydrate or rearrange to anhydro or lactone forms. Minocycline is well absorbed orally to give high plasma and tissue levels. It has a very long serum half-life, resulting from slow urinary excretion and moderate protein binding. Doxycycline and minocycline, along with oxytetracycline, show the least in vitro calcium binding of the clinically available tetracyclines. The improved distribution properties of the 6-deoxytetracyclines have been attributed to a greater degree of lipid solubility.

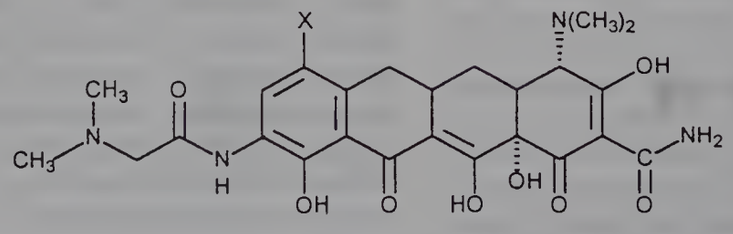


Minocycline

Perhaps the most outstanding property of minocycline is its activity toward gram-positive bacteria, especially staphylococci and streptococci. In fact, minocycline has been effective against staphylococcal strains that are resistant to methicillin and all other tetracyclines, including doxycycline.[186] Although it is doubtful that minocycline will replace bactericidal agents for the treatment of life-threatening staphylococcal infections, it may become a useful alternative for the treatment of less serious tissue infections. Minocycline has been recommended for the treatment of chronic bronchitis and other upper respiratory tract infections. Despite its relatively low renal clearance, partially compensated for by high serum and tissue levels, it has been recommended for the treatment of urinary tract infections. It has been effective in the eradication of *N. meningitidis* in asymptomatic carriers.

Investigational Tetracyclines

The remarkably broad spectrum of antimicrobial activity of the tetracyclines notwithstanding, the widespread emergence of bacterial genes and plasmids encoding tetracycline resistance has increasingly imposed limitations on the clinical applications of this antibiotic class in recent years.[164] This situation has prompted researchers at Lederle Laboratories to reinvestigate SARs of tetracyclines substituted in the aromatic (D) ring in an effort to discover analogues that might be effective against resistant strains. As a result of these efforts, the glycylcyclines, a class of 9-dimethylglycylamido-(DMG)-substituted tetracyclines exemplified by DMG-minocycline (DMG-MINO) and DMG-6-methyl-6-deoxytetracycline (DMG-DMDOT), were discovered.[187–189] The glycylcyclines retain the broad spectrum of activity and potency exhibited by the original tetracyclines against tetracycline-sensitive microbial strains and are highly active against bacterial strains that exhibit tetracycline resistance mediated by efflux or ribosomal protection determinants. If ongoing clinical evaluations of the glycylcyclines establish favorable toxicologic and pharmacokinetic profiles for these compounds, a new class of "second-generation" tetracyclines could be launched.



X = $N(CH_3)_2$ 9-(Dimethylglycylamino)minocycline (DMG-MINO)
X = H 9-(Dimethylglycylamino)-6-demethyl-6-deoxytetracycline (DMG-DMDOT)

THE MACROLIDES

Among the many antibiotics isolated from the actinomycetes is the group of chemically related compounds called the macrolides. In 1950, picromycin, the first of this group to be identified as a macrolide compound, was first reported. In 1952, erythromycin and carbomycin were reported as new antibiotics, and these were followed in subsequent years by other macrolides. Currently, more than 40 such compounds are known, and new ones are likely to appear in the future. Of all of these, only two, erythromycin and oleandomycin, have been available consistently for medical use in the United States. In recent years, interest has shifted away from novel macrolides isolated from soil samples (such as spiramycin, josamicin, and rosamicin), all of which thus far have proven to be clinically inferior to erythromycin and semisynthetic derivatives of erythromycin (such as clarithromycin and azithromycin), which have superior pharmacokinetic properties due to their enhanced acid stability and improved distribution properties.

CHEMISTRY

The macrolide antibiotics have three common chemical characteristics: (1) a large lactone ring (which prompted the name "macrolide"), (2) a ketone group, and (3) a glycosidically linked amino sugar. Usually, the lactone ring has 12, 14, or 16 atoms in it and is often unsaturated, with an olefinic group conjugated with the ketone function. (The polyene macrocyclic lactones, such as natamycin and amphotericin B; the ansamycins, such as rifampin; and the polypeptide lactones generally are not included among the macrolide antibiotics.) They may have, in addition to the amino sugar, a neutral sugar that is linked glycosidically to the lactone ring (see "Erythromycin," below). Because of the presence of the dimethylamino group on the sugar moiety, the macrolides are bases that form salts with pK_a values between 6.0 and 9.0. This feature has been employed to make clinically useful salts. The free bases are only slightly soluble in water but dissolve in somewhat polar organic solvents. They are stable in aqueous solutions at or below room temperature but are inactivated by acids, bases, and heat.

The chemistry of macrolide antibiotics has been the subject of several reviews.[190,191]

MECHANISM OF ACTION AND RESISTANCE

Some details of the mechanism of antibacterial action of erythromycin are known. It binds selectively to a specific site on the 50S ribosomal subunit to prevent the translocation step of bacterial protein synthesis.[192] It does not bind to mammalian ribosomes. Broadly based, nonspecific resistance to the antibacterial action of erythromycin among many species of gram-negative bacilli appears to be related, in large part, to the inability of the antibiotic to penetrate effectively the cell walls of these organisms.[193] In fact, the sensitivities of members of the *Enterobacteriaceae* family are *p*H-dependent, with MICs decreasing as a function of increasing *p*H. Furthermore, protoplasts from gram-negative bacilli, lacking cell walls, are sensitive to erythromycin. A highly specific resistance mechanism to the macrolide antibiotics occurs in erythromycin-resistant strains of *Staph. aureus.*[194,195] Such strains produce an enzyme that methylates a specific adenine residue at the erythromycin-binding site of the bacterial 50S ribosomal subunit. The methylated ribosomal RNA remains active in protein synthesis but no longer binds erythromycin. Bacterial resistance to the lincomycins apparently also occurs by this mechanism.

SPECTRUM OF ACTIVITY

The spectrum of antibacterial activity of the more potent macrolides, such as erythromycin, resembles that of penicillin. They are frequently active against bacterial strains that are resistant to the penicillins. The macrolides are generally effective against most species of gram-positive bacteria, both cocci and bacilli, and exhibit useful effectiveness against gram-negative cocci, especially *Neisseria* species. Many of the macrolides are also effective against *Treponema pallidum.* In contrast to penicillin, macrolides are also effective against *Mycoplasma, Chlamydia, Campylobacter,* and *Legionella* species. Their activity against most species of gram-negative bacilli is generally low and often unpredictable, though some strains of *H. influenzae* and *Brucella* spp. are sensitive.

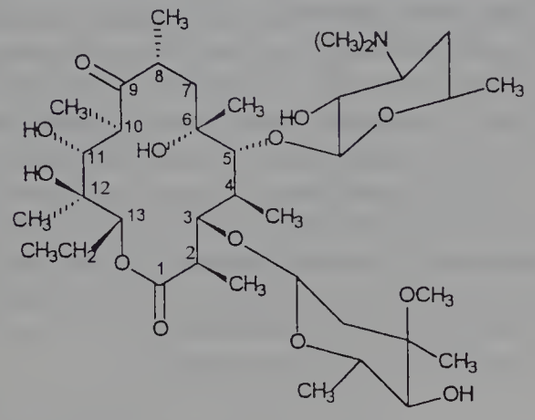
PRODUCTS

Erythromycin, USP (E-Mycin, Erythrocin, Ilotycin)

Early in 1952, McGuire et al.[196] reported the isolation of erythromycin from *Streptomyces erythreus.* It achieved rapid early acceptance as a well-tolerated antibiotic of value for the treatment of a variety of upper respiratory and soft-tissue infections caused by gram-positive bacteria. It is also effective against many venereal diseases, including gonorrhea and syphilis, and provides a useful alternative for the treatment of many infections in patients allergic to penicillins. More recently, erythromycin has been shown to be effective therapy for Eaton agent pneumonia (*Mycoplasma pneumoniae*), venereal diseases caused by chlamydiae, bacterial enteritis caused by *Campylobacter jejuni,* and legionnaires' disease.

The commercial product is erythromycin A, which differs from its biosynthetic precursor, erythromycin B, in having a hydroxyl group at the 12-position of the aglycone. The chemical structure of erythromycin A was reported by Wiley

et al.[197] in 1957 and its stereochemistry by Celmer[198] in 1965. An elegant synthesis of erythronolide A, the aglycone present in erythromycin A, has been described by Corey and associates.[199]



Erythromycin

The amino sugar attached through a glycosidic link to C-5 is desosamine, a structure found in a number of other macrolide antibiotics. The tertiary amine of desosamine (3,4,6-trideoxy-3-dimethylamino-D-*xylo*-hexose) confers a basic character to erythromycin and provides the means by which acid salts may be prepared. The other carbohydrate structure linked as a glycoside to C-3 is called cladinose (2,3,6-trideoxy-3-methoxy-3-C-methyl-L-*ribo*-hexose) and is unique to the erythromycin molecule.

As is common with other macrolide antibiotics, compounds closely related to erythromycin have been obtained from culture filtrates of *S. erythreus*. Two such analogues have been found, erythromycins B and C. Erythromycin B differs from erythromycin A only at C-12, at which a hydrogen has replaced the hydroxyl group. The B analogue is more acid-stable but has only about 80% of the activity of erythromycin. The C analogue differs from erythromycin by the replacement of the methoxyl group on the cladinose moiety with a hydrogen atom. It appears to be as active as erythromycin but is present in very small amounts in fermentation liquors.

Erythromycin is a very bitter, white or yellow-white, crystalline powder. It is soluble in alcohol and in the other common organic solvents but only slightly soluble in water. The free base has a pK_a of 8.8. Saturated aqueous solutions develop an alkaline *p*H in the range of 8.0 to 10.5. It is extremely unstable at a *p*H of 4 or lower. The optimum *p*H for stability of erythromycin is at or near neutrality.

Erythromycin may be used as the free base in oral dosage forms and for topical administration. To overcome its bitterness and irregular oral absorption (resulting from acid destruction and adsorption onto food), a variety of enteric-coated and delayed-release dose forms of erythromycin base have been developed. These forms have been fully successful in overcoming the bitterness but have solved only marginally problems of oral absorption. Chemical modifications of erythromycin have been made with primarily two different goals in mind: (1) to increase either its water or lipid solubility for parenteral dosage forms and (2) to increase its acid stability (and possibly its lipid solubility) for improved oral absorption. Modified derivatives of the antibiotic are of two types: acid salts of the dimethylamino group of the desosamine moiety (e.g., the glucoheptonate, the lactobionate, and the stearate) and esters of the 2′-hydroxyl group of the desosamine (e.g., the ethylsuccinate and the propionate, available as the laurylsulfate salt and known as the estolate).

The stearate salt and the ethylsuccinate and propionate esters are used in oral dose forms, intended to improve absorption of the antibiotic. The stearate releases erythromycin base in the intestinal tract, which is then absorbed. The ethylsuccinate and the estolate are absorbed largely intact and are hydrolyzed partially by plasma and tissue esterases to give free erythromycin. The question of bioavailability of the antibiotic from its various oral dosage and chemical forms has been the subject of considerable concern and dispute over the past two decades.[200–205] It is believed generally that the 2′-esters, of themselves, have little or no intrinsic antibacterial activity[206] and, therefore, must be hydrolyzed to the parent antibiotic in vivo. Although the ethylsuccinate is hydrolyzed more efficiently than the estolate in vivo and, in fact, provides higher levels of erythromycin following intramuscular administration, an equal dose of the estolate gives higher levels of the free antibiotic following oral administration.[201,205] Superior oral absorption of the estolate is attributed to both its greater acid stability and higher intrinsic absorption compared with the ethylsuccinate. Also, oral absorption of the estolate, unlike that of both the stearate and the ethylsuccinate, is not affected by food or fluid volume content of the gut. Superior bioavailability of active antibiotic from oral administration of the estolate over the ethylsuccinate, stearate, or erythromycin base cannot necessarily be assumed, however, because the estolate is protein-bound more extensively than erythromycin itself.[207] Measured fractions of plasma protein binding for erythromycin-2′-propionate and erythromycin base range from 0.94 to 0.98 for the former and from 0.73 to 0.90 for the latter, indicating a much higher level of free erythromycin in the plasma. Bioavailability studies comparing equivalent doses of the enteric-coated base, the stearate salt, the ethylsuccinate ester, and the estolate ester in human volunteers[203,204] showed delayed but slightly higher bioavailability for the free base than for the stearate, ethylsuccinate, or estolate.

One study, comparing the clinical effectiveness of recommended doses of the stearate, estolate, ethylsuccinate, and free base in the treatment of respiratory tract infections, failed to demonstrate substantial differences among them.[208] However, two other clinical studies, comparing the effectiveness of the ethylsuccinate and the estolate in the treatment of streptococcal pharyngitis, found the estolate to be superior.[209,210]

The water-insoluble ethylsuccinate ester is also available as a suspension for intramuscular injection. The glucoheptonate and lactobionate salts, however, are highly water-soluble derivatives that provide high plasma levels of the active antibiotic immediately after intravenous injection.

Aqueous solutions of these salts may also be administered by intramuscular injection, but this is not a common practice.

Erythromycin is distributed throughout the body water. It persists in tissues longer than in the blood. The antibiotic is concentrated by the liver and excreted extensively into the bile. Large amounts are excreted in the feces, in part due to poor oral absorption and in part due to biliary excretion. The serum half-life is 1.4 hr. Some cytochrome P450-catalyzed oxidative demethylation to a less-active metabolite may also occur. Erythromycin inhibits cytochrome P450-requiring oxidases, leading to a variety of potential drug interactions. Thus, toxic effects of theophylline, the hydroxycoumarin anticoagulants, the benzodiazepines alprazolam and midazolam, carbamazepine, cyclosporin, and the antihistaminic drugs terfenidine and aztemizole may be potentiated by erythromycin.

The toxicity of erythromycin is comparatively low. Primary adverse reactions to the antibiotic are related to its actions on the gastrointestinal tract and the liver. Erythromycin may cause stimulation of gastrointestinal motility following either oral or parenteral administration.[211] This dose-related, prokinetic effect can cause abdominal cramps, epigastric distress, and diarrhea, especially in children and young adults. Cholestalic hepatitis occurs occasionally with erythromycin, usually in adults and more frequently with the estolate.

Erythromycin Stearate, USP (Ethril, Wyamycin S, Erypar)

Erythromycin stearate is the stearic acid salt of erythromycin. Like erythromycin base, the stearate is acid-labile. It is film-coated to protect it from acid degradation in the stomach. In the alkaline *p*H of the duodenum, the free base is liberated from the stearate and absorbed. Erythromycin stearate occurs as a crystalline powder that is practically insoluble in water but soluble in alcohol and ether.

Erythromycin Ethylsuccinate, USP (EES, Pediamycin, Eryped)

Erythromycin ethylsuccinate is the ethylsuccinate mixed ester of erythromycin in which the 2′-hydroxyl group of the desosamine is esterified. It is absorbed as the ester and hydrolyzed slowly in the body to form erythromycin. It is somewhat acid-labile, and its absorption is enhanced by the presence of food. The ester is insoluble in water but soluble in alcohol and ether.

Erythromycin Estolate, USP (Ilosone)

Erythromycin propionate laurylsulfate is the laurylsulfate salt of the 2′-propionate ester of erythromycin. Erythromycin estolate is acid-stable and absorbed as the propionate ester. The ester undergoes slow hydrolysis in vivo. Only the free base binds to bacterial ribosomes. However, there is some evidence to suggest that the ester is taken up by bacterial cells more rapidly than the free base and undergoes hydrolysis by bacterial esterases within the cells. The incidence of cholestatic hepatitis is reportedly higher with the estolate than with other erythromycin preparations.

Erythromycin estolate occurs as long needles that are sparingly soluble in water but soluble in organic solvents.

Erythromycin Gluceptate, Sterile, USP

Erythromycin glucoheptonate (Ilotycin Gluceptate) is the glucoheptonic acid salt of erythromycin. It is a crystalline substance that is freely soluble in water and practically insoluble in organic solvents. Erythromycin gluceptate is intended for intravenous administration for the treatment of serious infections, such as Legionnaires' disease, or when oral administration is not possible. Solutions are stable for 1 week when refrigerated.

Erythromycin Lactobionate, USP

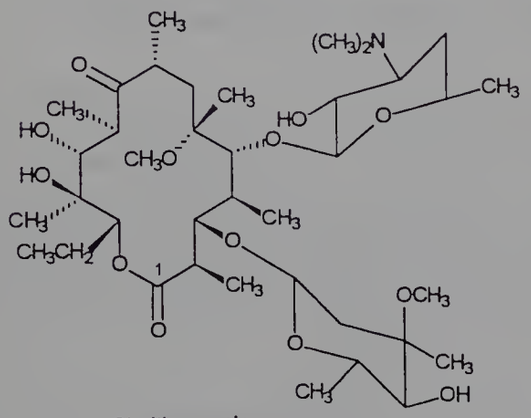
Erythromycin lactobionate is a water-soluble salt prepared by reacting erythromycin base with lactobiono-δ-lactone. It occurs as an amorphous powder that is freely soluble in water and alcohol and slightly soluble in ether. It is intended, after reconstitution in sterile water, for intravenous administration to achieve high plasma levels in the treatment of serious infections.

Clarithromycin USP (Biaxin)

Clarithromycin is the 6-methyl ether of erythromycin. The simple methylation of the 6-hydroxyl group of erythromycin creates a semisynthetic derivative that fully retains the antibacterial properties of the parent antibiotic, while markedly increasing acid stability and oral bioavailability and reducing gastrointestinal side effects associated with erythromycin.[212] Acid-catalyzed dehydration of erythromycin in the stomach initiates as a sequence of reactions, beginning with $\Delta^{6,7}$-bond migration followed by formation of an 8,9-anhydro-6,9-hemiketal and terminating in a 6,9:9,12-spiroketal. Since neither the hemiketal nor the spiroketal exhibits significant antibacterial activity, unprotected erythromycin is inactivated substantially in the stomach. Furthermore, evidence suggests that the hemiketal may be largely responsible for the gastrointestinal (prokinetic) side effects associated with oral erythromycin.[211]

Clarithromycin is well absorbed following oral administration. Its oral bioavailability is estimated to be 50% to 55%. The presence of food does not significantly affect its

absorption. Extensive metabolism of clarithromycin by oxidation and hydrolysis occurs in the liver. The major metabolite is the 14-hydroxyl derivative, which retains antibacterial activity. The amount of clarithromycin excreted in the urine ranges from 20% to 30%, depending on the dose, while 10% to 15% of the 14-hydroxy metabolite is excreted in the urine. Biliary excretion of clarithromycin is much less than that of erythromycin. Clarithromycin is widely distributed into the tissues, which retain much higher concentrations than the plasma. Protein-binding fractions in the plasma range from 65% to 70%. The plasma half-life of clarithromycin is 4.3 hr.



Clarithromycin

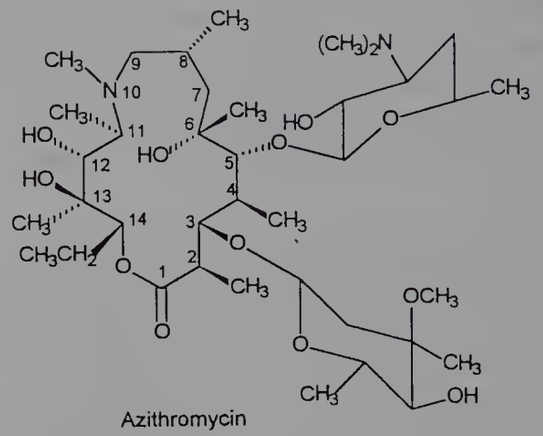
Some of the microbiologic properties of clarithromycin also appear to be superior to those of erythromycin. It exhibits greater potency against *Myc. pneumoniae, Legionella* spp., *Chlamydia pneumoniae, H. influenzae,* and *Mor. catarrhalis* than does erythromycin. Also of interest is the activity of clarithromycin against unusual pathogens such as *Borrelia burgdorferi*, the cause of Lyme disease, and the *Mycobacterium avium* complex (MAC). Clarithromycin is significantly more active than erythromycin against group A streptococci, *Streptococcus pneumoniae*, and the viridans group of streptococci in vivo because of its superior oral bioavailability. However, the greater cost of clarithromycin compared with erythromycin must be weighed against its potentially greater effectiveness.

Adverse reactions to clarithromycin are rare. The most common complaints relate to gastrointestinal symptoms, but these seldom require discontinuance of therapy. Clarithromycin, like erythromycin, inhibits cytochrome P450 oxidases and, thus, can potentiate the actions of drugs metabolized by these enzymes.

Clarithromycin occurs as a white crystalline solid that is practically insoluble in water, sparingly soluble in alcohol, and freely soluble in acetone. It is provided in the form of 250- and 500-mg oral tablets and as granules for the preparation of aqueous oral suspensions containing 25 or 50 mg/mL.

Azithromycin USP (Zithromax)

Azithromycin is a semisynthetic derivative of erythromycin, prepared by Beckman rearrangement of the corresponding 6-oxime, followed by *N*-methylation and reduction of the resulting ring-expanded lactam. It is a prototype of a series of nitrogen-containing, 15-membered ring macrolides known as azalides.[213] Removal of the 9-keto group coupled with incorporation of a weakly basic tertiary amine nitrogen function into the macrolide ring increases the stability of azithromycin to acid-catalyzed degradation. These changes also increase the lipid solubility of the molecule, thereby conferring unique pharmacokinetic and microbiologic properties.[214]



Azithromycin

The oral bioavailability of azithromycin is good, nearly 40%, provided the antibiotic is administered at least 1 hr before or 2 hr after a meal. Food decreases its absorption by as much as 50%. The pharmacokinetics of azithromycin are characterized by rapid and extensive removal of the drug from the plasma into the tissues followed by a slow release. Tissue levels far exceed plasma concentrations, leading to a highly variable and prolonged elimination half-life of up to 5 days. The fraction of azithromycin bound to plasma proteins is only about 50% and does not exert an important influence on its distribution. Available evidence indicates that azithromycin is largely excreted in the feces unchanged, with a small percentage appearing in the urine. Extensive enterohepatic recycling of the drug occurs. Azithromycin apparently is not metabolized to any significant extent. In contrast to the 14-membered ring macrolides, azithromycin does not significantly inhibit cytochrome P450 enzymes to create potential drug interactions.

The spectrum of antimicrobial activity of azithromycin is similar to that observed for erythromycin and clarithromycin but with some interesting differences. In general, it is more active against gram-negative bacteria and less active against gram-positive bacteria than its close relatives. The greater activity of azithromycin against *H. influenzae, Mor. catarrhalis*, and *Myc. pneumoniae* coupled with its extended half-life permits a 5-day dosing schedule for the treatment of respiratory tract infections caused by these pathogens. The clinical efficacy of azithromycin in the treatment of urogenital and other sexually transmitted infections caused by *Chlamydia trachomatis, N. gonorrhoeae, Hemophilus ducreyi*, and *Ureaplasma urealytecum* suggest that single-dose therapy with the antibiotic for uncomplicated urethritis or cervicitis may have advantages over other antibiotics.

Dirithromycin (Dynabac)

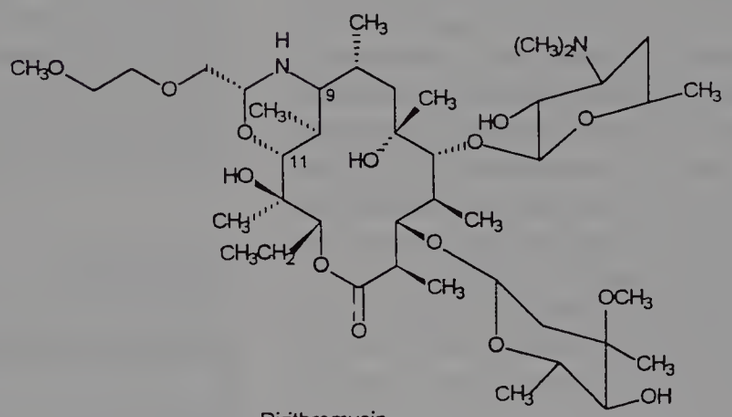
Dirithromycin is a more lipid-soluble prodrug derivative of 9S-erythromycyclamine prepared by condensation of the latter with 2-(2-methoxyethoxy)acetaldehyde.[215] The 9*N*,11*O*-oxazine ring thus formed is a hemi-aminal that is unstable under both acidic and alkaline aqueous conditions and undergoes spontaneous hydrolysis to form erythromycyclamine. Erythromycyclamine is a semisynthetic derivative of erythromycin wherein the 9-keto group of the erythronolide ring has been converted to an amino group. Erythromycyclamine retains the antibacterial properties of erythromycin in vitro but exhibits poor bioavailability following oral administration. The prodrug, dirithromycin, is provided as enteric-coated tablets to protect it from acid-catalyzed hydrolysis in the stomach.

Orally administered dirithromycin is absorbed rapidly into the plasma, largely from the small intestine. Spontaneous hydrolysis to erythromycyclamine occurs in the plasma. Oral bioavailability is estimated to be around 10%, but food does not affect absorption of the prodrug.

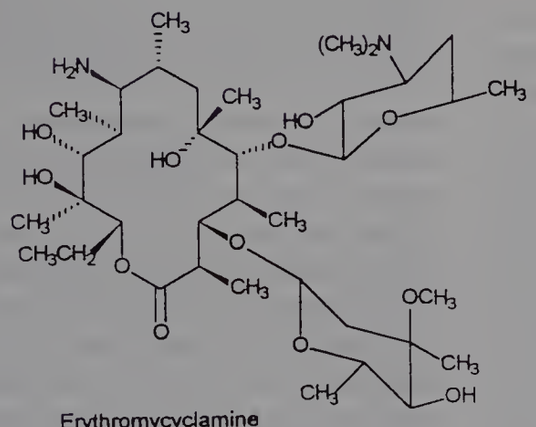
The low plasma levels and large volume of distribution of erythromycyclamine are believed to result from its rapid distribution into well-perfused tissues, such as lung parenchymal, bronchial mucosal, nasal mucosal, and prostatic tissues. The drug also concentrates in human neutrophils. The elimination half-life is estimated to be 30 to 44 hr. The majority of the prodrug and its active metabolite (62% to 81% in normal human subjects) is excreted in the feces, largely via the bile, following either oral or parenteral administration. Urinary excretion accounts for less than 3%.

The incidence and severity of gastrointestinal side effects associated with dirithromycin are similar to those seen with oral erythromycin. Preliminary studies indicate that dirithromycin and erythromycyclamine do not interact significantly with cytochrome P450 oxygenases. Thus, the likelihood of interference in the oxidative metabolism of drugs, such as phenytoin, theophylline, and cyclosporin, by these enzymes may be less with dirithromycin than with erythromycin.

Dirithromycin is recommended as an alternative to erythromycin for the treatment of bacterial infections of the upper and lower respiratory tracts, such as pharyngitis, tonsillitis, bronchitis, and pneumonia, and for bacterial infections of other soft tissues and the skin. The once-daily dosing schedule for dirithromycin is advantageous in terms of better patient compliance. Its place in therapy remains to be fully assessed.[216]



Dirithromycin

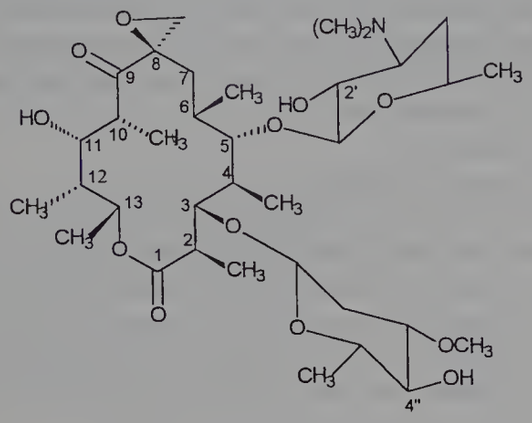


Erythromycyclamine

Troleandomycin

Triacetyloleandomycin (TAO). Oleandomycin, as its triacetyl derivative troleandomycin, remains available as an alternative to erythromycin for limited indications permitting use of an oral dosage form. Oleandomycin was isolated by

Sobin and associates.[217] The structure of oleandomycin was proposed by Hochstein et al.[218] and its absolute stereochemistry elucidated by Celmer.[219] The oleandomycin structure consists of two sugars and a 14-member lactone ring designated "oleandolide." One of the sugars is desosamine, also present in erythromycin; the other is L-oleandrose. The sugars are linked glycosidically to the 5- and 3-positions, respectively, of oleandolide.



Oleandomycin

Oleandomycin contains three hydroxyl groups that are subject to acylation, one in each of the sugars and one in the oleandolide. The triacetyl derivative retains the in vivo antibacterial activity of the parent antibiotic but possesses superior pharmacokinetic properties. It is hydrolyzed in vivo to oleandomycin. Troleandomycin achieves more rapid and higher plasma concentrations following oral administration than oleandomycin phosphate, and it has the additional advantage of being practically tasteless. Troleandomycin occurs as as a white, crystalline solid that is nearly insoluble in water. It is relatively stable in the solid state but undergoes chemical degradation in either aqueous acidic or alkaline conditions.

Because the antibacterial spectrum of activity of oleandomycin is considered to be inferior to that of erythromycin, the pharmacokinetics of troleandomycin have not been studied extensively. Oral absorption is apparently good, and detectable blood levels of oleandomycin persist up to 12 hr after a 500 mg dose of troleandomycin. Approximately 20% is recovered in the urine, with the majority excreted in the feces, primarily as a result of biliary excretion. There is some epigastric distress following oral administration with an incidence similar to that caused by erythromycin. Troleandomycin is the most potent inhibitor of cytochrome P450 enzymes of the commercially available macrolides. It may potentiate the hepatic toxicity of certain anti-inflammatory steroids and oral contraceptive drugs, as well as the toxic effects of theophylline, carbamazepine, and triazolam. Several allergic reactions, including cholestatic hepatitis, have also been reported with the use of troleandomycin.

Approved medical indications for troleandomycin are currently limited to the treatment of upper respiratory infections caused by such organisms as *Streptococcus pyogenes* and *Streptococcus pneumoniae*. It may be considered an alternative drug to oral forms of erythromycin. It is available in capsules and as a suspension.

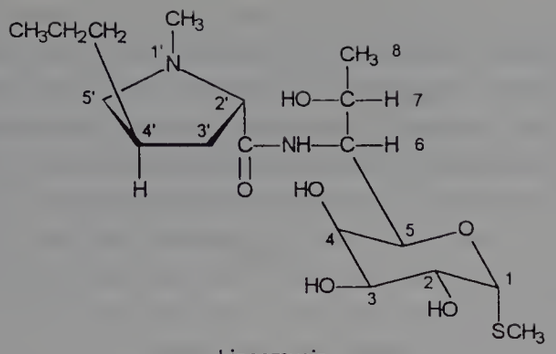
THE LINCOMYCINS

The lincomycins are sulfur-containing antibiotics isolated from *Streptomyces lincolnensis*. Lincomycin is the most active and medically useful of the compounds obtained from fermentation. Extensive efforts to modify the lincomycin structure to improve its antibacterial and pharmacologic properties resulted in the preparation of the 7-chloro-7-deoxy derivative clindamycin. Of the two antibiotics, clindamycin appears to have the greater antibacterial potency and better pharmacokinetic properties. Lincomycins resemble macrolides in antibacterial spectrum and biochemical mechanisms of action. They are primarily active against gram-positive bacteria, particularly the cocci, but are also effective against non–spore-forming anaerobic bacteria, actinomycetes, mycoplasma, and some species of *Plasmodium*. Lincomycin binds to the 50S ribosomal subunit to inhibit protein synthesis. Its action may be bacteriostatic or bactericidal depending on a variety of factors, which include the concentration of the antibiotic. A pattern of bacterial resistance and cross-resistance to lincomycins similar to that observed with the macrolides has been emerging.[195]

PRODUCTS

Lincomycin Hydrochloride, USP (Lincocin)

This antibiotic, which differs chemically from other major antibiotic classes, was isolated by Mason et al.[220] Its chemistry has been described by Hoeksema and co-workers,[221] who assigned the structure, later confirmed by Slomp and MacKellar,[222] given in the diagram below. Total syntheses of the antibiotic were accomplished independently in 1970 through research efforts in England and the United States.[223,224] The structure contains a basic function, the pyrrolidine nitrogen, by which water-soluble salts having an apparent pK_a of 7.6 may be formed. When subjected to hydrazinolysis, lincomycin is cleaved at its amide bond into *trans*-L-4-*n*-propylhygric acid (the pyrrolidine moiety) and methyl α-thiolincosamide (the sugar moiety). Lincomycin-related antibiotics have been reported by Argoudelis[225] to be produced by *S. lincolnensis*. These antibiotics differ in structure at one or more of three positions of the lincomycin structure: (1) the *N*-methyl of the hygric acid moiety is substituted by a hydrogen; (2) the *n*-propyl group of the hygric acid moiety is substituted by an ethyl group; and (3) the thiomethyl ether of the α-thiolincosamide moiety is substituted by a thioethyl ether.



Lincomycin

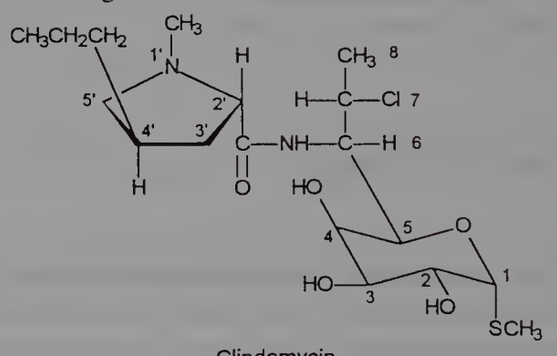
Lincomycin is employed for the treatment of infections caused by gram-positive organisms, notably staphylococci, β-hemolytic streptococci, and pneumococci. It is absorbed moderately well orally and distributed widely in the tissues. Effective concentrations are achieved in bone for the treatment of staphylococcal osteomyelitis but not in the cerebrospinal fluid for the treatment of meningitis. At one time, lincomycin was thought to be a nontoxic compound, with a low incidence of allergy (skin rashes) and occasional gastrointestinal complaints (nausea, vomiting, and diarrhea) as the only adverse effects. However, recent reports of severe diarrhea and the development of pseudomembranous colitis in patients treated with lincomycin (or clindamycin) have brought about the need for reappraisal of the position these antibiotics should have in therapy. In any event, clindamycin is superior to lincomycin for the treatment of most infections for which these antibiotics are indicated.

Lincomycin hydrochloride occurs as the monohydrate, a white, crystalline solid that is stable in the dry state. It is readily soluble in water and alcohol, and its aqueous solutions are stable at room temperature. It is degraded slowly in acid solutions but is absorbed well from the gastrointestinal tract. Lincomycin diffuses well into peritoneal and pleural fluids and into bone. It is excreted in the urine and the bile. It is available in capsule form for oral administration and in ampules and vials for parenteral administration.

Clindamycin Hydrochloride, USP

7(*S*)-Chloro-7-deoxylincomycin (Cleocin). In 1967, Magerlein et al.[226] reported that replacement of the 7(*R*)-hydroxy group of lincomycin by chlorine with inversion of configuration resulted in a compound with enhanced antibacterial activity in vitro. Clinical experience with this semisynthetic derivative, called clindamycin and released in 1970, has established that its superiority over lincomycin is even greater in vivo. Improved absorption and higher tissue levels of clindamycin and its greater penetration into bacteria have been attributed to its higher partition coefficient compared with that of lincomycin. Structural modifications at C-7, for example, 7(*S*)-chloro and 7(*R*)-OCH_3, and of the C-4 alkyl groups of the hygric acid moiety[227] appear to influence activity of congeners more through an effect on the partition coefficient of the molecule than through a stereospecific binding role. However, changes in the α-thiolincosamide portion of the molecule appear to decrease markedly activity, as evidenced by the marginal activity of 2-deoxylincomycin, its anomer, and 2-*O*-methyllincomycin.[227,228] Exceptions to this are fatty acid and phosphate esters of the 2-hydroxyl group of lincomycin and clindamycin, which are hydrolyzed rapidly in vivo to the parent antibiotics.

Clindamycin is recommended for the treatment of a wide variety of upper respiratory, skin, and tissue infections caused by susceptible bacteria. Its activity against streptococci, staphylococci, and pneumococci is undisputably high; and it is one of the most potent agents available against some non-spore-forming anaerobic bacteria, the *Bacteroides* species in particular. However, an increasing number of reports of clindamycin-associated gastrointestinal toxicity, which range in severity from diarrhea to an occasionally serious pseudomembranous colitis, have caused some clinical experts to call for a reappraisal of the appropriate position of this antibiotic in therapy. Clindamycin- (or lincomycin)-associated colitis may be particularly dangerous in elderly or debilitated patients and has caused deaths in such individuals. The colitis, which is usually reversible when the drug is discontinued, is now believed to result from an overgrowth of a clindamycin-resistant strain of the anaerobic intestinal bacterium *Clostridium difficile*.[229] Damage to the intestinal lining is caused by a glycoprotein endotoxin released by lysis of this organism.



Clindamycin

The glycopeptide antibiotic vancomycin has been effective in the treatment of clindamycin-induced pseudomembranous colitis and in the control of the experimentally induced bacterial condition in animals. Clindamycin should be reserved for staphylococcal tissue infections, such as cellulitis and osteomyelitis, in penicillin-allergic patients and for severe anaerobic infections outside the central nervous system. Ordinarily, it should not be used to treat upper respiratory tract infections caused by bacteria sensitive to other, safer antibiotics or in prophylaxis.

Clindamycin is absorbed rapidly from the gastrointestinal tract, even in the presence of food. It is available as the crystalline, water-soluble hydrochloride hydrate (hyclate) and the 2-palmitate ester hydrochloride salts in oral dosage forms and as the 2-phosphate ester in solutions for intramuscular or intravenous injection. All forms are chemically very stable in solution and in the dry state.

Clindamycin Palmitate Hydrochloride, USP (Cleocin Pediatric)

Clindamycin palmitate hydrochloride is the hydrochloride salt of the palmitic acid ester of cleomycin. The ester bond is to the 2-hydroxyl group of the lincosamine sugar. The ester serves as a tasteless prodrug form of the antibiotic, which hydrolyzes to clindamycin in the plasma. The salt form confers water solubility to the ester, which is available as granules for reconstitution into an oral solution for pediatric use. Although absorption of the palmitate is slower than that of the free base, there is little difference in overall bioavailability of the two preparations. Reconstituted solutions of the palmitate hydrochloride are stable for 2 weeks at room temperature. Such solutions should not be refrigerated because thickening occurs, making the preparation difficult to pour.

Clindamycin Phosphate, USP (Cleocin Phosphate)

Clindamycin phosphate is the 2-phosphate ester of clindamycin. It exists as a zwitterionic structure that is very soluble in water. It is intended for parenteral (intravenous or intramuscular) administration for the treatment of serious infections and when oral administration is not feasible. Solutions of clindamycin phosphate are stable at room temperature for 16 days and for up to 32 days when refrigerated.

THE POLYPEPTIDES

Among the most powerful bactericidal antibiotics are those possessing a polypeptide structure. Many of them have been isolated, but unfortunately, their clinical use has been limited by their undesirable side reactions, particularly renal toxicity. Another limitation is the lack of systemic activity of most peptides following oral administration. A chief source of the medicinally important members of this class has been *Bacillus* spp. The antitubercular antibiotics capreomycin and viomycin (see Chap. 7) and the antitumor antibiotics actinomycin and bleomycin (see Chap. 11) are peptides isolated from *Streptomyces* spp. The glycopeptide antibiotic vancomycin, which has become the most important member of this class, is isolated from a closely related actinomycete, *Amycolatopsis orientalis.*

Polypeptide antibiotics variously possess a number of interesting and often unique characteristics: (1) they frequently consist of several structurally similar but chemically distinct entities isolated from a single source; (2) most of them are cyclic, with a few exceptions (e.g., the gramicidins); (3) they frequently contain D-amino acids and/or "unnatural" amino acids not found in higher plants or animals; and (4) many of them contain non-amino acid moieties, such as heterocycles, fatty acids, sugars, etc. Polypeptide antibiotics may be acidic, basic, zwitterionic, or neutral depending on the number of free carboxyl and amino or guanidino groups in their structures. Initially, it was assumed that neutral compounds, such as the gramicidins, possessed cyclopeptide structures. Later, the gramicidins were determined to be linear and the neutrality was shown to be due to a combination of the formylation of the terminal amino group and the ethanolamine amidation of the terminal carboxyl group.[230]

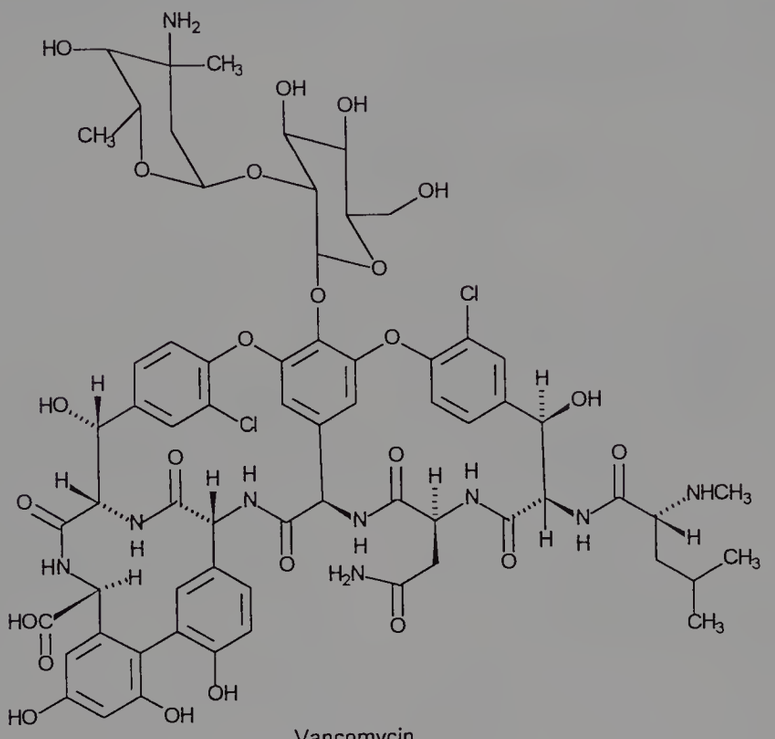
Antibiotics of the polypeptide class differ widely in their mechanisms of action and antimicrobial properties. Bacitracin and vancomycin interfere with bacterial cell wall synthesis and are effective only against gram-positive bacteria. Neither antibiotic apparently is able to penetrate the outer envelope of gram-negative bacteria. Both the gramicidins and the polymyxins interfere with cell membrane functions in bacteria. However, the gramicidins are effective primarily against gram-positive bacteria, while the polymyxins are effective only against gram-negative species. Gramicidins are neutral compounds that are largely incapable of penetrating the outer envelope of gram-negative bacteria. Polymyxins are highly basic compounds that penetrate the outer membrane of gram-negative bacteria through porin channels to act on the inner cell membrane.[231] The much thicker cell wall of gram-positive bacteria apparently serves as an effective barrier to penetration by the polymyxins.

Vancomycin Hydrochloride, USP (Vancocin, Vancoled).

The isolation of the glycopeptide antibiotic vancomycin from *Streptomyces orientalis* (renamed *Amycolatopsis orientalis*) was described in 1956 by McCormick et al.[232] The organism originally was obtained from cultures of an Indonesian soil sample and subsequently has been obtained from Indian soil. Vancomycin was introduced in 1958 as an antibiotic active against gram-positive cocci, particularly streptococci, staphylococci, and pneumococci. It is not active against gram-negative bacteria, with the exception of *Neisseria* spp. Vancomycin is recommended for use when infections fail to respond to treatment with the more common antibiotics or when the infection is known to be caused by a resistant organism. It is particularly effective for the treatment of endocarditis caused by gram-positive bacteria.

Vancomycin hydrochloride is a free-flowing, tan to brown powder that is relatively stable in the dry state. It is very soluble in water and insoluble in organic solvents. The salt is quite stable in acidic solutions. The free base is an amphoteric substance, the structure of which was determined on the basis of a combination of chemical degradation and nuclear magnetic resonance (NMR) studies and x-ray crystallographic analysis of a close analogue.[233] Slight stereochemi-

cal and conformational revisions in the originally proposed structure subsequently have been made.[234,235] Vancomycin is a glycopeptide containing two glycosidically linked sugars, glucose and vancosamine, and a complex cyclic peptide aglycon containing aromatic residues linked together in a unique resorcinol ether system.



Vancomycin

Vancomycin inhibits cell wall synthesis by preventing the synthesis of cell wall mucopeptide polymer. It does so by binding with the D-alanine-D-alanine terminus of the uridine diphosphate-*N*-acetylmuramyl peptides required for mucopeptide polymerization.[236] Details of the binding have been elucidated by the elegant NMR studies of Williamson et al.[237] The action of vancomycin leads to lysis of the bacterial cell. The antibiotic does not exhibit cross-resistance to β-lactams, bacitracin, or cycloserine, from which it differs in mechanism. Resistance to vancomycin among gram-positive cocci is rare. However, high-level resistance in clinical isolates of enterococci has been reported. Such resistance is associated with the inducible production of a protein, encoded by vancomycin A, that is an altered ligase enzyme that causes the incorporation of a D-alanine-D-lactate depsipeptide instead of the usual D-alanine-D-alanine dipeptide in the peptidoglycan terminus.[238] The resulting peptidoglycan can still undergo cross-linking but no longer binds vancomycin.

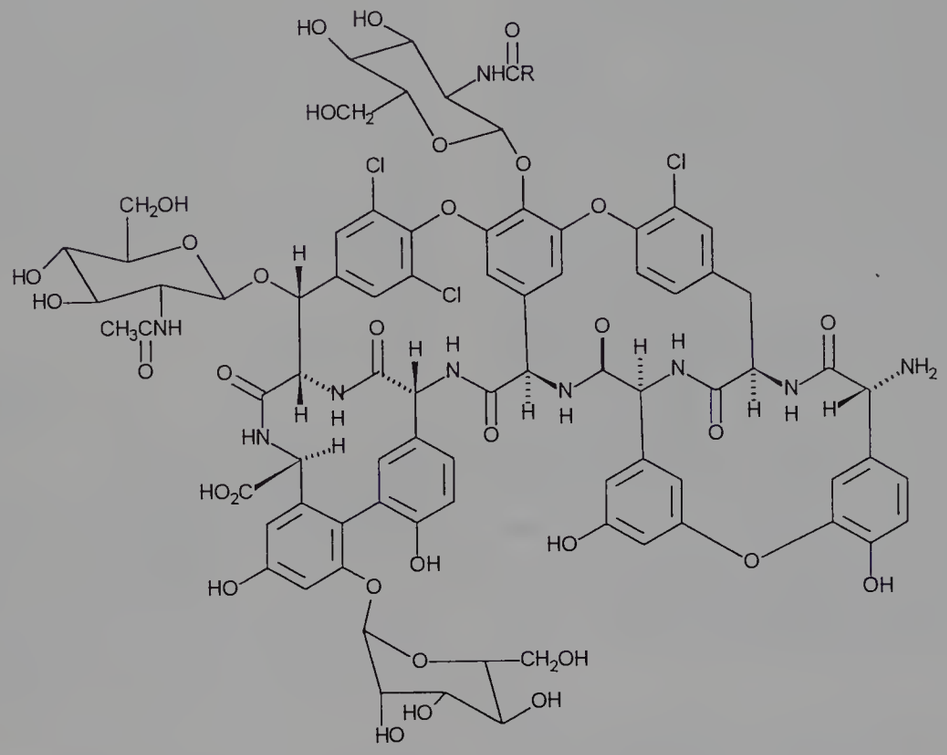
Vancomycin hydrochloride is always administered intravenously (never intramuscularly), either by slow injection or by continuous infusion, for the treatment of systemic infections. In short-term therapy, the toxic side reactions are usually slight, but continued use may lead to impairment of auditory acuity, renal damage, phlebitis, and rashes. Because it is not absorbed or significantly degraded in the gastrointestinal tract, vancomycin may be administered orally for the treatment of staphylococcal enterocolitis and for pseudomembranous colitis associated with clindamycin therapy. It is likely that some conversion to aglucovancomycin occurs in the low *p*H of the stomach. The latter retains about three-fourths of the activity of vancomycin.

Teicoplanin (Teichomycin A_2, Targocid)

Teicoplanin is a mixture of five closely related glycopeptide antibiotics produced by the actinomycete *Actinoplanes teichomyceticus*.[239,240] The teicoplanin factors differ only in the acyl group in the northernmost of two glucosamines glycosidically linked to the cyclic peptide aglycone. Another sugar, D-mannose is common to all of the teicoplanins. The structures of the teicoplanin factors were determined independently by a combination of chemical degradation[241] and spectroscopic[242,243] methods in three different groups in 1984.

The teicoplanin complex is similar to vancomycin structurally and microbiologically but has unique physical properties, which contribute some potentially useful advantages.[244] While retaining excellent water solubility, teicoplanin has significantly greater lipid solubility than vancomycin. Thus, teicoplanin is distributed rapidly into tissues and penetrates phagocytes well. The complex has a long elimination half-life, ranging from 40 to 70 hr, resulting from a combination of slow tissue release and a high fraction of

protein binding in the plasma (approximately 90%). Unlike vancomycin, teicoplanin is not irritating to tissues and may be administered by intramuscular or intravenous injection. Because of its long half-life, teicoplanin may be administered on a once-a-day dosing schedule. Orally administered teicoplanin is not absorbed significantly and is recovered 40% unchanged in the feces.



Teicoplanin Factor	R
1	—$CH_2CH_2CH{=}CH(CH_2)_4CH_3$
2	—$CH_2(CH_2)_5CH(CH_3)_2$
3	—$CH_2(CH_2)_7CH_3$
4	—$CH_2(CH_2)_5CH(CH_3)CH_2CH_3$
5	—$CH_2(CH_2)_6CH(CH_3)_2$

Teicoplanin exhibits excellent antibacterial activity against gram-positive organisms, including staphylococci, streptococci, enterococci, *Clostridium* and *Corynebacterium* spp., *Propionibacterium acnes,* and *Listeria monocytogenes*. It is not active against gram-negative organisms, including *Neisseria* and *Mycobacterium* spp. Teicoplanin impairs bacterial cell wall synthesis by complexing with the terminal D-alanine-D-alanine dipeptide of the peptidoglycan and thereby prevents cross-linking in a manner entirely analogous to the action of vancomycin.

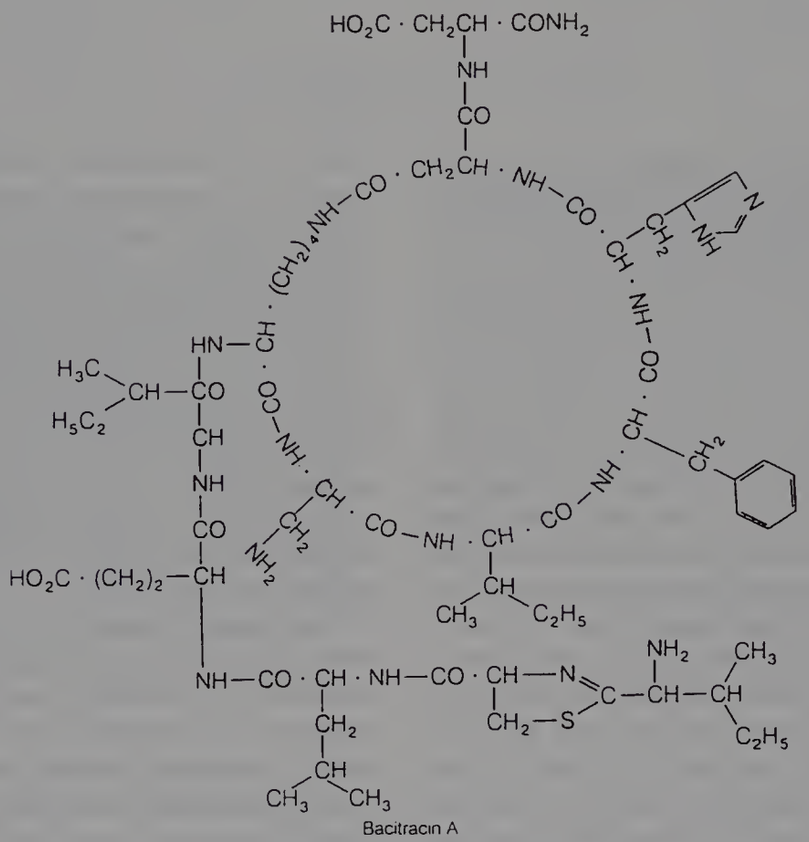
In general, teicoplanin appears to be less toxic than vancomycin. Unlike vancomycin, it does not cause histamine release following intravenous infusion. Teicoplanin apparently also has less potential for causing nephrotoxicity than vancomycin.

Bacitracin, USP

The organism from which Johnson et al.[245] produced bacitracin in 1945 is a strain of *Bacillus subtilis*. The organism had been isolated from debrided tissue from a compound fracture in the 7-year-old Margaret Tracy, hence the name "bacitracin." Production of bacitracin is now accomplished from the licheniformis group (*B. subtilis*). Like tyrothricin, the first useful antibiotic obtained from bacterial cultures, bacitracin is a complex mixture of polypeptides. So far, at least 10 polypeptides have been isolated by countercurrent distribution techniques: A, A^1, B, C, D, E, F_1, F_2, F_3, and G. The commercial product known as bacitracin is a mixture principally of A, with smaller amounts of B, D, E, and $F_{1\text{-}3}$.

The official product is a white to pale buff powder that is odorless or nearly so. In the dry state, bacitracin is stable, but it rapidly deteriorates in aqueous solutions at room temperature. Because it is hygroscopic, it must be stored in tight containers, preferably under refrigeration. The stability of aqueous solutions of bacitracin is affected by *p*H and temperature. Slightly acidic or neutral solutions are stable for as long as 1 year if kept at a temperature of 0° to 5°C. If the *p*H

rises above 9, inactivation occurs very rapidly. For greatest stability, the *p*H of a bacitracin solution is best adjusted at 4 to 5 by the simple addition of acid. The salts of heavy metals precipitate bacitracin from its solutions, with resulting inactivation. However, EDTA also inactivates bacitracin, leading to the discovery that a divalent ion (i.e., Zn^{2+}) is required for activity. In addition to being soluble in water, bacitracin is soluble in low-molecular-weight alcohols but insoluble in many other organic solvents, including acetone, chloroform, and ether.



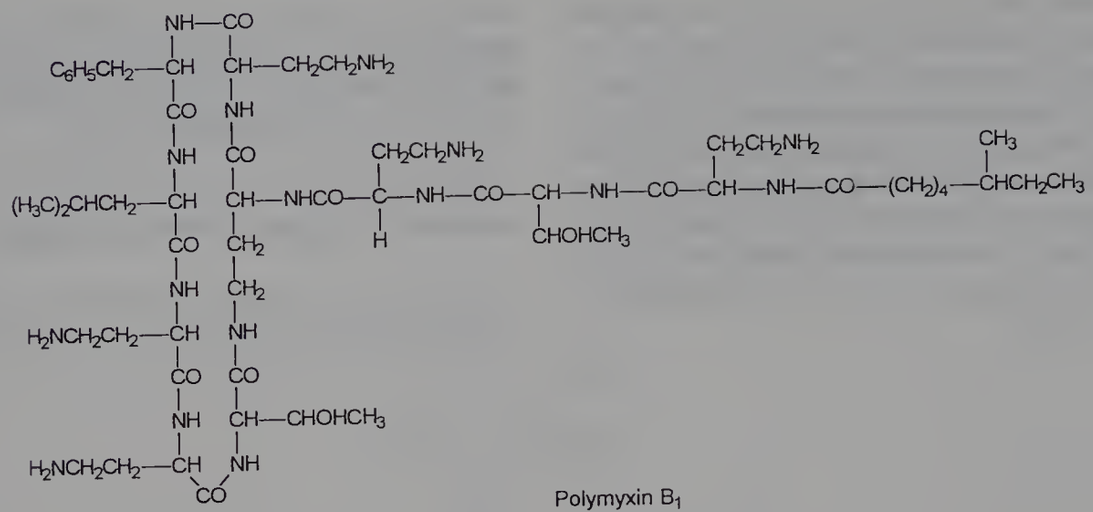
Bacitracin A

The principal work on the chemistry of the bacitracins has been directed toward bacitracin A, the component in which most of the antibacterial activity of crude bacitracin resides. The structure shown in the diagram is that proposed by Stoffel and Craig[246] and subsequently confirmed by Ressler and Kashelikar.[247]

The activity of bacitracin is measured in units per milligram. The potency per milligram is not less than 40 USP units/mg except for material prepared for parenteral use, which has a potency of not less than 50 units/mg. It is a bactericidal antibiotic that is active against a wide variety of gram-positive organisms, very few gram-negative organisms, and some others. It is believed to exert its bactericidal effect through an inhibition of mucopeptide cell wall synthesis. Its action is enhanced by zinc. Although bacitracin has found its widest use in topical preparations for local infections, it is quite effective in a number of systemic and local infections when administered parenterally. It is not absorbed from the gastrointestinal tract; accordingly, oral administration is without effect, except for the treatment of amebic infections within the alimentary canal.

Polymyxin B Sulfate, USP (Aerosporin)

Polymyxin was discovered in 1947 almost simultaneously in three separate laboratories in the United States and Great Britain.[248–250] As often happens when similar discoveries are made in widely separated laboratories, differences in nomenclature, referring to both the antibiotic-producing organism and the antibiotic itself, appeared in references to the polymyxins. Since it has been shown that the organisms first designated as *Bacillus polymyxa* and *B. aerosporus* Greer are identical species, the one name, *B. polymyxa*, is used to refer to all of the strains that produce the closely related polypeptides called polymyxins. Other organisms (e.g., see "Colistin" below) also produce polymyxins. Identified so far are polymyxins A, B_1, B_2, C, D_1, D_2, M, colistin A (polymyxin E_1), colistin B (polymyxin E_2), circulins A and B, and polypeptin. The known structures of this group and their properties have been reviewed by Vogler and Studer.[251] Of these, polymyxin B as the sulfate usually is used in medicine because, when used systemically, it causes less kidney damage than the others.



Polymyxin B_1

Polymyxin B sulfate is a nearly odorless, white to buff powder. It is freely soluble in water and slightly soluble in alcohol. Its aqueous solutions are slightly acidic or nearly neutral (*p*H 5 to 7.5) and, when refrigerated, stable for at least 6 months. Alkaline solutions are unstable. Polymyxin B has been shown to be a mixture by Hausmann and Craig,[252] who used countercurrent distribution techniques to obtain two fractions that differ in structure only by one fatty acid component. Polymyxin B_1 contains (+)-6-methyloctan-1-oic acid (isopelargonic acid), a fatty acid isolated from all of the other polymyxins. The B_2 component contains an isooctanoic acid, $C_8H_{16}O_2$, of undetermined structure. The structural formula for polymyxin B has been proved by the synthesis accomplished by Vogler et al.[253]

Polymyxin B sulfate is useful against many gram-negative organisms. Its main use in medicine has been in topical applications for local infections in wounds and burns. For such use, it frequently is combined with bacitracin, which is effective against gram-positive organisms. Polymyxin B sulfate is absorbed poorly from the gastrointestinal tract; therefore, oral administration is of value only in the treatment of intestinal infections such as pseudomonal enteritis or those due to *Shigella.* It may be given parenterally by intramuscular or intrathecal injection for systemic infections. The dosage of polymyxin is measured in USP units. One milligram contains not less than 6,000 USP units. Some additional confusion on nomenclature for this antibiotic exists as Koyama et al.[254] originally named the product colimycin, and that name is used still. Particularly, it has been the basis for variants used as brand names, such as Coly-Mycin, Colomycin, Colimycine, and Colimicina.

Colistin Sulfate, USP (Coly-Mycin S)

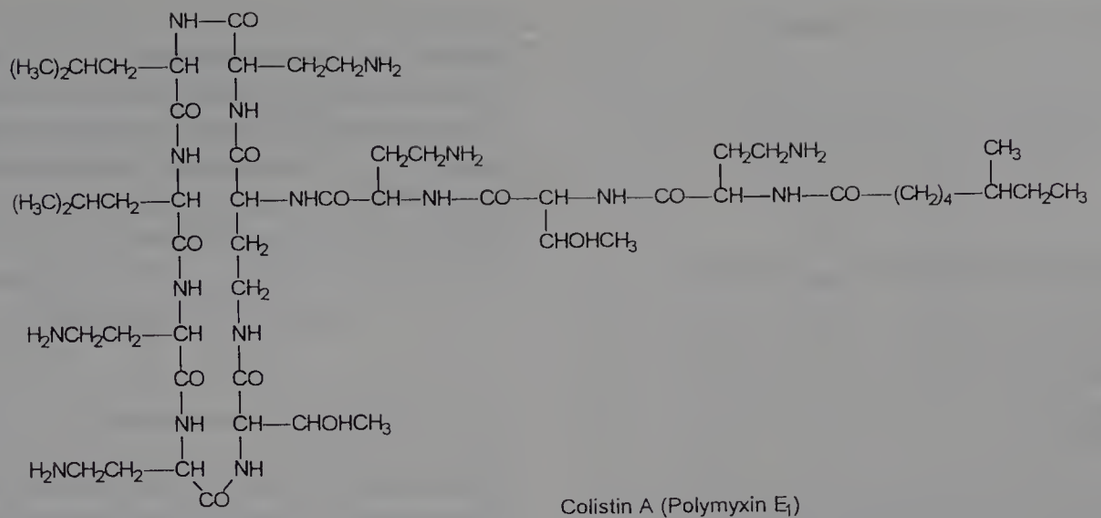
In 1950, Koyama and co-workers[254] isolated an antibiotic from *Aerobacillus colistinus* (*B. polymyxa* var. *colistinus*) that has been given the name colistin. It had been used in Japan and in some European countries for several years before it was made available for medicinal use in the United States. It is recommended especially for the treatment of refractory urinary tract infections caused by gram-negative organisms such as *Aerobacter, Bordetella, Escherichia, Klebsiella, Pseudomonas, Salmonella,* and *Shigella.*

Chemically, colistin is a polypeptide, reported by Suzuki et al.,[255] the major component being colistin A. They proposed the structure shown below for colistin A, which differs from polymyxin B only by the substitution of D-leucine for D-phenylalanine as one of the amino acid fragments in the cyclic portion of the structure. Wilkinson and Lowe[256] have corroborated the structure and have shown colistin A to be identical to polymyxin E_1.

Two forms of colistin have been made, the sulfate and methanesulfonate, and both forms are available for use in the United States. The sulfate is used to make an oral pediatric suspension; the methanesulfonate is used to make an intramuscular injection. In the dry state, the salts are stable, and their aqueous solutions are relatively stable at acid *p*H from 2 to 6. Above *p*H 6, solutions of the salts are much less stable.

Colistimethate Sodium, Sterile, USP

Pentasodium colistinmethanesulfonate; sodium colistimethanesulfonate (Coly-Mycin M). In colistin, five of the terminal amino groups of the α-aminobutyric acid fragment may be readily alkylated. In colistimethate sodium, the methanesulfonate radical is the attached alkyl group, and through each sulfonate, a sodium salt may be made. This provides a highly water-soluble compound that is very suitable for injection. In the injectable form, it is given intramuscularly and is surprisingly free from toxic reactions when compared with polymyxin B. Colistimethate sodium does not readily induce the development of resistant strains of microorganisms, and no evidence of cross-resistance with the common broad-spectrum antibiotics has been shown. It is used for the same conditions as those mentioned for colistin.

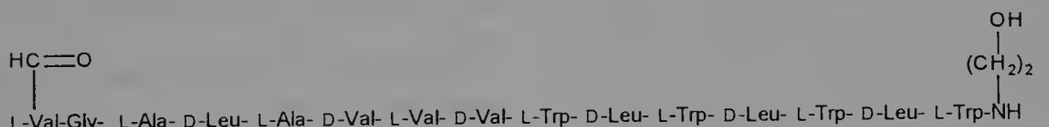


Colistin A (Polymyxin E_1)

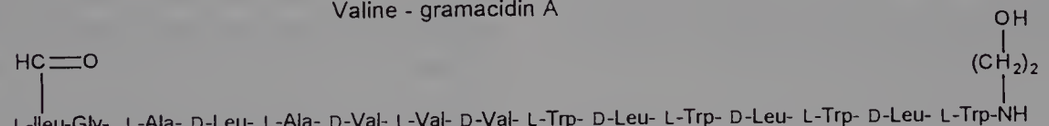
Gramicidin, USP

Gramicidin is obtained from tyrothricin, a mixture of polypeptides usually obtained by extraction of cultures of *Bacillus brevis*. Tyrothricin was isolated in 1939 by Dubos[257] in a planned search to find an organism growing in soil that would have antibiotic activity against human pathogens. Having only limited use in therapy now, it is of historical interest as the first in the series of modern antibiotics. Tyrothricin is a white to slightly gray or brown-white powder, with little or no odor or taste. It is practically insoluble in water and is soluble in alcohol and in dilute acids. Suspensions for clinical use can be prepared by adding an alcoholic solution to calculated amounts of distilled water or isotonic saline solutions.

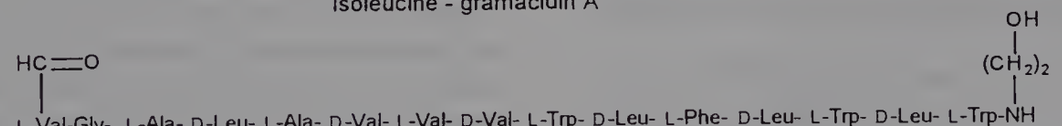
Tyrothricin is a mixture of two groups of antibiotic compounds, the gramicidins and the tyrocidines. Gramicidins are the more active components of tyrothricin, and this fraction, occurring in 10% to 20% quantities in the mixture, may be separated and used in topical preparations for the antibiotic effect. Five gramicidins, A_2, A_3, B_1, B_2, and C, have been identified. Their structures have been proposed and confirmed through synthesis by Sarges and Witkop.[230] The gramicidins A differ from the gramicidins B by having a tryptophan moiety substituted by an L-phenylalanine moiety. In gramicidin C, a tyrosine moiety substitutes for a tryptophan moiety. In both of the gramicidin A and B pairs, the only difference is the amino acid located at the end of the chain, which has the neutral formyl group on it. If that amino acid is valine, the compound is either valinegramicidin A or valinegramicidin B. If that amino acid is isoleucine, the compound is isoleucine-gramicidin, either A or B.



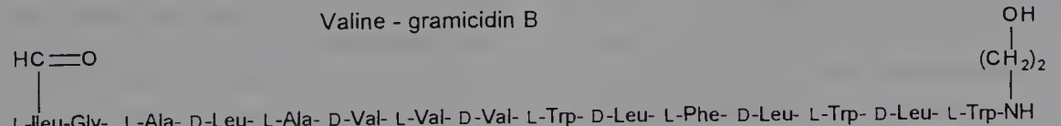
Valine - gramicidin A



Isoleucine - gramicidin A

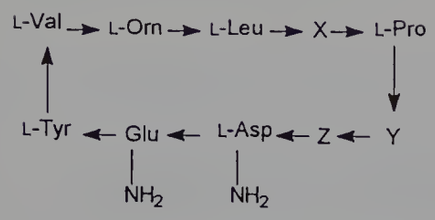


Valine - gramicidin B



Isoleucine-gramicidin B

Tyrocidine is a mixture of tyrocidines A, B, C, and D, whose structures have been determined by Craig and coworkers.[258,259] The synthesis of tyrocidine A has been reported by Ohno et al.[260]



	X	Y	Z
Tyrocidine A:	D-Phe	D-Phe	D-Phe
Tyrocidine B:	D-Phe	L-Tyr	D-Phe
Tyrocidine C:	D-Tyr	L-Tyr	D-Phe
Tyrocidine D:	D-Tyr	L-Tyr	D-Tyr

Gramicidin acts as an ionophore in bacterial cell membranes to cause the loss of potassium ion from the cell.[261] It exerts a bactericidal effect.

Tyrothricin and gramicidin are effective primarily against gram-positive organisms. Their use is restricted to local applications. The ability of tyrothricin to cause lysis of erythrocytes makes it unsuitable for the treatment of systemic infections. Its applications should avoid direct contact with the bloodstream through open wounds or abrasions. It is ordinarily safe to use tyrothricin in troches for throat infections, as it is not absorbed from the gastrointestinal tract. Gramicidin is available in a variety of topical preparations containing other antibiotics, such as bacitracin and neomycin.

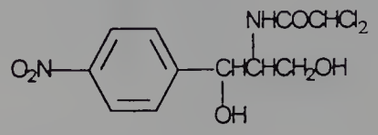
UNCLASSIFIED ANTIBIOTICS

Among the many hundreds of antibiotics that have been evaluated for activity are several that have gained significant clinical attention but do not fall into any of the previously considered groups. Some of these have quite specific activities against a narrow spectrum of microorganisms. Some have found a useful place in therapy as substitutes for other antibiotics to which resistance has developed.

Chloramphenicol, USP (Chloromycetin, Amphicol)

The first of the widely used broad-spectrum antibiotics, chloramphenicol, was isolated by Ehrlich et al.[262] in 1947. They obtained it from *Streptomyces venezuelae,* an organism that was found in a sample of soil collected in Venezuela. Since that time, chloramphenicol has been isolated as a product of several organisms found in soil samples from widely separated places. More importantly, its chemical structure was established quickly, and in 1949, Controulis et al.[263] reported its synthesis. This opened the way for the commercial production of chloramphenicol by a totally synthetic route. It was the first and still is the only therapeutically important antibiotic to be so produced in competition with microbiologic processes. Diverse synthetic procedures have been developed for chloramphenicol. The commercial process most generally used starts with *p*-nitroacetophenone.[264]

Chloramphenicol is a white, crystalline compound that is very stable. It is very soluble in alcohol and other polar organic solvents but is only slightly soluble in water. It has no odor but has a very bitter taste.



Chloramphenicol

Chloramphenicol possesses two asymmetric carbon atoms in the acylamidopropanediol chain. Biologic activity resides almost exclusively in the D-*threo*-isomer; the L-*threo*- and the D- and L-*erythro*-isomers are virtually inactive.

Chloramphenicol is very stable in the bulk state and in solid dosage forms. In solution, however, it slowly undergoes various hydrolytic and light-induced reactions.[265] The rates of these reactions are dependent on *p*H, heat, and light. Hydrolytic reactions include general acid-base–catalyzed hydrolysis of the amide to give 1-(*p*-nitrophenyl)-2-aminopropan-1,3-diol and dichloracetic acid and alkaline hydrolysis (above *p*H 7) of the α-chloro groups to form the corresponding α,α-dihydroxy derivative.

The metabolism of chloramphenicol has been investigated thoroughly.[266] The main path involves formation of the 3-*O*-glucuronide. Minor reactions include reduction of the *p*-nitro group to the aromatic amine, hydrolysis of the amide, and hydrolysis of the α-chloracetamido group, followed by reduction to give the corresponding α-hydroxyacetyl derivative.

Strains of certain bacterial species are resistant to chloramphenicol by virtue of the ability to produce chloramphenicol acetyltransferase, an enzyme that acetylates the hydroxy groups at the 1- and 3-positions. Both the 3-acetoxy and the 1,3-diacetoxy metabolites are devoid of antibacterial activity.

Numerous structural analogues of chloramphenicol have been synthesized to provide a basis for correlation of structure to antibiotic action. It appears that the *p*-nitrophenyl group may be replaced by other aryl structures without appreciable loss in activity. Substitution on the phenyl ring with several different types of groups for the nitro group, a very unusual structure in biologic products, does not cause a great decrease in activity. However, all such compounds yet tested are less active than chloramphenicol. As part of a QSAR study, Hansch et al.[267] reported that the 2-$NHCOCF_3$ derivative is 1.7 times as active as chloramphenicol against *E. coli.* Modifications of the side chain show it to possess a high degree of specificity in structure for antibiotic action. Conversion of the alcohol group on C-1 of the side chain to a keto group causes an appreciable loss in activity. The

relationship of the structure of chloramphenicol to its antibiotic activity will not be seen clearly until the mode of action of this compound is known. Brock[268] reports on the large amount of research that has been devoted to this problem. Chloramphenicol exerts its bacteriostatic action by a strong inhibition of protein synthesis. The details of such inhibition are as yet undetermined, and the precise point of action is unknown. Some process lying between the attachment of amino acids to sRNA and the final formation of protein appears to be involved.

The broad-spectrum activity of chloramphenicol and its singular effectiveness in the treatment of some infections not amenable to treatment by other drugs made it an extremely popular antibiotic. Unfortunately, instances of serious blood dyscrasias and other toxic reactions have resulted from the promiscuous and widespread use of chloramphenicol in the past. Because of these reactions, it is recommended that it not be used in the treatment of infections for which other antibiotics are as effective and not as hazardous. When properly used, with careful observation for untoward reactions, chloramphenicol provides some of the very best therapy for the treatment of serious infections.[269]

Chloramphenicol is recommended specifically for the treatment of serious infections caused by strains of gram-positive and gram-negative bacteria that have developed resistance to penicillin G and ampicillin, such as *H. influenzae, Salmonella typhi, Streptococcus pneumoniae, B. fragilis*, and *N. meningitidis*. Because of its penetration into the central nervous system, chloramphenicol represents a particularly important alternative therapy for meningitis. It is not recommended for the treatment of urinary tract infections because 5% to 10% of the unconjugated form is excreted in the urine. Chloramphenicol is also employed for the treatment of rickettsial infections, such as Rocky Mountain spotted fever.

Because it is bitter, this antibiotic is administered orally either in capsules or as the palmitate ester. Chloramphenicol palmitate is insoluble in water and may be suspended in aqueous vehicles for liquid dosage forms. The ester forms by reaction with the hydroxyl group on C-3. In the alimentary tract, it is hydrolyzed slowly to the active antibiotic. Parenteral administration of chloramphenicol is made by use of an aqueous suspension of very fine crystals or by use of a solution of the sodium salt of the succinate ester of chloramphenicol. Sterile chloramphenicol sodium succinate has been used to prepare aqueous solutions for intravenous injection.

Chloramphenicol Palmitate, USP

Chloramphenicol palmitate is the palmitic acid ester of chloramphenicol. It is a tasteless prodrug of chloramphenicol intended for pediatric use. The ester must hydrolyze in vivo following oral absorption to provide the active form. Erratic serum levels have been associated with early formulations of the palmitate, but it is claimed by the manufacturer that the bioavailability of the current preparation is comparable with that of chloramphenicol itself.

Chloramphenicol Sodium Succinate, USP

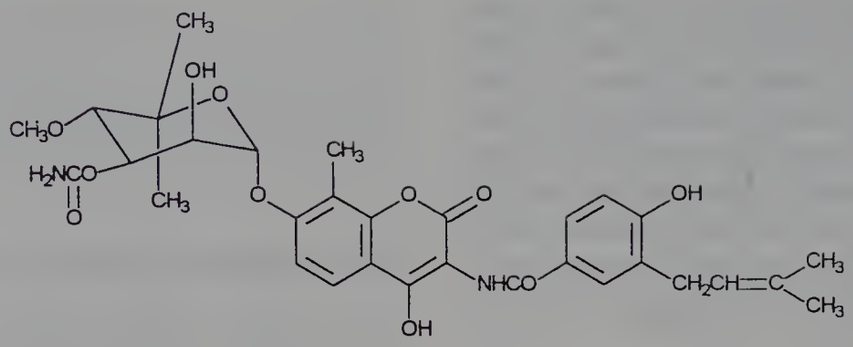
Chloramphenicol sodium succinate is the water-soluble sodium salt of the hemisuccinate ester of chloramphenicol. Because of the low solubility of chloramphenicol, the sodium succinate is preferred for intravenous administration. The availability of chloramphenicol from the ester following intravenous administration is estimated to be 70% to 75%; the remainder is excreted unchanged.[269,270] Poor availability of the active form from the ester following intramuscular injection precludes the attainment of effective plasma levels of the antibiotic by this route. Orally administered chloramphenicol or its palmitate ester actually gives higher plasma levels of the active antibiotic than does intravenously administered chloramphenicol sodium succinate.[270,271] Nonetheless, effective concentrations are achieved by either route.

Novobiocin Sodium, USP

Streptonivicin (Albamycin). In the search for new antibiotics, three different research groups independently isolated novobiocin from *Streptomyces* species. It was reported first in 1955 as a product of *S. spheroides* and *S. niveus*. Currently, it is produced from cultures of both species. Until the common identity of the products obtained by the different research groups was ascertained, confusion in the naming of this compound existed. Its chemical identity has been established as 7-[4-(carbamoyloxy)tetrahydro-3-hydroxy-5-methoxy-6,6-dimethylpyran-2-yloxyl-4-hydroxy-3-[4-hydroxy-3-(3-methyl-2-butenyl)benzamido]-8-methylcoumarin by Shunk et al.[272] and Hoeksema et al.[273] and confirmed by Spencer et al.[274,275]

Chemically, novobiocin has a unique structure among antibiotics, though, like several others, it possesses a glycosidic sugar moiety. The sugar in novobiocin, devoid of its carbamate ester, has been named "noviose" and is an aldose having the configuration of L-lyxose. The aglycon moiety has been termed "novobiocic acid."

Novobiocin is a pale yellow, somewhat photosensitive compound that crystallizes in two chemically identical forms with different melting points (polymorphs). It is soluble in methanol, ethanol, and acetone but is quite insoluble in less polar solvents. Its solubility in water is affected by *p*H. It is readily soluble in basic solutions, in which it deteriorates, and is precipitated from acidic solutions. It behaves as a diacid, forming two series of salts.



Novobiocin

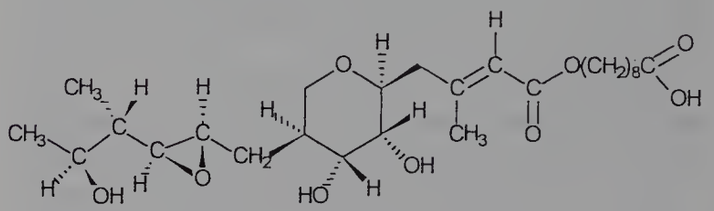
The enolic hydroxyl group on the coumarin moiety behaves as a rather strong acid (pK_a 4.3) and is the group by which the commercially available sodium and calcium salts are formed. The phenolic –OH group on the benzamido moiety also behaves as an acid but is weaker than the former, with a pK_a of 9.1. Disodium salts of novobiocin have been prepared. The sodium salt is stable in dry air but decreases in activity in the presence of moisture. The calcium salt is quite water-insoluble and is used to make aqueous oral suspensions. Because of its acidic characteristics, novobiocin combines to form salt complexes with basic antibiotics. Some of these salts have been investigated for their combined antibiotic effect, but none has been placed on the market, as no advantage is offered by them.

The action of novobiocin is largely bacteriostatic. Its mode of action is not known with certainty, though it does inhibit bacterial protein and nucleic acid synthesis. Studies indicate that novobiocin and related coumarin-containing antibiotics bind to the subunit of DNA gyrase possibly to interfere with DNA supercoiling[276] and energy transduction in bacteria.[277] The effectiveness of novobiocin is confined largely to gram-positive bacteria and a few strains of *Pr. vulgaris*. Its low activity against gram-negative bacteria is apparently due to poor cellular penetration.

Although cross-resistance to other antibiotics is reported not to develop with novobiocin, resistant *Staph. aureus* strains are known. Consequently, the medical use of novobiocin is reserved for the treatment of staphylococcal infections resistant to other antibiotics and sulfas and for patients allergic to these drugs. Another shortcoming that limits the usefulness of novobiocin is the relatively high frequency of adverse reactions, such as urticaria, allergic skin rashes, hepatotoxicity, and blood dyscrasias.

Mupirocin, USP (Pseudomonic Acid A, Bactroban)

Mupirocin is the major component of a family of structurally related antibiotics, pseudomonic acids A–D, produced by the submerged fermentation of *Pseudomonas fluorescens*. Although the antimicrobial properties of *Ps. fluorescens* were recorded as early as 1887, it was not until 1971 that Fuller et al.[278] identified the metabolites responsible for this activity. The structure of the major and most potent metabolite, pseudomonic acid A (which represents 90% to 95% of the active fraction from *Ps. fluorescens*), was later confirmed by chemical synthesis[279] to be the 9-hydroxynonanoic acid ester of monic acid.



Mupirocin

The use of mupirocin is confined to external applications.[280] Systemic administration of the antibiotic results in rapid hydrolysis by esterases to monic acid, which is inactive in vivo due to its inability to penetrate bacteria. Mupirocin has been employed for the topical treatment of impetigo, eczema, and folliculitis secondarily infected by susceptible bacteria, especially staphylococci and β-hemolytic streptococci. The spectrum of antibacterial activity of mupirocin is confined to gram-positive and gram-negative cocci, including staphylococci, streptococci, *Neisseria* spp., and *Mor. catarrhalis*. The activity of the antibiotic against most gram-negative and gram-positive bacilli is generally poor, with the exception of *H. influenzae*. It is also not effective against enterococci or anaerobic bacteria.

Mupirocin interferes with RNA synthesis and protein synthesis in susceptible bacteria.[281,282] It specifically and reversibly binds with bacterial isoleucyl transfer-RNA synthase to prevent the incorporation of isoleucine into bacterial proteins.[282] High-level, plasmid-mediated mupirocin resistance in *Staph. aureus* has been attributed to the elaboration of a modified isoleucyl t-RNA synthesis that does not bind mupirocin.[283] Inherent resistance in bacilli is likely due to poor cellular penetration of the antibiotic.[284]

Mupirocin is supplied in a water-miscible ointment containing 2% of the antibiotic in polyethylene glycols 400 and 3,350.

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CHAPTER 11

Antiviral Agents

Arnold R. Martin

PROPERTIES OF VIRUSES

Viruses are obligate cellular parasites composed of a nucleic acid core surrounded by a proteinaceous outer shell. As cellular parasites, they are dependent on the host cell for energy and the biochemical substrates required for replication and protein synthesis. They also utilize the biochemical apparatus of the cell to synthesize the virus-specific proteins required for production of the mature viral particle. Adult viruses possess only one type of nucleic acid (the genome may be either deoxyribonucleic acid [DNA] or ribonucleic acid [RNA]), and their organized structure is lost during replication of the genome in the host cell. These features differentiate viruses from other cellular parasites, such as the chlamydiae (which possess both DNA and RNA and replicate within the cell) and the more complex rickettsiae (which, in addition, have autonomous energy-generating and protein-synthesizing systems).

VIRAL CLASSIFICATION

Viral classification is based on various characteristics, such as nucleic acid content (RNA viruses, DNA viruses), morphology, site of replication in the cell (cytoplasm, nucleus), shell composition (nonenveloped, enveloped), and serologic typing. The RNA viruses include picornaviruses, togoviruses, flaviviruses, bunyaviruses, rhabdoviruses, myxoviruses, reoviruses, filoviruses, arenaviruses, and retroviruses. They cause such diseases as polio, colds, pneumonia, gastroenteritis, influenza, hepatitis, encephalitis, yellow fever, hemorrhagic fever, rubella, measles, mumps, rabies, and acquired immune deficiency syndrome (AIDS). The DNA-containing viruses include the herpesviruses, papoviruses, adenoviruses, poxviruses, and parvoviruses. Diseases caused by DNA viruses include cold sores, conjunctivitis, genital herpes, encephalitis, shingles, chickenpox, mononucleosis, hepatitis, smallpox, warts, and upper respiratory infections. It has been estimated that more than 60% of the infectious diseases that occur in developing countries are caused by viruses. Bacterial infections account for only 15%. A list of the more important viruses causing diseases in humans is provided in Table 11-1.

PREVENTION OF VIRAL INFECTION BY CHEMOPROPHYLAXIS

IMMUNIZATION

Prevention of viral infection by active immunization continues to be the primary approach for combating most viral diseases. Effective vaccines are available for prophylaxis of such diseases as polio, rubella, measles, mumps, influenza, yellow fever, encephalitis, rabies, smallpox (now considered eradicated worldwide), and hepatitis B. However, vaccines developed against herpesviruses, Epstein-Barr virus, cytomegalovirus (CMV), respiratory syncytial virus, and human immunodeficiency virus (HIV) thus far have proved to be either ineffective or unreliable. Development of a new vaccine effective against a disease-causing virus, such as the AIDS (HIV) virus, is not a simple task. The new vaccine must be sufficiently antigenic to induce an effective antibody response. At the same time, it must not cause disease, either the one it is designed to prevent or some other toxic manifestation. Some viruses undergo rapid mutation, leading to numerous serotypes, thereby confounding the production of a broadly effective vaccine.

ANTIVIRAL CHEMOTHERAPY: BIOCHEMICAL TARGETS

The discovery of useful antiviral agents historically has lagged behind progress in antibacterial chemotherapy. There

TABLE 11-1

CLASSIFICATION OF VIRUSES CAUSING DISEASE IN HUMANS

Family Agent	*Disease*	*Vaccine*	*Chemotherapy*
RNA Viruses			
Picornavirus			
Enterovirus	Polio; three serotypes cause meningitis, paralysis	Live and killed vaccines (very effective)	None
Coxsackie viruses	Variety of symptoms	None	None
Rhinovirus	Common cold, pneumonia (over 100 serotypes)	None	None
Hepatitis A virus	Hepatitis (usually mild and rarely chronic)	Inactivated virus (effective)	None
Calicivirus			
Norwalk virus	Gastroenteritis	None	None
Togavirus			
Alphaviruses (group A arboviruses)	Encephalitis, hemorrhagic fevers	Attenuated virus (generally effective)	None
Rubivirus	Rubella (German measles)	Attenuated virus	None
Flavivirus			
Flaviviruses (group B arboviruses)	Yellow fever, Dengue Encephalitis, hemorrhagic fevers	Attenuated virus (generally effective)	None
Hepatitis C virus	Hepatitis	None	None
Coronavirus	Respiratory infection	None	None
Rhabdovirus			
Rabies virus	Rabies	Inactivated virus (effective)	None
Vesicular stomatitis virus		None	None
Filovirus			
Marburg virus	Marburg disease	None	None
Ebola virus	Hemorrhagic fever	None	None
Paramyxovirus			
Parainfluenza virus	Respiratory infection	None	None
Respiratory syncytial virus	Respiratory infection	Attenuated virus (effectiveness uncertain)	Ribavirin
Morbillivirus	Measles (rubeola)	Attenuated virus (90% effective)	None
Mumps virus	Mumps	Attenuated virus	None
Orthomyxovirus			
Influenza virus	Influenza (A, B, C serotypes)	Attenuated virus (70% effective)	Amantadine
Bunyavirus			
Hantavirus	Fever, renal failure	None	None
Arboviruses	Encephalitis, hemorrhagic fever	None	None
Arenavirus			
Lymphocytic choriomeningitis virus	Meningitis	None	None
Junin, Machupo viruses	Hemorrhagic fever	None	None
Lassa virus	Hemorrhagic fever	None	Ribavirin
Reovirus			
Human rotavirus	Gastroenteritis in infants	None	None
Orbivirus	Colorado tick fever	Inactivated virus (effectiveness unknown)	None
Retrovirus			
Human immunodeficiency viruses (HIV-1, HIV-2)	AIDS and AIDS-related complex (ARC)	None	AZT, ddI, ddC, stavudine
Human T-cell lymphotropic viruses (HTLV-1, HTLV-2)	T-cell leukemia, lymphoma	None	None
DNA Viruses			
Herpesvirus			
Herpes simplex 1	Stomatitis, eye infections, encephalitis	Inactivated virus (efficacy uncertain)	Iudr, ara-A
Herpes simplex 2	Genital herpes, skin eruptions	None	Acyclovir
Varicella zoster	Chickenpox (children), shingles (adults)	None	Acyclovir
Cytomegalovirus	Infections in the immunocompromised, neonates	None	Ganciclovir, foscarnet
Epstein-Barr virus	Infectious mononucleosis, Burkitt's lymphoma	None	None
Papovavirus			
Papillomavirus	Warts	None	Podophyllin
Polyomavirus (JC virus)	Progressive leucoencephalopathy		None
Adenovirus			
Human adenovirus	Upper respiratory tract and eye infections	None	None
Hepadnavirus			
Hepatitis B virus	Hepatitis (may become chronic)	Inactivated subunit (very effective)	None
Poxvirus			
Variola	Smallpox	Vaccinia (cowpox) (very effective)	Methiazone
Parvovirus			
Human parvovirus B19	Erythema, hemolytic anemia	None	None

are a variety of reasons for this state of affairs. Unlike bacteria, viruses will not grow in synthetic culture media. Specialized cell culture techniques required for the screening of potential antiviral drugs have been developed only relatively recently. The comparative biochemical simplicity of viruses and their utilization of the biochemical processes of the host cell provide fewer targets for potential attack by chemotherapeutic agents. The spectacular success of immunization procedures for the prevention of certain viral diseases also may have contributed to the lack of interest in chemotherapy. Another problem that occurs in mild viral infections, such as the common cold, is that clinical symptoms do not appear until the infection is well established and the immune processes of the host have begun to mount a successful challenge. Thus, for many common viral infections, chemotherapy is not an appropriate choice of treatment. Chemotherapeutic agents are needed, however, against viruses that cause severe or chronic infections, such as encephalitis, AIDS, slow viral disease, and herpes, particularly in patients with depressed immune systems.

Despite their simplicity relative to bacteria, viruses possess a variety of biochemical targets for potential attachment by chemotherapeutic agents. An understanding of the specific biochemical events that occur during viral infection of the host cell should provide the basis for the future discovery of site-specific antiviral agents. The process of viral infection can be divided conveniently into seven stages: (1) *adsorption*, attachment of the virus to specific receptors on the surface of the host cell; (2) *entry*, penetration of the virus into the cell; (3) *uncoating*, release of viral nucleic acid from the protein coat; (4) *transcription*, production of viral messenger RNA from the viral genome; (5) *translation*, synthesis of viral proteins (coat proteins and enzymes for replication) and viral nucleic acid (i.e., the parental genome or complimentary strand); (6) *assembly* of the viral particle; and (7) *release* of the mature virus from the cell by budding from the cell membrane or rupture of the cell.

The initial attachment of viral particles to cells is believed to involve electrostatic interactions between components of the viral capsid or outer envelope and receptors on the cell surface. The high degree of specificity observed for such interactions appears to be responsible for the tissue tropism displayed by many viruses. There is good evidence that the cellular receptor for influenza viruses is *N*-acetylmuramic acid, which binds a protein molecule, hemagglutinin, projecting from the viral surface.[1] Initiation of HIV infection has been shown to involve the interaction of specific glycoprotein molecules (designated gp 120) that stud the viral cell surface with an antigenic CD4 receptor molecule on helper T lymphocytes.[2] Viruses are believed to enter cells by *endocytosis*, a process that involves fusion of the viral envelope with the cell membrane.

Before a virus can replicate within the cell, it must shed its outer envelope and capsid to release its nucleic acid genome. For complex DNA viruses such as vaccinia, the uncoating process occurs in two stages: (1) host cell enzymes partially degrade the envelope and capsid to reveal a portion of the viral DNA, which serves as a template for mRNA synthesis, and (2) the mRNAs direct the synthesis of viral enzymes, which then complete the degradation of the protein coat. Proteins of the viral envelope and capsule are the primary targets of the antibodies synthesized in response to immunization techniques. Protein synthesis inhibitors, such as cycloheximide and puromycin, inhibit the virus-uncoating process. However, they are not sufficiently selective to be useful as antiviral agents.

In the critical third state of infection, the virus commandeers the energy-producing and synthetic functions of the cell to replicate its genome and synthesize viral enzymes and structural proteins. Simple RNA viruses conduct both replication and protein synthesis in the cytoplasm of the cell. They contain specific RNA polymerases (RNA replicases) responsible for replication of the genome. Some single-stranded RNA viruses, such as poliovirus, have a (+)-RNA genome that serves the dual function of the messenger for protein synthesis and the template for the synthesis of a complementary strand of (−)-RNA, from which the (+)-RNA is replicated. In poliovirus, the message is translated as a single large protein that is cleaved enzymatically to specific viral enzymes and structural proteins. Other RNA viruses, such as influenza viruses, contain (−)-RNA, which serves as the template for the synthesis of a complementary strand of (+)-RNA. The (+)-RNA strand directs viral protein synthesis and provides the template for the replication of the (−)-RNA genome. Certain antibiotics, such as the rifamycins, inhibit viral RNA polymerases in vitro, but none has yet proved useful clinically. Bioactivated forms of the nucleoside analogue ribavirin variously inhibit ribonucleotide synthesis, RNA synthesis, or RNA capping in RNA viruses. Ribavirin has been approved for aerosol treatment of severe lower respiratory infections caused by respiratory syncytial virus (RSV).

Retroviruses are a special class of RNA viruses that possess an RNA-dependent DNA polymerase (*reverse transcriptase*) required for viral replication. In these viruses, a single strand of DNA is synthesized on the RNA genome (*reverse transcription*), duplicated, and circularized to a double-stranded proviral DNA. The proviral DNA is then integrated into the host cell chromosomal DNA to form the template (*aprovirus* or *virogene*) required for the synthesis of mRNAs and replication of the viral RNA genome. *Oncogenic* (cancer-causing) viruses, such as the human T-cell leukemia viruses (HTLV) and the related HIV, are retroviruses. Retroviral reverse transcriptase is inhibited by the triphosphates of certain dideoxynucleosides, such as 2′,3′-deoxy-3′-azidothymidine (AZT, zidovudine), 2,3-dideoxycytidine (ddC, zalcitabine), and 2′,3′-didehydrothymidine (d4T, stavudine), all of which have been approved for the treatment of AIDS. Such dideoxynucleoside triphosphates are incorporated into viral DNA in place of the corresponding 2′-deoxynucleoside (i.e., 2′-deoxythymidine, 2′-deoxycytidine, or 2′-deoxyadenosine) triphosphate.[3,4] This incor-

poration causes termination of the viral DNA chain since the incorporated dideoxynucleoside lacks the 3′-hydroxyl group required to form a 3′,5′-phosphodiester bond with the next 2′-deoxynucleotide triphosphate to be incorporated.

The DNA viruses constitute a heterogeneous group that use DNA as the genome and replicate in the nucleus of the host cell. Some DNA viruses are simple structures, consisting of a single DNA strand and a few enzymes surrounded by a capsule (e.g., parvovirus) or a lipoprotein envelope (e.g., hepatitis B virus). Others, such as the herpesviruses and poxviruses, are large, complex structures with double-stranded DNA genomes and several enzymes encased in a capsule and surrounded by an envelope consisting of several membranes. DNA viruses contain DNA-dependent RNA polymerases (transcriptases), DNA polymerases, and various other enzymes (depending on the complexity of the virus) that may provide targets for antiviral drugs. The more successful chemotherapeutic agents discovered thus far are directed against replication of herpesviruses. The nucleoside analogues idoxuridine, trifluridine, and vidarabine appear to block replication in herpesviruses by three general mechanisms: first, as the monophosphates, they inhibit the formation of precursor nucleotides required for DNA synthesis; second, as triphosphates, they inhibit DNA polymerase; and third, the triphosphates are incorporated into DNA that does not function normally. Acyclonucleosides (e.g., acycloguanosine) are bioactivated sequentially by viral and host cell kinases to the acyclonucleotide and the acyclonucleoside triphosphate, respectively. The latter inhibits viral DNA polymerase and causes termination of the viral DNA strand since no 3′-hydroxyl group is available for the subsequent formation of a 3′,5′-phosphodiester bond with the next nucleoside triphosphate.

Late stages in viral replication require crucial virus-specific processing of certain viral proteins by viral or cellular proteases. Retroviruses, such as HIV, express three genes as precursor polyproteins. Two of these gene products, designated the p55gag and p160gag-pol proteins for their location on the genome, undergo cleavage at several sites by a virally encoded protease to form structural (viral coat) proteins (P17, P24, P8, and P7) and enzymes required for replication (reverse transcriptase, integrase, and, autocatalytically, protease). The demonstration that HIV protease, a member of the aspartic protease family of enzymes, is essential for the maturation and infectivity of HIV particles[5] has stimulated unprecedented research efforts to develop safe and effective inhibitors. Such efforts have led to candidate inhibitors, which have shown significant promise in early clinical trials.

To complete replication, the viral components are assembled into the mature viral particle (*virion*). For simple, nonenveloped viruses (e.g., poliovirus), the genome and a few enzymes are encased by capsid proteins to complete the virion. Other, more complex viruses are enveloped by one or more membranes containing carbohydrate and lipoprotein components derived from the host cell membrane.

Once the mature virion has been assembled, it is ready for release from the cell. The release of certain viruses (e.g., poliovirus) is accompanied by lysis of the cell membrane and cell death. However, some enveloped viruses are released by *exocytosis*, a process involving fusion between the viral envelope and the cell membrane. The cell membrane remains intact under these conditions, and the cell may survive.

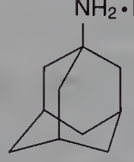
An alternative to active immunization for the prevention of viral infection is chemoprophylaxis. In principle, viral infection can be prevented at any stage prior to viral replication: (1) attachment of the virion to the host cell, (2) its entry into the cell, or (3) release of viral nucleic acid from the protein coat. At present, only a single class of agents, the adamantanamines (amantadine and rimantadine) have been approved for controlling influenza A infection. The adamantanamines appear to interfere with two stages of influenza A viral replication: preventing the early stage of viral uncoating and disturbing the late stage of viral assembly.[6] Clinical studies have shown that amantadine and rimantadine are effective in both prophylaxis and the treatment of influenza A infection.

Amantadine Hydrochloride, USP

1-Adamantanamine hydrochloride (Symmetrel, Symadine) is a white, bitter, crystalline powder that is soluble in water and insoluble in alcohol. It is useful in the prevention and treatment of influenza caused by influenza A virus. It is not effective against influenza B virus or other viruses.

Amantadine is estimated to be about 80% effective in the prevention of illness caused by strains of influenza A virus. When administered 24 to 48 hours after the onset of illness, it also reduces the duration of fever and other symptoms by preventing infection of additional cells. It is generally used in high-risk patients who have underlying debilitating disease or in close household or hospital contacts of patients with severe influenza A illness.

Amantadine is well absorbed following oral administration. It is distributed to all body fluids and tissues and excreted largely unchanged in the urine. It is supplied as capsules and in a syrup for oral administration. The drug also finds occasional use as an adjunct for the treatment of Parkinson's disease in patients who do not tolerate full therapeutic doses of levodopa.



Amantadine Hydrochloride

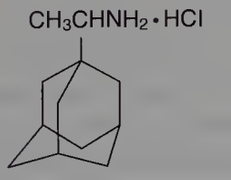
Side effects associated with amantadine therapy are related primarily to its ability to promote the release of dopamine from dopaminergic neurons (the basis for its use in Parkinson's disease). The most common side effects are

depression, dizziness, urinary retention, hallucinations, anxiety, and gastrointestinal upset.

Rimantadine Hydrochloride, USP

α-Methyl-1-adamantane methylamine hydrochloride (Flumadine) is a branched homologue of amantadine with similar antiviral properties. Like amantadine, rimantadine is effective for the prophylaxis and early treatment of infection caused by influenza A viral subtypes.

Rimantadine principally differs from amantadine in its pharmacokinetic properties. In contrast to amantadine, which is excreted largely unchanged in the urine, rimantadine is metabolized extensively following oral administration. Less than 15% of the dose is excreted unchanged, with more than 20% appearing in the urine as hydroxylated metabolites.[7] However, despite the extensive hepatic metabolism of rimantadine, its elimination half-life of 24 to 36 hours is approximately double that of amantadine, suggesting that it may not possess any advantage in patients with impaired renal function. The significantly reduced central nervous system side effects observed with rimantadine therapy, presumably related to its extensive biotransformation, may provide an important advantage over amantadine therapy in some patients.



Rimantadine Hydrochloride

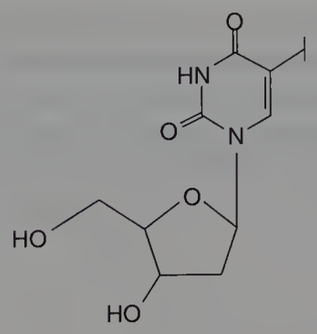
NUCLEOSIDE ANTIMETABOLITES

INHIBITORS OF DNA POLYMERASES

Idoxuridine, USP

2′-Deoxy-5-iodouridine; IUdR (Stoxil, Herplex). Idoxuridine was introduced in 1963 for the treatment of herpes simplex keratitis. At that time, it was the only suitable chemotherapy for this infection.

Idoxuridine is active only against DNA viruses such as herpes and vaccinia. It is phosphorylated by viral thymidylate kinase to the monophosphate, which is bioactivated further to the triphosphate. The triphosphate is believed to be both an inhibitor and a substrate of viral DNA polymerase, causing inhibition of viral DNA synthesis and producing the synthesis of DNA that contains the iodinated pyrimidine. This bogus DNA is more susceptible to strand breakage and to miscoded errors in RNA and protein synthesis. The ability of idoxuridylic acid to substitute for deoxythymidylic acid in the synthesis of DNA may be due to the similar van der Waals' radii of iodine (2.15 Å) and the methyl group (2.00 Å).



Idoxuridine

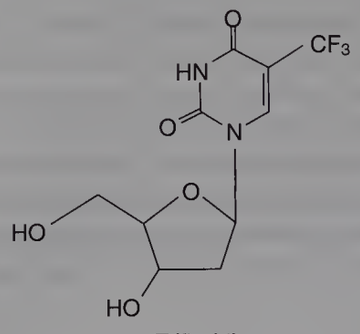
Idoxuridine occurs as a pale yellow, crystalline solid that is soluble in water and alcohol but poorly soluble in most organic solvents. It is a weak acid, with a pK_a of 8.25. Aqueous solutions are slightly acidic, having a *p*H of about 6, and are stable for up to 1 year if refrigerated. Idoxuridine is light- and heat-sensitive. Its solutions may not be autoclaved. It is supplied as a 0.1% ophthalmic solution and a 0.5% ophthalmic ointment.

Trifluridine, USP

α,α,α-Trifluorothymidine; 2-deoxy-5-(trifluoromethyl)uridine (Viroptic). This compound is similar to idoxuridine, having a trifluoromethyl group in place of the iodine atom at the 5-position. Trifluridine is employed as a 1% sterile ophthalmic solution in the treatment of keratoconjunctivitis caused by herpes simplex virus (HSV) types 1 and 2. It has been reported to be effective in cases resistant to idoxuridine.

The spectrum of antiviral activity and mechanism of action of trifluridine are similar to that of idoxuridine. However, the van der Waals' radius of the trifluoromethyl group is 2.44 Å, which is somewhat larger than that of the iodine atom.

Trifluridine solutions are heat-sensitive, requiring refrigeration before and after dispensing. The product will lose 10% of its potency in 1 month when kept at 25°C. Less than 10% of its potency is lost in 3 years when the solution is refrigerated at 4° to 8°C.

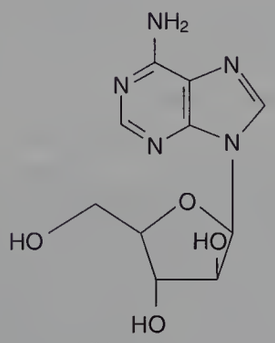


Trifluridine

Vidarabine, USP

9-β-D-Arabinofuranosyladenine; adenine arabinoside; ara-A (Vira-A). Originally synthesized in 1960 as a potential anticancer agent, ara-A later was found to have broad-spectrum

activity against DNA viruses.[8] First marketed in 1977 as an alternative to idoxuridine for the treatment of herpes simplex keratitis, it received FDA approval 1 year later for the treatment of herpes simplex encephalitis. Commercial vidarabine is now obtained as an antibiotic from cultures of *Streptomyces antibioticus*.



Vidarabine

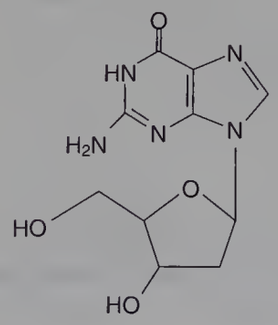
The antiviral action of vidarabine is confined to DNA viruses. It is converted by viral enzymes to the triphosphate, which is a potent inhibitor of ribonucleotide reductases and DNA polymerases. The triphosphate also may be incorporated into viral DNA.

In cases of viral encephalitis, vidarabine must be administered by continuous intravenous infusion because of its poor water solubility and relatively rapid metabolic conversion in vivo. The drug undergoes deamination, catalyzed by adenosine deaminase, to form the considerably less active hypoxanthine derivative hypoxanthine arabinoside (ara-H). These problems together with the availability of more effective and less toxic agents, such as acyclovir, have caused the withdrawal of parenteral vidarabine from the U.S. market.

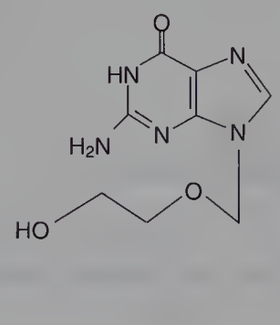
Vidarabine occurs as a white, crystalline monohydrate that is sparingly soluble (0.45 mg/mL at 25°C). It is available as a 3% ointment for the treatment of keratitis caused by HSV.

Acyclovir, USP

2-Amino-1,9-dihydro-9-[(2-hydroxyethoxy)methyl]-6*H*-purin-6-one; 9-[(2-hydroxyethoxy)methyl]guanine; acycloguanosine; ACV (Zovirax). Acyclovir is the most effective member of a series of acyclic nucleosides with antiviral activity. In contrast to true nucleosides, which have a ribose or a deoxyribose sugar attached to a purine or pyrimidine base, acyclic nucleosides have only a portion of the sugar present. The relationship between acyclovir (acycloguanosine) and 2′-deoxyguanosine is shown in the structural diagrams below.



Deoxyguanosine



Acyclovir

Acyclovir has potent activity against several DNA viruses, including HSV types 1 and 2, varicella-zoster virus (VZV), and Epstein-Barr virus. It has become the drug of choice for the treatment of genital herpes. The mode of action of acyclovir in DNA viruses has been studied extensively.[9] The acyclic nucleoside is transported into infected cells, where it is phosphorylated selectively by viral thymidylate kinase to the monophosphate. Further phosphorylation by cellular enzymes forms the triphosphate, which has an affinity 100 times greater for viral DNA polymerase than for human DNA polymerase. Viral DNA with acyclovir incorporated at its 3′-terminus is a potent inhibitor of viral DNA polymerase. Chain termination occurs because the 3′,5′-phosphodiester bond cannot form. The selectivity of acyclovir for herpesvirus results from the properties of viral DNA polymerase and the virus-specified thymidylate kinase.

Two systemic dosage forms of acyclovir are available, oral and parenteral. Oral acyclovir is employed in the initial treatment of genital herpes and to control mild recurrent episodes. It has been approved for the acute treatment of shingles and chickenpox caused by VZV. Intravenous administration is indicated for initial and recurrent infections in immunocompromised patients and for the prevention and treatment of severe recurrent episodes. The drug is absorbed slowly and incompletely from the gastrointestinal tract. Its oral bioavailability is only 15% to 30%. Acyclovir is widely distributed to tissues and biologic fluids. Most of it is excreted in the urine unchanged, but a small fraction (approximately 10%) is excreted as the carboxy metabolite.

Acyclovir occurs as a chemically stable, white, crystalline solid that is slightly soluble in water. Because of its amphoteric properties (pK_a values 2.27 and 9.25), solubility is increased by both strong acids and strong bases. The injectable form is the sodium salt, which is supplied as a lyophilized powder, equivalent to 50 mg of active ingredient dissolved in 10 mL of sterile water for injection. Because the resulting solution is strongly alkaline, with a *p*H of approximately 11, it must be administered by slow intravenous infusion to avoid irritation and thrombophlebitis at the injection site.

Adverse reactions associated with acyclovir are surprisingly few. Occasional gastrointestinal upset, dizziness, headache, lethargy, and joint pain are experienced by some patients. Thrombophlebitis following intravenous administration has been reported. An ointment containing 5% acyclovir in a polyethylene glycol base is available for the treatment of initial, mild episodes of herpes genitalis. It is not effective at preventing recurrent episodes.

Valacyclovir Hydrochloride (Valtrex)

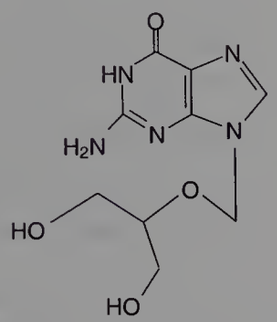
Valacyclovir is the hydrochloride salt of the L-valyl ester of acyclovir. It is a water-soluble, crystalline prodrug derivative intended to improve the oral bioavailability of acyclovir. Following oral administration, it is absorbed rapidly and converted efficiently by first-pass intestinal and/or hepatic enzymes to acyclovir. The absolute bioavailability of

acyclovir from orally administered valacyclovir is around 55%, or two to three times that of oral acyclovir alone.[10]

Valacyclovir has been approved for the treatment of herpes zoster (shingles) in immunocompromised patients. The nature and incidence of side effects observed with valacyclovir were found to be comparable to bioequivalent doses of acyclovir.

Ganciclovir, USP

9-[(1,3-Dihydroxy-2-propoxy)methyl]-guanine, DHPG (Cytovene). Ganciclovir is an analogue of acyclovir, having an additional hydroxymethyl group on the cyclic side chain. This structural modification, while maintaining the activity against HSV and VZV possessed by acyclovir, greatly enhances activity against CMV infection.



Ganciclovir

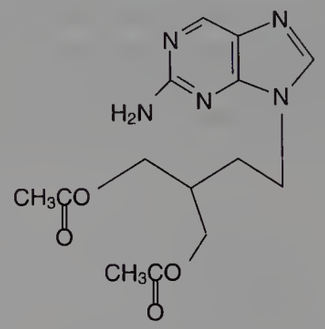
Ganciclovir is phosphorylated intracellularly by a virally encoded protein kinase to the monophosphate during CMV infection.[11] In the infected cell, host enzymes catalyze the formation of the triphosphate, which can achieve greater than 10-fold higher concentrations in infected than in uninfected cells. Ganciclovir triphosphate is a selective inhibitor of viral DNA polymerase, which is incorporated into viral DNA to cause eventually cessation of DNA elongation.[12]

Toxicity limits the clinical usefulness of ganciclovir to the treatment and suppression of sight-threatening CMV infection in immunocompromised patients and to the prevention of life-threatening CMV disease in at-risk transplant patients.[13] The most serious and more frequently observed adverse effects associated with ganciclovir therapy relate to its myelosuppressant properties, which may be associated with the inhibition of cellular γ-DNA polymerase by ganciclovir triphosphate.[12] These effects include neutropenia, thrombocytopenia, and anemia. Additional adverse effects associated with ganciclovir therapy include a variety of central nervous system symptoms, such as headaches, behavioral changes, convulsions, and some gastrointestinal intolerance. Ganciclovir is mutagenic, carcinogenic, and teratogenic in experimental animals.

Oral and parenteral forms of ganciclovir are available. Oral bioavailability is very poor. Since only 5% to 10% of an orally administered dose is absorbed, intravenous administration is preferred. More than 90% of the unchanged drug is excreted in the urine. Ganciclovir for injection is available as the lyophilized sodium salt for reconstitution in normal saline, 5% dextrose, or lactated Ringer's solution. Such solutions are strongly alkaline (pH ~11) and must be administered by slow intravenous infusion to avoid phlebitis.

Famciclovir

2-[2-(2-Amino-9H-purin-9-yl)ethyl]-1,3-propanediol diacetate (Famvir). Famciclovir is an oral prodrug derivative of penciclovir[14] [9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine]. Herpesviruses (HSV and VZV) selectively phosphorylate penciclovir to the monophosphate in infected cells by means of a virally encoded thymidine kinase.[15] Penciclovir monophosphate is bioactivated further by cellular kinases to the triphosphate, which tends to accumulate intracellularly. Penciclovir triphosphate is a potent inhibitor of viral DNA polymerase but, unlike acyclovir triphosphate, does not cause viral DNA termination.[15]



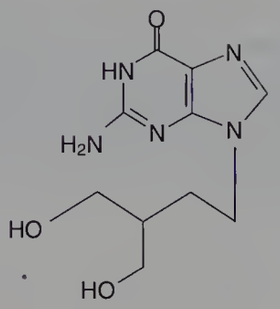
Famciclovir

Absolute oral bioavailability is estimated to be nearly 80%. It is bioactivated efficiently to penciclovir through the action of esterases (hydrolysis of the acetate esters) and aldehyde oxidase (oxidation of the purine-6-position). Penciclovir is excreted largely unchanged in the urine. Since the bioactivation of famciclovir does not require hepatic enzymes, no dosage adjustment is required in patients with liver disease.

Famciclovir has been approved in the United States for the treatment of acute VZV infection (shingles). Topical and parenteral formulations of penciclovir are currently undergoing clinical trials for the treatment of various herpesvirus infections. The toxicity of orally administered famciclovir is apparently very low.

Penciclovir

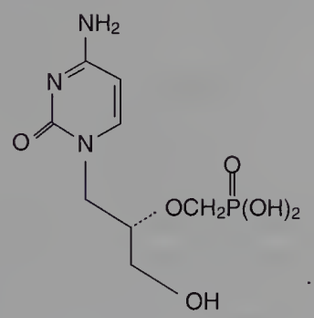
9-[(4-Hydroxy-3-hydroxymethyl)but-1-yl]guanine (Denvir). Penciclovir has been approved for the topical treatment of recurrent herpes labialis (cold sores) in adults. It is effective against HSV-1 and HSV-2.[16] It is available as a cream containing 10% penciclovir.



Penciclovir

Cidofovir

[(S) - 3 - Hydroxy - 2 - phosphonomethoxypropyl] cytosine (HPMPC, Vistide). Cidofovir is an acyclic nucleotide analogue that exhibits broad-spectrum activity against several DNA viruses.[16] Unlike other nucleotides (nucleoside phosphates), cidofovir is a phosphonic acid derivative that is not hydrolyzed by phosphatases *in vivo*. It is phosphorylated by cellular kinases to the diphosphate, which acts as an antimetabolite for deoxycytosine triphosphate (CTP). Cidofovir diphosphate is a competitive inhibitor of viral DNA[17] polymerase and may be incorporated into viral DNA to cause chain termination.



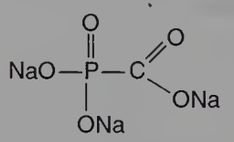
Cidofovir

Because of the high therapeutic index of cidofovir against CMV, it has been approved for the treatment of CMV-retinitis in AIDS patients. The drug is administered by slow intravenous infusion in a dose of 5 mg/kg body weight over a 1 hour period once a week for 2 weeks. This regimen is followed by a maintenance dose every 2 weeks. Unchanged cidofovir (80%) is excreted in the urine, with an elimination half-life of 2.4 to 3.2 hours. The diphosphate antimetabolite, in contrast, has a very long half-life (ranging from 17 to 30 hours).

The principal dose-related toxicity of cidofovir involves renal impairment, necessitating close monitoring of renal function. Pretreatment with probenecid and prehydration with intravenous normal saline solution can be employed to reduce the nephrotoxicity of the drug. Patients should be advised that cidofovir is not curative of CMV-retinitis. The disease may progress during or following treatment.

Foscarnet Sodium, USP

Trisodium phosphonoformate (Foscavir) is an analogue of pyrophosphate which inhibits replication of both herpesviruses (CMV, HSV, and VZV) and retroviruses (HIV).[18] Foscarnet inhibits viral DNA synthesis by binding to the pyrophosphate-binding sites of viral DNA polymerase and reverse transcriptase to prevent the incorporation of nucleoside triphosphates into DNA and concomitant release of pyrophosphate.[19] Since the inhibition is noncompetitive with respect to nucleoside triphosphate binding, foscarnet can synergize with nucleoside triphosphate antimetabolites (e.g., zidovudine and didanosine triphosphates) in the inhibition of viral DNA synthesis. Foscarnet does not require bioactivation by viral or cellular enzymes and, thus, can be effective against resistant viral strains that are deficient in virally encoded nucleoside kinases.[19]



Foscarnet Sodium

Foscarnet serves as a second-line agent for the treatment of retinitis caused by CMV in AIDS patients. Renal toxicity precludes its use in other infections caused by herpesviruses or as single-agent therapy for HIV infection. The metal ion-binding properties of foscarnet undoubtedly contribute to the electrolyte imbalances observed with use of this drug.[20] Thus, hypocalcemia, hypomagnesemia, hypokalemia, and both hypo- and hyperphosphatemia sometimes are seen in patients treated with foscarnet. Parathesias, tetany, seizures, and cardiac arrhythmias may result. Foscarnet also may enhance the actions of other nephrotoxic drugs, such as amphotericin B and pentamidine, which are employed frequently to control secondary infections in AIDS patients.

Foscarnet sodium is available as a sterile solution for slow intravenous infusion. The solution is compatible with normal saline and 5% dextrose but incompatible with calcium-containing buffers such as lactated Ringer's and total parenteral nutrition (TPN) solutions. It is incompatible chemically with drugs administered as acid salts, such as vancomycin, pentamidine, midazolam, and prochlorperazine. More than 80% of an injected dose of foscarnet is excreted in the urine unchanged.[18] The relatively long elimination half-life of foscarnet has been attributed to its sequestration into bone.[20]

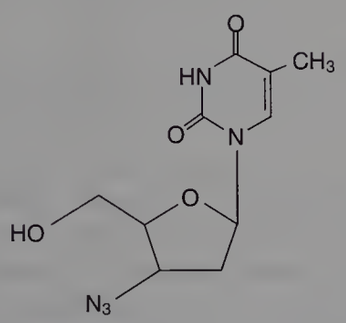
INHIBITORS OF REVERSE TRANSCRIPTASE

Zidovudine, USP

3′-Azido-3′,2′-deoxythymidine; azidothymidine; AZT (Retrovir). This nucleoside was synthesized in 1978 by Lin and Prusoff[21] as an intermediate in the preparation of amino acid analogues of thymidine. A screening program directed to-

ward the identification of agents potentially effective for the treatment of AIDS patients led to the discovery of its unique antiviral properties some 7 years later.[22] The following year, the clinical effectiveness of AZT in patients with AIDS and AIDS-related complex (ARC) was demonstrated.[23]

Zidovudine is active against retroviruses, a group of RNA viruses responsible for AIDS and certain types of leukemia. Retroviruses possess a reverse transcriptase, or RNA-directed DNA polymerase, that directs the synthesis of a DNA copy (proviral DNA) of the viral RNA genome that is duplicated, circularized, and incorporated into the DNA of the infected cell. The AZT is converted to the monophosphate by retroviral thymidylate kinase and eventually to the triphosphate, which is utilized by reverse transcriptase for incorporation into an incomplete proviral DNA.[24] The DNA chain terminates at the site of AZT incorporation because a 3′,5′-phosphodiester bond cannot form with another nucleoside triphosphate, thereby inhibiting the reverse transcriptase. Cellular α-DNA polymerase also is inhibited by AZT triphosphate but only at concentrations 100 times greater than those required for the viral DNA polymerase.[25] However, the γ-DNA polymerase of mitochondria is more sensitive to zidovudine and this sensitivity may contribute to the toxicity associated with its use.



Zidovudine

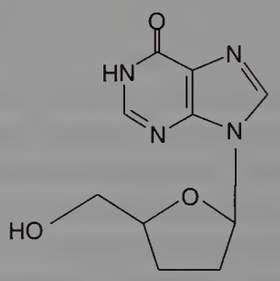
Resistance to the antiviral effects of AZT is commonly observed in AIDS patients undergoing long-term therapy. Such resistance has been attributed to the selective emergence of mutants of HIV having individual amino acid substitutions in viral reverse transcriptase, leading to reduced affinity for zidovudine triphosphate.[26] Another mechanism of resistance may be reduced bioactivation of the nucleoside by viral and/or cellular kinases.

Zidovudine is recommended for the management of adult patients with symptomatic human immune deficiency virus (HIV) infection (AIDS or ARC) who have a history of confirmed *Pneumocystis carinii* pneumonia or an absolute CD4 (T4-helper/inducer) lymphocyte count of fewer than 200/mm^3 before therapy. The hematologic toxicity of the drug precludes its use in asymptomatic patients. Anemia and granulocytopenia are the most common toxic effects associated with zidovudine.

For oral administration, the drug is supplied as 100 mg capsules and as a syrup containing 10 mg of drug/mL. The injectable form is a solution containing 10 mg/mL and is injected intravenously by infusion. It is absorbed rapidly from the gastrointestinal tract and distributed well into most body fluids and tissues, including the cerebrospinal fluid. It is metabolized rapidly to the inactive glucuronide metabolite in the liver. Only about 15% is excreted unchanged. Because AZT is an aliphatic azide, it is heat- and light-sensitive. It should be protected from light and stored at 15° to 25°C.

Didanosine, USP

2′,3′-Dideoxyinosine (Videx). Didanosine (ddI) is a synthetic purine nucleoside analogue that is bioactivated to 2′,3′-deoxyadenosine triphosphate (ddATP) by cellular enzymes.[27] ddATP accumulates intracellularly, where it inhibits its reverse transcriptase and is incorporated into viral DNA to cause chain termination in HIV-infected cells. Although the potency of ddI against HIV is less, it also causes less myelosuppression than AZT.[28] It is employed to treat patients with advanced HIV infection who have received prolonged therapy with AZT but have become intolerant to, or have experienced immunosuppression by, AZT. Combinations of AZT and ddI synergistically inhibit HIV replication in vitro, and ddI is effective against some AZT-resistant strains of HIV.[29]



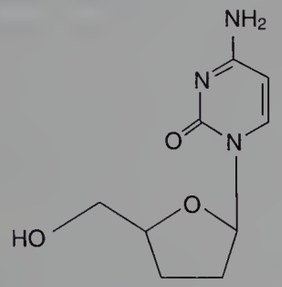
Didanosine

ddI is administered orally in the form of chewable tablets or as a solution prepared from the powder (sachet). Both oral preparations contain buffers to prevent acid destruction of the acid-labile didanosine to hypoxanthine in the stomach. Despite the buffering, oral bioavailability is low and highly variable, in part because food interferes with absorption. Although the biotransformation of ddI has not been studied thoroughly in humans, the small fraction (less than 20%) of unchanged drug excreted in the urine suggests extensive metabolism.[30] In dogs, ddI is degraded to the expected products of purine metabolism: allantoin (~60%), hypoxanthine, xanthine, and uric acid. High-dose therapy can cause hyperuricemia in some patients due to the increased purine load.[28]

Serious dose-related adverse effects of ddI somewhat limit its use. The most frequent and serious toxic effects are pancreatitis (nausea, abdominal pain, and elevated amylase) and peripheral neuropathy (tingling, numbness, and pain in the hands and feet).

Zalcitabine, USP

2′,3′-Dideoxycytidine (Hivid). Zalcitabine (ddC) is a pyrimidine antimetabolite of cytidine that inhibits retroviral reverse transcriptase as the triphosphate 2′,3′-dideoxycytidine triphosphate (ddCTP). ddC is phosphorylated by deoxycytidine kinase in the cell to ddCMP, which lowers cellular dCMP, and is phosphorylated further to ddCTP by cellular enzymes.[31] In HIV-infected cells, ddCTP competitively inhibits viral reverse transcriptase and causes viral DNA chain termination. It also inhibits mitochondrial DNA synthesis, and this effect probably contributes to the clinical toxicities associated with ddC.[32] Combinations of ddC and AZT synergistically inhibit HIV replication, and ddC is effective against some AZT-resistant strains.

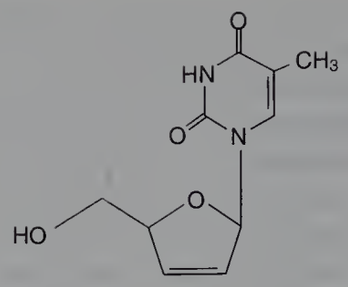


Zalcitabine

ddC is absorbed well orally.[33] Oral bioavailability is greater than 80% in adults but may be reduced in the presence of food. Orally administered ddC probably experiences very little metabolism since more than 75% of an intravenous dose is excreted unchanged in the urine. The elimination half-life of the drug ranges from 1 to 3 hours but can be prolonged greatly in renal insufficiency. The major dose-related toxic effect is peripheral neuropathy, which occurs in approximately 30% of patients. Pancreatitis, a potentially fatal toxic effect, is rare. ddC has been approved for the treatment of HIV infection in adults with advanced disease who are intolerant to AZT or who have disease progression while receiving AZT. The drug is combined with AZT for the treatment of advanced HIV infection.

Stavudine

2′,3′-Didehydro-2′-deoxythymidine, d4T (Zerit). Stavudine is an unsaturated pyrimidine nucleoside related to thymidine. It inhibits replication of HIV by a mechanism similar to that of its close analogue AZT.[34] It is bioactivated by cellular thymidine kinase to the monophosphate, which in turn is converted by cellular enzymes to the triphosphate. Stavudine triphosphate is a competitive inhibitor for the incorporation of thymidine triphosphate (TTP) into retroviral DNA by reverse transcriptase.[35] Stavudine also causes termination of viral DNA elongation through its incorporation into DNA.

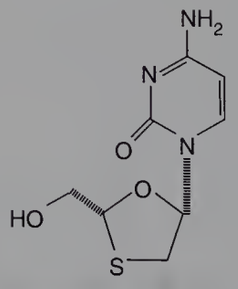


Stavudine

Stavudine is available as capsules for oral administration. It is acid-stable and absorbed well (approximately 90%) following oral administration. It has a short half-life (1 to 2 hours) in plasma and is excreted largely unchanged (85% to 90%) in urine.[36] As with ddC, the primary dose-limiting toxic effect is peripheral neuropathy. At recommended doses, approximately 15% to 20% of patients experience symptoms of peripheral neuropathy. Stavudine is recommended for the treatment of adults with advanced HIV infection who are intolerant of other approved therapies or who have experienced clinical or immunologic deterioration while receiving such therapies.

Lamivudine

(−)-2′,3′-Dideoxy-3′-thiacytidine, (−)-β-L-(2R,5S)-1,3-oxathiolanylcytosine, 3TC, (−)-SddC. Lamivudine is a synthetic nucleoside analogue that differs from 2′,3′-dideoxycytidine (ddC) by the substitution of a sulfur atom in place of a methylene group at the 3′-position of the ribose ring. In early clinical trials, it exhibited promising antiretroviral activity against HIV and low toxicity in the dosages studied.[37,38] Preliminary pharmacokinetic studies indicate that it exhibits good oral bioavailability (approximately 80%) and a plasma half-life of 2 to 4 hours.[37]



Lamivudine

Curiously, the unnatural stereoisomer (−)-SddC exhibits greater antiviral activity against HIV in vivo and causes less toxicity than the natural enantiomer, (+)-SddC.[39] Biochemical studies[40] have shown that both isomers are bioactivated by cellular kinases to the corresponding triphosphates. Both SddCTP isomers inhibit HIV-reverse transcriptase and are

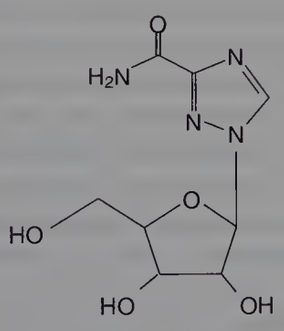
incorporated into viral DNA to cause chain termination. However, (+)-SddCTP inhibits cellular DNA polymerases much more potently than (−)-SddCTP, thus explaining the greater toxicity associated with (+)-SddC. Initial metabolic comparison of SddCTP isomers failed to explain the greater potency of the (−)-isomer against HIV. Thus, although the intracellular accumulation of (−)-SddCTP was two times greater than that of (+)-SddCTP, the latter was one and one-half times more potent as an inhibitor of HIV-reverse transcriptase and the two isomers were incorporated into viral DNA at comparable rates. Finally, the mystery was solved with the discovery of a cellular 3′,5′-exonuclease, which was found to cleave terminal (+)-SddCMP incorporated into viral DNA six times faster than (−)-SddCMP from the viral DNA terminus.

Rapid resistance to lamivudine develops as a result of mutation in codon 184 of the gene that encodes HIV-reverse transcriptase when the drug is employed as monotherapy for HIV.[38] However, when combined with AZT in a clinical trial of HIV-infected patients, lamivudine caused substantial increases in $CD4^+$ cell counts, which were sustained over the course of therapy.[41] The mutation that causes lamivudine resistance suppresses AZT resistance, making the virus more susceptible.[41]

MISCELLANEOUS NUCLEOSIDE ANTIMETABOLITES

Ribavirin, USP

1β-D-Ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide (Virazole). This synthetic nucleoside exhibits in vitro activity against a wide variety of DNA and RNA viruses,[42] including adenovirus, herpesvirus, vaccinia virus, myxoma virus, influenza virus, parainfluenza virus, and rhinovirus. Despite the broad spectrum of activity of ribavirin, the drug has been approved for only one therapeutic indication: the treatment of severe lower respiratory infections caused by RSV in carefully selected hospitalized infants and young children.



Ribavirin

The broad antiviral spectrum of ribavirin suggests multiple modes of action.[43] The nucleoside is bioactivated by viral and cellular kinases and cellular phosphorylating enzymes to the monophosphate (RMP) and the triphosphate (RTP). RMP inhibits inosine monophosphate (IMP) dehydrogenase, thereby preventing the conversion of IMP to xanthine monophosphate (XMP). XMP is required for guanosine triphosphate (GTP) synthesis. RTP inhibits viral RNA polymerases. It also prevents the capping of viral mRNA by inhibiting guanyl N^7-methyltransferase.

Ribavirin occurs as a white, crystalline, polymorphic solid that is soluble in water and chemically stable. It is supplied as a powder to be reconstituted in an aqueous aerosol containing 20 mg/mL of sterile water. The aerosol is administered with a special small-particle aerosol generator (SPAG). Deterioration of respiratory function, bacterial pneumonia, pneumothorax, and apnea have been reported in severely ill infants and children with RSV infection. The role of ribavirin in these events has not been determined. Anemia, headache, abdominal pain, and lethargy have been reported in patients receiving oral ribavirin. It is teratogenic in some animal species and embryocidal in others.

Unlabeled uses of ribavirin include aerosol treatment of influenzas A and B and oral treatment of hepatitis, genital herpes, measles, and Lassa fever. It does not protect cells against the cytotoxic effects of the AIDS virus.

AGENTS UNDER DEVELOPMENT FOR HIV INFECTION

The discovery of HIV as the causative agent of AIDS in 1983[44,45] stimulated an unprecedented level of research activity directed toward both the prevention and treatment of the disease. Information about the genomic structure and replication cycle of retroviruses has facilitated the identification of biochemical targets for attack by potential therapeutic agents for the treatment of HIV infection.[46] Many of these targets are key proteins involved in the HIV replication cycle that have been obtained through recombinant DNA technology and used in rapid-turnover, mechanism-based assays to complement tissue culture screens for whole virus. Thus, clinical candidates, acting at several critical stages of the HIV replicative cycle, have been discovered.[47,48]

Despite the advances outlined above, current clinical therapies (i.e., nucleoside antimetabolite inhibitors of reverse transcriptase) are unable to effect a cure of HIV infection. The picture has been clouded further by the emergence of HIV resistance to clinically proven drugs,[49] including the reverse transcriptase inhibitors and the HIV-protease inhibitors. Moreover, as information about the immunopathogenesis of HIV infection accumulates, researchers have become increasingly pessimistic about the ability of the damaged immune system in an infected patient to recover despite eradication of the virus. Early optimism concerning the development of a safe and effective vaccine for the prevention of HIV similarly has been tempered by the emerging realization

that AIDS is a complex, chronic disease caused by a rather enigmatic virus.[50]

VACCINE DEVELOPMENT

The spectacular successes in disease prevention achieved earlier in this century with traditional live attenuated- or killed whole-virus vaccines, and more recently with recombinant coat protein vaccines, primarily have involved acute viral diseases in which natural infection and recovery lead to long-lasting immunity. Such immunity is considered to be largely antibody-mediated. In contrast, AIDS is a chronic disease in which infection persists despite an apparently robust antibody response to the virus. HIV possesses the unique ability to evade the humoral (antibody) response to infection by first attacking and then eventually killing $CD4^+$ T cells, thus effectively destroying the body's immune system. Thus, cellular responses are critical to the prevention and treatment of HIV infection, and an effective vaccine must elicit an appropriate cellular immune response in addition to the antibody response.

Early efforts in vaccine development focused on variations of HIV envelope glycoprotein (gp 120) obtained by recombinant DNA technology. This focus was based on safety concerns associated with live attenuated-virus vaccines and the discovery that a portion of gp 120 provides the primary target for neutralizing antibodies associated with HIV infection.[51] Unfortunately, the results of early limited clinical trials of these first-generation vaccines were sufficiently disappointing to cause the National Institutes of Health to suspend plans for massive clinical trials for their efficacy in high-risk individuals.[52] The general lack of effectiveness of the first-generation HIV vaccines can be attributed to a number of factors,[53] including the existence of multiple subtypes of HIV throughout the world; the capability of the virus to infect by means of cell-free as well as cell-associated forms; the demonstrated immunosuppressive, immunopathologic, and/or infection-enhancing properties of various portions of the envelope glycoprotein; and the inability of the vaccines to stimulate and maintain sufficient levels of immune responses to be effective.

The projected failure of the first-generation vaccines has led to a reappraisal of the entire AIDS vaccine effort.[53] As a result, criteria for the ''ideal'' AIDS vaccine have been formulated for the guidance of manufacturers. Briefly, the ''ideal'' AIDS vaccine should (1) be safe, (2) elicit a protective immune response in a high proportion of vaccinated individuals, (3) stimulate both cellular and humoral (antibody) responses of the immune system, (4) protect components against all major HIV subtypes, (5) induce long-lasting protection, (6) induce local immunity in both genital and rectal mucosa, and (7) be practical for worldwide delivery and administration. It is too early to judge the extent to which the second-generation AIDS vaccines satisfy the above criteria or to predict when an AIDS vaccine will achieve approval.

NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS

The widespread use of HIV-1 reverse transcriptase in the pharmaceutical industry for the random screening of chemical inventories has led to the discovery of several non-nucleoside reverse transcriptase inhibitors (NNRTIs) of the enzyme, representing structurally distinct classes of compounds. Examples of NNRTIs that have shown sufficient promise in preclinical efficacy, toxicity, and pharmacokinetic screening to reach clinical trials include the tetrahydroimidazobenzodiazepenone (TIBO) analogue R82913,[54] the tricyclic compound nevirapine (Viramune),[55] the bis(heteroaryl)pipcrazine (BHAP) derivatives ateviridine and delaviridine (Rescriptor),[56] the pyridinone derivative L-702,007,[57] and the α-anilinophenylacetamide analogue R89439[58] (Fig. 11-1).

As a group, the NNRTIs share a number of common biochemical and pharmacologic properties.[48,59] Unlike the nucleoside antimetabolites, the NNRTIs do not require bioactivation. They bind to an allosteric site distinct from the substrate (nucleoside triphosphate)-binding site of reverse transcriptase to cause a noncompetitive inhibition of the enzyme. The NNRTIs are extremely potent in cell culture assays and inhibit HIV-1 at nanomolar concentrations. Inhibition of HIV-1 reverse transcriptase by the non-nucleoside compounds is selective; they do not inhibit reverse transcriptases of other retroviruses, including HIV-2 (the other primary subtype of HIV) and SIV (simian immunodeficiency virus) enzymes. The NNRTIs have high therapeutic indexes (in contrast to the nucleosides) and do not inhibit mammalian DNA polymerases. The non-nucleoside and nucleoside reverse transcriptase inhibitors are expected to exert an additive, or possibly synergistic, action against HIV. The major shortcoming of the NNRTIs is the rapid emergence of resistance among HIV isolates.[49] Cross-resistance between structurally different NNRTIs is more common than between NNRTIs and nucleoside antimetabolites. Future clinical trials of the NNRTIs are expected to utilize combinations with the nucleosides to reduce the incidence of toxicity to the latter, to take advantage of additive or synergistic effects, and to discourage the emergence of viral resistance.[49,59] In fact, the tricyclic compound nevirapine (Viramune) and the BHAP derivative delaviradine (Rescriptor) have been approved for use in combination with nucleoside reverse transcriptase inhibitors, such as AZT, for the treatment of HIV.

HIV-PROTEASE INHIBITORS

Biochemical and structural characterization[60] of purified HIV-protease expressed in *Escherichia coli* has established

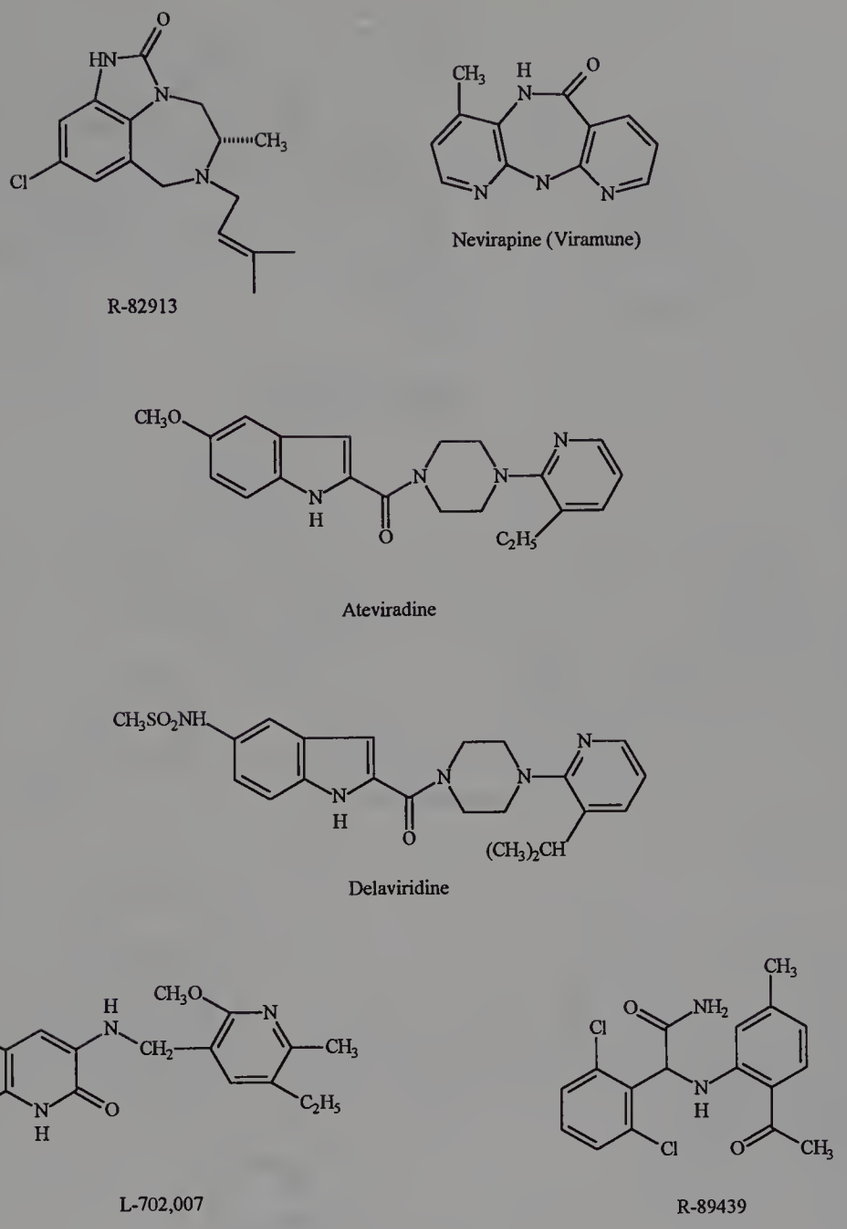
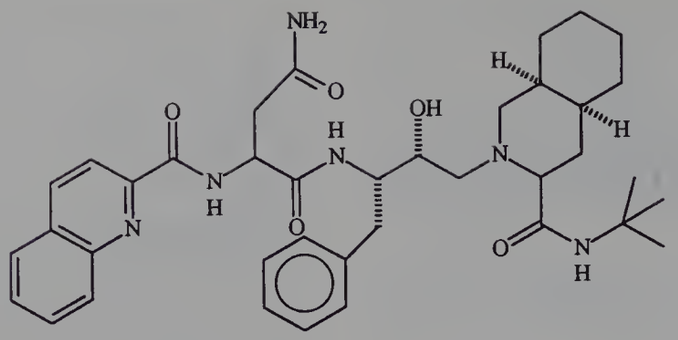


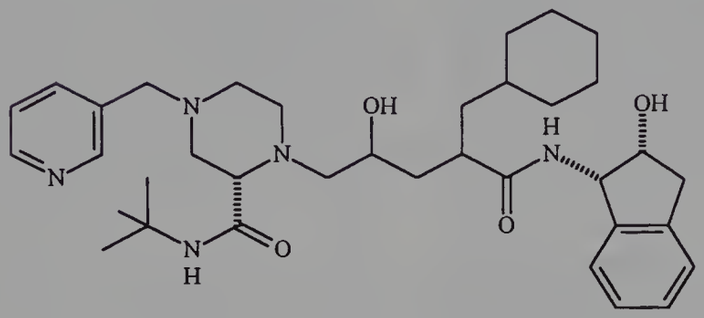
FIG. 11-1. *Non-nucleoside reverse transcriptase inhibitors.*

that the catalytically active enzyme exists as a symmetric dimer of two identical 99 amino acid subunits, each contributing an Asp-Thr-Gly- (DTG) triad at the active site. The homodimeric structure of HIV-protease distinguishes it from the monomeric mammalian aspartic proteases, such as renin, pepsin, and cathepsin D, which also have different substrate specificities. The design of inhibitors of HIV-protease[60] has exploited the C_2-symmetry of the enzyme, its specificity for Tyr(Phe)-Pro cleavage in Ser(Thr)-Xaa-Xaa-Tyr(Phe)-Pro motifs (where Xaas are variable amino acid residues) in the Pr55 gag and Pr160 gag-pol HIV polyproteins, and previous experience gained in application of the transition state analogue concept for the design of inhibitors of the related aspartic protease renin. Such analogue inhibitors resemble a small portion of the substrate protein structure (usually a tripeptide or a tetrapeptide) but contain an isosteric replacement for the scissible (hydrolyzable) peptide bond that mimics the transition state for the hydrolysis of that bond but is not scissible. Hundreds of inhibitors of HIV protease, many of them exhibiting excellent selectivity and high potency, have been designed and synthesized by application of the above principles.[60] Very few of these inhibitors, however, are likely to possess the pharmacokinetic properties required to be successful clinical candidates for the treatment of HIV. Since HIV-protease inhibitors are aimed at arresting replication of the virus at the step of maturation to thereby prevent the spread of cellular infection, they should possess good oral bioavailability and a relatively long duration of action. A long elimination half-life is also desirable because of the known development of resistance by HIV under selective antiviral pressure.[48,49]

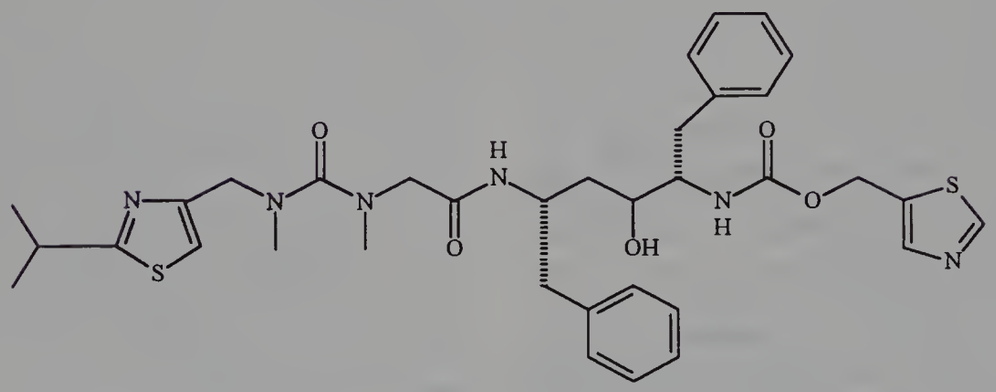
The majority of early-generation inhibitors are high-molecular-weight, peptide-like structures generally having low water solubility. The oral bioavailability of such compounds



Saquinovir (Invirase)



Indinavir (Crixivan)



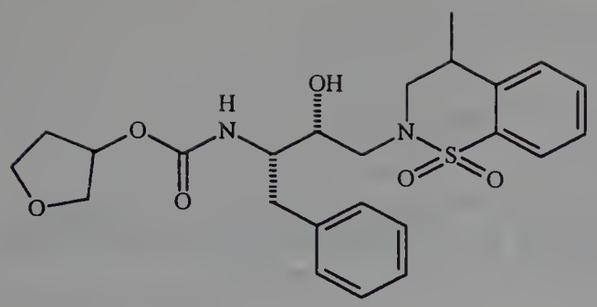
Ritonavir (Norvir)

FIG. 11-2. *Approved HIV protease inhibitors.*

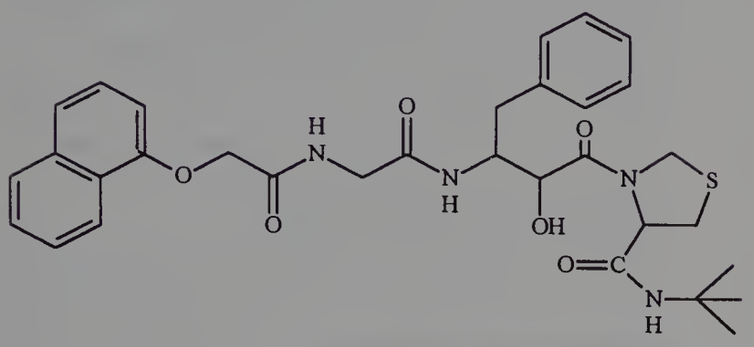
is consequently low, and their elimination half-lives are usually short due to peptide hydrolysis or hepatic metabolism.[61] Strategies aimed at enhancing water solubility and increasing metabolic stability have led to the identification of several promising clinical candidates. Three of these, saquinovir (Invirase),[62] indinavir (Crixivan),[63] and ritonavir (Norvir),[64] have been approved for use in the treatment of HIV-infected patients. Several additional compounds—for example, VX-478,[65] KN,[66] and AG1343[67]—have shown sufficient promise in preclinical evaluations to have entered into early clinical trials (Fig. 11-2).

Numerous nonpeptide inhibitors of HIV-protease have been discovered as a result of the application of two strikingly different approaches. For example, the C_2-symmetry of the active site of the enzyme was exploited in the structure-based design of the symmetric cyclic urea derivative DPM 323.[68] This inhibitor exhibited potent inhibition of the protease in vitro, excellent anti-HIV activity in cell culture, and promising bioavailability in experimental animals. However, in phase I clinical trials, the bioavailability of DPM 323 was poor and highly variable, possibly due to its low water solubility and susceptibility to hepatic metabolism. Random screening of chemical inventories led to the discovery of the pyrone-based inhibitor U-96988.[69] This compound, in addition to having acceptable potency against HIV-protease and anti-HIV activity in cell culture, exhibits excellent bioavailability in experimental animals.

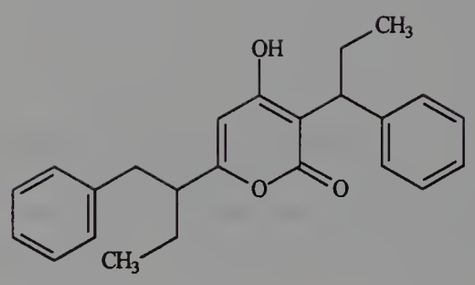
Early clinical evaluation of saquinovir[70] revealed that it is well tolerated following oral administration. Although its bioavailability is low, food greatly enhances its oral absorp-



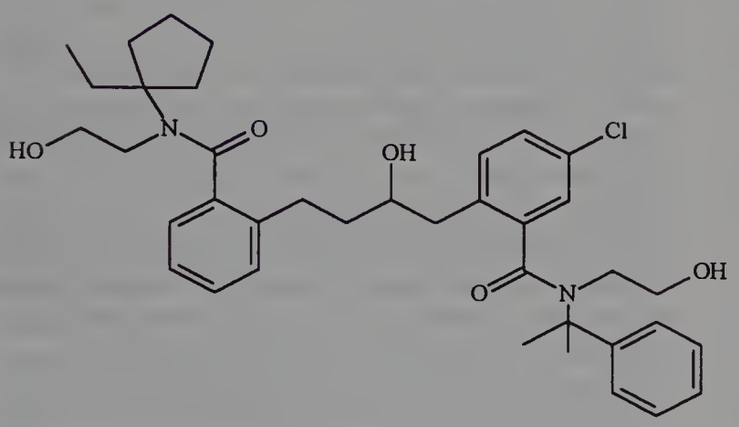
VX-478



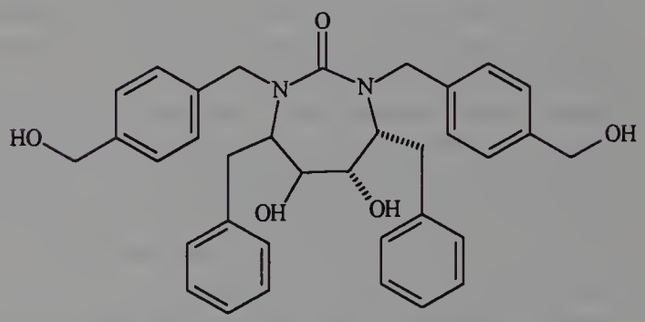
KNI 272



U-96988



AG1343



DPM 323

FIG. 11-3. *Investigational HIV protease inhibitors.*

tion. Blood levels exceeding the retroviral ED_{90} were achieved on a 600 mg three-times-daily dosing schedule. Saquinovir lowered p24 antigen levels in HIV-infected patients, elevated CD4 counts, and exerted a synergistic antiviral effect when combined with the reverse transcriptase inhibitors AZT and ddC.[71,72] Although HIV-1 resistance to saquinovir and other HIV-protease inhibitors is known to occur in vivo,[48,49] it is believed to be less intense and less frequent than resistance to the reverse transcriptase inhibitors.[73] Nonetheless, cross-resistance between different HIV-protease inhibitors appears to be common and additive,[74] suggesting that the use of combinations of such inhibitors would not be rational.

Cell kinetic studies of HIV-1 production and CD4 cell turnover using the HIV-protease inhibitor ritonavir[75,76] and the reverse transcriptase inhibitor nevirapine[75] indicate that replication of the virus is continuous and highly productive and drives the rapid turnover of CD4 lymphocytes. The rapid turnover of HIV permits creation of genetic diversity, leading to increased opportunities for viral escape from therapeutic intervention. If therapeutic strategies are to have a dramatic impact against HIV, they must be instituted as early in the course of infection as possible.[75,76]

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CHAPTER 12

Antineoplastic Agents

William A. Remers

The chemotherapy of neoplastic disease has become increasingly important in recent years. An indication of this importance is the establishment of a medical specialty in oncology, wherein the physician practices various protocols of adjuvant therapy. Most cancer patients now receive some form of chemotherapy, even though it is merely palliative in many cases.

Cancer chemotherapy has received no spectacular breakthrough of the kind that the discovery of penicillin provided for antibacterial chemotherapy. However, there has been substantial progress in many aspects of cancer research. In particular, an increased understanding of tumor biology has led to elucidation of the mechanisms of action for antineoplastic agents. It also has provided a basis for the more rational design of new agents. Recent advances in clinical techniques, including large cooperative studies, are allowing more rapid and reliable evaluation of new drugs. The combination of these advantages with improved preliminary screening systems is enhancing the emergence of newer and more potent compounds.

At present, at least 10 different neoplasms can be "cured" by chemotherapy in the majority of patients. Cure is defined here as an expectation of normal longevity. These neoplasms are acute leukemia in children, Burkitt's lymphoma, choriocarcinoma in women, Ewing's sarcoma, Hodgkin's disease, lymphosarcoma, mycosis fungoides, rhabdomyosarcoma, retinoblastoma in children, and testicular carcinoma.[1] Unfortunately, only these relatively rare neoplasms are readily curable. Considerable progress is being made in the treatment of breast cancer by combination drug therapy. However, for carcinoma of the pancreas, colon, liver, or lung (except small cell carcinoma), the outlook is bleak. Short-term remissions are the best that can be expected for most patients with these diseases.

There are cogent reasons why cancer is more difficult to cure than bacterial infections. One is that there are qualitative differences between human and bacterial cells. For example, bacterial cells have distinctive cell walls, and their ribosomes are different from those of human cells. In contrast, the differences between normal and neoplastic human cells are mostly quantitative. Another difference is that immune mechanisms and other host defenses are very important in killing bacteria and other foreign cells, whereas they play a negligible role in killing cancer cells. By their very nature, the cancer cells have eluded or overcome the immune surveillance system of the body. Thus, it is necessary for chemotherapeutic agents to kill every single clonogenic malignant cell, because even one can reestablish the tumor. This kind of kill is extremely difficult to effect because antineoplastic agents kill cells by first-order kinetics. That is, they kill a constant fraction of cells. Suppose that a patient had a trillion leukemia cells. This amount would cause a serious debilitation. A potent anticancer drug might reduce this population 10,000-fold, in which case the symptoms would be alleviated and the patient would be in a state of remission. However, the remaining hundred million leukemia cells could readily increase to the original number after cessation of therapy. Furthermore, a higher proportion of resistant cells would be present, which would mean that retreatment with the same agent would achieve a lesser response than before. For this reason, multiple drug regimens are used to reduce drastically the number of neoplastic cells. Typical protocols for leukemia contain four different anticancer drugs, usually with different modes of action.

TUMOR CELL PROPERTIES

The basic differences between cancer cells and normal cells are uncontrolled cell proliferation, decreased cellular differentiation, ability to invade surrounding tissue, and ability to establish new growth at ectopic sites (metastasis). Contrary to popular belief, not all tumor cells are rapidly proliferating. Proliferation rates vary widely with the cell type. Thus, lymphomas and normal intestinal mucosa both proliferate faster than solid tumors. Acute leukemia cells actually proliferate

more slowly than the corresponding precursors in normal bone marrow. The accumulation of tumor cells is the result of defective homeostasis that causes an imbalance in the rates of cell production and cell loss. Current research is directed toward understanding programmed cell death (apoptosis) and how tumor cells block it to allow tumor growth through excess cell survival.[2]

The concept of a cell cycle is based on experiments using [^{3}H] thymidine radiography and flow cytometry. These experiments showed that DNA synthesis, as measured by incorporation of [^{3}H] thymidine, takes place at a specific period, known as the S phase, in the life cycle of a dividing cell. Periods between the S phase and cell division (mitosis or M phase) are termed G_1 and G_2. A circular pictorial model (Fig. 12-1) was derived for the clockwise progression of the cell cycle. The duration of each phase in the cell cycle varies considerably with the cell type and within a single tumor. Typical durations are as follows: S, 10 to 20 hr, G_2, 2 to 10 hr, and M, 0.5 to 1 hr. G_1 is highly variable as the result of another phase, G_0, in which the cell is not active in cell division. Most anticancer drugs block the biosynthesis or transcription of nucleic acids or they prevent cell division by interfering with mitotic spindles. Cells in the DNA synthesis or mitosis phases are highly susceptible to these agents. In contrast, cells in the resting state are resistant to many agents. Slow-growing tumors characteristically have many cells in the resting state.[3]

Antitumor agents are classified by their effects on cell survival as a function of dose. For many drugs, including alkylating agents, cell survival is exponentially related to dose, and a plot of log cell survival against drug concentration (Fig. 12-2) gives a straight line. They exert their cytotoxicity regardless of the cell cycle phase and are termed non–cell cycle phase–specific. Other drugs, including antimetabolites and mitotic inhibitors, which act at one phase of the cell cycle (cell cycle phase–specific), show a plateau after an initial low-dose exponential region.

The proportion of labeled cells in tissue after a specific interval (usually 1 hr) following injection of [^{3}H] thymidine or 5-bromodeoxyuridine is known as the labeling index (LI). Comparison of the LI with the proportion of proliferating

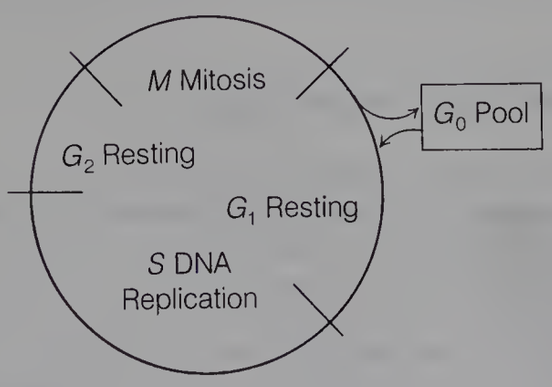


FIG. 12-1. *The cell life cycle.*

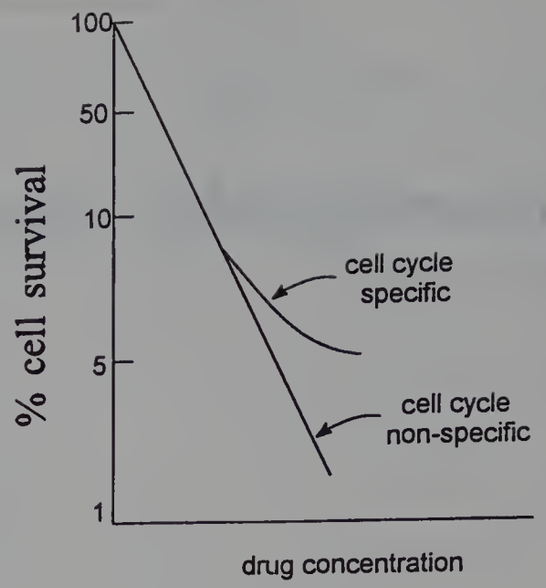


FIG. 12-2. *Cell cycle specificity.*

cells in DNA synthesis provides the growth fraction. Doubling times for tumor growth are calculated from the growth fraction and cell cycle times. They rarely are as rapid as predicted because of tumor cell loss through necrosis, metastasis, and differentiation.

The cell-kill hypothesis states that the effects of antitumor drugs on tumor cell populations follow first-order kinetics. This means that the number of cells killed is proportional to the dose. Thus, chemotherapy follows an exponential or log-kill model in which a constant proportion, not a constant number, of cancer cells are killed.[4] The fractional reductions possible with cancer chemotherapy can never theoretically reduce tumor populations to zero. Complete eradication requires another effect such as the immune response. A modified form of the first-order log-kill hypothesis holds that tumor regressions produced by chemotherapy are described by the relative growth fraction present in the tumor at the time of treatment. This idea is consistent with the finding that very small and very large tumors are less responsive than tumors of intermediate size.[5]

Stem cells are the cells of origin of a cell line, which maintain the potential to regenerate the cell population and from which the differentiated cells are derived. They are important in the chemotherapy of human tumors because they must be eradicated completely to effect a cure. Treatments that afford substantial reductions in tumor burdens can produce remissions, but the tumor may recur if some of the stem cells remain. Their eradication is difficult because many are in the G_0 phase of the cell cycle.[6]

Drug resistance to chemotherapy usually involves the selection of certain cell populations. Populations of drug-resistant cells can be inherently produced by clonal evolution or mutation. Drug-resistant cells in tissue culture are generated at a frequency consistent with known rates of genetic mutation. Mutagenic agents increase the frequency of generation

of drug-resistant cells. This effect may have clinical importance because many antitumor agents are mutagenic. Intracellular effects that cause drug resistance may be secondary to cellular adaptation or altered enzyme levels or properties. For example, resistance to methotrexate involves increased levels of the target enzyme, dihydrofolate reductase.[7] Other modes of resistance to antimetabolites include reduced drug transport into cells, reduced affinity of the molecular target, stimulation of alternate biosynthetic pathways, and impaired activation or increased metabolism of the drug. A major factor in resistance to alkylating agents is the ability of tumor cells to repair DNA lesions such as cross-links and breakage of DNA strands caused by alkylation. Cells selected for resistance to one drug may show cross-resistance to other drugs, even if their chemical structures are quite different; however, most of these drugs are derived from natural products. One type of molecular explanation for this form of multiple drug resistance is overexpression of membrane glycoproteins termed P-glycoproteins, as well as LRP and MRP forms of proteins, which function as drug efflux pumps. This overexpression is associated with gene amplification.[2]

Most antineoplastic drugs are highly toxic to the patient and must be administered with extreme caution. Some of them require a clinical setting where supportive care is available. The toxicity usually involves rapidly proliferating tissues such as bone marrow and the intestinal epithelium. However, individual drugs produce distinctive toxic effects on the heart, lungs, kidneys, and other organs. Chemotherapy is seldom the initial treatment used against cancer. If the cancer is well defined and accessible, surgery is the preferred method. Skin cancers and certain localized tumors are treated by radiotherapy. Generally, chemotherapy is important where the tumor is inoperable or where metastasis has occurred. Chemotherapy is finding increasing use as an "adjuvant" after surgery to insure that few cells remain to regenerate the parent tumor.

The era of chemotherapy of malignant disease was born in 1941, when Huggins demonstrated that the administration of estrogens produced regressions of metastatic prostate cancer.[8] In the following year, Gilman and others began clinical studies on the nitrogen mustards and discovered that mechlorethamine was effective against Hodgkin's disease and lymphosarcoma.[9] These same two diseases were treated with cortisone acetate in 1949, and dramatic, although temporary, remissions were observed.[10] The next decade was marked by the design and discovery of antimetabolites: methotrexate in 1949, 6-mercaptopurine in 1952, and 5-fluorouracil in 1957. Additional alkylating agents such as melphalan and cyclophosphamide were developed during this period, and the activity of natural products such as actinomycin, mitomycin C, and the vinca alkaloids was discovered. During the 1960s, progress continued in all of these areas with the discovery of cytosine arabinoside, bleomycin, doxorubicin, and carmustine. Novel structures such as procarbazine, dacarbazine, and cis-platinum complexes were found to be highly active. In 1965, Kennedy reported that remissions occurred in 30% of postmenopausal women with metastatic breast cancer upon treatment with high doses of estrogen.[11]

Much of the leadership and financial support for the development of antineoplastic drugs derives from the National Cancer Institute (NCI). In 1955, this organization established the Cancer Chemotherapy National Service Center (now the Division of Cancer Treatment) to coordinate a national voluntary cooperative cancer chemotherapy development program. By 1958, this effort had evolved into a targeted drug development program. A massive screening system was established to discover new lead compounds, and thousands of samples have been submitted to it. The current highly automated NCI tumor cell culture screening system achieved operational status in 1990. It emphasizes rigorous end points such as net cell killing and tumor regression, rather than earlier growth-inhibitory end points, and it uses a wide variety of specific types of cancer, including many solid tumor models, in the initial stage of screening. New drug candidates are being screened at a rate of ~20,000 per year, with input divided about equally between pure compounds and extracts or fractions from natural products. The present in vitro screening panel contains 60 human tumor cell lines arranged in seven subpanels that represent diverse histologies: leukemia, melanoma, lung, colon, kidney, ovary, and brain. For routine evaluation, each sample is tested in a 2-day continuous drug exposure protocol using five $\log_{10}$-spaced concentrations starting at 10^{-4} M for pure compounds and 100 μg/ml for extracts. Antitumor activities are compared at three different levels of response: GI_{50} is the drug concentration producing 50% inhibition in cell proliferation relative to the control, tumor growth inhibition (TGI) is the drug concentration at which there is no net proliferation, and LC_{50} is the lethal concentration of drug producing a 50% reduction in the number of tumor cells relative to the control.[12]

The primary NCI screening data is reported in a mean-graph format (Fig. 12-3), in which a vertical reference bar, obtained by averaging the negative $\log_{10} GI_{50}$ values for all of the cell lines tested, is plotted along the drug concentration axis and then horizontal bars are plotted for the individual negative $\log_{10} GI_{50}$'s of each line with respect to the vertical reference bar. This graphical representation provides a characteristic fingerprint for a given compound, displaying the individual cell lines that are more sensitive than average (bars to the right of the reference) or less sensitive than average (bars to the left of the reference). Thus, Fig. 12-3 shows that colon cancer cell lines are more sensitive than average to 5-fluorouracil, whereas CNS cancer cell lines are more resistant than average to it.[12]

A secondary stage of preliminary screening on selected compounds is performed in vivo in xenograft models using a subset of cell lines found to be active in the primary in vitro screen. Two xenograft models in current use are the severe combined immunodeficiency (SCID) mouse and the athymic nude mouse. Both of these mouse models have deficient immune responses, which permit human tumor cells to be transplanted into them without rejection. Consequently,

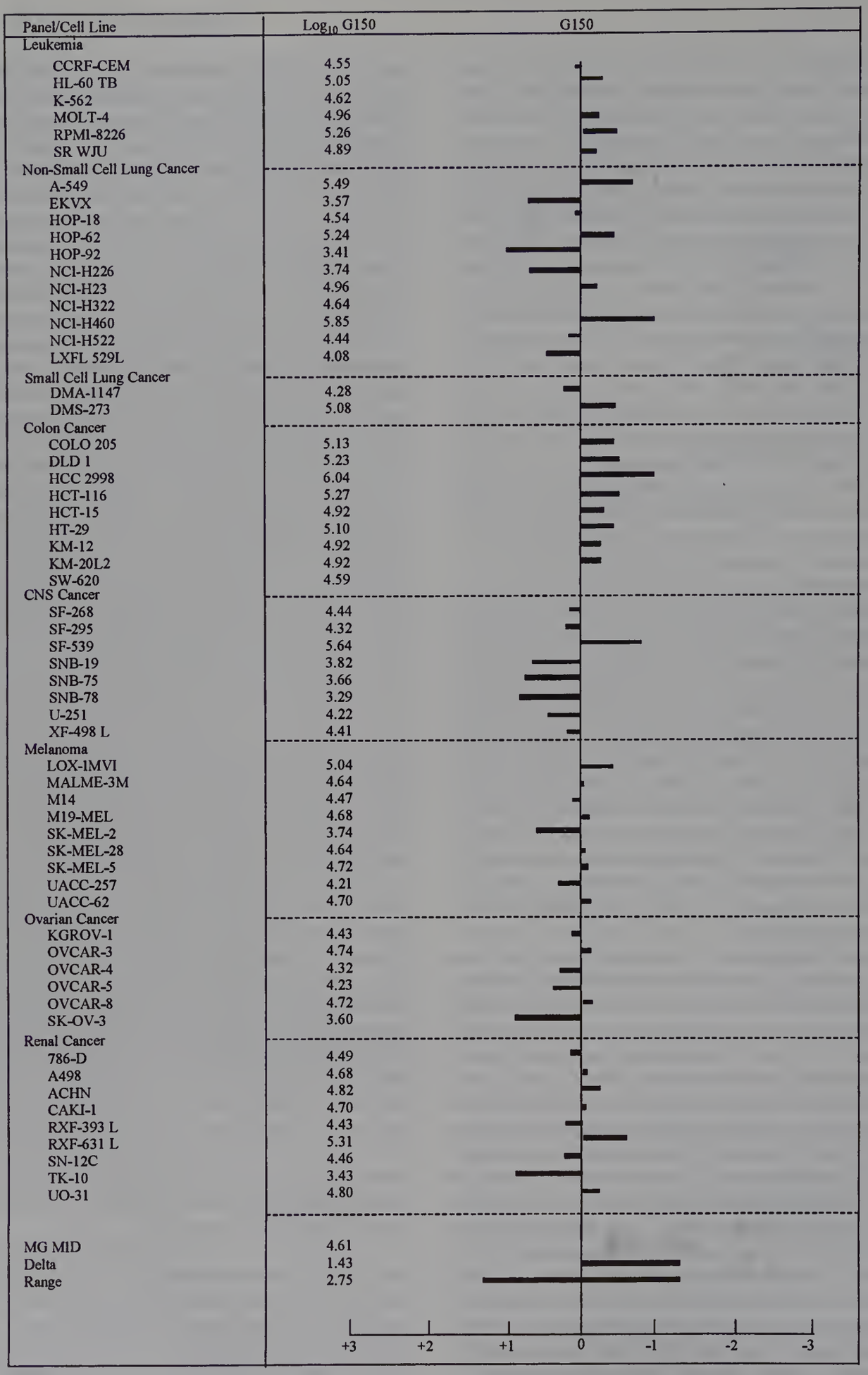


FIG. 12-3. *NIH testing result profile for 5-FU.*

potential antitumor drugs may be tested against human tumors in an in vivo model. These models are better predictors of human clinical tumor responses than were the older allograft models, which were based on transplanting mouse tumors such as P388 leukemia into the same strain of mice (syngeneic tumors). The important antitumor drug paclitaxel was discovered using a xenograft model.

An in vitro system that is a good predictor of human clinical activity is the human-tumor-colony-forming assay (HTCFA). This system uses fresh human tumor tissue from individual patients.[13] It is thought to be most valuable in selecting chemotherapeutic agents for individual tumor types and occasionally specific patients, but its use in large-scale primary screening has not been feasible.

Compounds with significant antitumor activity are subjected to preclinical pharmacology and toxicology evaluation in mice and dogs. Clinical trials may be underwritten by the NCI. They involve three discrete phases. Phase I is the clinical pharmacology stage. The dosage schedule is developed, and toxicity parameters are established in it. Phase II involves the determination of activity against a "signal" tumor panel, which includes both solid and hematological types.[8] A broad-based multicenter study usually is undertaken in Phase III. It features randomization schemes designed to statistically validate the efficacy of the new drug in comparison to alternative modalities of therapy. As might be anticipated, the design of clinical trials for antineoplastic agents is very complicated, especially in the matter of controls. Ethical considerations do not permit patients to be left untreated if any reasonable therapy is possible.

It should be mentioned that a number of pharmaceutical industry laboratories and foreign institutions have made significant contributions to the development of anticancer drugs. Organizations such as the United Kingdom's Cancer Research Campaign, the European Organization for Research on the Treatment of Cancer, and the Japanese Foundation for Cancer Research have broadened international cooperation in anticancer drug research.

ALKYLATING AGENTS

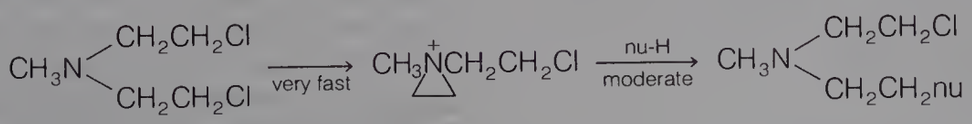
Toxic effects of sulfur mustard and ethylenimine on animals were described in the 19th century.[14] The powerful vesicant action of sulfur mustard led to its use in World War I, and medical examination of the victims revealed that tissues were damaged at sites distant from the area of contact.[15] Such systemic effects included leukopenia, bone marrow aplasia, lymphoid tissue suppression, and ulceration of the gastrointestinal tract. Sulfur mustard was shown to be active against animal tumors, but it was too nonspecific for clinical use. A variety of nitrogen mustards were synthesized between the two world wars. Some of these compounds, for example mechlorethamine, showed selective toxicity, especially to lymphoid tissue. This observation led to the crucial suggestion that nitrogen mustards be tested against tumors of the lymphoid system in animals. Success in this area was followed by cautious human trials that showed methchlorethamine to be useful against Hodgkin's disease and certain lymphomas. This work was classified during World War II but was finally published in a classical paper by Gilman and Phillips in 1946.[9] In that paper, the chemical transformation of nitrogen and sulfur mustards to cyclic "onium" cations was described, and the locus of their interaction with cancer cells was established to be the nucleus. The now familiar pattern of toxicity to rapidly proliferating cells in bone marrow and the gastrointestinal tract was established.

Alkylation is defined as the replacement of hydrogen on an atom by an alkyl group. The alkylation of nucleic acids or proteins involves a substitution reaction in which a nucleophilic atom (nu) of the biopolymer displaces a leaving group from the alkylating agent.

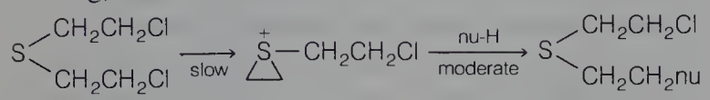
$$\text{nu-H} + \text{alkyl-Y} \rightarrow \text{alkyl-nu} + \text{H}^+ + \text{Y}^-$$

The reaction rate depends on the nucleophilicity of the atom (S, N, O), which is greatly enhanced if the nucleophile is ionized. A hypothetical order of reactivity at physiological pH would be ionized thiol, amine, ionized phosphate, and ionized carboxylic acid.[16] Rate differences among various amines would depend on the degree to which they are protonated and their conjugation with other functional groups. The N-7 position of guanine in DNA (see Scheme 5 below) is strongly nucleophilic.

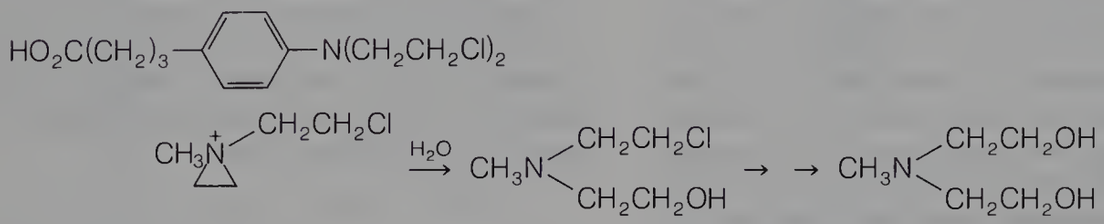
Reaction orders depend on the structure of the alkylating agent. Methanesulfonates, epoxides, and aziridines give second-order reactions that depend on concentrations of the alkylating agent and nucleophile. The situation is more complex with β-haloalkylamines (nitrogen mustards) and β-haloalkylsulfides (sulfur mustards) because these molecules undergo neighboring-group reactions in which the nitrogen or sulfur atom displaces the halide to give strained, three-membered "onium" intermediates. These "onium" ions react with nucleophiles in second-order processes. However, the overall reaction kinetics depend on the relative rates of the two steps. In the case of mechlorethamine, the aziridinium ion is rapidly formed in water, but reaction with biological nucleophiles is slower. Thus, the kinetics will be second order.[17]



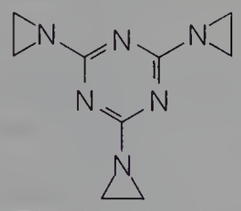
In contrast, sulfur mustard forms the less stable episulfonium ion more slowly than this ion reacts with biological nucleophiles. Thus, the neighboring-group reaction is rate limiting, and the kinetics are first order.[18]



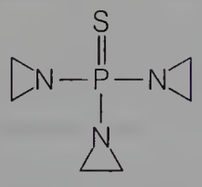
Aryl-substituted nitrogen mustards such as chlorambucil are relatively stable toward aziridinium ion formation, because the aromatic ring decreases the nucleophilicity of the nitrogen atom. These mustards react according to first-order kinetics.[18] The stability of chlorambucil allows it to be taken orally, whereas mechlorethamine is given by intravenous administration of freshly prepared solutions. The requirement for freshly prepared solutions is based on the gradual decomposition of the aziridinium ion by interaction with water.



Ethylenimines and epoxides are strained ring systems, but they do not react as readily as aziridinium or episulfonium ions with nucleophiles. Their reactions are second order and are enhanced by the presence of acid.[16] Examples of antitumor agents containing ethylenimine groups are triethylene melamine and thiotepa.

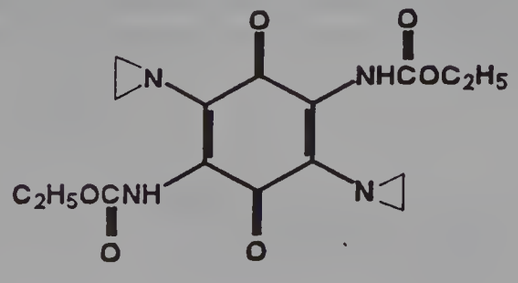


Triethylene Melamine



Thiotepa

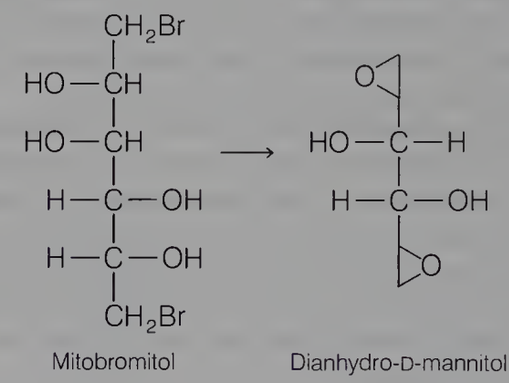
Diaziquone is an investigational benzoquinone substituted with ethylenimine groups and carbamate groups, both of which are cancerostatic.[19] Following activation by reduction of the quinone ring to a hydroquinone, the ethyleneimine groups alkylate DNA to produce cross-links. Some DNA-protein cross-links also are formed.



Diaziquone

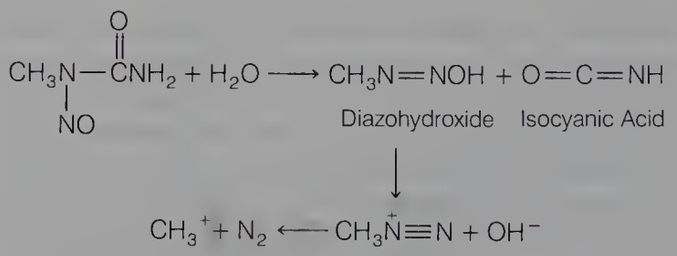
The use of epoxides as cross-linking agents in textile chemistry suggested that they be tried in cancer chemotherapy. Simple diepoxides such as 1,2:3,4-diepoxybutane showed clinical activity against Hodgkin's disease,[20] but none of these compounds became an established drug. Dibromomannitol (mitobromitol) gives the corresponding diepoxide upon continuous titration at pH 8. This diepoxide (1,2:5,6-dianhydro-D-mannitol) shows potent alkylating activity against experimental tumors.[21] Thus, it is supposed that dibromomannitol and related compounds such as dibromodulcitol act by way of the diepoxides.

A somewhat different type of alkylating agent is the N-alkyl-N-nitrosourea. Compounds of this class are unstable in aqueous solution under physiological conditions. They produce carbonium ions (also called carbenium ions) that can alkylate and isocyanates that can carbamoylate. For example, methylnitrosourea decomposes initially to form isocyanic acid and methyldiazohydroxide. The latter species decomposes further to methyldiazonium ion and finally to methyl carbonium ion, the ultimate alkylating species.[22]



Mitobromitol

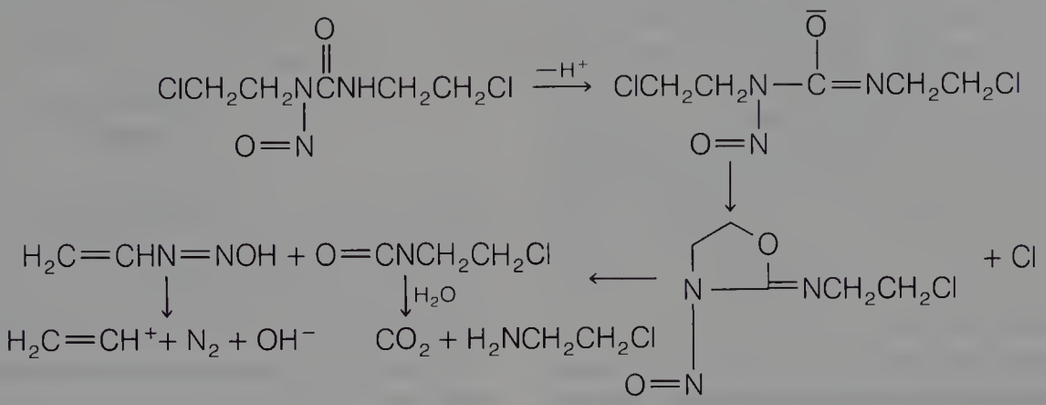
Dianhydro-D-mannitol



Substituents on the nitrogen atoms of the nitrosourea influence the mechanism of decomposition in water, which determines the species generated and controls the biological effects. Carmustine (BCNU) undergoes an abnormal base-catalyzed decomposition in which the urea oxygen displaces a chlorine to give a cyclic intermediate (Scheme 1). This intermediate decomposes to vinyl diazohydroxide, the pre-

cursor to vinylcarbonium ion, and 2-chloroethylisocyanate. The latter species gives 2-chloroethylamine, an additional alkylating agent.[22]

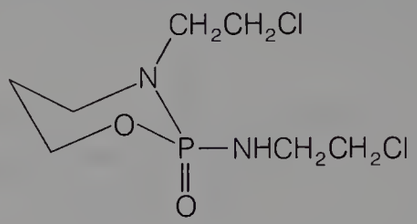
Cyclophosphamide has been resolved, and the enantiomers have been tested against tumors. The levorotatory form has twice the therapeutic index of the dextrorotatory form.[24]



Scheme 1. *Decomposition of carmustine.*

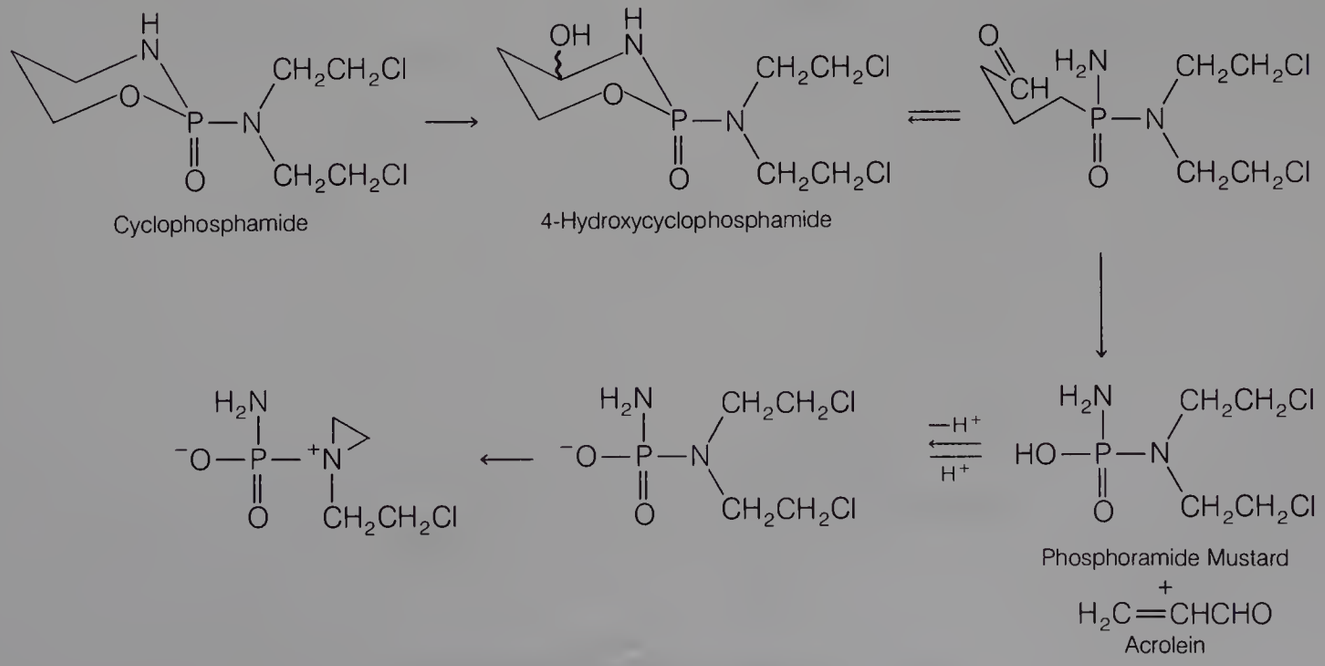
Some clinically important alkylating agents are not active until they have been transformed by metabolic processes. The leading example of this group is cyclophosphamide, which is converted by hepatic cytochrome P-450 into the corresponding 4-hydroxy derivative by way of the 4-hydroperoxy intermediate (Scheme 2). The 4-hydroxy derivative is a carbinolamine in equilibrium with the open-chain aminoaldehyde form. Nonenzymatic decomposition of the latter form generates phosphoramide mustard and acrolein. Studies based on ^{31}P NMR have shown that the conjugate base of phosphoramide mustard cyclizes to an aziridinium ion,[23] which is the principal cross-linking alkylator formed from cyclophosphamide. The maximal rate of cyclization occurs at pH 7.4. It was suggested that selective toxicity toward certain neoplastic cells might be based on their abnormally low pH. This would afford a slower formation of aziridinium ions, and they would persist longer because of decreased inactivation by hydroxide ions.[22]

Ifosfamide, an isomer of cyclophosphamide in which one of the 2-chloroethyl substituents is on the ring nitrogen, also has potent antitumor activity. It must be activated by hepatic enzymes, but its metabolism is slower than that of cyclophosphamide[25] and involves substantially more dechloroethylation, yielding a chloroacetate metabolite.

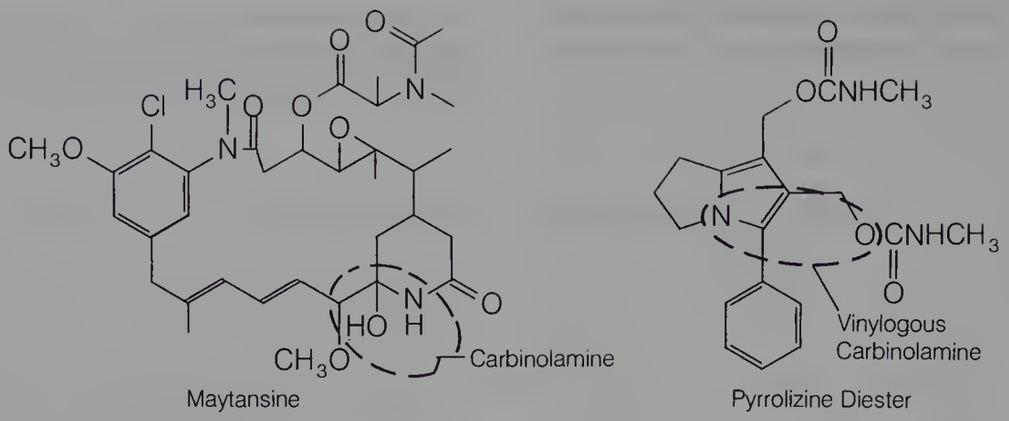


Ifosfamide

Other examples of alkylating species are afforded by carbinolamines as found in maytansine and vinylogous carbinolamines as found in certain pyrrolizine diesters.[26]



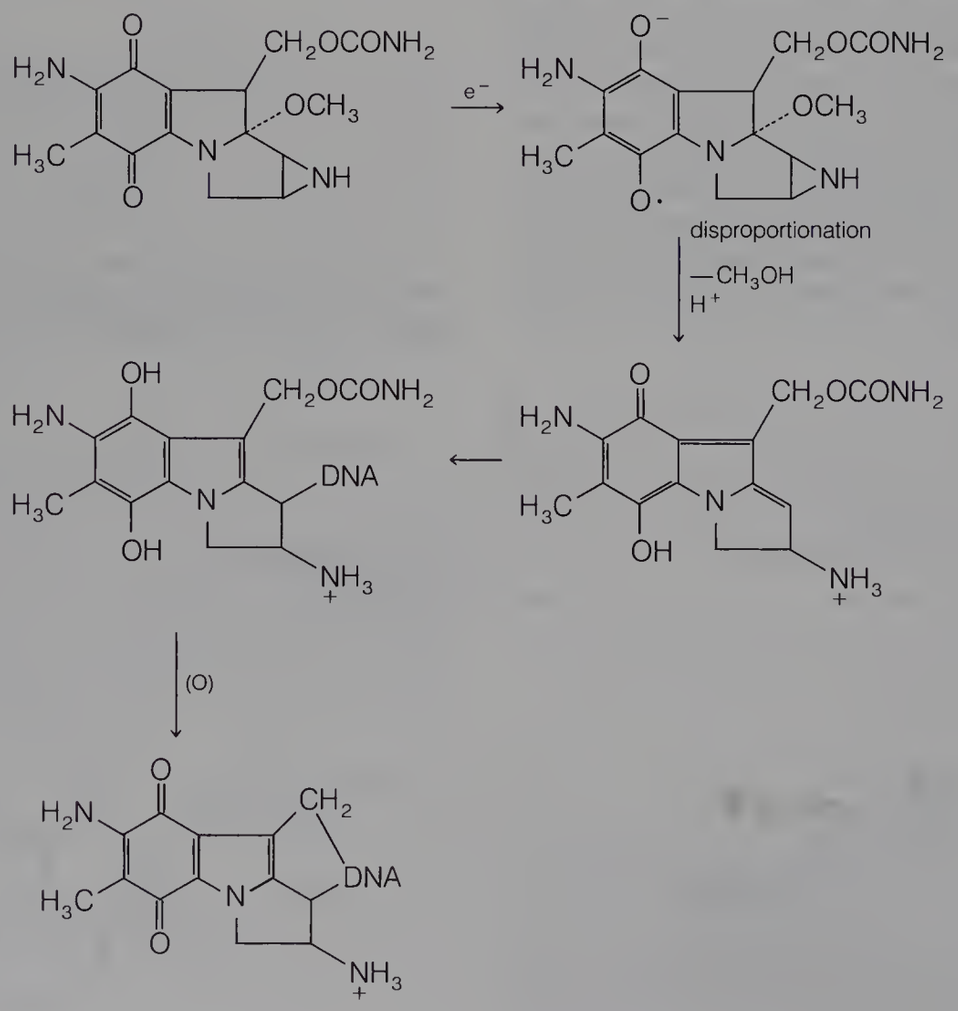
Scheme 2. *Activation of cyclophosphamide.*



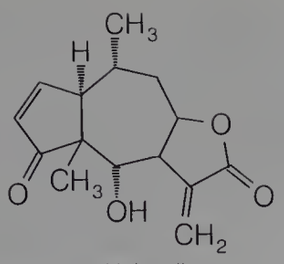
When mitomycin C is reduced enzymatically to its semiquinone radical, disproportion and spontaneous elimination of methanol affords the vinylogous carbinolamine system. Loss of the carbamoyloxy group from this system gives a stabilized carbonium ion that is capable of alkylating DNA (Scheme 3). The first alkylation step results from opening of the aziridine ring, and, together with the vinylogous carbinolamine, it allows mitomycin C to cross-link double helical DNA.[27] Molecules like mitomycin C are said to act by "bioreductive alkylation."[28]

Another type of alkylating species occurs in α, β-unsaturated carbonyl compounds. These compounds can alkylate nucleophiles by conjugate addition. Although there are no established clinical agents of this type, many natural products active against experimental tumors contain α-methylene lactone or α, β-unsaturated ketone functionalities. For example, the sesquiterpene helenalin has both of these systems.[29]

Alkylation can also occur by free radical reactions. The methylhydrazines are a chemical class prone to decomposi-



Scheme 3. *Mitomycin C activation and DNA alkylation.*

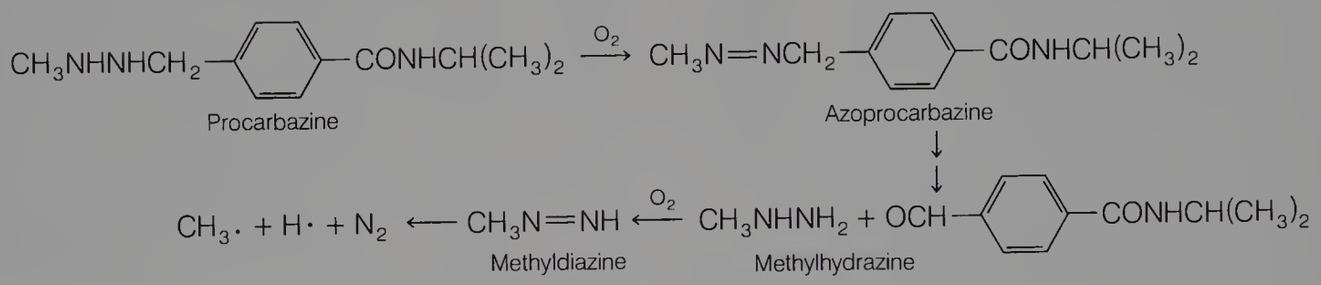


Helenalin

tion in this manner. These compounds were tested as antitumor agents in 1963, and one of them, procarbazine, was found to have a pronounced, but rather specific effect on Hodgkin's disease.[30] Procarbazine is relatively stable at pH 7, but air oxidation to azoprocarbazine occurs readily in the presence of metalloproteins. Isomerization of this azo compound to the corresponding hydrazone, followed by hydrolysis, gives methylhydrazine and p-formyl-N-isopropylbenzamide. The formation of methylhydrazine from procarbazine has been demonstrated in living organisms.[31] Methylhydrazine is known to be oxidized to methyldiazine, which can decompose to nitrogen, methyl radical, and hydrogen radical.[32] The methyl group of procarbazine is incorporated intact into cytoplasmic RNA.[33] However, it has not been conclusively established that the methyl radical is the methylating species.

Dacarbazine was originally thought to be an antimetabolite because of its close resemblance to 5-aminoimidazole-4-carboxamide, an intermediate in purine biosynthesis. However, it now appears to be an alkylating agent.[34] The isolation of an N- demethyl metabolite suggested that there might be a sequence in which this metabolite was hydrolyzed to methyldiazohydroxide, a precursor to methylcarbonium ion,[35] but it was found that this metabolite was less active than starting material against the Lewis lung tumor. An alternative mode of action was proposed in which dacarbazine undergoes acid-catalyzed hydrolysis to a diazonium ion, which can react in this form or decompose to the corresponding carbonium ion (Scheme 4). Support for the latter mechanism was afforded by a correlation between the hydrolysis rates of phenyl-substituted dimethyltriazines and their antitumor activities.[36]

The interaction of alkylating agents with macromolecules such as DNA and RNA has been studied extensively. However, no mode of action for the lethality to cancer cells has been conclusively established. A good working model has been developed for the alkylation of bacteria and viruses, but there are uncertainties in extrapolating it to mammalian cells. The present working hypothesis is that most alkylating agents produce cytotoxic, mutagenic, and carcinogenic effects by reacting with cellular DNA. They also react with RNA and proteins, but these effects are thought to be less significant.[37] The most active clinical alkylating agents are bifunctional compounds capable of cross-linking DNA. Agents such as methylnitrosourea that give simple alkylation are highly mutagenic relative to their cytotoxicity. The cross-linking process can be either interstrand or intrastrand. Interstrand links can be verified by a test based on the thermal denaturation and renaturation of DNA. When double helical

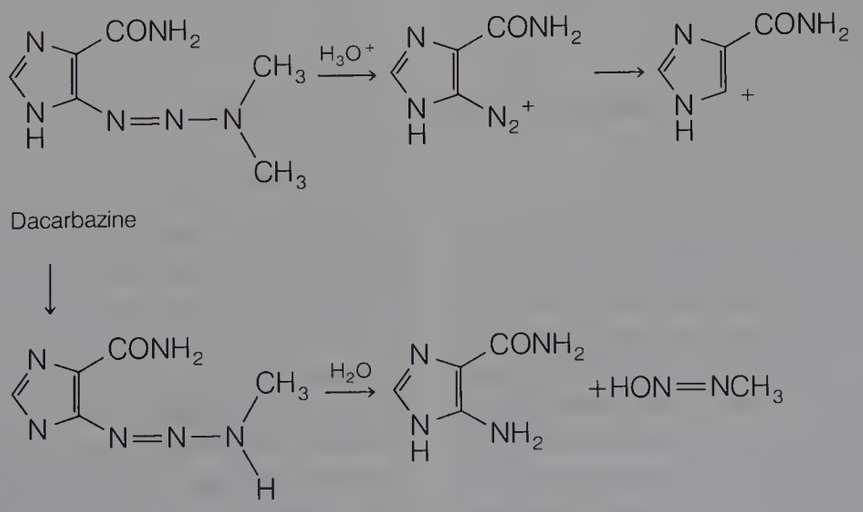


Procarbazine

Azoprocarbazine

Methyldiazine

Methylhydrazine

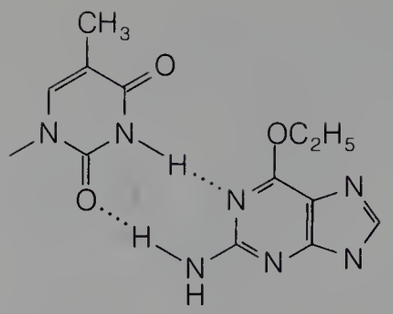


Dacarbazine

Scheme 4. *Activation of dacarbazine.*

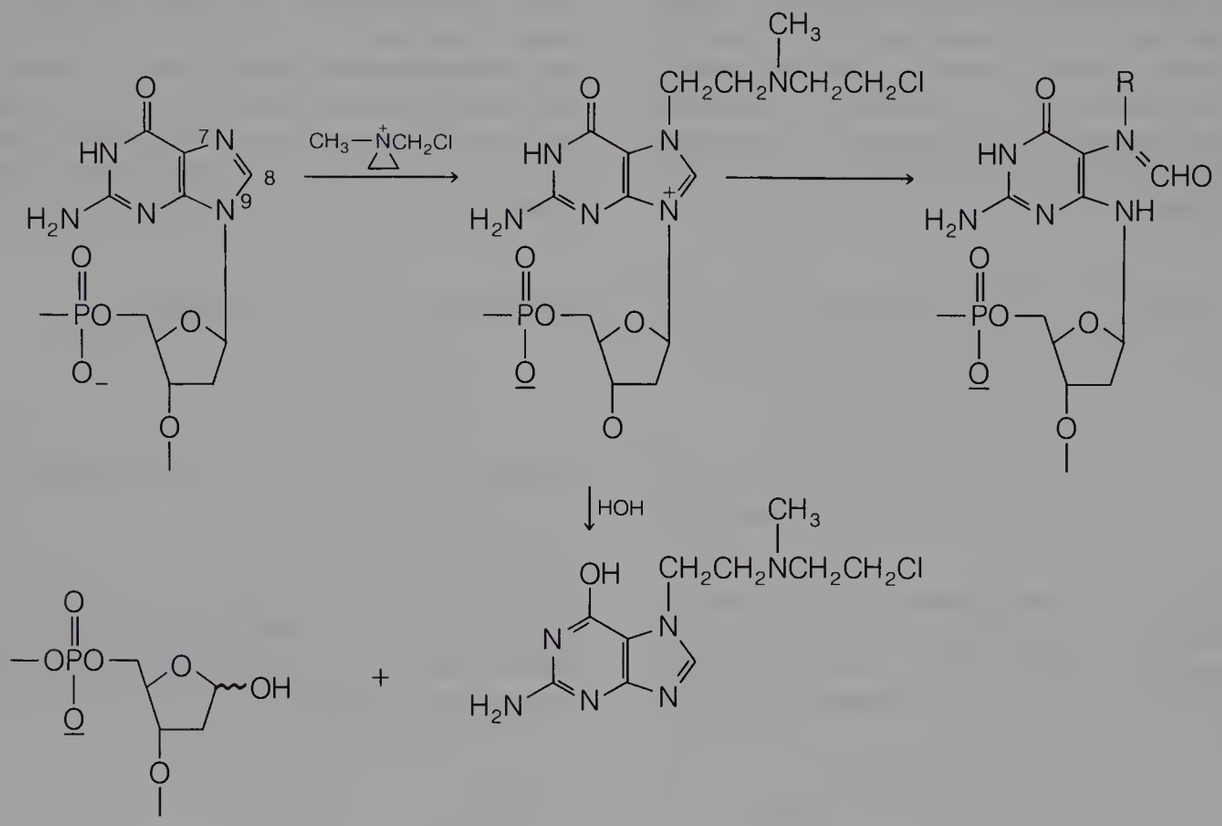
DNA is heated in water, it unwinds and the strands separate. Renaturation, in which the strands recombine in the double helix, is slow and difficult. In contrast, if the two strands are cross-linked, they cannot separate. Hence, they renaturate rapidly on cooling. Interstrand cross-linking occurs with mechlorethamine and other "two-armed" mustards, but busulfan appears to give intrastrand links according to this test.[38]

In DNA, the 7-position (nitrogen) of guanine is especially susceptible to alkylation by mechlorethamine and other nitrogen mustards (Scheme 5).[39] The alkylated structure has a positive charge in its imidazole ring, which renders the guanine-ribose linkage susceptible to cleavage. This cleavage results in the deletion of guanine, and the resulting "apurinic acid" ribose-phosphate link is readily hydrolyzable. Alkylation of the imidazole ring also activates it to cleavage of the 8,9-bond.[16]



Other base positions of DNA attacked by alkylating agents are N-2 and N-3 of guanine, N-3, N-1, and N-7 of adenine, 0-6 of thymine, and N-3 of cytosine. The importance of these minor alkylation reactions is difficult to assess. The phosphate oxygens of DNA are alkylated to an appreciable extent, but the significance of this feature is unknown.[42]

Guanine is also implicated in the cross-linking of double helical DNA. Di(guanin-7-yl) derivatives have been identified among the products of reaction with mechlorethamine.[43] Busulfan alkylation has given 1′,4′-di(guanin-7-yl)-butane, but this product is considered to have resulted from intrastrand linking.[38] Enzymatic hydrolysis of DNA cross-linked by mitomycin C has given fragments in which the antibiotic is covalently bound to the 2-amino groups of two guanosine residues, presumably from opposite strands of the double helix.[40]



Scheme 5. *Alkylation of guanine in DNA.*

Other consequences of the positively charged purine structure are facile exchange of the 8-hydrogen, which can be used as a probe for 7-alkylation,[40] and a shift to the enolized pyrimidine ring as the preferred tautomer. The latter effect has been cited as a possible basis for abnormal base pairing in DNA replication, but this has not been substantiated. One example in which alkylation of guanine does lead to abnormal base pairing is the 0-6-ethylation produced by ethyl methanesulfonate. This ethyl derivative pairs with thymine, whereas guanine normally pairs with cytosine.[41]

Alkylating agents also interact with enzymes and other

proteins. Thus, the repair enzyme, DNA nucleotidyltransferase of L1210 leukemia cells, was inhibited strongly by carmustine (BCNU), lomustine (CCNU), and 2-chloroethyl isocyanate. Because 1-(2-chloroethyl)-1-nitrosourea was a poor inhibitor of this enzyme, it was concluded that the main interaction with the enzyme was carbamoylation by the alkyl isocyanates generated in the decomposition of carmustine and lomustine.[44]

Alkylating agents can damage tissues with low mitotic indices, but they are most cytotoxic to rapidly proliferating tissues that have large proportions of cells in cycle. Nucleic acids are especially susceptible to alkylation when their structures are changed or unpaired in the process of replication. Thus, alkylating agents are most effective in the late G_1 or S phases. Alkylation may occur to some degree at any stage in the cell cycle, but the resulting toxicity is usually expressed when cells enter the S phase (Fig. 12-1). Progression through the cycle is blocked at G_2, the premitotic phase, and cell division fails.[45]

If cells can repair damage to their DNA before the next cell division, the effects of alkylation will not be lethal. Cells have developed a complex mechanism to accomplish this repair. Initially, a recognition enzyme discovers an abnormal region in the DNA. This recognition brings about the operation of an endonuclease, which makes a single-strand breakage in the DNA. An exonuclease then removes a small segment of DNA containing the damaged bases. Finally, the DNA is restored to its original structure by replacing the bases and rejoining the strand.[46] It is evident that tumor cells with efficient repair mechanisms will be relatively resistant to alkylating agents. Tumor cells outside the cell cycle in the resting phase (G_0) will have a rather long time to repair their DNA. Thus, slow-growing tumors should not respond well to alkylating agents. This limitation is observed clinically.

PRODUCTS

Mechlorethamine Hydrochloride, USP. Mustargen, nitrogen mustard, HN_2, NSC-762, 2,2-dichloro-N-methyldiethylamine hydrochloride. This compound is prepared by treating 2,2′-(methylimino)diethanol with thionyl chloride.[47] It occurs as hygroscopic leaflets that are very soluble in water. The dry crystals are stable at temperatures up to 40°C. They are very irritating to mucous membranes and harmful to eyes. The compound is supplied in rubber-stoppered vials containing a mixture of 10 mg of mechlorethamine hydrochloride and 90 mg of sodium chloride. It is diluted with 10 ml of sterile water immediately prior to injection into a rapidly flowing intravenous infusion. Intracavity injections are sometimes given to control malignant effusions.

The aziridinium ion formed from mechlorethamine in body fluids is highly reactive. It acts on various cellular components within minutes of administration. Less than 0.01% is recovered unchanged in the urine, but >50% is excreted in urine as inactive metabolites in the first 24 hr.

Mechlorethamine is effective in Hodgkin's disease. Current practice is to give it in combination with other agents. The combination with vincristine (Oncovin), procarbazine, and prednisone, known as the MOPP regimen, was considered the treatment of choice. Other lymphomas and mycosis fungoides can be treated with mechlorethamine. The most serious toxic reaction is bone marrow depression, which results in leukopenia and thrombocytopenia. Emesis is prevalent and lasts ~8 hr. Nausea and anorexia persist longer. These gastrointestinal effects may be prevented by administering the antiemetic ondansetron. Inadvertent extravasation produces intense local reactions at the site of injection. If it occurs, the immediate application of sodium thiosulfate solution can protect the tissues, because thiosulfate ion reacts very rapidly with the aziridinium ion formed from mechlorethamine.

Cyclophosphamide, USP. Cytoxan, NSC-26271, N,N-bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine-2-oxide. It is prepared by treating bis(2-chloroethyl)-phosphoramide dichloride with propanolamine.[48] The monohydrate is a low-melting solid that is very soluble in water. It is supplied as 25- and 50-mg white tablets, as 50-mg unit-dose cartons, and as a powder (100, 200, or 500 mg) in sterile vials. For reconstitution, Sterile Water for Injection, USP is added 5 ml/100 mg.

The oral dose of cyclophosphamide is 90% bioavailable, with an 8% first-pass loss. It must be metabolized by liver microsomes to become active. Among the metabolites, phosphoramide mustard has antitumor activity, and acrolein is toxic to the urinary bladder. The acrolein toxicity can be decreased by intravenous or oral administration of the sodium salt of 2-mercaptoethanesulfonic acid (mesna), whose sulfhydryl group gives conjugate addition to the double bond of acrolein.[49] In the plasma, mesna forms a disulfide that is converted selectively to the active sulfhydryl in renal tubules.

Cyclophosphamide has advantages over other alkylating agents in that it is active orally and parenterally and can be given in fractionated doses over prolonged periods of time. It is active against multiple myeloma, chronic lymphocytic leukemia, and acute leukemia of children. In combination with other chemotherapeutic agents, it has given complete remissions and even cures in Burkitt's lymphoma and acute lymphoblastic leukemia in children.[50] The most frequently encountered toxic effects are alopecia, nausea, and vomiting. Leukopenia occurs, but thrombocytopenia is less frequent than with other alkylating agents. Sterile hemorrhagic cystitis may result and even be fatal. Gonadal suppression has been reported in a number of patients.

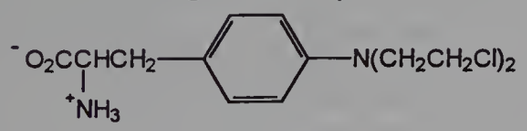
Ifosfamide. IFEX, Holoxan, NSC-109724, 3-(2-Chloroethyl)-2-[(2-chloroethyl)amino]-tetrahydro-2*H*-1,3,2-oxazaphosphorine-2-oxide, isophosphamide. This compound is prepared from 3-[(2-chloroethyl)amino]propanol by treatment with phosphorus oxychloride followed by 2-chloroethylamine.[51] It is supplied in 1- and 3-g vials as an off-white lyophilized powder. The intact vials may be stored at room temperature or under refrigeration for prolonged times. At temperatures above 35°C, it liquifies and decomposition is more rapid.

Ifosfamide usually is administered in a short infusion in 5% dextrose or normal saline. Use within 8 hr of reconstitution is recommended. Pharmacokinetic studies indicate that it is handled in the same way as cyclophosphamide, except that metabolism is less extensive. There is an apparent half-life of 7 hr and a urinary recovery of 73%.[52]

The FDA-approved indication for ifosfamide is in combination therapy of germ cell testicular cancer.[53] Combination salvage regimens are effective against soft tissue sarcoma, ovarian and breast carcinomas, and leukemia. Its limiting toxicity is in the urinary tract, especially hemorrhagic cystitis, which results from the excretion of alkylating metabolites in the urinary bladder.[54] Vigorous hydration and/or administration of mesna are needed to prevent bladder damage. Other toxicities include nausea and vomiting, alopecia, and CNS effects.

Melphalan, USP. Alkeran, L-sarcolysin, L-mustard, NSC-8808, 4-[bis(2-chloroethyl)amino]-L-phenylalanine. This compound is prepared by treating L-N-phthalimido-p-aminophenylalanine ethyl ester with ethylene oxide, followed by phosphorus oxychloride, and finally hydrolysis with hydrochloric acid.[55] Scored 2-mg tablets are available for oral administration. Oral absorption is erratic and incomplete, with absolute bioavailability ranging from 25% to 89%. A preparation kit is provided for parenteral formulation. It contains 100 mg of melphalan, which is dissolved in 1 ml of acid-alcohol solution, and then combined with final diluent containing 108 mg of dipotassium phosphate, 5.4 ml of propylene glycol and Sterile Water for Injection, USP to give 9 ml of solution. This preparation should be used promptly.

There is no significant first-pass effect with melphalan, but the drug is gradually inactivated by nonenzymatic hydrolysis to monohydroxy and dihydroxy derivatives.[56] Elimination is biphasic, with half-lives of 6 to 8 min and 40 to 60 min. Most of the drug is cleared by nonrenal mechanisms.



Melphalan

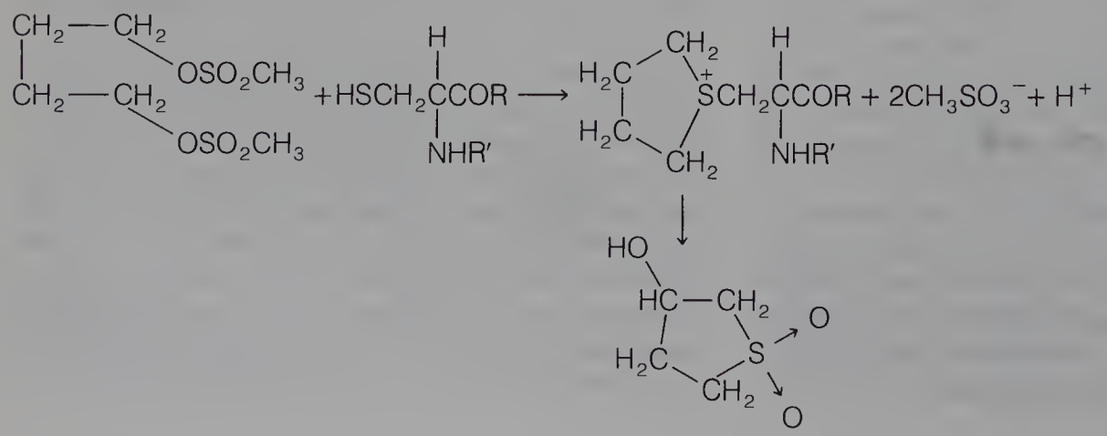
Melphalan is active against multiple myeloma. It also is active against breast, testicular, and ovarian carcinoma.[57] The clinical toxicity is mainly hematological, which means that the blood count must be carefully followed. Nausea and vomiting are infrequent, but alopecia does not occur.

Chlorambucil, USP. Leukeran, chloraminophene, NSC-3088, p-(di-2-chlorethyl)-aminophenylbutyric acid. It is prepared by treating p-aminophenyl butyric acid with ethylene oxide, followed by thionyl chloride.[58] Chlorambucil is soluble in ether and aqueous alkali. Its oral absorption is efficient and reliable. Sugar-coated 2-mg tablets are supplied.

Chlorambucil acts most slowly and is the least toxic of any nitrogen mustard derivative in use. It is indicated especially in treatment of chronic lymphocytic leukemia (CLL) and primary macroglobulinemia. Other indications are lymphosarcoma and Hodgkin's disease.[59] Many patients develop progressive, but reversible, lymphopenia during treatment. Most patients also develop a dose-related and rapidly reversible neutropenia. For those reasons, weekly blood counts are made to determine the total and differential leukocyte levels. The hemoglobin levels are also determined to monitor both toxicity (low counts) and efficacy in CLL (raised counts).

Busulfan, USP. Myleran, NSC-750, 1,4-di(methanesulfonyloxy)butane. This compound is synthesized by treating 1,4-butanediol with methanesulfonyl chloride in the presence of pyridine.[60] It is obtained as crystals that are soluble in acetone and alcohol. Although practically insoluble in water, it dissolves slowly on hydrolysis. However, it is stable in dry form. It is supplied as scored 2-mg tablets.

Busulfan is well absorbed orally and metabolized rapidly. Much of the drug undergoes a process known as "sulfur stripping" in which its interaction with thiol compounds such as glutathione or cysteine results in loss of two equivalents of methanesulfonic acid and formation of a cyclic sulfonium intermediate involving the sulfur atom of the thiol.[61] Such sulfonium intermediates are stable in vitro, but in vivo they are readily converted into the metabolite 3-hydroxy thiolane-1,1-dioxide.[62] That the sulfur atom of this thiolane does not come from a methanesulfonyl group was shown by the nearly quantitative isolation of labeled methanesulfonic acid in the urine when busulfan ^{35}S is administered to animals.[63]



Oral doses of busulfan are generally well tolerated. The absorption has zero-order kinetics, with a mean log time of 36 min and a 2-hr duration to the end of absorption.[64] Mean plasma concentration × time values are dose dependent, with peak levels of 24 to 130 ng/ml for 2 to 6 mg doses. The half-life is 2.1 to 2.6 hr.

The main therapeutic use of busulfan is in chronic granulocytic leukemia. Remissions in 85% to 90% of patients are observed after the first course of therapy. However, it is not curative. It is used in preparative regimens (bone marrow ablative) for bone marrow transplantation in patients with various leukemias. Toxic effects are mostly limited to myelosuppression in which the depletion of thrombocytes may lead to hemorrhage. Blood counts should be taken not less than weekly. The rapid destruction of granulocytes can cause hyperuricemia, which might result in kidney damage. This complication is prevented by using allopurinol, a xanthine oxidase inhibitor.[65]

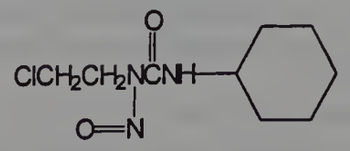
Carmustine. BiCNU, BCNU, NSC-409,962, 1,3-bis(2-chloroethyl)-1-nitrosourea. This compound is synthesized by treating 1,3-bis(2-chloroethyl)urea with sodium nitrite and formic acid.[66] It is a low-melting white powder that changes to an oily liquid at 27°C. This change is considered a sign of decomposition, and such samples should be discarded. Carmustine is most stable in petroleum ether or water at pH 4. It is administered intravenously because metabolism is very rapid. However, some of the degradation products have prolonged half-lives in plasma. Carmustine is supplied as 100-mg quantities of lyophilized powder. When it is diluted with 3 ml of the supplied sterile diluent, ethanol, and further diluted with 27 ml of sterile water, a 10% ethanolic solution containing 3.3 mg/ml is obtained.

Biotransformation of carmustine is rapid and extensive, with most of a dose recovered in urine as metabolites. The half-life has an α phase of 6.1 min and a β phase of 21.5 min.[67]

Because of its ability to cross the blood-brain barrier, carmustine is used against brain tumors and other tumors, such as leukemias, that have metastasized to the brain.[68] It also is used as secondary therapy in combination with other agents for Hodgkin's disease and other lymphomas. Multiple myeloma responds to a combination of carmustine and prednisone. Delayed myelosuppression is the most frequent and serious toxicity. This condition usually occurs 4 to 6 weeks after treatment. Thrombocytopenia is the most pronounced effect, followed by leukopenia. Nausea and vomiting frequently occur ~2 hr after treatment.

Carmustine is given as a single dose by intravenous injection at 100 to 200 mg/m^2. A repeat course is not given until the blood elements return to normal levels. This process requires ~6 weeks.

Lomustine. CeeNU, CCNU, NSC-79037, [1-(2-chloroethyl)]-3-cyclohexyl-1-nitrosourea. This compound is synthesized by treating ethyl 5-(2-chloroethyl)-3-nitrosohydantoate with cyclohexylamine, followed by renitrosation of the resulting intermediate, [1-(2-chloroethyl)]-3-cyclohexylurea.[69] It is sufficiently stable to metabolism to be administered orally. The high lipid solubility of lomustine allows it to cross the blood-brain barrier rapidly. Levels in the CSF are 50% higher than those in plasma. Lomustine is supplied in dose packs that contain two each of color-coded 100-, 40-, and 10-mg capsules. The total dose prescribed is obtained by appropriate combination of these capsules.



Lomustine

Oral absorption of lomustine is nearly complete within 30 min. It is rapidly converted into *cis*- and *trans*-4-OH metabolites by liver microsomes. The half-life of the parent drug is 1.3 to 2.9 hr, and the peak concentration of metabolites is reached 2 to 4 hr after dosing.

Lomustine is used against both primary and metastatic brain tumors and as secondary therapy in relapsed Hodgkin's disease. The most common adverse reactions are nausea and vomiting, thrombocytopenia, and leukopenia. As in the case of carmustine, the myelosuppression caused by lomustine is delayed.[70]

The recommended dosage of lomustine is 130 mg/m^2 orally every 6 weeks. A reduced dose is given to patients with compromised bone marrow function.

Thiotepa, USP TSPA, NSC-6396, N,N′,N″-triethylenethiophosphoramide, tris(1-aziridinyl)phosphine sulfide. This compound is prepared by treating trichlorophosphine sulfide with aziridine[71] and is obtained as a white powder that is soluble in water. It is supplied in vials containing 15 mg of thiotepa, 80 mg of sodium chloride, and 50 mg of sodium bicarbonate. Sterile water is added to make an isotonic solution. Both the vials and solutions must be stored at 2°C to 8°C. The solutions may be stored 5 days without loss of potency.

Thiotepa blood levels decline in a rapid biphasic manner. It is converted into TEPA by oxidative desulfurization, and TEPA levels exceed those of thiotepa 2 hr after administration. Aziridine metabolism also occurs with liberation of ethanolamine.

Thiotepa has been tried against a wide variety of tumors, and it has given palliation in many types, although with varying frequency. The most consistent results have been obtained in breast, ovarian, and bronchogenic carcinomas and malignant lymphomas. It is a mainstay of high-dose regimens in treating solid tumors when followed by autologous bone marrow transplantation. It also is used to control intracavity effusions resulting from neoplasms. Thiotepa is highly toxic to bone marrow, and blood counts must be made during therapy.

Procarbazine Hydrochloride, USP. Matulane, MIH, NSC-77213, N-isopropyl-α-(2-methylhydrazine)-p-toluamide. It is prepared from N-isopropyl-p-toluamide in a process involving condensation with diethyl azodicarboxylate,

methylation with methyl iodide and base, and acid hydrolysis.[72] Although soluble in water, it is unstable in solution. Capsules containing the equivalent of 50 mg of procarbazine as its hydrochloride are supplied.

Procarbazine is rapidly and completely absorbed following oral administration. It readily decomposes by chemical and metabolic routes, with a half-life of 7 to 10 min, to produce highly reactive species including methyl diazonium ion, methyl radicals, hydrogen peroxide, formaldehyde, and hydroxyl radicals.[73]

Procarbazine has demonstrated activity against Hodgkin's disease. For this condition, it is used in combination with other agents such as mechlorethamine, vincristine, and predinisone (MOPP program). Toxic effects such as leukopenia, thrombocytopenia, nausea, and vomiting occur in a majority of patients. Neurological and dermatological effects also occur. Concurrent intake of alcohol, certain amine drugs, and foods containing high tyramine levels is contraindicated. The weak monoamine oxidase-inhibiting properties of procarbazine may potentiate catechol amines to produce hypertension.

Dacarbazine. DTIC-Dome, DIC, DTIC, NSC-45388, 5-(3,3-dimethyl-1-triazenyl)-1H-imidazole-4-carboxamide. This compound is prepared by treating the diazonium salt, prepared from 5-aminoimidazole-4-carboxamide, with dimethylamine in methanol.[74] It is obtained as a colorless to ivory-colored solid that is very sensitive to light. It does not melt but decomposes explosively when heated above 250°C. Water solubility is good, but solutions must be protected from light. Dacarbazine is supplied in vials containing either 100 or 200 mg. When reconstituted with 9.9 and 19.7 ml, respectively, of sterile water, these samples give solutions containing 10 mg/ml at pH 3.0 to 4.0. Such solutions may be stored at 4°C for 72 hr.

Injected dacarbazine disappears rapidly from plasma because of hepatic metabolism. The half-life is ~40 min. Excretion is by the renal tubules, and in the 6-hr excretion fraction, 50% of the drug is intact and 50% is the N-demethylated metabolite.[75]

Dacarbazine is indicated for the treatment of metastatic malignant melanoma.[76,77] Combination with other antineoplastic drugs is superior to its use as a single agent. Anorexia, nausea, and vomiting are the most frequent toxic reactions. However, leukopenia and thrombocytopenia are the most serious effects.[78] Blood counts should be made and therapy temporarily suspended if the counts are too low. Dacarbazine is also used in combination therapy for Hodgkin's disease.

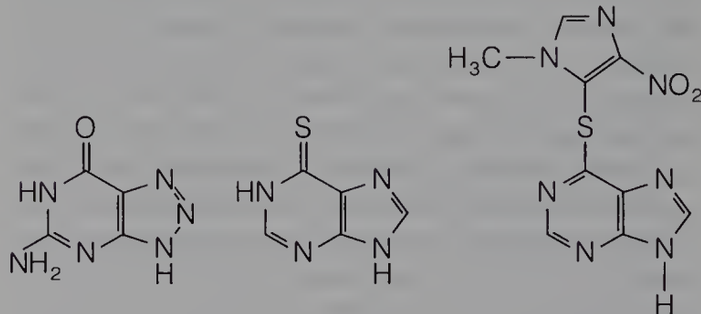
The recommended daily dosage is 2 to 4.5 mg/kg for 10 days with repetition at 4-week intervals. Extravasation of the drug during injection may result in severe pain.

ANTIMETABOLITES

Antimetabolites are compounds that prevent the biosynthesis or utilization of normal cellular metabolites. They usually are closely related in structure to the metabolite that is antagonized. Many antimetabolites are enzyme inhibitors. In this capacity, they may combine with the active site as if they are the substrate or cofactor.[79] Alternatively, they may bind to an allosteric regulatory site, especially when they resemble the end product of a biosynthetic pathway under feedback control.[16] Sometimes, the antimetabolite must be transformed biosynthetically (anabolized) into the active inhibitor. For example, 6-mercaptopurine is converted into the corresponding ribonucleotide, which is a potent inhibitor of the conversion of 5-phosphoribosyl pyrophosphate into 5-phosphoribosylamine, a rate-controlling step in the de novo synthesis of purines[80] (Scheme 6). An antimetabolite and its transformation products may inhibit a number of different enzymes. Thus, 6-mercaptopurine and its anabolites interact with >20 enzymes. This multiplicity of effects makes it difficult to decide which ones are crucial to the antitumor activity.

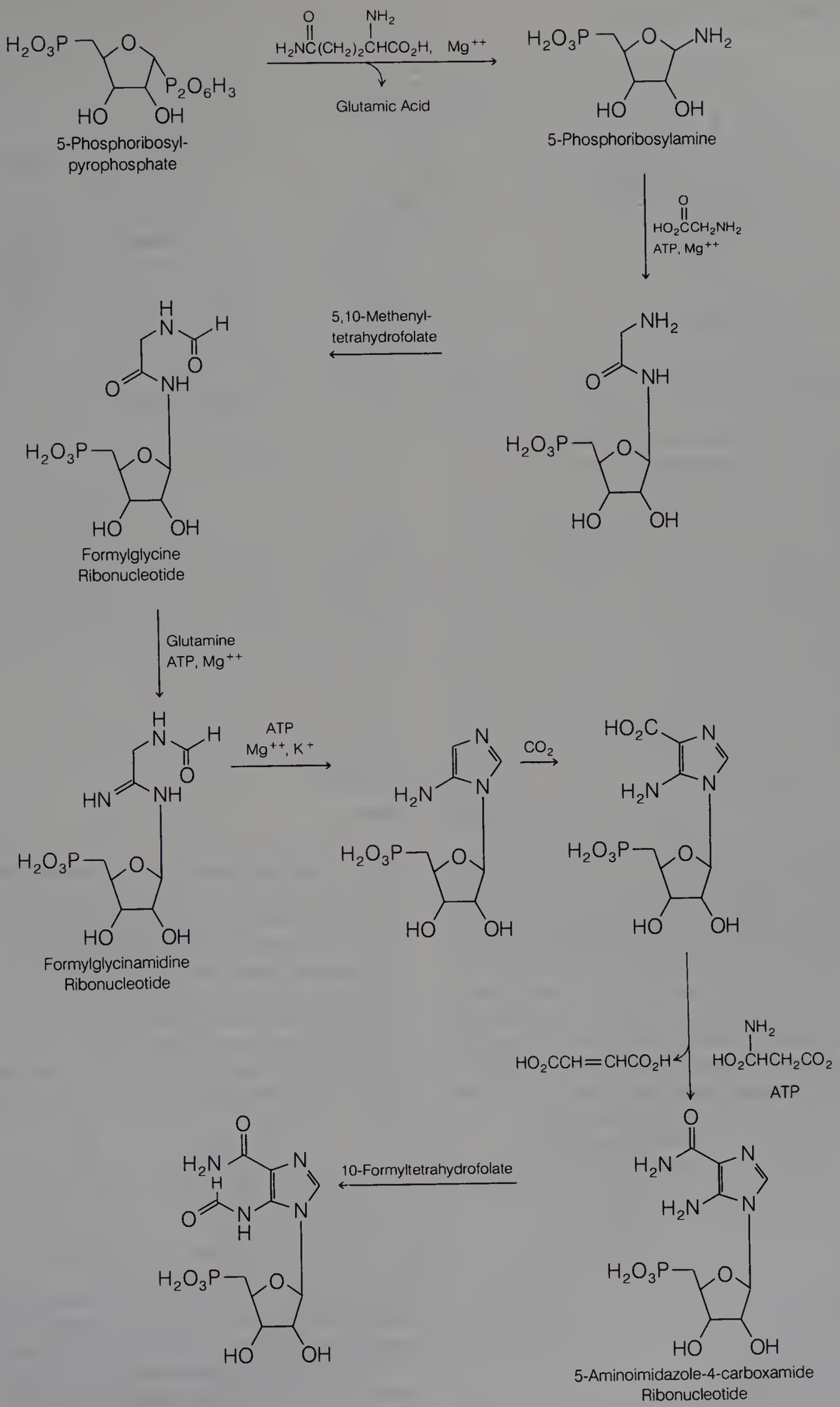
The anabolites of purines and pyrimidine antagonists may be incorporated into nucleic acids. In this event, part of their antitumor effect might result from malfunction of further macromolecular synthesis because of the abnormal nucleic acids.[81] Although antimetabolites of every type have been tested against neoplasms, nearly all of the clinically useful agents are related to metabolites and cofactors in the biosynthesis of nucleic acids.

Following the formulation of the antimetabolite theory by Woods and Fildes in 1940,[82] antimetabolites based on a variety of known nutrients were prepared. The first purine analog to show antitumor activity in mice, 8-azaguanine, was synthesized by Roblin in 1945.[83] This compound was introduced into clinical trials but was abandoned in favor of newer and more effective agents such as 6-mercaptopurine and 6-thioguanine developed by Elion and Hitchings.[84] 6-Mercaptopurine was synthesized in 1952[85] and was shown to be active against human leukemia in the following year.

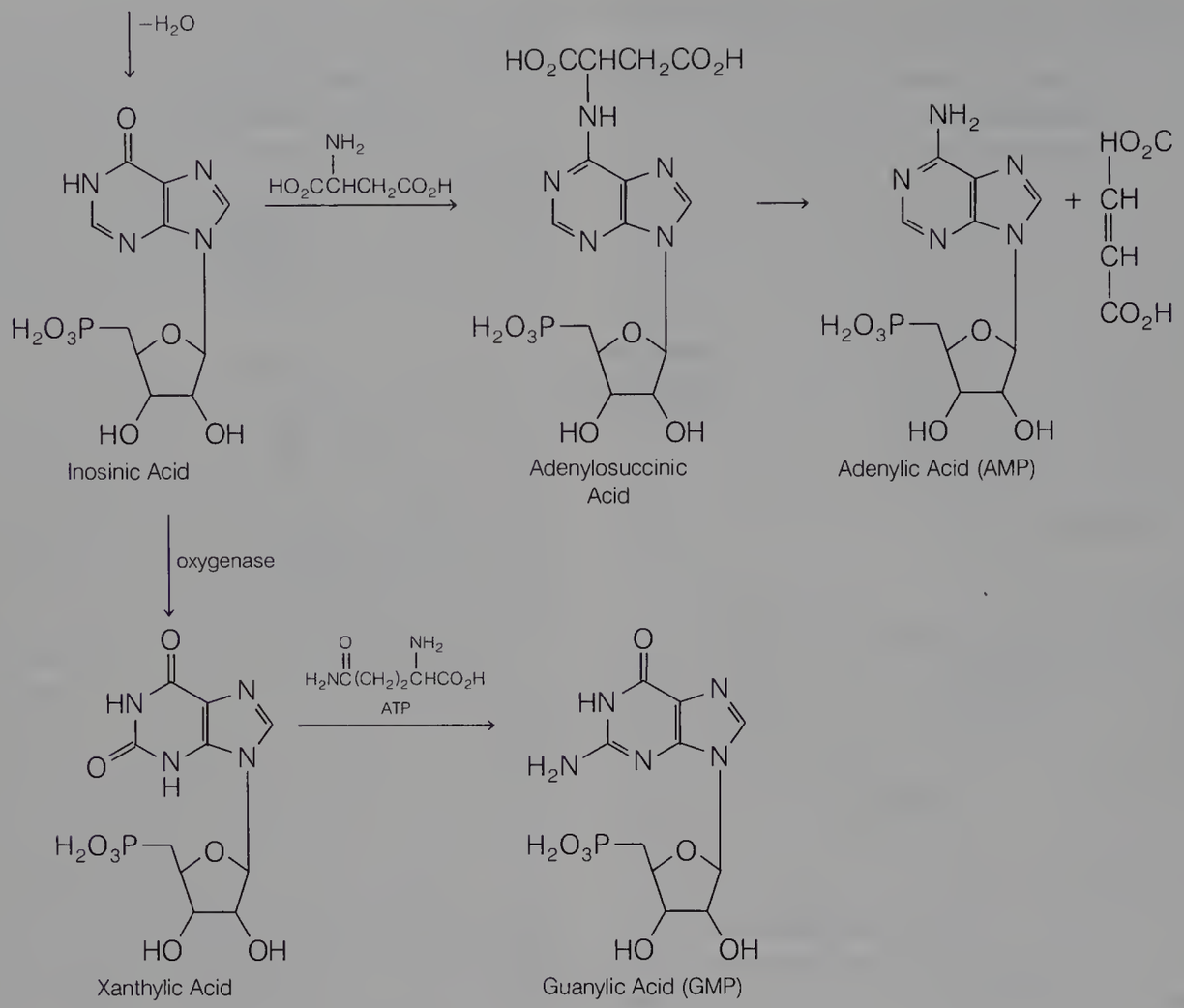


8-Azaguanine 6-Mercaptopurine Azathioprine

In order to be active against neoplasms, 6-mercaptopurine must be converted into its ribonucleotide, 6-thioinosinate, by the enzyme hypoxanthine-guanine phosphoribosyltransferase. Neoplasms that lack this enzyme are resistant to the drug.[86] 6-Thioinosinate is a potent inhibitor of the conversion of 5-phosphoribosyl pyrophosphate into 5-phosphoribosylamine, as mentioned above. It also inhibits the conversion of inosinic acid to adenylic acid at two stages: (a) the reaction of inosinic acid with aspartate to give adenylosuccinic acid, and (b) the loss of fumaric acid from adenylosuccinic acid

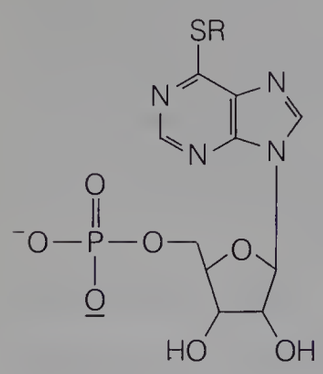


Scheme 6. *De novo synthesis of nucleotides (simplified).*



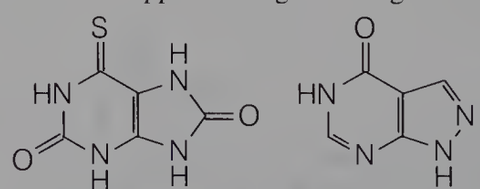
Scheme 6. *Continued.*

to give adenylic acid.[87] Furthermore, it inhibits the oxidation of inosinic acid to xanthylic acid.[88] The mode of action of 6-mercaptopurine is further complicated by the fact that its ribose diphosphate and triphosphate anabolites are also active enzyme inhibitors, and the triphosphate can be incorporated into DNA and RNA to inhibit further chain elongation.[87] Still more complex is the ability of 6-thioinosinate to act as a substrate for a methyl transferase, requiring S-adenosylmethionine, that converts it into 6-methylthioinosinate. The latter compound is responsible for certain of the antimetabolite activities of 6-mercaptopurine.[89]



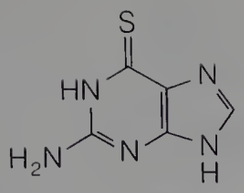
6-Thioinosinate (R = H)
6-Methylthioinosinate (R = CH_3)

Metabolic degradation (catabolism) of 6-mercaptopurine by guanase gives 6-thioxanthine, which is oxidized by xanthine oxidase to afford 6-thiouric acid.[90] Allopurinol, an inhibitor of xanthine oxidase, increases both the potency and the toxicity of 6-mercaptopurine. However, its main importance is as an adjuvant to chemotherapy because it prevents the uric acid kidney toxicity caused by the release of purines from destroyed cancer cells. Heterocyclic derivatives of 6-mercaptopurine such as azathioprine (Imuran) were designed to protect it from catabolic reactions.[91] Although azathioprine has antitumor activity, it is not significantly better than 6-mercaptopurine. However, it has found an important role as an immunosuppressive agent in organ transplants.[84]



6-Thiouric Acid Allopurinol

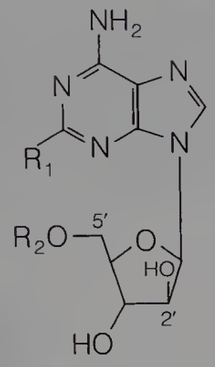
Thioguanine is converted into its ribonucleotide by the same enzyme that acts on 6-mercaptopurine. It is converted further into the di- and triphosphates.[92] These species inhibit most of the same enzymes that are inhibited by 6-mercaptopurine. Thioguanine is also incorporated into RNA, and its 2′-deoxy metabolite is incorporated into DNA. The significance of these "fraudulent" nucleic acids in lethality to neoplasms is uncertain.[93]



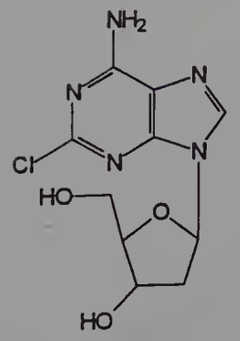
6-Thioguanine

Adenine arabinoside (Vidarabine) was first prepared by chemical synthesis[94] and later isolated from cultures of *Streptomyces antibioticus*.[95] It has a sugar, D-arabinose, that is epimeric with D-ribose at the 2′-position. This structural change makes it a competitive inhibitor of DNA polymerase.[96] In addition to its antineoplastic activity, adenosine arabinoside has potent antiviral action. Adenine arabinoside and some of its derivatives are limited in their antitumor effect by susceptibility to adenosine deaminase. This enzyme converts them into hypoxanthine arabinoside derivatives. The resistance of certain tumors correlates with their levels of adenosine deaminase.[97]

In contrast to the susceptibility of adenosine arabinoside to adenosine deaminase, its 2-fluoro derivative, named fludarabine, is stable to this enzyme. Fludarabine is prepared as the 5′-monophosphate. Fludarabine has good activity against chronic lymphocytic leukemia. It is converted into the corresponding triphosphate,[98] which inhibits ribonucleotide reductase.[99] 2-Chloro-2′-deoxyadenosine (cladribine) also is resistant to adenosine deaminase. It is phosphorylated in cells to the triphosphate by cytidine kinase and the triphosphate inhibits enzymes required for DNA repair.[100] Cladribine is highly effective against hairy cell leukemia.

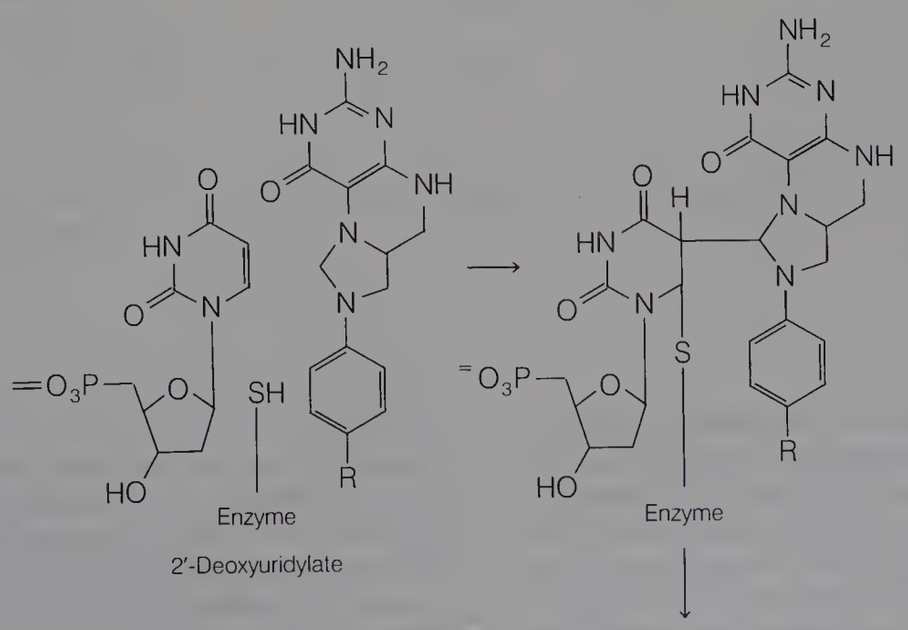


$R_1 = R_2 = H$; Vidarabine
$R_1 = F$, $R_2 = HOPO_2$; Fludarabine

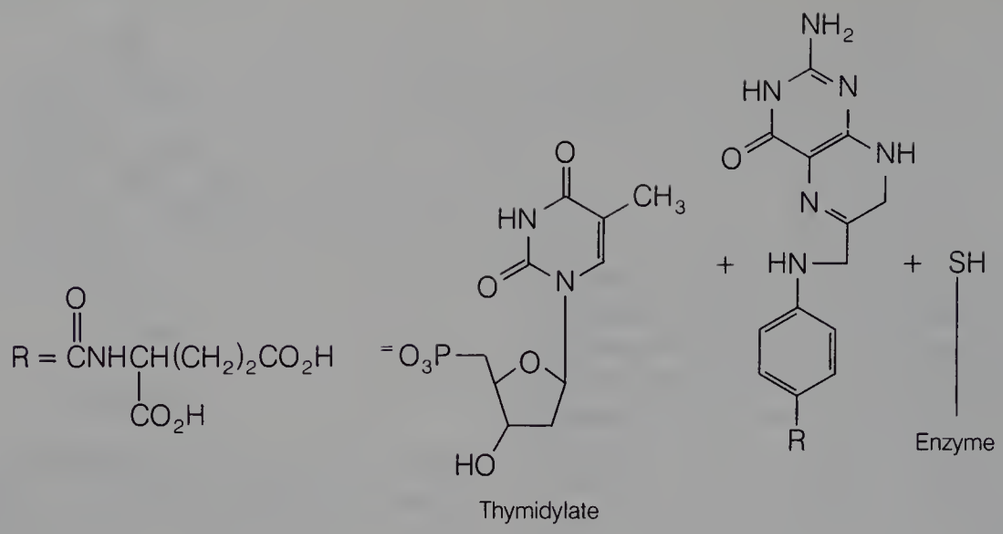


Cladribine

The invention of 5-fluorouracil as an antimetabolite of uracil by Heidelberger in 1957 provided one of our foremost examples of rational drug design.[101] Starting with the observation that in certain tumors uracil was used more than orotic acid, the major precursor for nucleic-acid pyrimidine biosynthesis in normal tissue, he decided to synthesize an antimetabolite of uracil with only one modification in the structure. The 5-position was chosen for a substituent in order to block the conversion of uridylate to thymidylate (Scheme 7), thus diminishing DNA biosynthesis. Fluorine was chosen as the substituent because the increased acidity caused by its inductive effect was expected to cause the molecule to bind strongly to enzymes. These choices were well founded, as 5-fluorouracil soon became one of the most widely used antineoplastic agents. It is a mainstay in the therapy of adenocarcinoma of the colon and rectum. Side effects are both dose and schedule dependent. They include myelosuppression on bolus administration and mucositis on prolonged infusions. Otherwise, the drug is well tolerated.



Scheme 7. *Conversion of uridylate into thymidylate.*

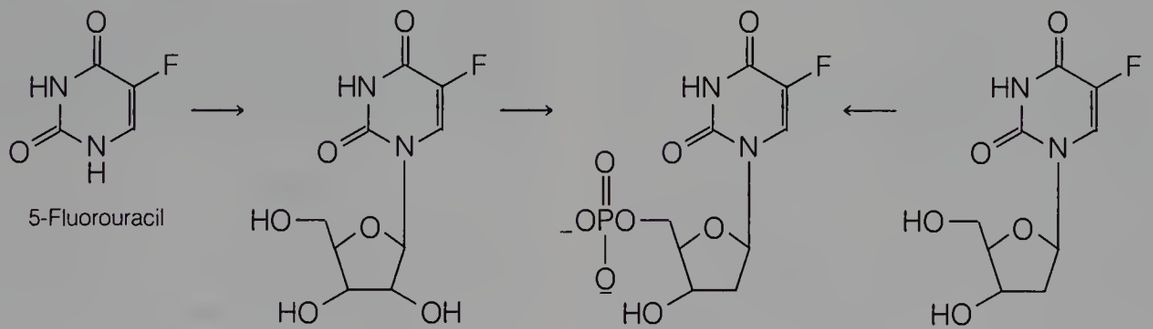


Thymidylate

5-Fluorouracil is activated by anabolism to 5-fluoro-2′-deoxyuridylic acid. This conversion may proceed by two routes. In one route, 5-fluorouracil reacts with ribose-1-phosphate to give its riboside, which is phosphorylated by uridine kinase.[102] The resulting compound, 5-fluorouridylic acid, is converted into its 2′-doxy derivative by ribonucleotide reductase. 5-Fluorouracil also may be transformed directly into 5-fluorouridylic acid by a phosphoribosyltransferase, which is present in certain tumors.[103] An alternative pharmaceutical based on 5-fluorouracil is its 2-deoxyriboside (floxuridine).[101] This compound is phosphorylated by 2′-deoxyuridine kinase.

narily, this step would be followed by the transfer of the 5-hydrogen of uracil to the methylene group, resulting in the formation of thymidylate and dihydrofolate. However, the 5-fluorine is stable to transfer, and a terminal product results involving the enzyme, cofactor, and substrate, all covalently bonded. Thus, 5-fluoro-2′-deoxyuridylic acid would be classified as a K_{cat} inhibitor.[105]

The rate-determining enzyme in 5-fluorouracil catabolism is dihydropyrimidine dehydrogenase. Inhibition of this enzyme by 5-ethynyluracil increases the plasma concentration-time curve of 5-fluorouracil to the extent that its therapeutic index is raised two- to fourfold.

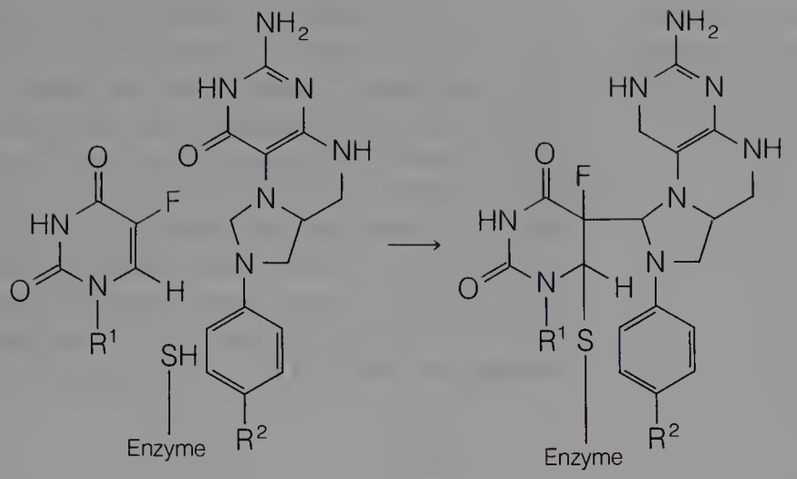


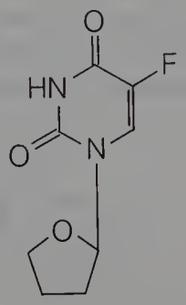
5-Fluorouracil
5-Fluorouracil Riboside
5-Fluorodeoxyuridylic Acid
5-Fluorouracil 2-Deoxyriboside

5-Fluoro-2′-deoxyuridylic acid is a powerful competitive inhibitor of thymidylate synthetase, the enzyme that converts 2′-deoxyuridylic acid to thymidylic acid. This blockade is probably the main lethal effect of 5-fluorouracil and its metabolites.[104] In the inhibiting reaction, the sulfhydryl group of a cysteine residue in the enzyme adds to the 6-position of the fluorouracil moiety. The 5-position then binds to the methylene group of 5,10-methylenetetrahydrofolate. Ordi-

The tetrahydrofuranyl derivative of 5-fluorouracil, known as Tegafur (florafur), was prepared in Russia.[106] It is active in clinical cancer and less myelosuppressive than 5-fluorouracil. However, it has gastrointestinal and CNS toxicity. Tegafur is slowly metabolized to 5-fluorouracil; thus, it may be considered a prodrug.[107]

Trifluorothymidine (Trifluridine) was designed by Heidelberger as an antimetabolite of thymine.[101] The riboside is

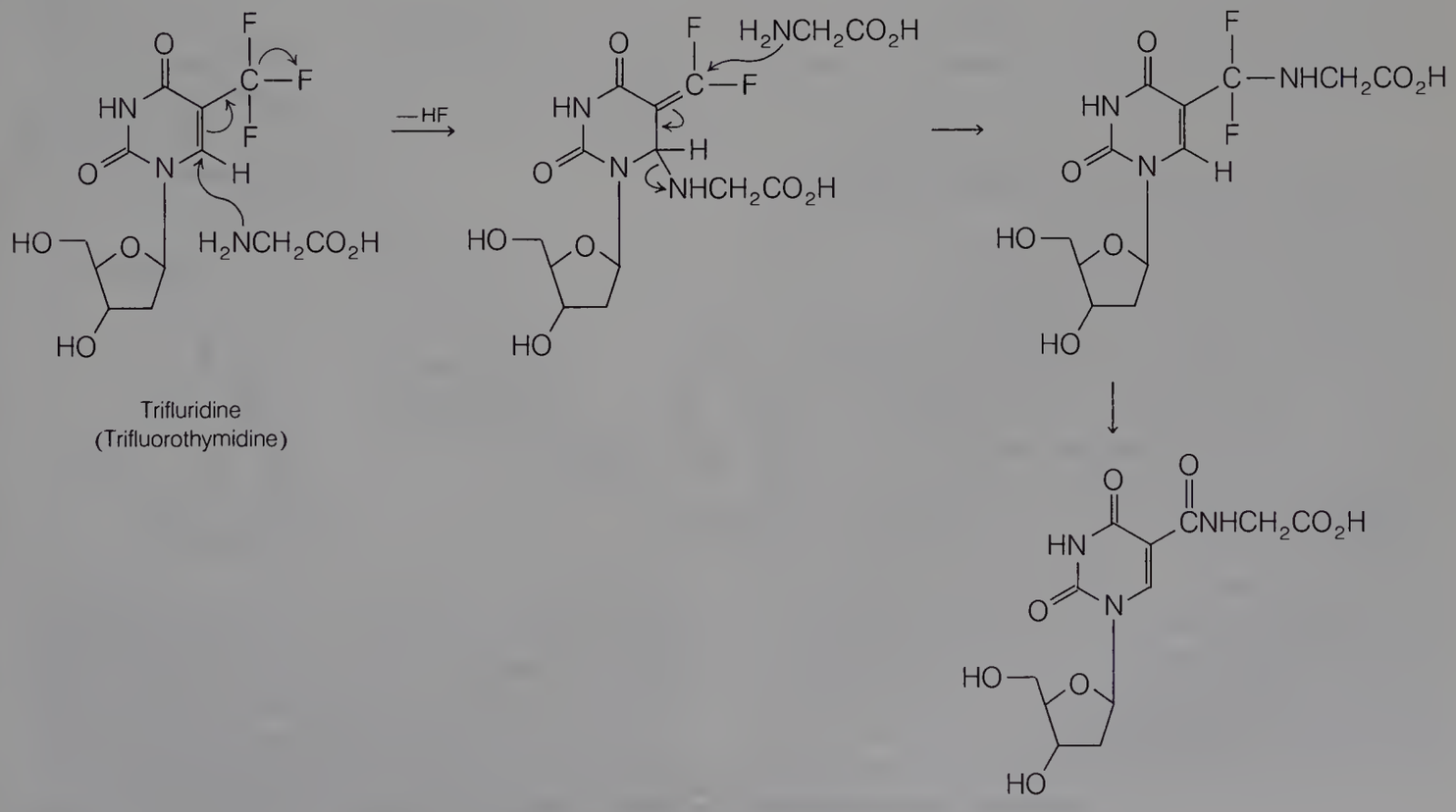




Tegafur
(Fuorafur)

essential because mammalian cells are unable to convert thymine and certain analogs into thymidine and its analogs. Thymidine kinase converts trifluorothymidine into trifluorothymidylic acid, which is a potent inhibitor of thymidylate synthetase.[101] In contrast to the stability of most trifluoromethyl groups, the one of trifluorothymidylic acid is extraordinarily labile. It reacts with glycine to give an amide at neutral pH.[108] Kinetic studies have shown that this reaction involves initial nucleophilic attack at position-6 followed by loss of HF to give the highly reactive difluoromethylene group.[109] Glycine then adds to this group and hydrolysis of the remaining two fluorine atoms follows (Scheme 8). The interaction of trifluorothymidylic acid with thymidylate synthetase apparently follows a similar course. Thus, after preincubation it becomes irreversibly bound to the enzyme, and the kinetics are noncompetitive.[101]

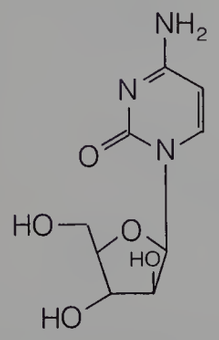
Cytosine arabinoside was synthesized in 1959[110] and later found as a fermentation product.[111] It is noteworthy structurally in that its arabinose moiety is epimeric at the 2′-position with ribose. This modification, after anabolism to the triphosphate, causes it to inhibit the conversion of cytidylic acid to 2′-deoxycytidylic acid.[112] For a number of years, this inhibition was believed to be the main mode of action of cytosine arabinoside triphosphate; however, it was shown recently that various deoxyribonucleosides were just as effective as cytosine arabinoside in reducing cellular levels of 2′-deoxycytidylic acid.[113] Other modes of action include the inhibition of DNA-dependent DNA polymerase[114] and miscoding following incorporation into DNA and RNA.[115] Cytosine arabinoside is readily transported into cells and phosphorylated by deoxycytidine kinase. It acts predominantly in the S phase of the cell cycle. Tumor-cell resistance is



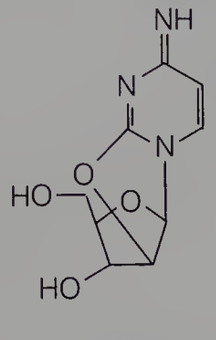
Trifluridine
(Trifluorothymidine)

Scheme 8. *Reaction of trifluorothymidine with glycine.*

based on low levels of deoxycytidine kinase and the elaboration of deaminases that convert cytosine arabinoside into uridine arabinoside.[116] Cytidine deaminase has been partially purified and found to be inhibited by tetrahydrouridine.[117]



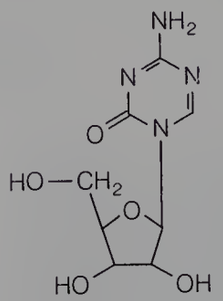
Cytarabine
(Cytosine arabinoside)



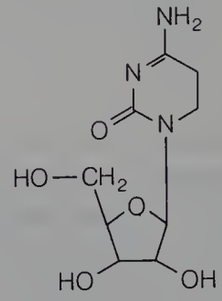
Ancitabine
(Cyclocytidine)

A new analog of cytosine arabinoside is cyclocytidine (ancitabine). This analog apparently is a prodrug that is slowly converted into cytosine arabinoside. It is reported to be resistant to deamination and to have a therapeutic index superior to that of the parent compound.[118]

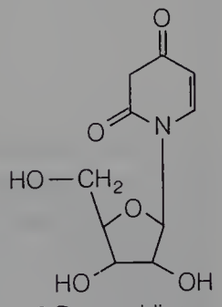
A number of pyrimidine nucleoside analogs have one more or one less nitrogen in the heterocyclic ring. They are known as azapyrimidine or deazapyrimidine nucleosides. One of these antimetabolites is 5-azacytidine. It was synthesized in 1964 by Sórm in Czechoslovakia[119] and later was isolated as an antibiotic by Hanka.[120] The mode of action of this compound is complex, involving anabolism to phosphate derivatives and deamination to 5-azauridine. In certain tumor systems, it is incorporated into nucleic acids that possibly cause misreading.[121] One of its main effects is the inhibition of orotidylate decarboxylase (Scheme 9), which prevents the new synthesis of pyrimidine nucleotides.[122] Tumor resistance is based on decreased phosphorylation of the nucleoside, decreased incorporation into nucleic acids, and increased RNA and DNA polymerase activity.[123]



Azacitidine
(5-Azacytidine)

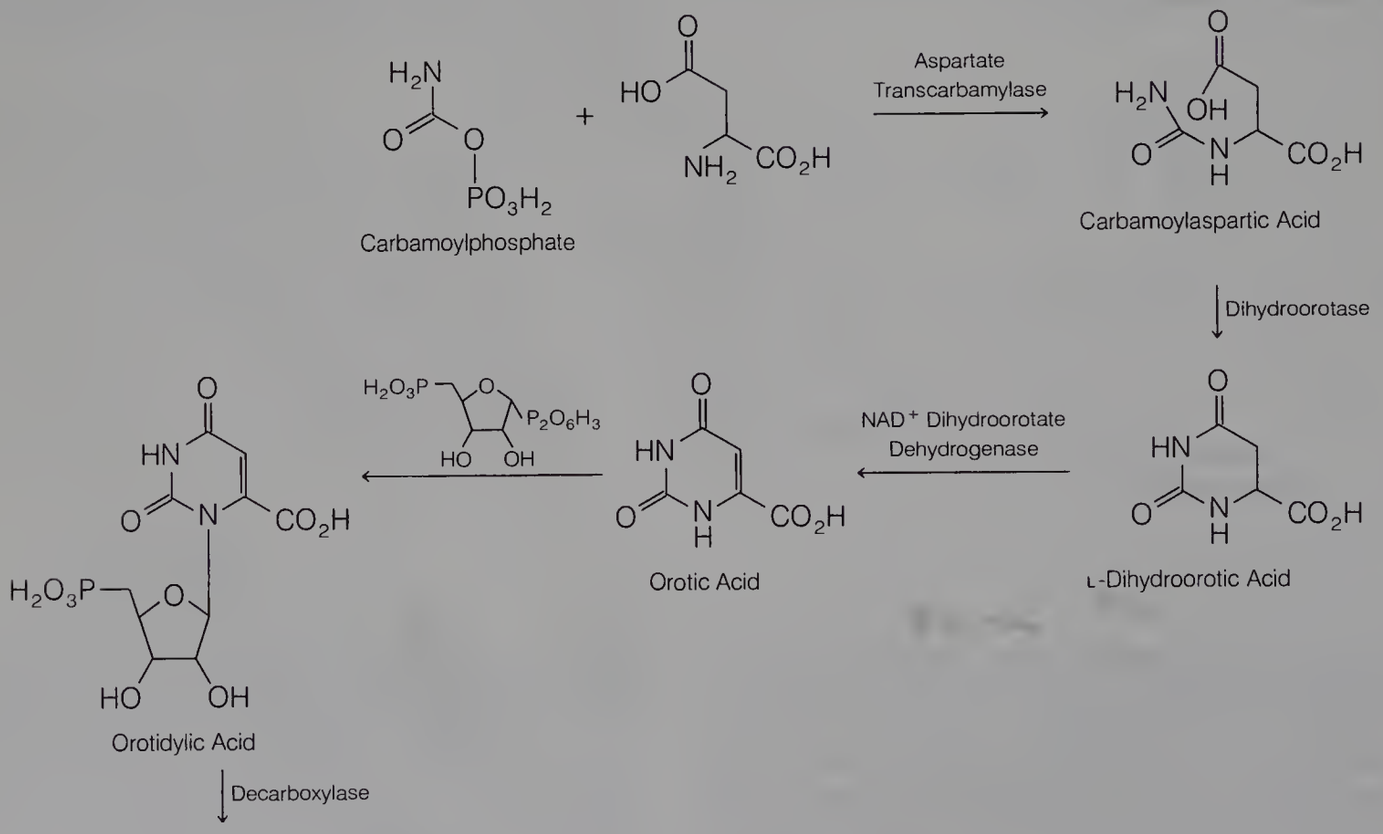


Dihydro-5-azacytidine

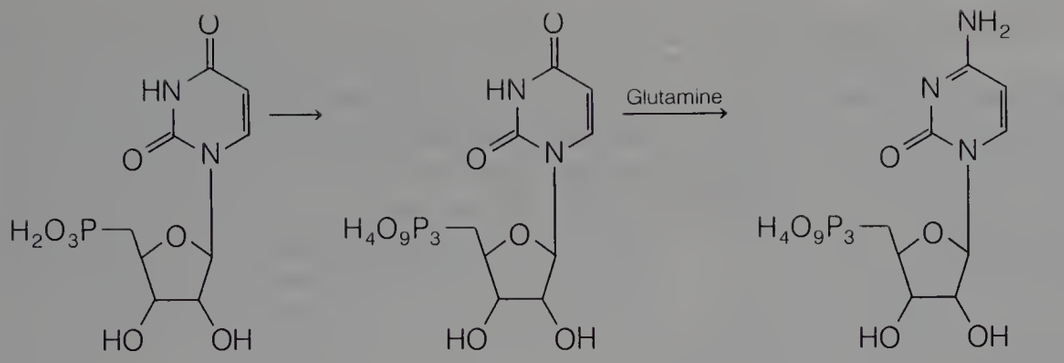


3-Deazauridine

Pyrimidine nucleoside antagonists active against experimental tumors and which have received clinical study include dihydro-5-azacytidine and 3-deazauridine.[98]

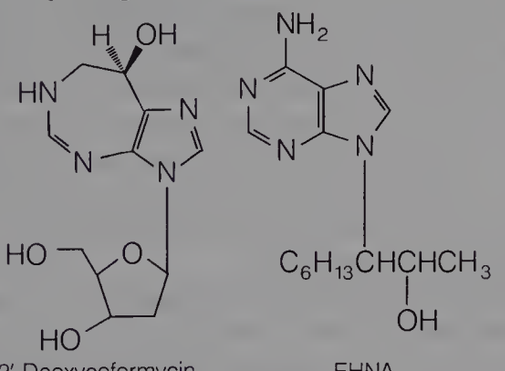


Scheme 9. *De novo synthesis of pyrimidine nucleotides (simplified).*



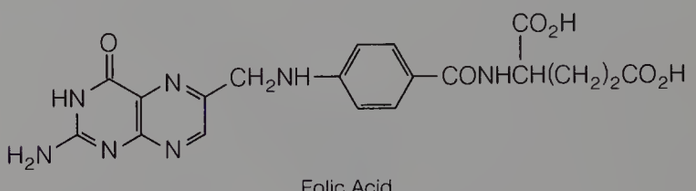
Uridylic Acid — Uridine Triphosphate — Cytidine Triphosphate

The resistance to purine and pyrimidine antimetabolites such as adenosine arabinoside and cytosine arabinoside by neoplastic cells that produce deaminases has stimulated a search for compounds that might inhibit these deaminases. In principle, a potent deaminase inhibitor would produce a synergistic effect on the antitumor activity of the antimetabolite, even though it might not be active itself. Two types of deaminase inhibitors have emerged recently. One type is the purine analog in which the pyrimidine ring has been expanded to a seven-membered ring. The first example of this type was 2′-deoxycoformycin (pentostatin), an unusual nucleoside produced in the same cultures as the antibiotic formycin.[124] It strongly synergized the action of formycin against organisms that produce deaminases. In clinical trials it showed a synergistic effect on the activities of adenine arabinoside and cytosine arabinoside. A second type of adenosine deaminase inhibitor has the adenine portion unchanged, but is modified in the ribose moiety. Such modifications have been designed to probe the active site of the enzyme and take advantage of strong binding to adjacent lipophilic regions.[125] The compound EHNA is an example of a rationally designed inhibitor.

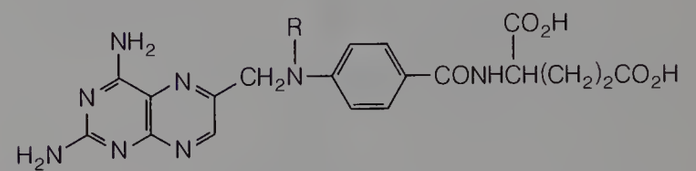


2′-Deoxycoformycin — EHNA

Following the discovery of folic acid, a number of analogs based on its structure were synthesized and tested as antimetabolites. The N^{10}-methyl derivative of folic acid was found to be an antagonist, but it had no antitumor activity. Antitumor activity finally was found for the 4-amino-4-deoxy derivative, aminopterin, and its N^{10}-methyl homolog, methotrexate (amethopterin).[126]



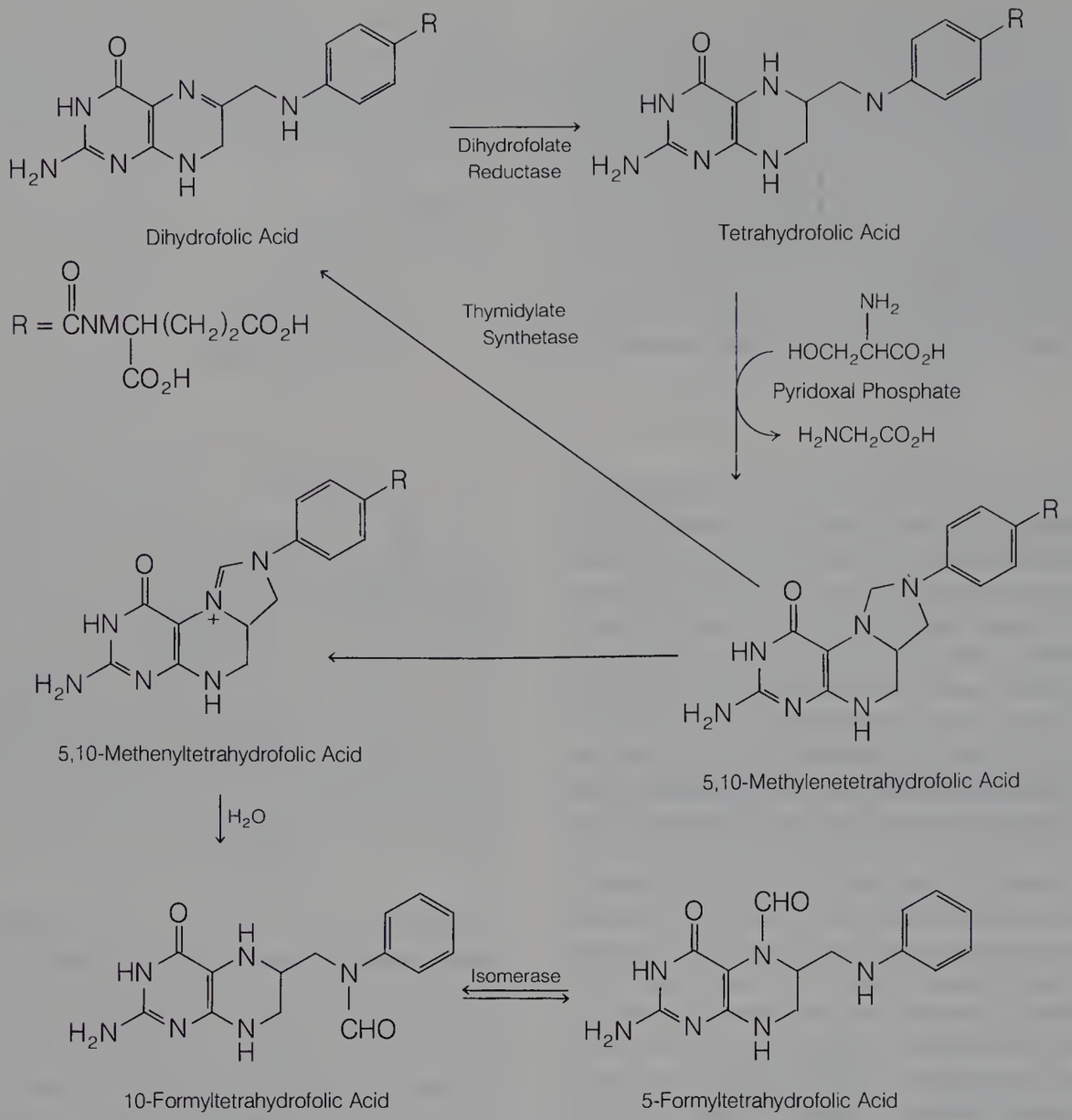
Folic Acid



Aminopterin, R = H
Methotrexate, R = CH_3

Methotrexate and related compounds inhibit the enzyme dihydrofolate reductase. They bind so tightly to it that their inhibition has been termed "pseudoirreversible." The basis of this binding strength is in the diaminopyrimidine ring, which is protonated at physiological pH. At pH 6, methotrexate binds stoichiometrically with dihydrofolate reductase (K_I 10^{-10} M), but at higher pH the binding is weaker and competitive with the substrate.[127]

Folate acid antagonists kill cells by inhibiting DNA synthesis in the S phase of the cell cycle. Thus, they are most effective in the log growth phase.[128] Their effect on DNA synthesis results partially from the inhibition of dihydrofolate reductase, which depletes the pool of tetrahydrofolic acid. Folic acid is reduced stepwise to dihydrofolic acid and tetrahydrofolic acid, with dihydrofolic reductase thought to catalyze both steps.[129] As shown in Scheme 10, tetrahydrofolic acid accepts the β-carbon atom of serine, in a reaction requiring pyridoxal phosphate, to give N^5,N^{10}-methylenetetrahydrofolic acid. The last compound transfers a methyl group to 2′-deoxyuridylate to give thymidylate in a reaction catalyzed by thymidylate synthetase. Dihydrofolic acid is generated in this reaction and it must be reduced back to tetrahydrofolic acid before another molecule of thymidylate can be synthesized. It is partly by their effect in limiting thymidylate synthesis that folic acid analogs prevent DNA synthesis and kill cells. This effect has been termed the "thymineless death."[130]



Scheme 10. *Interconversions of folic acid derivatives.*

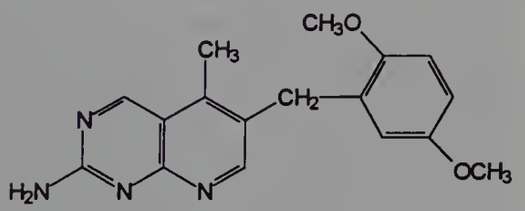
The inhibition of dihydrofolate reductase produces other limitations on nucleic acid biosynthesis. Thus, N^5,N^{10}-methylenetetrahydrofolic acid is oxidized to the corresponding methenyl derivative, which gives N^{10}-formyltetrahydrofolic acid on hydrolysis (Scheme 11). The latter compound is a formyl donor to 5-aminoimidazole-4-carboxamide ribonucleotide in the biosynthesis of purines.[131] N-Formyltetrahydrofolic acid, also known as leucovorin and citrovorum factor, is interconvertible with the N^{10}-formyl analog by way of an isomerase-catalyzed reaction. It carries the formimino group for the biosynthesis of formiminoglycine, a precursor of purines (Scheme 5). Leucovorin is utilized in "rescue therapy" with methotrexate. It prevents the lethal effects of methotrexate on normal cells by overcoming the blockade of tetrahydrofolic acid production. In addition, it inhibits the active transport of methotrexate into cells and stimulates its efflux.[132]

Recently it has been shown that giving thymidine with methotrexate to mice bearing L1210 leukemia increased their survival time. This finding contradicts the idea that thymine deficiency is the most lethal effect of methotrexate on tumors. It suggests that the blockade of purine biosynthesis might have greater effects on tumor cells than normal cells.[133] Consequently, the administration of thymidine might protect the normal cells relative to the tumor cells. Unfortunately, the use of such "thymidine rescue" in clinical trials was disappointing.[134]

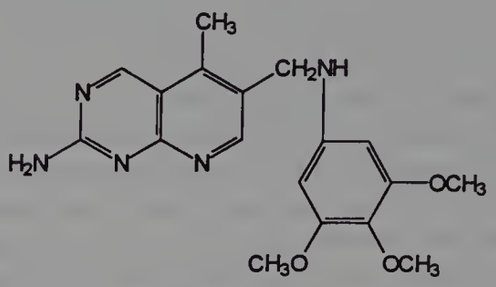
Numerous compounds closely related to methotrexate have been prepared and tested against neoplasms. Most structural variations, such as alkylation of the amino groups, partial reduction, and removal or relocation of heterocyclic nitrogens, lead to decreased activity. Piritrexim and trimetrexate are analogs of methotrexate in which one or two nitrogens in the pyridine ring are replaced by carbons and the benzoylglutamic acid chain is replaced by a more lipophilic group. As with methotrexate, both compounds inhibit dihydrofolate reductase; however, they do not interact with the reduced folate transport system used by methotrexate. Con-

sequently, they are active in vitro against some forms of methotrexate resistance. Their increased lipophilicity allows rapid transport by simple diffusion.[135,136]

Although the active sites of dihydrofolic reductases from normal and neoplastic cells are identical, Baker proposed that regions adjacent to the active sites of these enzymes might be different. He designed inhibitors to take advantage of these differences, thus affording species specificity. One of these inhibitors, known as "Baker's antifol," shows activity against experimental tumors that are resistant to methotrexate.[137]



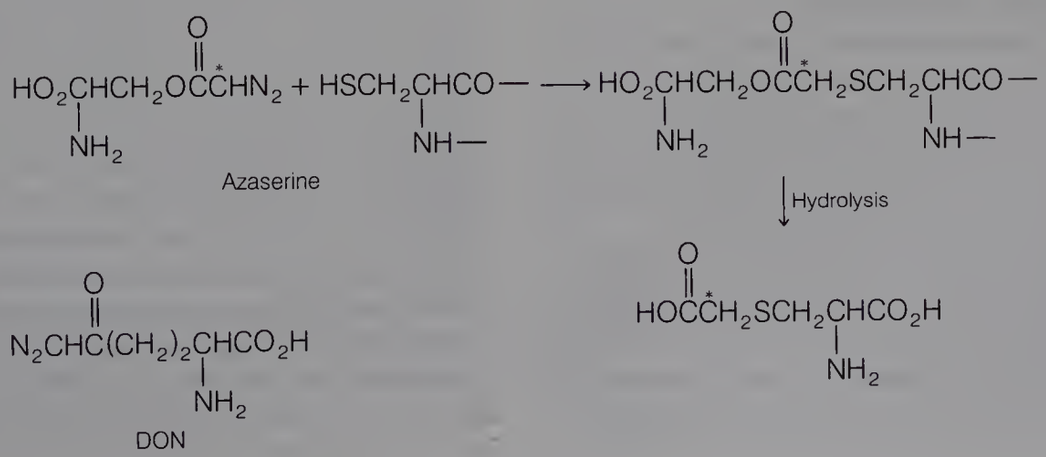
Piritrexim



Trimetrexate

Glutamine and glutamate are the donors of the three- and nine-nitrogen atoms of purines and the two-amino group of guanine.[138] They also contribute the three-nitrogen atom and the amino group of cytosine[139] (Schemes 5 and 8). Thus, they are involved at five different sites of nucleic acid biosynthesis. Although glutamine is not an essential nutrient for normal cells, many tumors are dependent upon exogenous sources of it. This provides a rationale for the selective action of agents that interfere with the uptake, biosynthesis or functions of glutamine.

In 1954, azaserine was isolated from a streptomyces species.[140] It was found to antagonize many of the metabolic processes involving glutamine, with the most important effect being the conversion of formylglycine ribonucleotide into formyglycinamidine ribonucleotide (Scheme 5).[141] A related compound, 6-diazo-5-oxo-L-norleucine (DON), was isolated in 1956 and found to produce similar antagonism.[142] A study involving incubation with azaserine ^{14}C followed by digestion with proteolytic enzymes and acid hydrolysis produced S-carboxymethylcysteine ^{14}C, which showed that azaserine had reacted covalently with a sulfhydryl group of cysteine on the enzyme.[143] DON is a more potent inhibitor than azaserine of this enzyme and of the enzyme that converts uridine nucleosides into cytidine nucleosides.[144] Although both compounds show good antitumor activity in animal models, they have been generally disappointing in clinical trials.



PRODUCTS

Mercaptopurine, USP. Purinethol, 6-mercaptopurine, MP, Lukerin, Mercaleukin, NSC-755, 6-purinethiol. This compound is prepared by treating hypoxanthine with phosphorus pentasulfide[145] and is obtained as yellow crystals of the monohydrate. Solubility in water is poor. It dissolves in dilute alkali but undergoes slow decomposition. Scored 50-mg tablets are supplied. The injectible formulation is in vials containing 500 mg of the sodium salt of 6-mercaptopurine, which is reconstituted with 49.8 ml of Sterile Water for Injection, USP.

Mercaptopurine is not active until it is anabolized to the phosphorylated nucleotide. In this form, it competes with endogenous ribonucleotides for enzymes that convert inosinic acid into adenine- and xanthine-based ribonucleotides. Furthermore, it is incorporated into RNA, wherein it inhibits further RNA synthesis. One of its main metabolites is 6-methylmercaptopurine ribonucleotide, which also is a potent inhibitor of the conversion of inosinic acid into purines.[146]

Despite poor absorption, low bioavailability, and first-pass metabolism by the liver, mercaptopurine has oral activity. Peak plasma levels of ~70 ng/ml occur 1 to 2 hr after injection of a 75 mg/m^2 oral dose. After a bolus injection, plasma levels of 5,000 ng/ml are reached within minutes. Renal excretion is rapid. Mercaptopurine is metabolized by S-methylation followed by 8-hydroxylation. It also is oxidized to 6-thiouric acid.

Mercaptopurine is used primarily for treating acute leukemia. A higher proportion of children than adults respond.[147] The chief toxic effect is leukopenia. Thrombocytopenia and bleeding occur in high doses. Because the leukopenia is delayed, it is important to discontinue the drug temporarily at the first sign of an abnormally large drop in the white cell count.

The tolerated dose varies with the individual patient. Allopurinol potentiates the effect of mercaptopurine by inhibiting its metabolism. However, it also increases its toxicity. If allopurinol is given for potentiation or reduction of hyperuricemia resulting from the killing of leukemia cells, the doses of mercaptopurine must be decreased.[148]

Thioguanine, USP. Thioguanine Tabloic, 6-thioguanine, TG, NSC-752, 2-aminopurine-6-thiol. The preparation of this compound is by treating guanine with phosphorus pentasulfide in pyridine.[149] Scored 40-mg tablets are supplied. Oral thioguanine is poorly absorbed. An injectible form is supplied in 75-mg vials. Reconstitution is made by adding 5 ml of Sodium Chloride for Injection, USP.

Thioguanine is converted by hypoxanthine-guanine phosphoribosyl transferase into a nucleotide form that inhibits a number of reactions in RNA and DNA synthesis, including the activity of phosphoribosylpyrophosphate amidotransferase, the initial enzyme involved in purine biosynthesis.[150] The 2′-deoxyribose triphosphate anabolite of thioguanine is extensively incorporated into DNA in place of the natural substrate. Thioguanine is metabolized to methylthioguanine, thiouric acid, methylthioxanthine, and thioxanthine.

Thioguanine is used in treating acute leukemia, especially in combination with cytarabine.[151] Cross-resistance exists between thioguanine and mercaptopurine. The chief toxic effect is delayed bone marrow depression, resulting in leukopenia and eventually thrombocytopenia and bleeding.

The usual initial dose is 2 mg/kg daily by the oral route. If there is no clinical improvement or leukopenia after 4 weeks the dosage is increased to 3 mg/kg daily. In contrast to mercaptopurine, thioguanine may be continued in the usual dose when allopurinol is used to inhibit uric acid formation.

Cladribine. Leustatin, 2-CdA, NSC-105014-F, 2-chlorodeoxyadenosine, 2-chloro-2′-deoxy-β-D-adenosine. This compound is prepared by a multistep procedure from 2,8-dichloroadenine and D-xylose,[152] and is supplied as a 1 mg/ml sterile solution in 0.9% sodium chloride for injection, USP. The desired dose is removed from the vial and diluted with normal saline for infusion over 24 hr. These solutions are stable for 72 hr. Infusion is continued for 5 to 7 days.

Half-lives of 35 min (α) and 6.7 hr (β) were found for cladribine.[153] It is completely cleared from plasma in 1 to 3 days after the infusion is stopped. Cladribine is phosphorylated in cells to the active triphosphate by deoxycytidine kinase. It acts by inhibiting several enzymes required for DNA repair, and it is resistant to adenosine deaminase.

The current FDA approval for cladribine is hairy cell leukemia, in which it exhibits a very high percentage of complete responses, even in pretreated patients.[154] It has activity in a variety of other lymphoid malignancies. The limiting toxicity in the majority of patients is a temporary decrease in neutrophils, which commonly leads to infections. There is also a prolonged suppression of helper lymphocytes.

Fludarabine Phosphate. Fludara, 2F-ara-AMP, FLAMP, NSC-321887, 9-β-D-arabinofuranosyl-2-fluoroadenosine 5′-monophosphate. It is prepared by treating fludarabine with triethyl phosphate and phosphorus oxychloride,[155] and it is supplied in 6-ml sterile vials containing 50 mg of fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust the pH to 7.7. The intact vials should be stored at 2°C to 8°C. Each vial is reconstituted with 2 ml of sterile water, and this solution is stable for 8 hr at 25°C. It should be discarded after this time because the vial contains no antibacterial agent. Administration is by short intravenous infusion or as a rapid loading dose/continuous infusion.

Following infusion, fludarabine phosphate is rapidly dephosphorylated by serum phosphatases and converted into 2-fluoroadenosine arabinoside (2-FLAA). The levels of 2-FLAA decline biexponentially with half-lives of 0.6 hr and 9.3 hr. 2-FLAA enters cells by a carrier-mediated process

and undergoes intracellular phosphorylation by deoxycytidine kinase to the active form, 2-F-ara-ATP.[98]

Fludarabine phosphate has good activity in chronic lymphocytic leukemia. In ongoing clinical trials, it also shows activity against low-grade lymphomas and mycosis fungoides. The dose-limiting toxic effect is myelosuppression. Gastrointestinal and central nervous system toxicity also occur.

Fluorouracil, USP. Fluorouracil Ampuls, Fluoroplex, Efudex, 5-FU, 5-fluoro-2,4(1H,3H)-pyrimidinedione, 2,4-dioxo-5-fluoropyrimidine. This compound is prepared by condensing S-ethylisothiouronium bromide with the potassium salt (enolate) of ethyl 2-fluoro-2-formylacetate.[156] Recently the preparation of fluorouracil by direct fluorination of uracil was demonstrated.[157] Fluorouracil is supplied in 10-ml ampuls containing 500 mg of fluorouracil in a water solution at pH 9. These ampuls should be stored at room temperature and protected from light. Topical formulations of fluorouracil are Efudex Solution, which contains 2% or 5% fluorouracil compounded with propylene glycol, tris (hydroxymethyl)aminomethane, hydroxypropyl cellulose, methyl and propyl parabens and disodium edetate, and Efudex Cream, which contains 5% fluorouracil in a vanishing cream base consisting of white petrolatum, stearyl alcohol, propylene glycol, polysorbate 60, and methyl and propyl parabens.[158]

Fluorouracil is anabolized to its 2′-deoxyribose monophosphate, a potent inhibitor of thymidylate synthetase. It also is converted into fluorouridine triphosphate, which is incorporated into RNA and DNA.[104] There is cell cycle phase-specificity for the S phase.

Plasma levels of fluorouracil are erratic after oral dosing. High plasma levels are obtained after parenteral administration, but the pharmacokinetic characteristics are not linear. Fluorouracil is extensively metabolized in the liver, and the main metabolite is dihydrofluorouracil.[159] Most of an administered dose is excreted in urine as α-fluoro-β-alanine.

Fluorouracil is effective in the palliative management of carcinoma of the breast, colon, pancreas, rectum, and stomach in patients who cannot be cured by surgery or other means.[160] The topical formulations are used with favorable results for the treatment of premalignant keratoses of the skin and superficial basal-cell carcinomas.[161] Parenteral administration almost invariably produces toxic effects. Leukopenia usually follows every course of therapy, with the lowest white blood cell counts occurring between days 9 and 14 after the first course. Gastrointestinal hemorrhage may occur and may even be fatal. Stomatitis, esophagopharyngitis, diarrhea, nausea, and vomiting are commonly seen; alopecia and dermatitis also occur. Therapy must be discontinued if leukopenia or gastrointestinal toxicity becomes too severe. Topical administration is contraindicated in patients who develop hypersensitivity. Prolonged exposure to ultraviolet radiation may increase the intensity of topical inflammatory reactions.

Floxuridine, USP. FUDR, fluorodeoxyuridine, NSC-27640, 2′-deoxy-5-fluorouridine, 1-(2-deoxy-β-D-ribofuranosyl)-5-fluorouracil. This compound is prepared by condensing monomercuri-5-fluorouracil with 3,5-di-O-p-toluyl-2-deoxyribosyl-1-chloride followed by alkaline hydrolysis.[162] It is supplied in 5-ml vials containing 500 mg of floxuridine as sterile powder. Reconstitution is by the addition of 5 ml of sterile water. The resulting solutions should be stored under refrigeration for not more than 2 weeks.

Floxuridine is used for palliation of gastrointestinal adenocarcinoma metastatic to the liver in patients who are considered incurable by surgery or other means.[163] It is administered by continuous regional intra-arterial infusion. When given in this manner, it has significant advantages over fluorouracil. Because floxuridine is rapidly catabolized to fluorouracil, it gives the same toxic reactions as fluorouracil.

Cytarabine, USP. Cytosar-U, ara-c, cytosine arabinoside, NSC-63878, 1-β-D-arabinofuranosylcytosine. It is synthesized from uracil arabinoside in a route involving acetylation, treatment with phosphorus pentasulfide, and heating with ammonia.[164] It is supplied as the freeze-dried solid in vials containing 100 or 500 mg. The 100-mg sample is reconstituted with 5 ml of sterile water containing 0.9% benzyl alcohol to give 20 mg/ml of cytarabine, whereas the 500-mg sample is reconstituted with 10 ml of sterile water containing 0.9% benzyl alcohol to give 50 mg/ml of cytarabine. These solutions may be stored at room temperature for 48 hr.

Sequential actions of deoxycytidine kinase anabolize cytarabine to the triphosphorylated nucleotide, which acts as a competitive inhibitor of DNA polymerase after incorporation into DNA chains.[165] It is specific for the S phase of the cell cycle. Cytarabine is not orally active because of the extensive deamination to inactive uracil arabinoside catalyzed by the enzyme cytidine deaminase.

Plasma levels of 0.01 to 0.15 μg/mL are required for cytotoxic effects of cytarabine, and they are achievable with the use of continuous or sequential bolus doses of 100 to 200 mg/m^2.[166]

Cytarabine is indicated primarily for inducing the remission of acute granulocytic leukemia of adults. It also is used for other acute leukemias of adults and children.[167] Remissions have been brief unless followed by maintenance therapy or given in combination with other antineoplastic agents.[168] Side effects include severe leukopenia, thrombocytopenia, and anemia. Gastrointestinal disturbances also are relatively frequent.

Pentostatin. 2′-Deoxycoformycin, dCF, NSC-218321, (R)-3-(2-deoxy-β-D-erythropentofuranosyl)-3, 6, 7, 8-tetrahydroimidazo[4,5-d][1,3]diazepin-8-ol. This compound is obtained from extracts of *Streptomyces antibioticus*[169] and formulated in 10-mg vials as a lyophilized powder. Mannitol (50 mg) and sodium hydroxide (to adjust pH) are included. It is administered intravenously, usually as short infusions in isotonic solutions. Pentostatin exhibits first-order, two-compartment excretion behavior.[170]

Pentostatin is an irreversible inhibitor of the enzyme aden-

osine deaminase. The resulting accumulation of deoxyadenosine and its phosphorylated congeners inhibits DNA synthesis. It is most effective against leukemias and lymphomas, especially hairy cell leukemia.[171] The dose-limiting effects include renal dysfunction, neurological toxicity, and reversible granulocytopenia.

Methotrexate, USP. Amethopterin, methyl aminopterin, NSC-740, 4-amino-N^{10}-methyl-pteroylglutamic acid, L-(+)-N-[p-[[(2,4-diamino-6-pteridinyl)methyl]methylamino] benzoyl]glutamic acid. This compound is prepared by combining 2,4,5,6-tetrahydropyrimidine, 2,3-dibromopropionaldehyde, disodium p-(methylamino)-benzoylglutamate, iodine, and potassium iodide, followed by heating with lime water.[126] It is isolated as the monohydrate, a yellow solid. Recent studies indicate that the commercial preparation contains a number of impurities, including 4-amino-N^{10}-methylpteroic acid and N^{10}-methylfolic acid.[172] Methotrexate is soluble in alkaline solutions but decomposes in them. It is supplied as 25-mg tablets and in vials containing either 5 mg or 50 mg of methotrexate sodium in 2 ml of solution. The 5-mg sample contains 0.90% of benzyl alcohol as preservative, 0.63% of sodium chloride, and sodium hydroxide to give pH 8.5. The 50-mg sample contains 0.90% of benzyl alcohol, 0.26% of sodium chloride, and sodium hydroxide to give pH 8.5. A preservative-free lyophilized preparation is recommended for intrathecal administration to prevent or treat tumor cells within the CNS.

Following oral administration, methotrexate is rapidly but incompletely absorbed. Approximately 50% to 60% of the absorbed drug is bound to plasma proteins. Cytotoxic levels are found in cerebrospinal fluid when high doses are given. Most of the drug is excreted in the urine unchanged, although some 7-hydroxymethotrexate is found following high-dose therapy. Plasma level decay is biphasic or possibly triphasic.

Methotrexate binds tightly to dihydrofolate reductase, blocking the reduction of dihydrofolate to tetrahydrofolate, the active form of the coenzyme.[173] It is specific for the S phase of the cell cycle. Methotrexate undergoes polyglutamation intracellularly, forming a pool of compounds that is retained for months. Resistance to methotrexate develops by an increase in dihydrofolate reductase, which results from gene amplification, or by defective transport into tumor cells.[174]

Methotrexate was the first drug to produce substantial (although temporary) remissions in leukemia.[175] It is still used for this purpose against acute lymphocytic leukemia and acute lymphoblastic leukemia. Because it has some ability to enter the CNS, it is used in the treatment and prophylaxis of meningeal leukemia. The discovery that methotrexate afforded a high percentage of apparently permanent remissions in choriocarcinoma in women justified the use of the term "cure" in cancer chemotherapy.[176] Methotrexate is used in combination chemotherapy for palliative management of breast cancer, epidermoid cancers of the head and neck, and lung cancer. It also is used against severe, disabling psoriasis. The most common toxic reactions are ulcerative stomatitis, leukopenia, and abdominal distress. A high dose of methotrexate combined with leucovorin "rescue" produces some responses in osteogenic sarcoma, but it can cause renal failure in some patients. This condition is thought to result from crystallization of the drug or its metabolites in acidic urine, and it is countered by hydration and alkalinization.[177]

Azathioprine, USP. Imuran, 6-[(1-methyl-4-nitroimidazole-5-yl)thio]purine. This compound is prepared from 6-mercaptopurine and 5-chloro-1-methyl-4-nitroimidazole.[178] It is supplied as 50-mg scored tablets. The injectable sodium salt is available in 20-ml vials containing 100 mg of azathioprine.

Azathioprine is well absorbed when taken orally. It is converted extensively to 6-mercaptopurine. The main indication for azathioprine is as an adjunct to prevent the rejection of renal heterotransplants. It is contraindicated in patients who show hypersensitivity to it. The chief toxic effects are hematological, expressed as leukopenia, anemia, and thrombocytopenia. Complete blood counts should be performed at least weekly, and the drug should be discontinued if there is a rapid fall or persistent decrease in leukocytes. Patients with impaired renal function might have slower elimination of the drug, which requires appropriate reduction of the dose. Azathioprine should not be taken with allopurinol, which blocks its metabolism by xanthine oxidase.

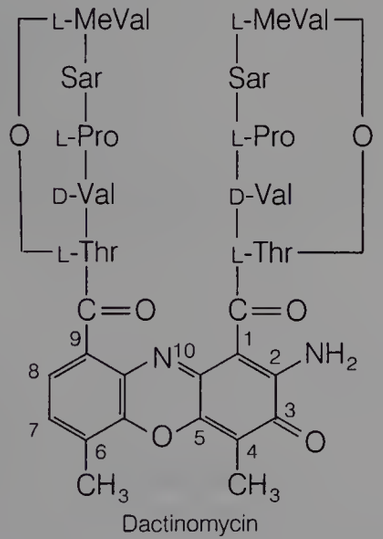
ANTIBIOTICS

Eight different antibiotics now are established clinical anticancer agents, and a number of other antibiotics are undergoing clinical development. Most of these agents have been approved recently. However, some of the compounds in this class have been known for a long time. For example, dactinomycin (actinomycin D) was first isolated in 1940 by Waksman and Woodruff,[179] although its activity against neoplasms was not described until 1958. Furthermore, plicamycin, originally discovered as aureolic acid in 1953, had to be rediscovered twice before its antitumor activity was established in 1962.[180] These compounds were originally rejected as antibacterial agents because of their cytotoxicity. Only later was it found that this toxicity could be turned to an advantage in the chemotherapy of cancer. The discovery of antitumor activity is much simpler today, and some laboratories routinely screen extracts of microorganism cultures for antitumor activity in cell cultures.

The production of antitumor agents from microbial fermentations has some special advantages and disadvantages over chemical synthesis. In some cases, the biosynthesis can be controlled to afford novel analogs. This has been true for actinomycins[181] and bleomycins.[182] Strain selection and fermentation conditions can optimize the formation of a particular component of an antibiotic mixture. Thus, *Streptomyces parvullus* produces dactinomycin almost exclusively, in contrast to other species that form complex mixtures of acti-

nomycins. The fermentation in *Streptomyces caespitosus* has been developed similarly to produce almost all mitomycin C. In some cases, such as with doxorubicin, improvement of the antibiotic yield has been difficult. This results in an expensive product and intensive research on chemical synthesis.

The actinomycins comprise a large number of closely related structures. All of them contain the same chromophore, a substituted 3-phenoxazone-1,9-dicarboxylic acid known as actinocin. Each of the carboxyl groups is bonded to a pentapeptide lactone by way of the amino group of an L-threonine unit of this pentapeptide. The hydroxyl group of the L-threonine forms part of the lactone along with L-methylvaline, the fifth amino acid from the chromophore. D-Valine or D-alloisoleucine is the second amino acid and the fourth amino acid usually is sarcosine. The third amino acid is more variable, consisting of L-proline, L-hydroxyproline, L-oxoproline, or others produced by controlled biosynthesis. Actinomycins that have two identical pentapeptide lactones are called isoactinomycins, whereas those with different pentapeptide lactones are called anisoactinomycins. The individual pentapeptide lactones are designated α and β, depending on their attachment to the 9- or 1-carboxylic acids, respectively. Dactinomycin (actinomycin D, actinomycin C_1) is an isoactinomycin with an amino acid sequence of L-threonine, D-valine, L-proline, sarcosine, and L-N-methylvaline. Actinomycin C_3, which is used in Germany, differs from actinomycin D by a D-alloisoleucine unit instead of D-valine in both the α and β chains.[183]



Dactinomycin

The mode of action of actinomycins has been studied extensively, and it now is generally accepted that they intercalate into double helical DNA. In the intercalation process, the helix unwinds partially to permit the flat phenoxazone chromophore to fit in between successive base pairs (Fig. 12-4). Adjacent G-C pairs are especially suitable because the 2-amino groups of the guanines can hydrogen bond with the carbonyl groups of threonines in the actinomycin. This bonding reinforces the π-bonding between the heterocyclic chromophores. Additional stability is conferred by the interaction between the pentapeptide lactone chains and DNA. These chains lie in the minor groove of the double helix, running in opposite directions to each other, and they make numerous van der Waals interactions with the DNA.[184]

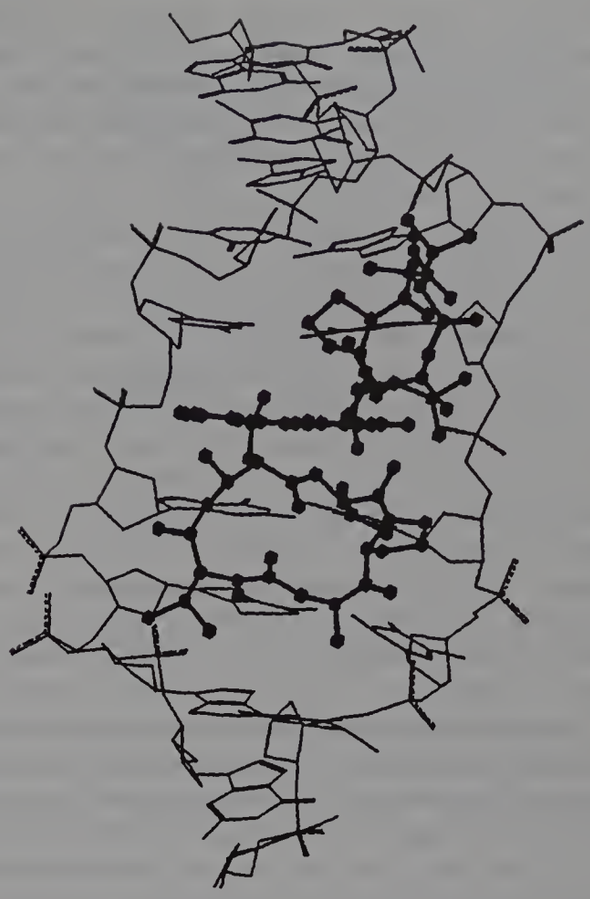


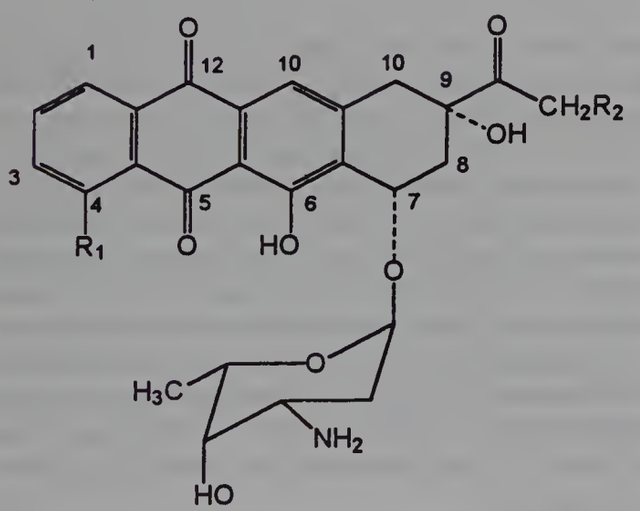
FIG. 12-4. *Dactinomycin intercalating DNA.*

Intercalation into DNA changes its physical properties in characteristic ways. Thus, the length, viscosity, and melting temperature increase, whereas the sedimentation coefficient decreases.[185,186] Changes in substituents on the actinomycins influence their binding to DNA, usually by making it less effective. Opening of a lactone ring or changing the stereochemistry of an amino acid abolishes activity, and replacement of the 4- and 6-methyl groups by other substituents reduces it. Replacement of the 2-amino group also reduces activity.[187]

Actinomycins, anthracyclines, and certain other intercalating agents, including mitoxantrone and amsacrine, inhibit the enzyme topoisomerase II. Topoisomerases regulate the topological state of DNA by unwinding and unlinking coiled double-strand DNA molecules. They are thought to be critical for DNA replication and transcription, and they act by cleaving and rejoining one or both strands of the phosphodiester backbone of DNA. Topoisomerase I cuts one of the two DNA strands, allowing the other to act as a swivel about which the winding or unwinding can take place.[188] In contrast, topoisomerase II cleaves both strands, allowing complete rotation or, as has been suggested, passage of part of the intact double strand through the gap. Drugs that inhibit

topoisomerases bind to and trap the covalent complex formed between phosphate groups on the DNA and tyrosine residues on the enzyme, preventing subsequent reclosure of the broken strand or strands. The extent to which drug-topoisomerase-DNA complexes are formed does not necessarily correlate with cytotoxicity.[188]

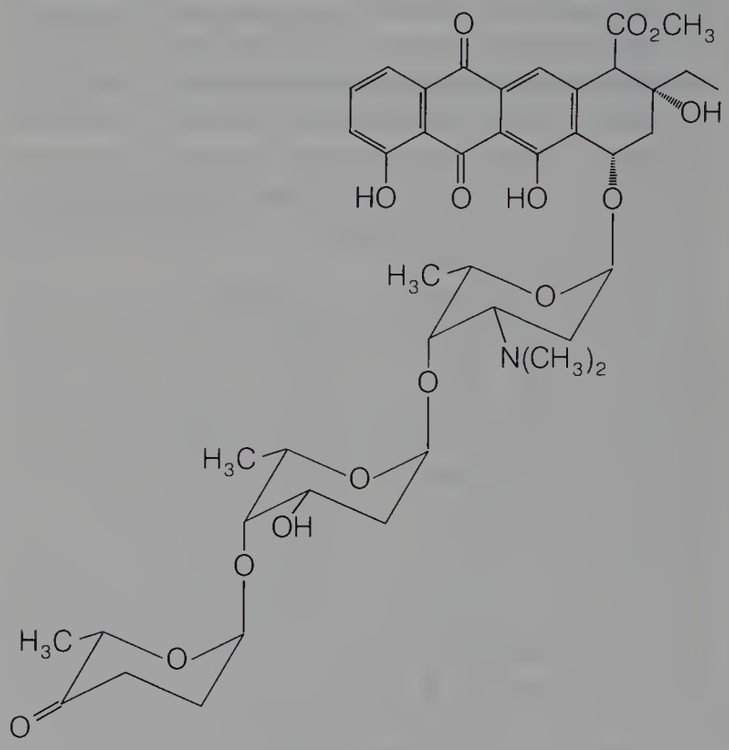
Anthracyclines are another large and complex family of antibiotics. Many members of this family were investigated before a useful antitumor agent, daunorubicin, was isolated from *Streptomyces caeruleorubidis* and *S. peucetius*. This significant discovery was made independently in France and Italy in 1963.[189,190] Daunorubicin proved to be active against acute leukemias, and it became an established clinical agent. However, it was pushed into the background by the discovery of doxorubicin (Adriamycin) in *S. peucetius var caesius* in 1969.[191] Doxorubicin is active against a broad spectrum of tumors, including both solid and hematological types. It is presently one of the most widely used antineoplastic agents.[192] A third anthracycline, which was recently approved for clinical use in the United States, is idarubicin. This compound is the 4-demethoxy analog of daunorubicin. It has enhanced antitumor potency, and it appears to be less cardiotoxic than daunorubicin and doxorubicin. Epirubicin, the 4′-hydroxy epimer of doxorubicin, is available in Europe. It also is considered to be less toxic than doxorubicin with equal or greater antitumor activity. The 4-hydroxy analog of daunorubicin, carminomycin, isolated from *Actinomadura carminata*, has been evaluated in Russia.[193]



Daunorubicin:	$R_1 = OCH_3$, $R_2 = H$
Doxorubicin:	$R_1 = OCH_3$, $R_2 = OH$
Idarubicin:	$R_1 = H$, $R_2 = OH$
Carminomycin:	$R_1 = OH$, $R_2 = H$

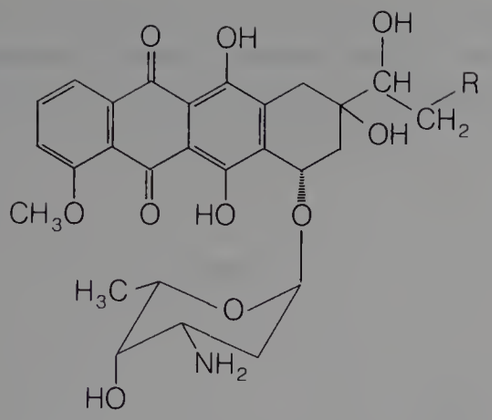
Many anthracyclines, including all of those with antitumor activity, occur as glycosides of the anthracyclinones. The glycosidic linkage usually involves the 7-hydroxyl group of the anthracyclinone and the β-anomer of a sugar with L-configuration. Anthracyclinone refers to an aglycone containing the anthraquinone chromophore within a linear hydrocarbon skeleton related to that of the tetracyclines.[194] The anthracyclinones differ from each other in the number and location of phenolic hydroxyl groups, the degree of oxidation of the two-carbon side chain at position 9, and the presence of a carboxylic acid ester at position 10. Thus, daunorubicin is a glycoside formed between daunomycinone and L-daunosamine, whereas doxorubicin is its 14-hydroxy analog.[195] In contrast, aclacinomycin A has aklavinone in combination with a trisaccharide chain.[196]

Daunorubicin and doxorubicin exhibit biological effects similar to those of actinomycin, and they are thought to intercalate into double helical DNA and inhibit topoisomerase II.[197] Reduction of doxorubicin followed by intercalation causes DNA-strand scission. This scission is thought to result from the attack of hydroxyl radicals generated from redox cycles involving doxorubicin.[198] In contrast to daunorubicin, aclacinomycin and related compounds do not induce lysogenic phage in bacteria. They are believed to interfere with RNA syntheses more than with DNA synthesis. Aclacinomycin lacks the cardiotoxicity shown by daunorubicin and doxorubicin.[196]

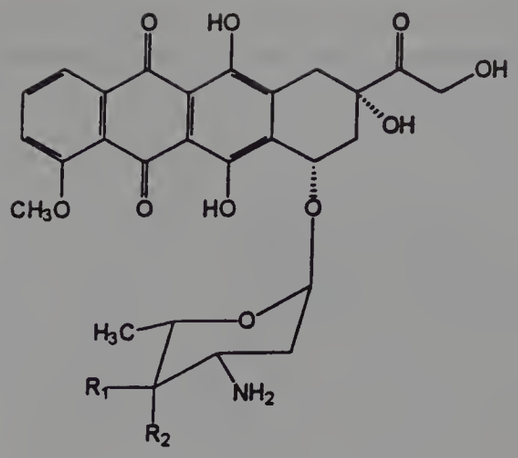


Aclarubicin
(Aclacinomycin A)

In contrast to the actinomycins, anthracyclines are metabolized in the liver. Daunorubicin is readily converted into its 13-hydroxy analog, daunorubicinol, which is further cleaved to the aglycone.[199] The 14-hydroxyl group of doxorubicin makes it less susceptible to reduction of the 13-carbonyl group. However, the 13-hydroxy derivative, adriamycinol, is found among the metabolites, along with the 4-demethyl-4-sulfate. Both daunomycinol and adriamycinol are active against neoplastic cells, but their rates of uptake are low.[200]



Daunomycinol, R = H
Adriamycinol, R = OH



Esorubicin: $R_1 = R_2 = H$

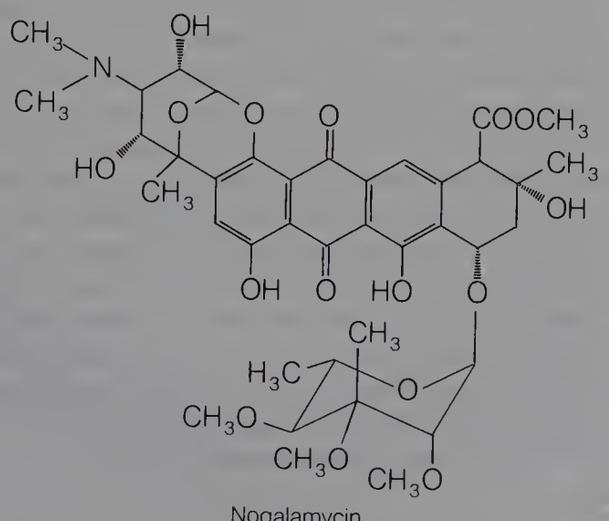
Epirubicin: $R_1 = OH, R_2 = H$

Pirarubicin: $R_1 = H, R_2 = O$

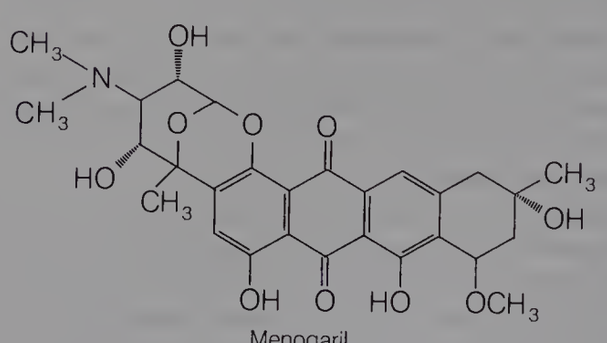
Many analogs of doxorubicin with changes in the sugar moiety have been prepared. They include 4′-deoxydoxorubicin (esorubicin), 4′-epidoxorubicin (epirubicin), and 4′-O-tetrahydropyranyl doxorubicin (pirarubicin). Pirarubicin accumulates more rapidly than doxorubicin in tumor cells and shows superior activity in animal models.[201]

Another anthracycline with significant antitumor activity is nogalamycin, which is obtained from *Streptomyces nogalater*.[202] It differs from other anthracyclines in that the aminosugar is joined to the nucleus by a carbon-carbon bond and a cyclic acetal linkage. However, there is a non-amino sugar, nogalose, at the usual 7-position. Although nogalamycin itself is not an established antineoplastic drug, a semisynthetic analog, menogaril, has received Phase II clinical trials and is under consideration for approval. Menogaril has the nogalose moiety replaced by a methoxy group and reversed chirality at the 7-position. It also differs from nogalamycin by the absence of the 10-carbomethoxy group.[203] These structural changes result in a change in the mode of action from intercalation into DNA, as found for nogalamycin, to some other site and type of cytotoxic process. Thus, menogaril localizes in the cytoplasm, rather than the nucleus, and it has very little effect on DNA and RNA synthesis at cytotoxic doses.[204] Menogaril is not more effective than doxorubicin against tumors, but its much lower cardiotoxicity and potential oral activity might offer clinical advantages.

The aureolic acid group of antitumor antibiotics includes aureolic acid (plicamycin, mithramycin), the olivomycins, the chromomycins, variamycin, and related compounds. Plicamycin is the only member approved for clinical use in the United States. It is restricted to testicular carcinoma and hypercalcemia that is resistant to other drugs. However, chromomycin A_3 is used in Japan, and olivomycin A is used in Russia.[187] Aureolic acid group compounds have complex structures consisting of an aglycone and two carbohydrate chains. The aglycones are tetrahydroanthracene derivatives with phenolic hydroxyl groups at positions 6, 8, and 9 and



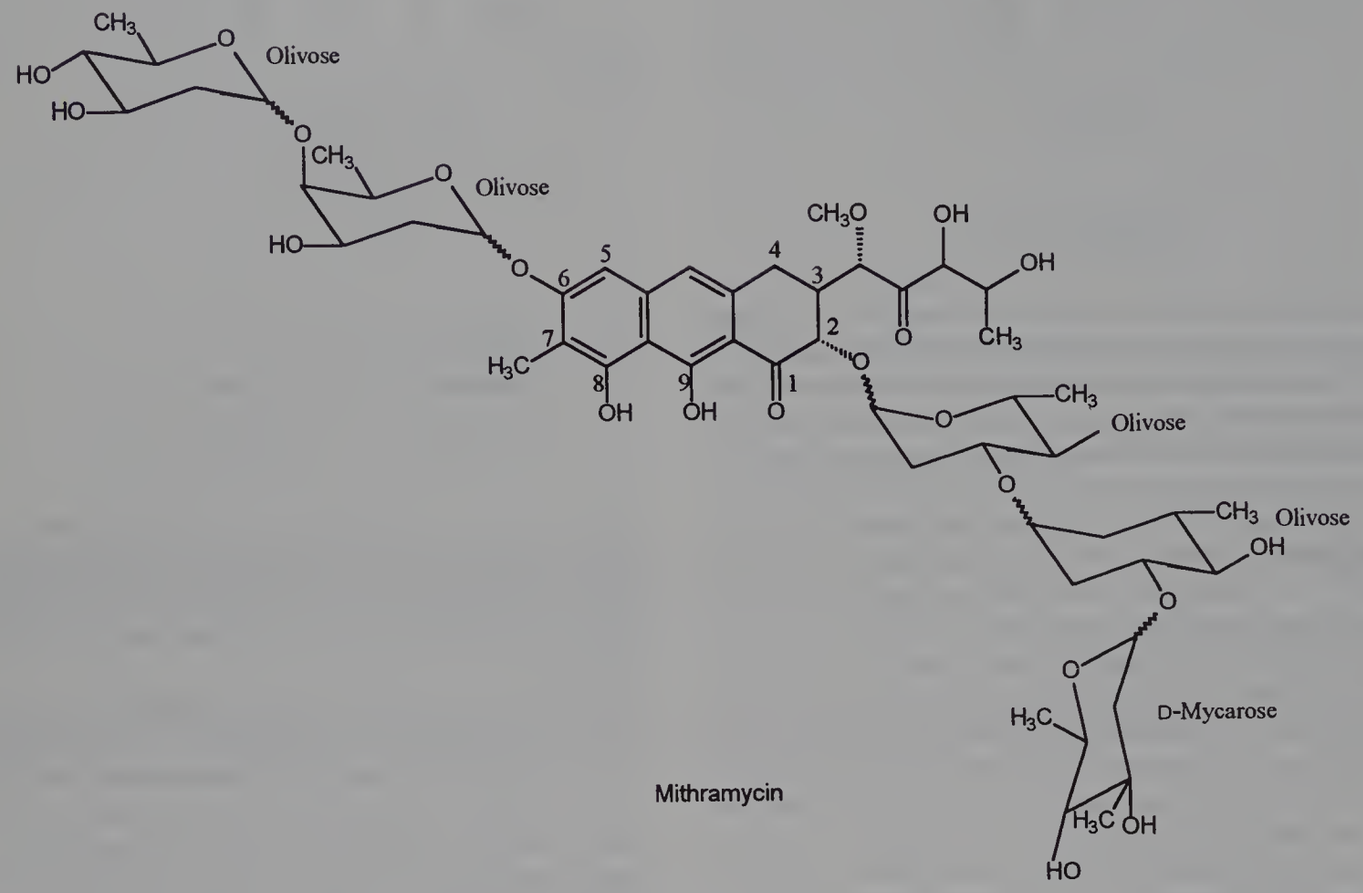
Nogalamycin



Menogaril

a pentanyl side chain that is highly oxygenated. The carbohydrate chains contain either two or three 2,6-dideoxy sugars of novel structures.[205]

the phleomycins (which differ from bleomycins in having one thiazole ring partly reduced), zorbamycin and the zorbonamycins, antibiotic YA-56, victomycin, the tallysomycins,

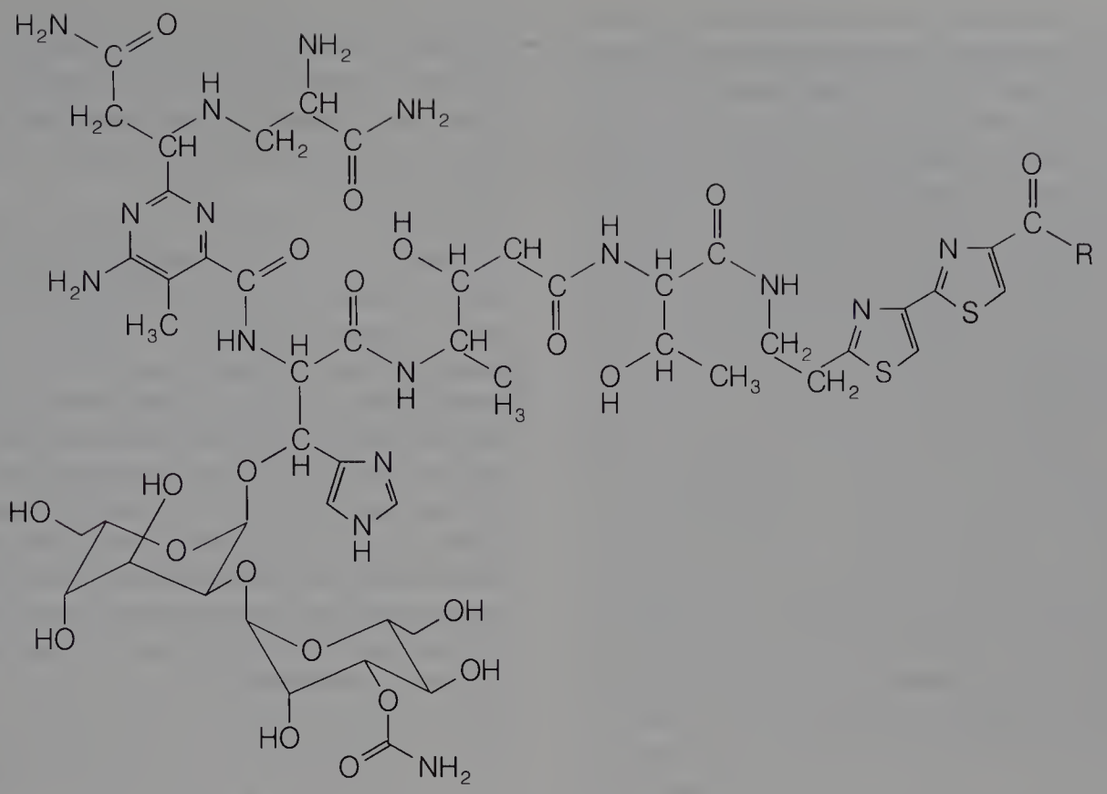


Mithramycin

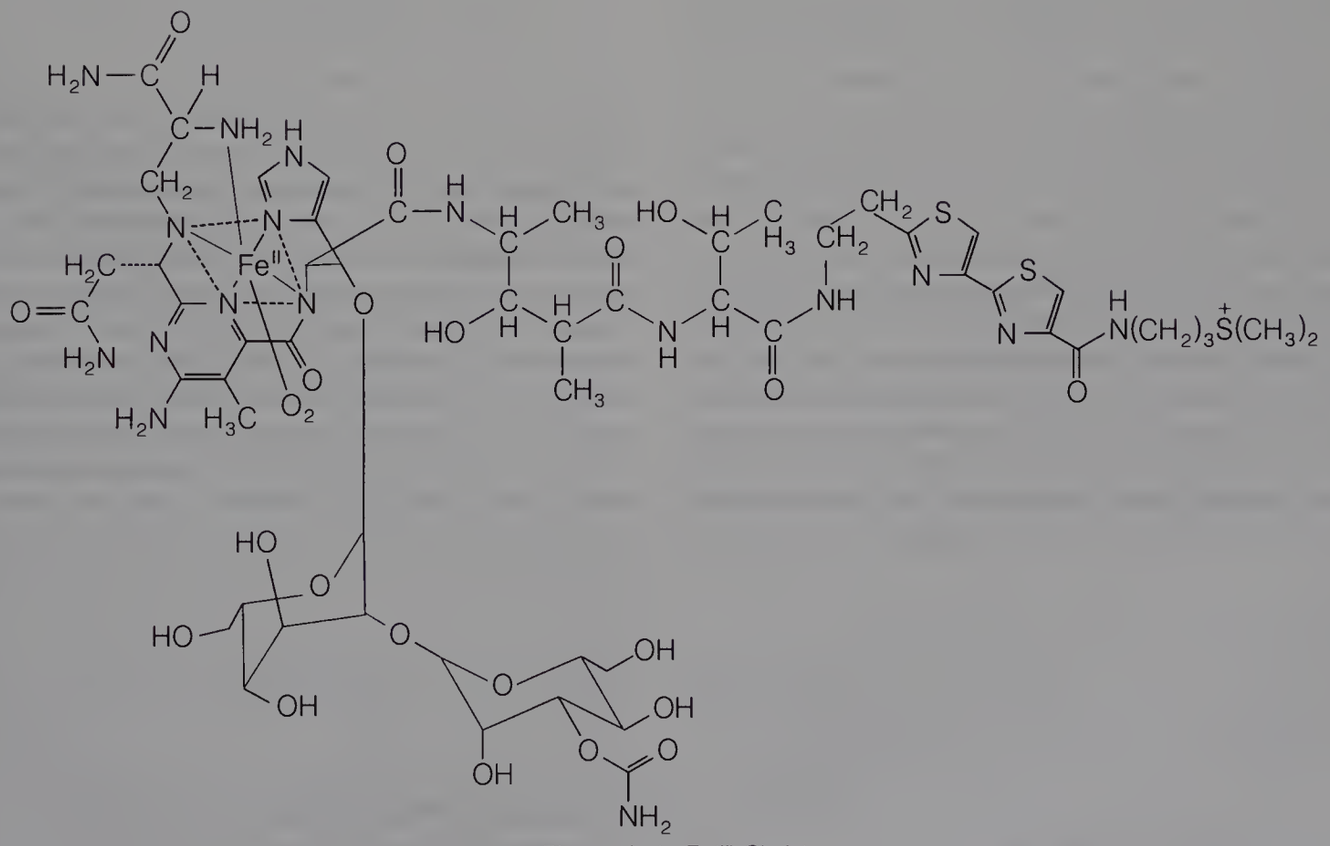
Plicamycin and related compounds are weakly acidic owing to the phenolic groups ($pK_a = 5$). They readily form sodium salts that show brilliant yellow fluorescence.[206] The chromophore is responsible for complex formation with divalent metals such as magnesium and calcium. Such complex formation is required before aureolic acids can bind with DNA.[207] The nature of this DNA binding is uncertain at the present time. Intercalation has been suggested, but the evidence for this process is incomplete.[208] Whatever the exact nature of the binding, plicamycin and other aureolic acids inhibit DNA-dependent RNA polymerase, and this effect leads to cell death.[209]

The discovery of bleomycin in 1966 resulted from the establishment by H. Umezawa of a program for screening microbial-culture filtrates against experimental tumors.[210] Bleomycin is a mixture of closely related compounds that is partly resolved before formulation for clinical use.[211] The presently used commercial product, Blenoxane, contains bleomycins A_2 and B_2. A variety of other antibiotics have structures similar to those of the bleomycins. They include and the platomycins.[187] New bleomycin analogs also have been prepared by controlled biosynthesis from bleomycinic acid.

Bleomycins and their analogs occur naturally as blue copper chelates. Removal of the copper by chemical reduction or complexing agents affords the antibiotics as white solids.[212,213] Copper-free bleomycin is the active species for chemotherapy, and it has decreased toxicity. The complexation of bleomycin with metal ions occurs readily and is a key factor in its mode of action. Inside the cell, bleomycin forms a chelate with Fe(II) that has square pyramidal geometry.[214] Nitrogen atoms from bleomycin occupy five of the positions in this structure. The sixth position may be occupied by the carboxyl group of the carbamate function, but this group is readily displaced by molecular oxygen. The resulting complex may give rise to hydroxyl radicals and superoxide radicals. These highly reactive radicals are generated close to the double helix, and they cause cleavage of the phosphodiester bonds. This degradation of DNA strands is thought to be the lethal event in cells.[215]



Bleomycinic Acid, R = OH
Bleomycin A_2, R = $NH(CH_2)_3\overset{+}{S}(CH_3)_2$
Bleomycin B_2, R = $NH(CH_2)_4NHC(=NH)NH_2$



Bleomycin A_2 Fe(II) Chelate

Bleomycin is inactivated by an intracellular enzyme named bleomycin hydrolase, an aminopeptidase that hydrolyzes the carboxamide group of the β-aminoalanine carboxamide residue to the corresponding carboxylate. This structural change increases the pKa of the α-amino group from 7.3 to 9.4, which results in poorer binding to DNA.[213] Chelation with Fe(II) still occurs, but the production of hydroxyl radicals is drastically reduced.[215] Bleomycin hydrolase levels in tumor cells help to determine their resistance to bleomycin. Thus, squamous-cell carcinoma is characterized by ready uptake of bleomycin and low levels of the hydrolase. It is especially sensitive to bleomycin.[216]

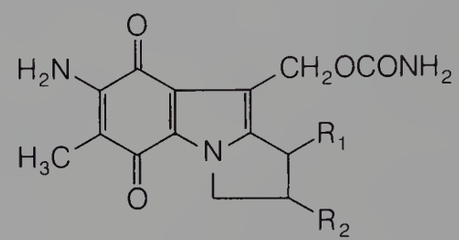
Bleomycins undergo two different inactivating reactions under mildly alkaline conditions. One is migration of the carbamoyl group to an adjacent hydroxyl group of the mannose residue. The resulting product is called an isobleomycin.[217] Copper-chelated bleomycins do not undergo this reaction. However, they are slowly transformed into epibleomycins, which are racemized at the carbon atom substituted at the 2-position of the pyrimidine ring.[218] Epibleomycins retain ~25% of the antitumor activity of the parent bleomycins.

Bleomycinic acid is obtained by chemical degradation of bleomycin A_2 or enzymatic degradation of bleomycin B_2. It can be readily transformed into semisynthetic bleomycins such as PEP-bleomycin (pepleomycin), which possesses reduced pulmonary toxicity.[211]

The mitomycins were discovered in Japan in the late 1950s, and one of them, mitomycin C, was rapidly developed as an anticancer drug.[219] However, the initial clinical experience with this compound in the United States was disappointing. It was not approved until 1974, following extensive studies and the establishment of satisfactory dosage schedules. Porfiromycin, the N-methyl homolog of mitomycin C, was discovered at the Upjohn Company.[220] It has received clinical study, but it is not yet an approved agent.

Structures of the mitomycins were elucidated at Lederle Laboratories. These compounds have an unusual combination of three different carcinostatic functions: quinone, carbamate, and aziridine.[221] They are arranged in such a way that the molecule is relatively unreactive in its natural state. However, chemical or enzymatic reduction to the corresponding hydroquinone is followed by the loss of methanol (water from mitomycin B), and the resulting indolohydroquinone becomes a bifunctional alkylating agent capable of cross-linking double helical DNA (Scheme 3).[222] Mitomycins bound to DNA may undergo successive redox cycles, each of which results in the generation of hydrogen peroxide. This potent oxidizing agent can cause single-strand cleavage of the DNA.[223]

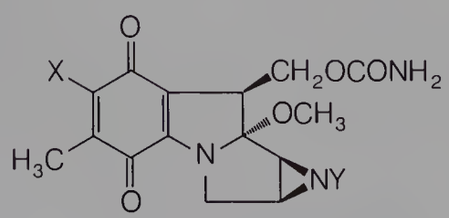
Mitomycins are unstable in both acids and bases. Mild acid hydrolysis results in opening of the aziridine ring and loss of methanol or water to give mitosenes such as 2,7-diamino-1-hydroxymitosene.[224] Catalytic hydrogenation followed by reoxidation gives aziridinomitosenes, which retain a significant amount of antitumor activity in animals.[225] Many mitomycin analogs have been prepared by partial synthesis, and two of them have received clinical trials.[226,227] However, unexpected toxicity has led to their withdrawal. The present clinical candidates, BMY-25067 and KT 6149, contain disulfide substituents on the 7-amino group. Control of the quinone reduction potential is especially stressed in analog studies, because reduction is the key step in bioactivation of these molecules.[228]



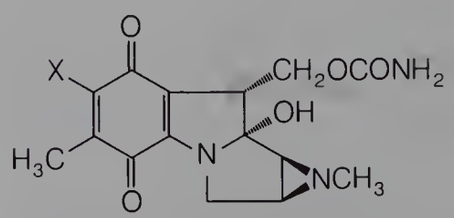
2,7-Diamino-1-hydroxymitosene, $R^1 = OH$, $R^2 = NH_2$

1,2-Aziridino-7-aminomitosene, $R^1, R^2 = \rangle NH$

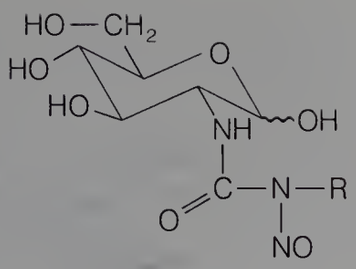
Streptozocin was isolated from *Streptomyces achromogenes* in 1960.[229] It is the nitrosomethylurea derivative of 2-deoxyglucose.[230] The simplicity of its structure and the cost of preparing it by fermentation have led to the development of practical syntheses from 2-amino-2-deoxyglucose.[231] Streptozocin is an alkylating agent similar in reactivity to other nitrosomethylureas, except that its glucose moiety causes it to be especially taken up in the pancreas. This effect is detrimental in that it produces diabetes, but it makes the molecule especially effective against malignant insulinomas.[232] It is an approved clinical agent for this specific use. The chloroethyl analog of streptozotocin, called chlorozotocin, shows good antitumor activity in animals and is not diabetogenic.[233]



Mitomycin A, X = CH_3O, Y = H
Mitomycin C, X = H_2N, Y = H
Porfiromycin, X = H_2N, Y = CH_3
BMY-25067, X = $O_2NC_6H_4SS(CH_2)_2NH$

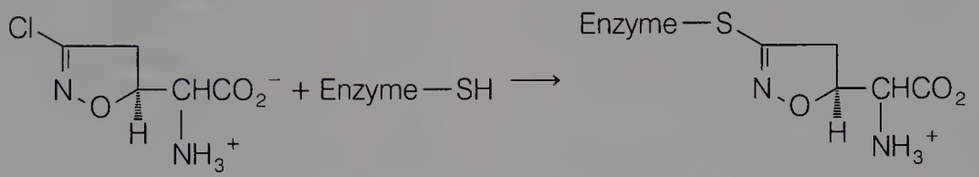


Mitomycin B, X = CH_3O
Mitomycin D, X = H_2N



Streptozocin, R = CH_3
Chlorozotocin, R = CH_2CH_2Cl

Acivicin is another antibiotic that has received clinical study. It is obtained from *Streptomyces svicens*, and it functions as an inhibitor of the amidotransferases involved in purine and pyrimidine biosynthesis.[234] The structure of acivicin shows a chlorine atom that can be replaced readily because it is located on an imine group. A cysteine residue at the active site of an amidotransferase replaces this chlorine, affording alkylation and irreversible inhibition of the enzyme. Phase I clinical studies revealed CNS toxicity for acivicin. It is thought that conversion of the antibiotic to ibotenic acid, a known CNS toxin found in mushrooms, by exchange of the chlorine for a hydroxyl group, might be responsible for this toxicity.



Acivicin

In 1978 scientists at the Upjohn Company reported the isolation of CC-1065 from *Streptomyces zelensis*.[235] This compound is composed of three pyrrolo [3,2-e]indoline units joined by amide bonds. Two of these subunits are virtually identical, but the third has a cyclopropane ring. The structure is curved and twisted in such a manner that it makes a precise fit in the minor groove of double helical DNA.[236] It prefers DNA sequences rich in adenine and thymine, where the cyclopropane ring can alkylate N(3) of an adenine (Fig. 12-5).[237] CC-1065 has remarkable antitumor potency, but delayed liver toxicity in mice prevented its clinical development. Numerous analogs of CC-1065 were synthesized, and one of them, adozelesin, has been introduced into clinical trials.[238] This analog retains intact the subunit bearing the cyclopropane ring, but the other two subunits are simplified. It retains significant antitumor activity without the delayed toxicity.

Compounds in the enediyne class of antibiotics show antitumor potencies in the microgram per kilogram range in mice, and they have a remarkable mode of DNA cleavage. Although the gross structures of these compounds differ widely, they have the common feature of a medium-sized ring (9 or 10 carbons) containing one olefinic bond and two acetylenic bonds.[239] Upon activation, this system is converted into a benzene diradical, which is able to simultaneously cleave two strands of double helical DNA. This process is illustrated for the neocarzinostatin A chromophore in Fig. 12-6. Activation begins with the addition of a thiol to the double bond of the chromophore, which causes a rearrangement of bonds and epoxide opening to give a conjugated system containing acetylene, alkene, and cumulene groups. In the rearranged system, the acetylene and cumulene groups are close enough that they form the benzene diradical. This diradical can remove hydrogen radicals from the 5′-methylene carbon of 2′-deoxyribose residues in DNA, which leads to cleavage of the strands.[240]

Neocarzinostatin is obtained from cultures of *Streptomyces carzinostaticus*,[241] and it occurs as a combination of the chromophore with an apoprotein of 113 amino acids. This globular protein stabilizes the chromophore to heat and light and augments its activity.[242] Large clinical trials of neocarzinostatin in Japan have indicated activity against both leukemia and solid tumors. Other enediynes of interest as potential antitumor drugs include the calicheamicins, esperamicins, and dynemicin.[239]

PRODUCTS

Dactinomycin, USP. Cosmegen, Actinomycin D, Actinomycin C_1, actinomycin IV, NSC-3053. This compound is obtained from the fermentation of selected strains of *Streptomyces parvullus*. It is soluble in alcohols and alcohol-water mixtures; however, these solutions are very sensitive to light. Vials containing 0.5 mg of lyophilized powder of the drug and 20 mg of mannitol are supplied. For reconstitution, 1.1 ml of Sterile Water for Injection, USP is added to the vial. The resulting solution is stable for 2 to 5 months at room temperature.

Only minimal metabolism of dactinomycin occurs. Its prolonged half-life may be explained by significant retention in lymphocytes and granulocytes. Dactinomycin intercalates between the base pairs of DNA and inhibits topoisomerase II. It selectively inhibits the synthesis of DNA-dependent ribosomal RNA and messenger RNA.[243]

Dactinomycin is used against rhabdomyosarcoma and Wilms' tumor in children.[244] It can be lifesaving for women with choriocarcinoma resistant to methotrexate. In combination with vincristine and cyclophosphamide, it has received some use in solid tumors in children. Toxic reactions include

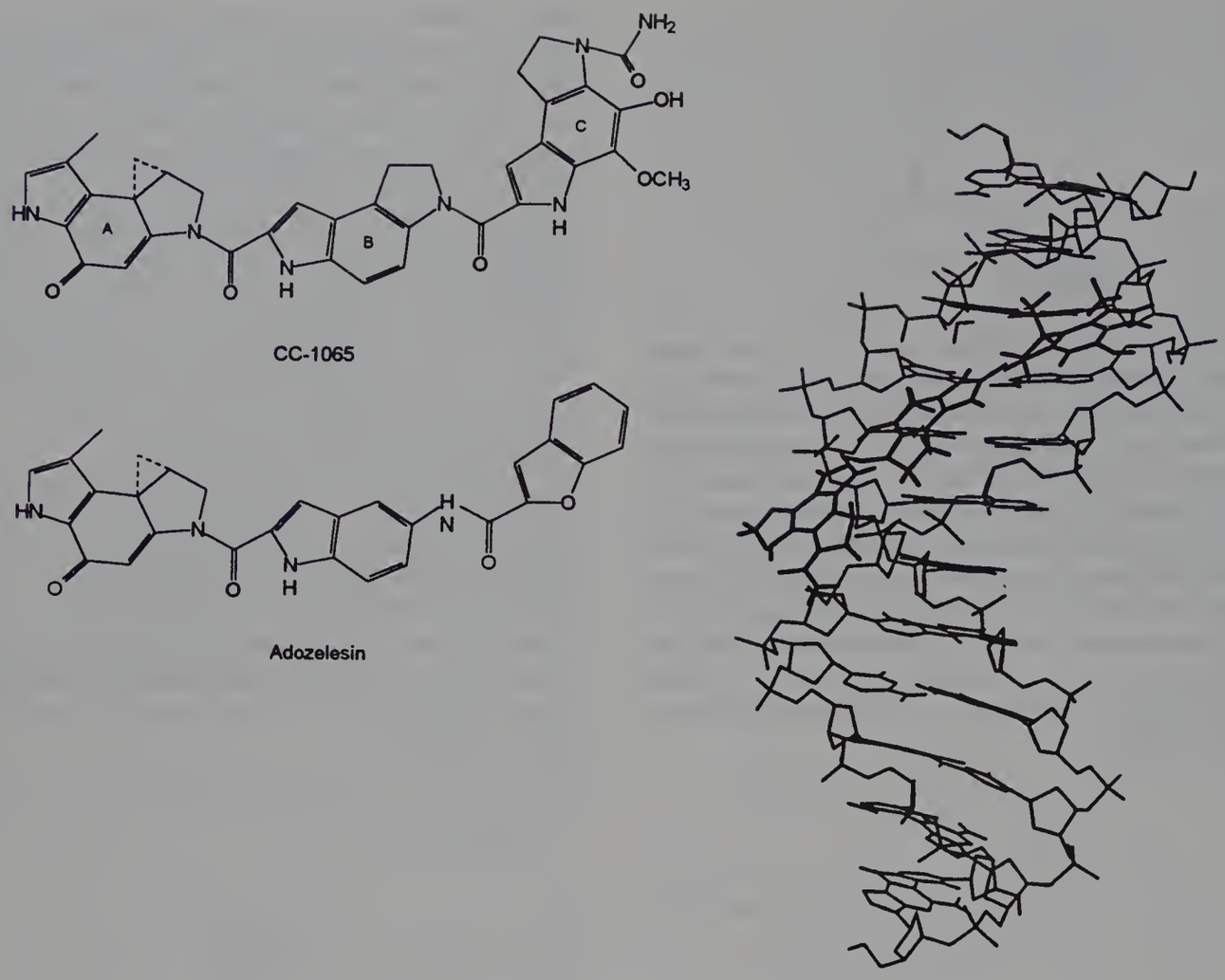


FIG. 12-5. *CC-1065 binding DNA.*

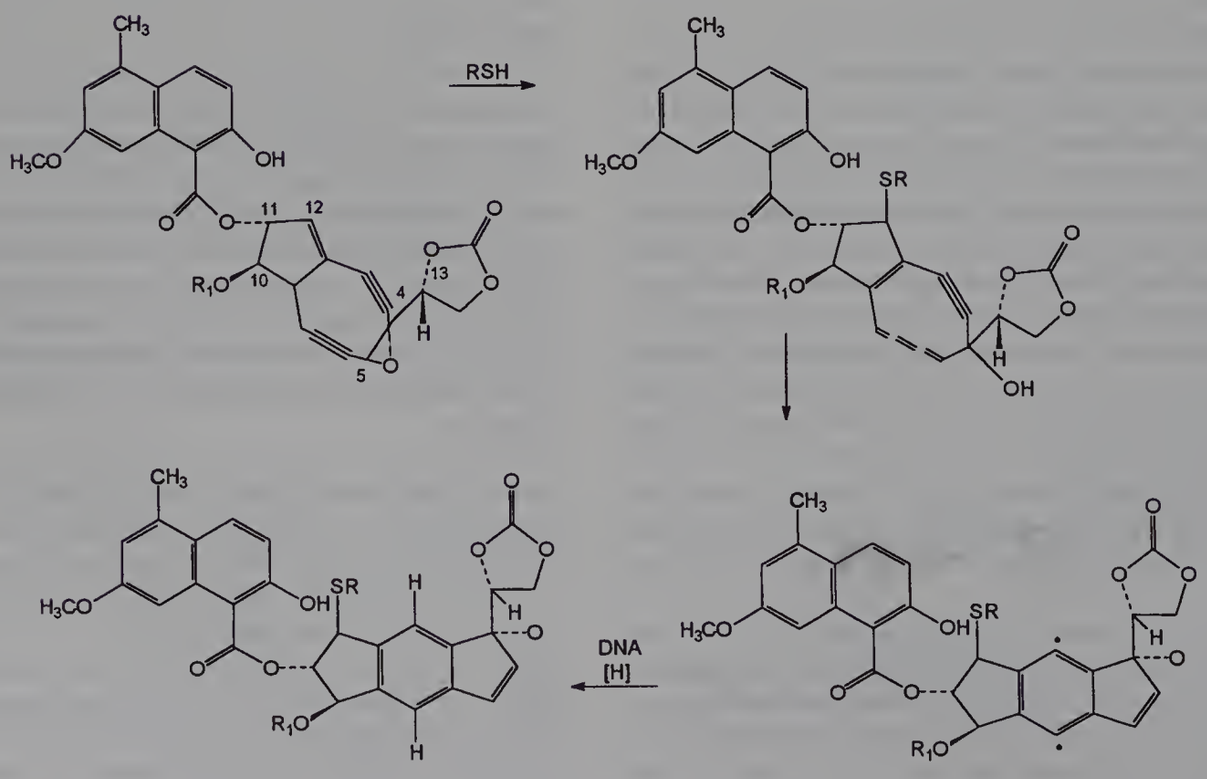


FIG. 12-6. *Activation of neocarzinostatin A chromophore.*

anorexia, nausea, and vomiting. Bone marrow depression, resulting in pancytopenia, may occur within a week after therapy. Alopecia, erythema, and tissue injury at the site of injection may occur.

Daunorubicin Hydrochloride. Cerubidin, daunomycin, rubidomycin, NSC-82151. It is obtained from the fermentation of *Streptomyces peucetius.*[190] The hydrochloride salt is a red crystalline compound that is soluble in water and alcohols. Daunorubicin hydrochloride is available as lyophilized powder in 20-mg vials. In this form, it is stable at room temperature, but after reconstitution with 5 to 10 ml of sterile water it should be used within 6 hr. A new liposomal formulation of daunorubicin known as DaunoXome is in phase II clinical trial. Significantly reduced toxicity, including cardiotoxicity, has been claimed for it.

The long terminal plasma half-life of daunorubicin results from extensive tissue binding. It is readily metabolized to daunorubicinol by reduction of its 13-keto group. This metabolite is one-tenth as active as daunorubicin. The drug and its metabolite are eliminated by hepatobiliary excretion.

A number of cellular lesions may contribute to the antitumor effects of daunorubicin. It intercalates into DNA and inhibits the ligase activity of topoisomerase II, resulting in decreased synthesis of both DNA and RNA. Redox cycling of the quinone functionality generates hydroxyl and superoxide radicals, which peroxidize lipids and damage cellular membranes. This effect may produce cardiotoxicity because heart cells are relatively deficient in antioxidant defenses.[245]

Daunorubicin is used in the treatment of acute lymphocytic and granulocytic leukemias.[246] Toxic effects include bone marrow depression, stomatitis, alopecia, and gastrointestinal disturbances. At higher doses, cardiac toxicity may develop. Severe and progressive congestive heart failure may follow initial tachycardia and arrhythmias.

The usual dose of daunorubicin is 30 to 45 mg/m^2 daily for 3 days. It is administered intravenously, taking care to prevent extravasation.

Doxorubicin Hydrochloride, USP. Adriamycin, NSC-123127, 14-hydroxy-daunomycin. This compound is obtained from cultures of *Streptomyces peucetius* variety caesius.[247] The orange-red needles are soluble in water and alcohols. Doxorubicin hydrochloride is supplied as a freeze-dried powder in two different sizes: 10 mg plus 50 mg of Lactose, USP, and 50 mg plus 250 mg of Lactose, USP. These amounts are reconstituted with 5 ml and 25 ml, respectively, of Sodium Chloride Injection, USP.

Following administration, doxorubicin is rapidly distributed to body tissues, with ~75% of it binding to plasma proteins. It is extensively metabolized and eliminated primarily as glucuronide conjugates of the parent aglycone or the 13-hydroxyl reduction product, doxorubicinol. A small amount of the 7-deoxyaglycone also is formed. Disposition and elimination can be explained by a two-compartment or three-compartment model. Liposome encapsulated doxorubicin (LED) is available in several formulations for clinical trials.

The modes of action of doxorubicin are similar to those described for daunorubicin.

Doxorubicin is one of the most effective antitumor agents. It has been used successfully to produce regressions in acute leukemias, Hodgkin's disease and other lymphomas, Wilms' tumor, neuroblastoma, soft-tissue and bone sarcomas, breast carcinoma, ovarian carcinoma, transitional-cell bladder carcinoma, thyroid carcinoma, and small-cell bronchogenic carcinoma.[248] Combination chemotherapy with a variety of other agents is being developed for specific tumors. The dose-limiting toxicities are myelosuppression and cardiotoxicity. There is a high incidence of bone marrow depression, primarily of leukocytes, that usually reaches its nadir at 10 to 14 days. Red blood cells and platelets also may be depressed. Thus, careful blood counts are essential. Acute left ventricular failure has occurred, particularly in patients receiving a total dose exceeding the currently recommended 550 mg/m^2. Cardiomyopathy and congestive heart failure may be encountered several weeks after discontinuing Adriamycin. Toxicity is augmented by impaired liver function, because this is the site of metabolism. Thus, evaluation of liver function by conventional laboratory tests is recommended before individual dosing.

The recommended dosage schedule is 60 to 75 mg/m^2 intravenously at 21-day intervals. This dose is decreased if liver function or bone marrow reserves are inadequate. Care must be taken to avoid extravasation.

Idarubicin Hydrochloride. Idamycin, IDA, 4-DMR, 4DDM, NSC-256439, 4-Demethoxydaunorubicin. This compound has been prepared by a number of synthetic routes.[249] The hydrochloride salt is formulated in single-dose vials containing 5 or 10 mg of orange lyophilized powder. It is reconstituted with 5 and 10 ml of sodium chloride for injection. These solutions are stable at least 7 days under refrigeration. Administration is intravenous, with care taken to avoid extravasation because of the potent vesicant action.

Idarubicin differs from daunorubicin by the lack of a methoxy group at the 4-position. Like daunorubicin, it intercalates DNA and inhibits topoisomerase II. Intravenous idarubicin is approved for therapy of acute nonlymphocytic leukemia in combination with cytarabine. It also is active against the blast phase of chronic myelogenous leukemia. The main dose-limiting toxicity is myelosuppression, especially leukopenia. It appears to be less cardiotoxic than doxorubicin and daunorubicin.[250]

Bleomycin Sulfate, Sterile, USP. Blenoxane. NSC-125066. This product is a mixture of cytotoxic glycopeptides isolated from a strain of *Streptomyces verticillus.*[251] The main component is bleomycin A_2 (~65%) and bleomycin B_2 (~20% to 30%) also is present. Bleomycin is a whitish powder that is readily soluble in water. It occurs naturally as a blue copper complex, but the copper is removed from the pharmaceutical form. It is supplied in ampuls containing 15 units of sterile bleomycin sulfate. The bleomycin unit is based on inhibitory activity against *Mycobacterium smegmatis* in culture: 0.1 mg of bleomycin equals 1 unit. Bleomy-

cin sulfate is reconstituted by dissolution in 1 to 5 ml of sterile water, D5W, or normal saline for injection.

Bleomycin undergoes rapid initial distribution with a half-life of 10 to 20 min, which is followed by an elimination half-life of 2 to 3 hr. It is inactivated readily in the liver and kidney and excreted in the urine.

The mode of bleomycin action involves binding to DNA followed by single- or double-strand cleavage. Transfer RNA also may be cleaved. This cleavage is caused by active oxygen species that are generated in a stepwise process from bleomycin-iron-oxygen complexes. The process is cell cycle–specific with the main effect in the G_2 and M phases.

Resistance to bleomycin is afforded by the cytosolic enzyme bleomycin hydrolase, which removes an amino acid from the molecule. This is especially a problem with sarcomas because they have high levels of the hydrolase.

Bleomycin is used for the palliative treatment of squamous cell carcinomas of the head and neck, esophagus, skin, and genitourinary tract, including penis, cervix, and vulva.[252] It also is used against testicular carcinoma, especially in combination with cisplatin and vinblastine.[253] The principal toxicities of bleomycin are in skin and lungs. Other tissues contain an aminopeptidase that rapidly inactivates it. Bleomycin has very little bone marrow toxicity; thus, it may be used in combination with myelosuppressive agents. Pulmonary toxicity is induced in ~10% of treated patients, with pulmonary fibrosis and death occurring in ~1%. Thus, cumulative doses of >400 units are not recommended. Skin or mucous membrane toxicity occurs in about half of the patients. Anaphylactoid reactions are possible in lymphoma patients.

The recommended dosage is 0.25 to 0.50 units/kg (10 to 20 units/m^2) given intravenously, intramuscularly, or subcutaneously once or twice weekly. For maintenance of Hodgkin's disease patients in remission, a dose of 1 unit daily or 5 units weekly is given. Blenoxane is stable for 24 hr at room temperature in sodium chloride or 5% dextrose solutions for injection.

Mitomycin, USP. Mutamycin, mitomycin C, NSC-26980. This compound is obtained from cultures of *Streptomyces caespitosus* as blue-violet crystals.[254] It is soluble in water and polar organic solvents. Vials containing either 5 mg of mitomycin and 10 mg of mannitol or 20 mg of mitomycin and 40 mg of mannitol are supplied. The unreconstituted product is stable at room temperature for at least 2 years. Reconstitution is effected by adding 10 ml of Sterile Water for Injection, USP, and administration is intravenous or intravesicle. The drug is rapidly cleared from the vascular compartment, and liver metabolism is the primary means of elimination.

Although it is a relatively stable compound, mitomycin C is activated by reduction to a bifunctional alkylating agent, which cross-links complementary DNA strands, resulting in inhibition of DNA synthesis. The 2-amino groups of guanine residues are alkylated, and the preferred DNA sequence is CpG.[255] There is no cell cycle specificity.

Mitomycin is useful in treating disseminated breast, gastric, pancreatic, or colorectal adenocarcinomas in combination with fluorouracil and Adriamycin (FAM program). It is used in combination with cyclophosphamide and Adriamycin for lung cancer. Recently, complete remissions of superficial transitional cell carcinomas of the bladder have been obtained in 60% of patients given intravesical mitomycin C instillations.[256] The dose-limiting toxicity is myelosuppression, characterized by delayed, cumulative pancytopenia. Fever, anorexia, nausea, and vomiting also occur.

Mitomycin at 10 to 20 mg/m^2 is given as a single dose by intravenous catheter. No repeat dose should be given until the leukocyte and platelet counts have recovered (~8 weeks).

Plicamycin, USP. Mithracin, aureolic acid, mithramycin, NSC-24559. This antibiotic is obtained from *Streptomyces plicatus*[206] or *Streptomyces argillaceus* as a yellow solid. It is soluble in polar organic solvents and aqueous alkali; however, it is susceptible to air oxidation in alkali. Mithramycin readily forms complexes with magnesium and other divalent metal ions, and these complexes have drastically altered optical rotations. Vials containing 2.5 mg of mithramycin as a freeze-dried powder, together with 100 mg of mannitol and disodium phosphate sufficient to give pH 7 when diluted with water are supplied. The drug is reconstituted by injecting 4.9 ml of Sterile Water for Injection, USP. Short intravenous infusions are used clinically.

Plicamycin is used in the treatment of advanced embryonal tumors of the testes.[232] However, it has been largely superseded by newer agents such as bleomycin and cisplatin. The main present use of mithramycin is in Paget's disease, in which it gives reduction of alkaline-phosphatase activity and relief of bone pain.[257] It also is useful in treating patients with severe hypercalcemia or hypercalciuria resulting from advanced metastatic cancer involving bones. Plicamycin may produce severe hemorrhaging. Bone marrow, liver, and kidney toxicity also occur. The lower total dose used for hypercalcemia results in less toxicity.

Streptozocin. Zanosar, NSC-85998, 2-(3-methyl-3-nitrosoureido)-2-deoxy-D-glucopyranose. This compound is obtained from cultures of *Streptomyces achromogenus* subspecies *streptozoticus*[258] or synthesized from D-glucosamine.[259] It is readily soluble in water or saline. Vials containing 1.0 g of lyophilized powder are supplied. They should be refrigerated at 35°F to 46°F and protected from light. The drug is reconstituted by adding 9.5 ml of either normal saline or Sterile Water for Injection, USP.

Unchanged drug is rapidly cleared from plasma after an intravenous bolus. Metabolites demonstrate triphasic plasma clearance with a short initial phase. Streptozocin undergoes spontaneous decomposition to form methylcarbonium ions, which alkylate DNA and inhibit new DNA synthesis.

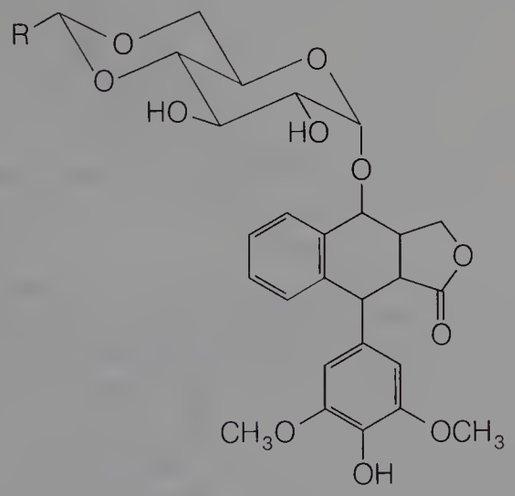
Streptozocin is indicated only for metastatic islet cell carcinoma of the pancreas.[260] Therapy is limited to patients with symptomatic or progressive disease, because of inherent renal toxicity of the drug. Up to two-thirds of patients treated with it experience renal toxicity. Adequate hydration

is recommended to reduce this toxicity. Nausea and vomiting occur in >90% of patients, which occasionally requires discontinuation of drug therapy. Liver dysfunction also occurs. It has been found to be mutagenic, carcinogenic, and teratogenic in animals. Carcinogenesis following topical exposure is a possible hazard. After rapid injection, unchanged drug is rapidly cleared from the plasma. The half-life is 35 min.

PLANT PRODUCTS

The use of higher plants in treating neoplastic disease dates to antiquity. Dioscorides described the use of colchicine for this purpose in the first century. In more recent years, scientists have attempted to select and screen systematically plants reputed to have antitumor activity. If the presence of activity is established for one member of a plant family, other members of this family are selected and tested. A major impetus to this research was given by Hartwell at the NCI, who established an extensive system of plant collection, screening, and isolation.[261] More than 100,000 plants have been screened under this program.

Resin of the may apple, *Podophyllum peltatum*, has long been used as a remedy for warts. One of its constituents, podophyllotoxin, has antineoplastic activity, but it is highly toxic.[262] This lignan inhibits mitosis by destroying the structural organization of the mitotic apparatus.[263] Early derivatives of podophyllotoxin showed poor clinical activity, but newer analogs, such as the epipodophyllotoxin derivatives etoposide and teniposide, are much better. Both of these analogs differ from podophyllotoxin in that they are inhibitors of topoisomerase II rather than microtubule assembly.[264]



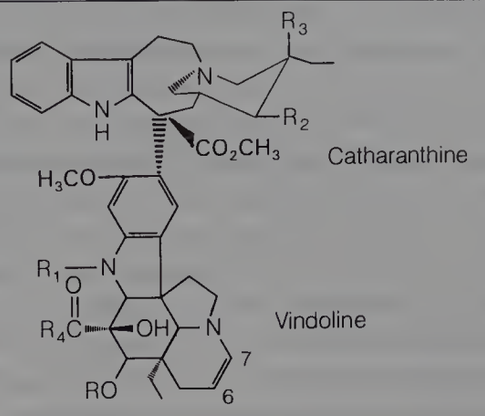
Etoposide, R = CH_3

Teniposide, R = 

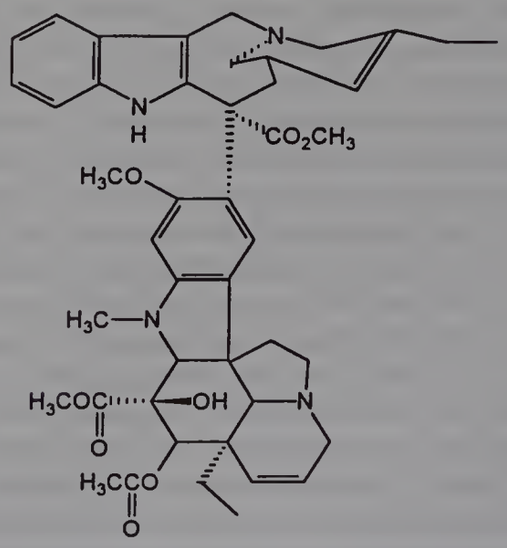
The vinca alkaloids are a family of important antitumor agents from plants. These compounds were isolated from the periwinkle *Catharanthus rosea* at the Eli Lilly Company.[265] They have complex structures composed of an indole-containing moiety, catharanthine, and an indoline-containing moiety, vindoline (Table 12-1).[266] Four closely related compounds have antitumor activity: vincristine, vinblastine, vinrosidine, and vinleurosine. Among this group, vincristine and vinblastine are proven clinical agents. These two compounds are used against different types of tumors, despite the similarity of their structures. A number of semisynthetic compounds have been prepared. Among them, vinglycinate and 6,7-dihydrovinblastine have clinical potential.[267] Vindesine has undergone Phase II clinical studies. It is considered to resemble vincristine but to be less neurotoxic.[268] A related vinca, vinorelbine, has activity in advanced lung cancer.

TABLE 12-1

VINCA ALKALOIDS AND THEIR ANALOGUES



	R	R_1	R_2	R_3	R_4
Vincristine	CH_3CO	CHO	H	OH	OCH_3
Vinblastine	CH_3CO	CH_3	H	OH	OCH_3
Vinrosidine	CH_3CO	CH_3	OH	H	OCH_3
Vinleurosine	CH_3CO	CH_3	Oxide		OCH_3
Vinglycinate	$(CH_3)_2NCH_2CO$	CH_3	H	OH	OCH_3
Vindesine	H	CH_3	H	OH	NH_2

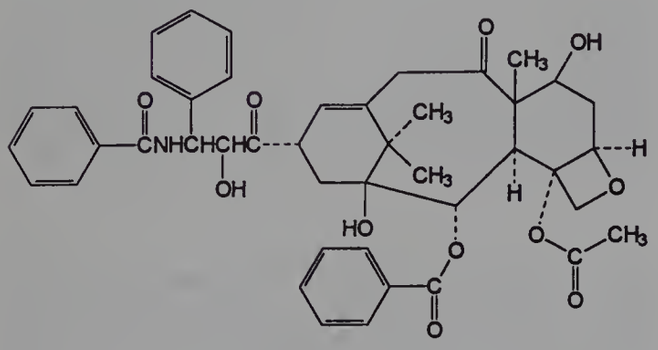


Vinorelbine

Vinca alkaloids cause mitotic arrest by promoting the dissolution of microtubules in cells. Microtubule crystals containing the alkaloids are formed in the cytoplasm.[269] Vin-

blastine is the most active compound, whereas vincristine is the only compound to cause irreversible inhibition of mitosis.[270] Cells can resume mitosis following brief exposure to other vinca alkaloids after these compounds are withdrawn.[271]

A plant product of high current interest in cancer chemotherapy is paclitaxel (Taxol). This compound was isolated from the bark of the Pacific yew tree, *Taxus brevifolia* by Wani et al. in 1971[272] and was found to have antitumor activity; however, there was little enthusiasm for its further development until recently, when its potential for human clinical activity was suggested by screening against human tumors in immunodeficient mice. It is now approved by the FDA and considered to be active against refractory ovarian cancer, metastatic breast cancer, metastatic melanoma, and non–small cell lung cancer.

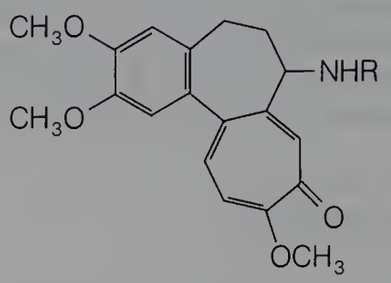


Paclitaxel

Paclitaxel inhibits mitosis by acting as a spindle poison; however, it acts by a unique mechanism in promoting the assembly of microtubules and stabilizing them against depolymerization.[273] This mechanism is in contrast to compounds like the vinca alkaloids, which prevent the assembly of microtubules.

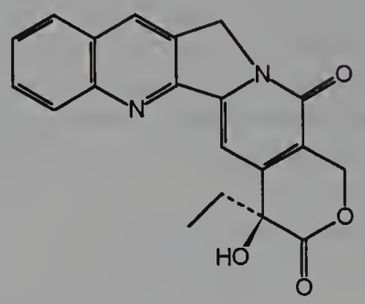
At the present time, paclitaxel is obtained by extracting the bark of *Taxus brevifolia*, a slow-growing tree containing only a small amount of the drug. This process is expensive and a threat to forest ecology. Consequently, the manufacturer, Bristol-Myers Squibb, has developed a route based on partial synthesis from 10-deacetylbaccatin III, which is obtained from the needles of *Taxus baccata*, a European yew tree. Because needles are rapidly regenerated, this is a less destructive method for obtaining paclitaxel. Furthermore, 10-deacetylbaccatin III is an important intermediate for the synthesis of analogs. One such analog, taxotere, has been prepared at Rhône-Poulenc Rorer. It is more water soluble than paclitaxel and reported to be more potent against solid tumors, but it is relatively more toxic than paclitaxel.[274]

As noted above, colchicine, obtained from the crocus *Colchicum autumnale*, has long been known for its antitumor activity. However, it is not now used clinically for this purpose. Its main use is in terminating acute attacks of gout. Among colchicine derivatives, demecolcine (colcemid) is active against myelocytic leukemia, but only at near-toxic doses. Colchicines have an unusual tricyclic structure containing a tropolone ring. They inhibit mitosis at metaphase by disorienting the organization of the spindle and asters.[275]

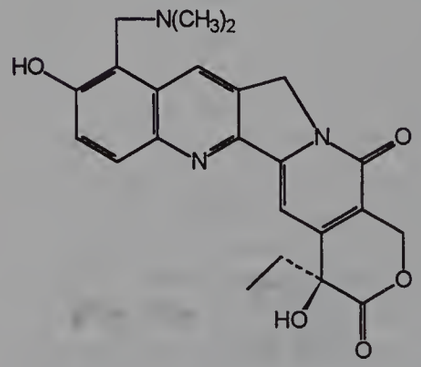


Colchicine, R = $COCH_3$
Colcemid, R = CH_3

One of the more important compounds under current investigation is topotecan, which is a semisynthetic analog of camptothecin.[276] Camptothecin was isolated from *Camptotheca acuminata*, an ornamental tree found in China.[277] It is very insoluble in water, but its sodium salt, prepared by alkaline hydrolysis of the lactone ring, showed promising antitumor activity. Clinical trials were eventually discontinued because of unpredictable toxic effects. Topotecan has a basic tertiary amine group, which can be protonated to solubilize the drug. The lactone ring remains intact and increases activity above that of the ring-opened sodium salt. Camptothecin and topotecan are inhibitors of topoisomerase I. They cause single-strand breaks in DNA, which results in lethal DNA damage during replication.[276]



Camptothecin



Topotecan

Many other plant constituents show significant antitumor activity in animals and have been given clinical evaluation. Some of the more important compounds are homoharringtonine, anguidine, and maytansine.[278]

PRODUCTS

Etoposide. VePesid, VP-16,213, NSC-141540. This compound is a semisynthetic derivative of podophyllotoxin. It is supplied in 5-ml ampules containing 20 mg/ml of the drug plus 30 mg benzyl alcohol, 80 mg polysorbate 80, 650 mg of polyethylene glycol 300, and absolute alcohol. This mixture is diluted with either 5% dextrose or 0.9% saline to give a final concentration of 0.2 or 0.4 mg/ml. Etoposide also is supplied as 50-mg capsules also containing sorbitol. They must be stored at 36°F to 46°F.

The pharmacokinetics of etoposide fit a two-compartment model. A terminal half-life of 7 hr is independent of the dose and method of administration. About 43% of a dose is recovered in the urine, of which 66% is unchanged drug.[279] The primary metabolites found in plasma are picro hydroxy acids and picro lactone, whereas the major urinary metabolite is 4′-demethylepipodophyllic acid. Oral bioavailability is ~50%.

Etoposide has marked schedule dependence with cytotoxic effects in the G_2 phase. It causes protein-linked DNA strand breaks by inhibiting topoisomerase II. Although etoposide does not bind directly to the DNA, it stabilizes a covalent intermediate form of the DNA–topoisomerase II complex.[280]

Etoposide in combination with other chemotherapeutic agents is the first choice treatment for small cell lung cancer. It also is effective in combination with other agents for refractory testicular tumors, and it has been used alone or in combination against acute nonlymphocytic leukemias, Hodgkin's disease, non-Hodgkin's lymphomas, and Kaposi's sarcoma. It is contraindicated in patients who develop hypersensitivity. Dose-limiting bone marrow suppression is the most significant toxicity, and reversible alopecia occurs frequently. Nausea and vomiting are usually controlled with standard therapy. On intravenous administration, the disposition of etoposide is biphasic with a distribution half-life of ~1.5 hr and an elimination half-life of 4 to 11 hr.

Teniposide. Vumon, ETP, VM-26, NSC-122819, 4′-demethylepipodophyllotoxin-β-thenylidine glucoside. This compound is prepared by treating epipodophyllotoxin with thiophene-2-carboxaldehyde.[281] It is supplied in 5-ml ampules in which each milliliter contains 10 mg of teniposide, 30 mg of benzyl alcohol, 60 mg of N,N-dimethylacetamide, 500 mg of Cremophor EL, maleic acid to adjust the pH to 5.1, and absolute alcohol to adjust the total volume to 1 ml. This preparation is stable for 4 years at room temperature. It is diluted with at least five equivalents of sodium chloride solution prior to intravenous infusion.

Teniposide is highly protein bound to albumin and displays biexponential decay. Most of the urinary excretion is as metabolites.

As a single agent, teniposide is active against Kaposi's sarcoma, lymphomas, multiple myeloma, cervical cancer, and small cell lung cancer.[282] It is active in combination with cytarabine against refractory acute lymphocytic leukemia, and this indication is the only one approved by the FDA. The dose-limiting toxicity is leukopenia. Thrombocytopenia is also observed. Chemical phlebitis at the injection site is common. As with etoposide, prolonged treatment with teniposide may cause secondary acute myelogenous leukemia.[283]

Vinblastine Sulfate, USP. Velban, vincaleucoblastine, VLB, NSC-49842. This antitumor alkaloid is isolated from *Vinca rosea* Linnaeus, the periwinkle plant.[265] It is soluble in water and alcohol. Vials containing 10 mg of vinblastine sulfate as a lyophilized plug are supplied. It is reconstituted by the addition of sodium chloride solution for injection preserved with phenol or benzyl alcohol.

Intravenous vinblastine is rapidly cleared from plasma and eliminated in a triphasic pattern. The apparent volume of distribution is three to four times the blood volume. A large portion (73%) is retained in the body, but some is excreted intact in urine and bile.[284] There is some metabolism to deacetyl vinblastine, which is more active than the parent compound.

The mode of action is tubulin binding, which results in inhibition of microtubule assembly and microtubule spindle formation. This binding causes the accumulation of cells in metaphase.

Vinblastine has been used for the palliation of a variety of neoplastic diseases. It is one of the most effective single agents against Hodgkin's disease, and it may be used in combination chemotherapy for patients who have relapses after treatment by the MOPP program. Advanced testicular germinal cell tumors respond to vinblastine alone or in combination. Beneficial effects are also obtained against lymphocytic lymphoma, histiocytic lymphoma, mycosis fungoides, Kaposi's sarcoma, Letterer-Siwe disease, resistant choriocarcinoma, and carcinoma of the breast. The limiting toxicity is leukopenia, which reaches its nadir in 5 to 10 days after the last dose. Gastrointestinal and neurological symptoms occur and are dose dependent. Extravasation during injection can lead to cellulitis and phlebitis.

Vincristine Sulfate, USP. Oncovin, leurocristine VCR, LCR, NSC-67574. This alkaloid is isolated from *Vinca rosea* Linnaeus.[265] The sulfate is a crystalline solid that is soluble in water. It is supplied in vials containing either 1 mg of vincristine sulfate and 10 mg of lactose, or 5 mg of vincristine and 50 mg of lactose. Each size has an accompanying vial of 10 ml of bacteriostatic sodium chloride solution containing 90 mg of sodium chloride and 0.9% benzyl alcohol. The reconstituted pharmaceutical may be stored 14 days in a refrigerator.

Following administration, vincristine is rapidly distributed to tissues and bound to formed blood elements. Elimination is triphasic, with more than half of the drug cleared

within 20 min. The primary mode of elimination is hepatic extraction with secretion into bile.

Vincristine binds reversibly to tubulin, producing a halt in microtubule assembly that arrests cell division in metaphase.[285] This temporary arrest causes a cell cycle block called strathmokinesis. Resistance to vincristine results from increased cellular levels of P-glycoprotein.

Vincristine is effective against acute leukemia. In combination with prednisone it produces complete remission in 90% of children with acute lymphoblastic leukemia.[167] It is used in the MOPP program of combination chemotherapy for Hodgkin's disease.[286] Other tumors that respond to vincristine in combination with other antineoplastic agents include lymphosarcoma, reticulum-cell sarcoma, rhabdomyosarcoma, neuroblastoma, and Wilms' tumor. Although the tumor spectra of vinblastine and vincristine are similar, there is a lack of cross-resistance between the two. Because vincristine is less myelosuppressive than vinblastine, it is preferred in combination with myelotoxic agents. The most serious clinical toxicity of vincristine is neurological, with paresthesias, loss of deep-tendon reflexes, pain, and muscle weakness occurring. These symptoms can usually be reversed by lowering the dose or suspending therapy. Constipation and alopecia also occur. The rapid action of vincristine in destroying cancer cells may result in hyperuricemia. This complication can be prevented by administering allopurinol.

Vinorelbine Tartrate. Navelbine. This compound is a new semisynthetic vinca alkaloid derived from vinblastine by loss of one carbon from ring C′ and dehydration in ring D′, both in the catharanthine moiety. It is named 3′,4′-didehydro-4′-deoxy-C′-norvincaleukoblastine. Navelbine is supplied in vials containing 10 mg/ml solution in a volume of 1 ml of Water for Injection or 10 mg/ml solution in a volume of 5 ml Water for Injection. Unopened vials are stable at room temperature for up to 72 hr. It is diluted to a concentration of 0.5 to 2 mg/ml with 0.9% Sodium Chloride Injection or 5% Dextrose Injection for intravenous infusion or slow intravenous push administration.

The primary mechanism of action of vinorelbine is binding to tubulin, resulting in inhibition of microtubule assembly. It may be more specific than other vinea alkaloids for mitotic microtubules. Vinorelbine has been approved by the FDA for unresectable advanced non–small cell lung cancer. The most important side effect is granulocytopenia.

Paclitaxel. Taxol. This diterpene is obtained from the needles and bark of the western yew, *Taxus brevifolia*,[272] or by partial synthesis from closely related compounds obtained from similar species. It is formulated as a concentrated sterile solution containing 30 mg of paclitaxel in a 5-ml ampule containing a mixture of 50% polyoxylated castor oil, Cremophor EL, and 50% dehydrated alcohol, USP. It is usually reconstituted in 50 ml of D5W. Solutions should be used within 24 hr of reconstitution. It is administered by intravenous infusion only.

Disposition of paclitaxel from plasma follows a biphasic elimination pattern. Approximately 97.5% of it is bound to plasma proteins. Clearance is triphasic and results mainly from hepatic extraction and biliary excretion. Eleven metabolites have been detected in plasma, but not identified.[287]

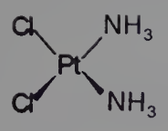
Paclitaxel is a mitotic spindle poison that acts by a unique process. It promotes assembly of microtubules and stabilizes them against depolymerization. This process blocks cycle traverse in mitosis.[273] Paclitaxel is highly active against refractory ovarian cancer and effective against metastatic breast cancer, metastatic melanoma, and non–small cell lung cancer. It is used in combinations containing doxorubicin, cisplatin, and filgrastim (a human granulocyte colony-stimulating factor produced by recombinant DNA technology). Hypersensitivity occurs in some patients within 10 min of starting an infusion. It has been suggested that the allergen is the Cremophor EL diluent.[288] Reversible peripheral neuropathy is common with prolonged infusions. Bradycardia, gastrointestinal disturbances, flu-like symptoms, and total body alopecia also occur.

MISCELLANEOUS COMPOUNDS

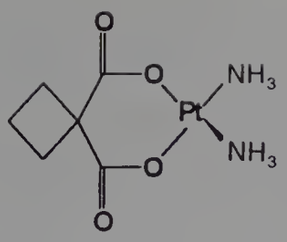
In 1965, Rosenberg investigated the effects of electrical fields on bacteria and found that *Escherichia coli* formed long filaments instead of dividing.[289] He subsequently discovered that this effect was caused not by the electrical current, but by a complex, $[Pt(Cl)_4(NH_3)_2]^\circ$, formed from the platinum electrode in the presence of ammonium and chloride ions.[290] This discovery was followed by testing a variety of platinum neutral complexes against tumors, with the result that cis-dichlorodiammineplatinum II (cisplatin) eventually became established as a clinical agent.[291]

This platinum complex is a potent inhibitor of DNA polymerase. Its activity and toxicity resemble those of the alkylating agents. Considerable evidence has been obtained for DNA binding by the platinum complex, wherein the two chlorides are displaced by nitrogen or oxygen atoms of purines. This evidence includes facilitated renaturation, increased sedimentation coefficient, hyperchromicity of the DNA ultraviolet spectrum, and selective reaction of the complex with guanine over other bases.[292]

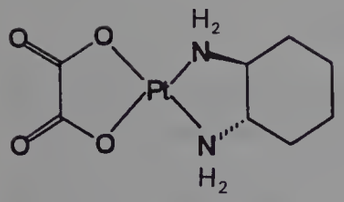
Many other platinum complexes have been found active against tumors. Generally, they fall into the classification of cis-isomers in which one pair of ligands are monodentate anions of intermediate leaving ability (such as chloride) or bidentate anions (such as malonate), and the other pair are mono- or bidentate amines.[293] Among the more significant analogs is carboplatin, which is approved by the FDA for treatment of ovarian cancer and which also is used against lung, genitourinary, and head and neck cancer.[294,295]



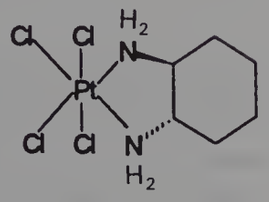
Cisplatin



Carboplatin



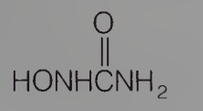
Oxaliplatin



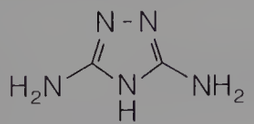
Ormaplatin

Other platinum complexes of current interest are oxaliplatin, which has oxalate and 1,2-diaminocyclohexane as ligands[296] and ormaplatin, a Pt (IV) complex whose six ligands include four chlorides and 1,2-diaminocyclohexane. Ormaplatin must be reduced to dichloro-1,2-diaminocyclohexane Pt (II) for activation.[297]

Hydroxyurea has been known for >100 years, but its antitumor activity was not discovered until 1963.[297] It is active against rapidly proliferating cells in the synthesis phase, during which it prevents the formation of deoxyribonucleotides from ribonucleotides. The mode of its action is inhibition of ribonucleotide diphosphate reductase, an enzyme consisting of two protein subunits.[298] It does this by interfering with the iron-containing portion of one of these subunits.[299]



Hydroxyurea



Guanazole

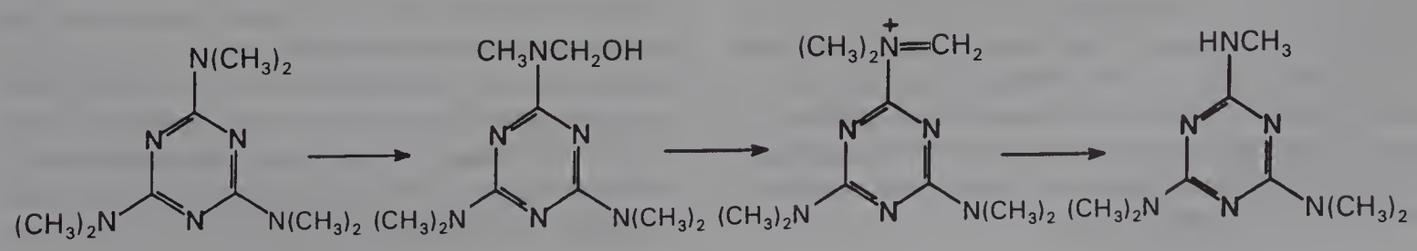
Another very old compound recently found active against tumors is guanazole.[300] This diaminotriazole resembles hydroxyurea in its ability to limit DNA synthesis by inhibiting the reduction of ribonucleotides. It is clinically active in inducing remissions of acute adult leukemia.[301]

In 1953, Kidd found that injections of guinea pig serum caused regressions of certain transplanted tumors in mice and rats.[302] Subsequent investigation revealed that these tumors required L-asparagine as a nutrient, but the presence of the enzyme L-asparaginase in the guinea pig serum created a deficiency in this amino acid.[303] The practical preparation of L-asparaginase for clinical trials follows the discovery that *Escherichia coli* produces a form of it that has antineoplastic activity.[304] Thus, mass cultures are harvested and treated with ammonium sulfate to rupture the cells, and the liberated enzyme is isolated by solvent extraction and chromatography. Very pure material is obtained by gel filtration or affinity chromatography, followed by crystallization. The *E. coli* enzyme has a molecular weight of 120,000 to 141,000 daltons, an isoelectric point of 4.9 to 5.2, and a K_m of 1.2×10^{-5}.[305]

Earlier preparations of L-asparaginase contained endotoxicins from *E. coli*, but these are absent in the purer new preparations. Clearance of the enzyme from plasma is due to an immunological reaction in which it combines with protein. This reaction may lead to sensitization in some patients. Patients who cannot tolerate L-asparaginase from *E. coli* might be treated by the preparation from *Erwinia carotovora*.[306] Tumor resistance is based on the development of asparagine synthetase by the tumor cells.[307]

Altretamine (hexamethylmelamine) is approved by the FDA for use as a single agent for resistant ovarian cancer.[308] It is rapidly metabolized to pentamethylmelamine, tetramethylmelamine, and seven other compounds. Pentamethylmelamine also has antitumor activity. A suggested mode of action for altretamine is hydroxylation of one of the methyl groups to give the corresponding hydroxymethyl compound.[309] This compound is a carbinolamine that can lose hydroxide ion to form an immonium ion capable of either alkylation of a macromolecule or hydrolysis to pentamethylmelamine. This process could be repeated in converting pentamethylmelamine to an alkylating agent or to tetramethylmelamine.

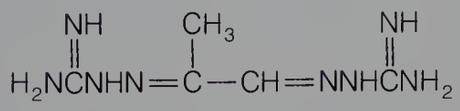
Methylglyoxal bis(guanylhydrazone) (mitoguazone) has antitumor activity in humans. It interferes with polyamine synthesis to block nuclear and mitochondrial metabolism.[310] Many of its actions are related to the functions of spermidine, which it resembles in structure. Thus, it competes with spermidine for the transport carrier and intracellular binding site. It also inhibits spermidine biosynthesis. Its antiproliferative



Hexamethylmelamine

Pentamethylmelamine

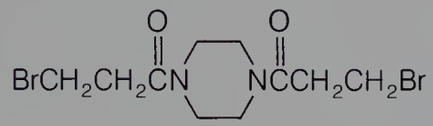
effects on cells can be prevented by administering spermidine.[311] Many other bis(guanylhydrazones) have been prepared, but none has proven superior to the methylglyoxal derivative.



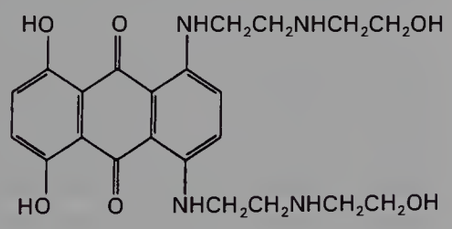
Mitoguazone
[Methylglyoxal Bis(guanylhydrazone)]

Spermidine

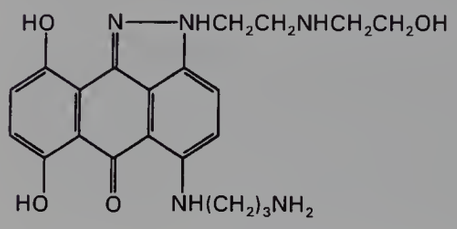
Pipobroman is used against hematological cancers, especially polycythemia vera and chronic granulocytic leukemia.[312] Its mode of action is unknown, although it has sometimes been included among the alkylating agents. It has not been possible to demonstrate alkylating properties in vitro under physiological conditions.



Pipobroman



Mitoxantrone

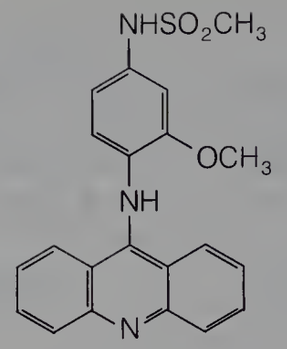


Piroxantrone

Among the newer antineoplastic drugs, 4-[(9-acridinyl) amino]methanesulfon-m-anisidide (m-AMSA) showed a wide spectrum of activity in early clinical trials. It afforded some remissions in refractory cases of breast cancer, malignant melanoma, and acute myelocytic leukemia. Leukopenia is the limiting toxicity.[313]

m-AMSA (amsacrine) is an acridine derivative that is thought to bind to DNA through intercalation. However, it does not affect DNA synthesis.[314] This compound was rationally designed as one member of a group of acridinylaminomethanesulfonamides.[315] Previously, a number of other acridine derivatives had shown antitumor activity.

The clinical importance of anthracyclines stimulated the synthesis and screening of anthraquinones with partial anthracycline structures. One of the best of these analogs is mitoxantrone, which has two hydroxyl and two 2-[(2-hydroxyethylamino)ethyl]amino substituents on the anthraquinone nucleus.[316] Like doxorubicin, mitoxantrone intercalates into DNA and inhibits DNA topoisomerase II;[317] however, it is not a substrate for reductases and does not form oxygen-free radicals in a redox cycling process. Consequently, it is less cardiotoxic than doxorubicin. Mitoxantrone is approved for inducing remissions in acute nonlymphocytic leukemia, usually in combination with cytarabine.



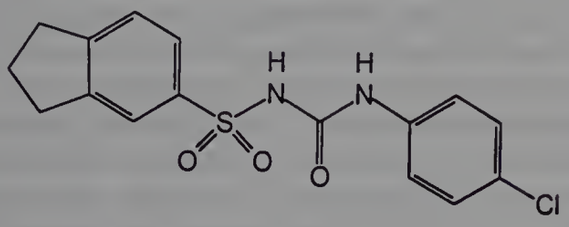
Amsacrine
(m-AMSA)

Piroxantrone is an anthrapyrazole structurally related to mitoxantrone in having two phenolic hydroxyls and side chains containing amino groups; however, its quinone ring is modified to form part of a pyrazole ring with a nitrogen atom on the next ring.[318] The mode of action of piroxantrone is intercalation and interference with DNA synthesis by template inhibition. Cardiotoxicity is low because it does not undergo redox cycling.[319] Piroxantrone is presently in clinical trial.

The antiparasitic drug, suramin sodium (Chap. 7), has long been used to treat trypanosomal and filarial infections. It also inhibits reverse transcriptase in RNA tumor viruses. Antitumor activity has been demonstrated in hormonally refractive prostate cancer[320] and advanced ovarian carcinoma. Suramin acts by a variety of biological mechanisms, including inhibition of hyaluronidase, urease, hexokinase, RNA polymerase, DNA topoisomerase II, and lysosomal enzymes.[321] It affects ATP synthesis and degradation and inhibits mitochondrial enzymes. Signal transduction in cells is inhibited by binding to tumor growth factors and protein

kinases.[322] Suramin may also inhibit angiogenesis and induce normal cellular differentiation by increasing tissue glycosaminoglycans.[323]

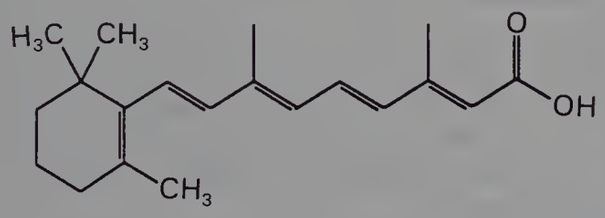
Sulofenur is a diarylsulfonurea with broad antitumor activity against mouse tumors.[324] In phase I clinical studies, it has given responses in patients with refractory ovarian cancer, head and neck cancer, and refractive seminoma.[325] The mode of action of sulofenur is not known, although it has been suggested that it promotes apoptosis in tumor cells.[326]



Sulofenur

Although antitumor activity was found for gallium nitrate in phase II clinical trials,[327] its approved use is for the treatment of cancer-related hypercalcemia.[328] It has proven to be superior to calcitonin and etidronate in this use. The clinical material is the nonahydrate of $Ga(NO_3)_3$.

Tretinoin (*trans*-retinoic acid) is a normal metabolite of retinol (vitamin A). It can induce normal differentiation in a variety of malignant cells, especially acute promyelocytic leukemia cells, and its differentiating effects are augmented by a variety of other agents, including cytotoxic drugs, cytokines, and polar solvents.[329] Topical tretinoin produces regression of basal cell carcinoma in most patients and reduces the size of cutaneous lesions in AIDS-related Kaposi's sarcoma.[330]



Tretinoin

Retinoid effects are mediated by retinoic acid cytosolic receptors, which aid in their transfer into the nucleus. In the nucleus, there are three types of retinoic acid receptors that control transcription of genes involved in cellular growth inhibition and differentiation by interacting directly with DNA sequences termed retinoic acid response elements.[331]

Sargramostim (GM-CSF, Leukine) is a natural human protein produced by recombinant DNA techniques using yeast or bacteria as the host organism. This partially glycosylated protein contains 120 to 127 amino acids and two internal disulfide bridges. The yeast-derived product is approved by the FDA to promote bone marrow recovery in patients with leukemias or lymphomas undergoing autologous bone marrow transplantation.[332]

Filgrastim also promotes the production of neutrophil precursors in the bone marrow. This granulocyte colony stimulating factor (G-CSF) is a 175–amino acid protein manufactured by recombinant DNA technology. It is identical in sequence with the natural protein except for an N-terminal methionine necessary for expression in *E. coli*. It has no glycosylation because of its production in *E. coli*.

PRODUCTS

Cisplatin. Platinol, NSC-119875, CDDP. This compound is prepared by treating potassium chloroplatinite with ammonia.[291] It is a water-soluble white solid supplied in amber vials containing 10 mg of cisplatin as a lyophilized powder. For reconstitution, 10 ml of sterile water is added, and the resulting solution is diluted in 2 L of 5% dextrose in 0.5 or 0.33 N saline containing 37.5 g of mannitol.[333]

Cisplatin has a triphasic disappearance curve with the following half-lives: α, 20 min; β, 48 to 70 min; γ, 24 hr. Glomerular filtration and tubular secretion in the kidney removes 90% of the dose.

Interaction with DNA is the primary mode of cisplatin activity. Intrastrand cross-links are produced, and they cause changes in DNA conformation that affect replication.[334]

Cisplatin is used in combination with bleomycin and vinblastine for metastic testicular tumors. This combination represents a significant improvement over previous treatments.[335] As a single agent or in combination with doxorubicin, cisplatin is used for the remission of metastic ovarian tumors. Other tumors that have shown sensitivity to cisplatin include penile cancer, bladder cancer, cervical cancer, head and neck cancer, and small-cell cancer of the lung. The major dose-limiting toxicity is cumulative renal insufficiency associated with renal tubular damage. Hydrating patients with intravenous fluids prior to and during cisplatin treatment significantly reduces the incidence of renal toxicity.[336] Myelosuppression, nausea and vomiting, and ototoxicity also occur frequently.

The usual dosage for metastatic testicular tumors is 20 mg/m^2 intravenously daily for 5 days, once every 3 weeks for three courses. Metastatic ovarian tumors are treated with 50 mg/m^2 intravenously once every three weeks. Pretreatment hydration is recommended for both regimens.[333]

Carboplatin. Paraplatin, CBDCA, JM8, NSC-241240, *cis*-diammine(1,1-cyclobutanedicarboxalato)platinum (II). This compound is prepared by treatment of *cis*-Pt $(NH_3)_2I_2$ with silver sulfate followed by the barium salt of 1,1-cyclobutanedicarboxylic acid.[337] It is ~10 times as soluble in water as cisplatin, and its rate of hydrolysis is much slower than that of cisplatin. Hydrolysis of the carboxalato bonds yields transient aquated intermediates that bind to DNA. Carboplatin is supplied in vials containing 50, 150, and 450

mg of sterile lyophilized powder plus an equivalent amount of mannitol. The vials have a shelf-life of 3 years. For reconstitution, the drug typically is diluted with 500 ml of sodium chloride for infusion or D5W, and it is administered by infusion.

Plasma clearance of carboplatin is biphasic with up to 65% excreted in the urine. There is little bound to plasma proteins and no true metabolism.

Carboplatin is approved by the FDA for treatment of advanced ovarian cancer. It is cross-resistant with cisplatin in this tumor. Activity also has been reported in non–small cell lung cancer, head and neck cancer, and testicular cancer. The usual dose-limiting toxicity is bone marrow suppression, especially thrombocytopenia. Nephrotoxicity is much less common than with cisplatin.[338]

Hydroxyurea, USP. Hydrea, hydroxycarbamide, NSC-32065. This compound is prepared from hydroxylamine hydrochloride and potassium cyanide.[339] It is a crystalline solid with good solubility in water. Capsules containing 500 mg of hydroxyurea are supplied.

Following passive diffusion into cells, hydroxyurea inhibits ribonucleotide reductase, and this action results in decreased levels of deoxyribonucleotides.[340] Hydroxyurea may interfere with the function of the enzyme by chelating with its ferrous iron cofactor.

Hydroxyurea is well absorbed after oral administration, and it produces peak serum levels of 0.3 to 2.0 mM ~1 to 2 hr later. Approximately 50% of a dose is degraded in the liver and excreted as urea and CO_2. Acetohydroxamic acid is a major metabolite in humans.

Hydroxyurea is active against melanoma, chronic myelocytic leukemia, and metastatic ovarian carcinoma. It is used in combination with radiotherapy for head and neck cancer. The main toxicity is bone marrow depression expressed as leukopenia, anemia, and, occasionally, thrombocytopenia. Gastrointestinal toxicity and dermatological reactions also occur.

Pipobroman, USP. Vercyte, NSC-25154, 1,4-bis(3-bromopropionyl)piperazine. This compound is prepared from piperazine and 3-bromopropionyl bromide.[341] It is supplied as 10- or 25-mg grooved tablets.

Pipobroman is used primarily for treating polycythemia vera. It also is used in patients with chronic granulocytic leukemia refractory to busulfan. Nausea, vomiting, abdominal cramping, diarrhea, and skin rash occur.

Asparaginase. L-Asparaginase EC 3.5.1.1, colaspase, L-ASP, L-asnase, L-asparaginase amidohydrolase, NSC-109229 (*E. coli*), Erwinia asparaginase (NSC-106997). Asparaginase is an enzyme isolated commercially from *E. coli* and *Erwinia caratovora*. It has four subunits each with a molecular weight of 32,000 to 34,000 and one active site per subunit.[342] The isolate is not pure, and the potency varies with the batch. Consequently, batch potencies are rated in terms of the international asparaginase unit (IU), which is the enzyme activity sufficient to release 1 μmol of ammonia from L-asparagine in 1 min under the test conditions. Lyophilized asparaginase is provided in 10,000-IU vials also containing 80 mg of mannitol (*E. coli*) or 20 mg of dextrose and 0.6 mg of sodium chloride (*E. caratovora*). It should be stored under refrigeration. Reconstitution is with 2 to 5 ml of normal saline or sterile water.

The reconstituted drug is given by infusion or intramuscular injection. Allergic reactions can occur in up to 25% of patients. They include life-threatening anaphylactic shock. Skin testing before administration is recommended by the manufacturer. Patients allergic to the *E. coli* preparation may be switched to the Erwinia preparation; however, the cross-over anaphylactoid rate is ~25% in children. L-Asparaginase conjugated with polyethylene glycol (Peg-Asparaginase) exhibits minimal immunogenicity, although gastrointestinal toxicity may be greater. Asparaginase has very poor extravascular tissue penetration and is slowly and unpredictably cleared from plasma. Biphasic elimination with an initial half-life of 4 to 9 hr and a terminal half-life of 1.4 to 1.8 days was described.[343]

Asparagine is required for the biosynthesis of proteins. Although normal cells have the ability to synthesize asparagine, tumors such as acute lymphoblastic leukemia (ALL) lack this ability and depend on exogenous compound. Administration of asparaginase reduces the concentration of asparagine in plasma, making it unavailable to the leukemia cells.[344] Asparaginase is used in combination chemotherapy to induce remissions in ALL, and Peg-asparagine has shown activity in non-Hodgkin's lymphoma. In addition to hypersensitivity, side effects include gastrointestinal damage, hepatic toxicity, and pancreatitis.

Altretamine. Hexalen, hexamethylmelamine; NSC-13875, N,N,N′,N′,N″,N″-hexamethyl-1,3,5-triazine-2,4,6-triamine. It is prepared from dimethylamine and cyanuric chloride[76] and formulated as 50-mg hard gelatin capsules that also contain lactose and calcium stearate. They should be stored at room temperature in a tightly sealed bottle containing a desiccant.

Administration is orally, and there is considerable variation in the bioavailability and pharmacokinetics. It is rapidly metabolized by N-demethylation through hepatic microsomes. The main metabolites include pentamethylmelamine and tetramethylmelamine. A possible mode of action involves hydroxylation of a methyl group to the corresponding hydroxymethyl derivative, a carbinolamine that can lose hydroxide ion to form an immonium ion capable of either alkylation or hydrolysis to the monomethylamine.[77]

The FDA approval for altretamine is as a single agent for resistant ovarian cancer.[78] It also is active in combination regimens against this tumor. Gastrointestinal toxicity, manifested as anorexia, nausea, and vomiting, is dose limiting. Neurotoxicity occurs, but it is usually reversible.

Mitoxantrone Hydrochloride. Novantrone, DHAD, NSC-301379, 1,4-Dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)

amino]ethyl]amino]-9,10-anthracenedionedihydrochloride, DHAQ (free base). This compound is prepared from 2,3-dihydroxyquinazarine by treatment with 2-(2-amino-ethylamino)ethanol followed by chloranil oxidation.[345] It is supplied in vials containing 10, 12.5, or 15 ml of a 2 mg/ml sterile solution that is stable for years at room temperature. Sodium chloride, sodium acetate, and acetic acid are present as inactive ingredients. These preparations are diluted with at least 50 ml of sodium chloride for injection or 5% dextrose for injection and administered as an infusion.

Mitoxantrone is bound up to 78% to plasma proteins. The serum concentration-time profile is fit by a three-compartment model that has an α-phase half-life of 2.4 to 15 min corresponding to distribution into formed blood elements, a β-phase of 17 min to 3 hr corresponding to redistribution into blood and various tissues, and a γ-phase of 2.9 to 298 hr.[346] Highest concentrations of the drug are found in the liver, pancreas, thyroid, spleen, heart, and bone marrow. Large amounts of drug may be retained in these organs for prolonged times.

The mode of action of mitoxantrone involves intercalation and inhibition of topoisomerase II.[347] In contrast to doxorubicin, it does not undergo redox cycling to form oxygen-free radicals, because its redox potential is outside the reductive capability of mammalian reductases. Mitoxantrone is approved for remission-induction therapy in acute nonlymphocytic leukemia, where it typically is used with cytarabine.[348] It also is active against other leukemias, breast cancer, and ovarian cancer. The dose-limiting toxic effect is myelosuppression, which usually involves leukopenia. Other toxic effects include nausea and vomiting. Cardiac toxicity can occur with long-term administration of high doses of mitoxantrone.

Gallium Nitrate. Ganite, NSC-15200. This compound is prepared by the reaction of gallium metal with nitric acid. The clinical material is supplied as 500 mg of the nonahydrate $Ga(NO_3)_3 \cdot 9H_2O$ in a 20-ml single dose flip-top vial. Also present is 28.75 mg of sodium citrate dihydrate and sodium hydroxide for pH adjustment to 6.0 to 7.0. The daily dose is diluted in 1,000 ml of 0.9% Sodium Chloride Injection, USP or in 5% Dextrose Injection, USP for intravenous infusion.

The approved use of gallium nitrate is for treating cancer-related hypercalcemia.[328] It showed antitumor activity for patients with lymphoma in phase II trials.[327] Gallium nitrate probably works in hypercalcemia by inhibiting calcium resorption from bone, although the precise mechanism is unknown. Major side effects include hypocalcemia and nephrotoxicity. On continuous infusion, the drug has a biphasic elimination with an α-half life of 8.3 to 26 min and a β-half life of 6.3 to 96 hr. Between 69% and 91% of the dose was recovered in the urine.[349]

Sargramostim Leukine (yeast-derived, Immunex), Prokine (Hoechst-Roussel), Leukomax (*E. coli*–derived, Schering), granulocyte-macrophage colony-stimulating factor (GM-CSF). Sargramostim is produced by recombinant DNA methods using as host organisms *Saccharomyces cerevisiae* (yeast) or *E. coli.* It contains 120 to 127 amino acids, and the tertiary structure is maintained by two disulfide bridges. Two arginine sites are variably glycosylated in the yeast-derived preparation. Leukine differs from native sargramostim by substitution of leucine at position 23 and by a different carbohydrate makeup. It has a specific activity of ~5 $\times$ 10^7 U/mg of protein. Leukine is available commercially as lyophilized powder in 250- and 500-μg amounts. It is reconstituted with 1.0 ml of Sterile Water for Injection, USP to yield a clear isotonic solution at pH 7.4. Further dilutions in 0.9% sodium chloride should include 0.1% (v/v) of human serum albumin to reduce absorption to the glass surface. Vials should be discarded within 6 hr of reconstitution because there is no antibacterial preservative. Prokine also is supplied in 250- and 300-μg vials and is reconstituted like Leukine. Leukomax is available for investigational use from the Schering corporation. Most doses of sargramostim are administered by infusion, although it is active by the subcutaneous route.

Sargramostim is used to promote bone marrow recovery in patients undergoing autologous bone marrow transplantation.[332] It also reduces the severity and duration of neutropenia following standard chemotherapy with myelosuppressive agents. The mode of action of sargramostim is an interaction with high-affinity cell receptors on neutrophils. Signal transduction may involve coupling to a G-protein.[350] Following treatment with sargramostim, there is a biphasic increase in circulating leukocytes, including an initial increase that peaks after 4 to 5 days and a second increase over the next 5 days.[351] The dose-limiting toxicities are pericarditis, fluid retention, and venous thromboses. Other side effects include a flu-like syndrome and bone pain. The latter is managed with nonsteroidal anti-inflammatory agents.

Filgrastim. Neupogen granulocyte-colony stimulating factor (G-CSF). This compound is manufactured by recombinant DNA methods using *E. coli* as the host organism. It contains 175 amino acids identical with those in the natural protein except for an N-terminal methionine necessary for expression in *E. coli.* Neupogen is supplied in single-use vials, each containing 300 mcg/ml of filgrastim at a specific activity of 1.0 $\pm$ 0.6 $\times$ 10^8 U/mg. It is formulated in 10 mM sodium acetate buffer at pH 4.0 containing 50 mg of mannitol, 0.004% Tween 80, and 1.0 ml of Water for Injection. This formulation is stable for 24 months at 36°F to 46°F. The recommended dosage is 5 mcg/kg/day administered subcutaneously or intravenously.

Filgrastim is indicated to decrease the incidence of infection in patients with nonmyeloid malignancies receiving myelosuppressive anticancer drugs associated with a significant incidence of severe neutropenia with fever. A complete blood count and platelet count should be obtained prior to

chemotherapy and twice weekly during therapy with filgrastim. The only consistently observed adverse reaction to filgrastim is bone pain.

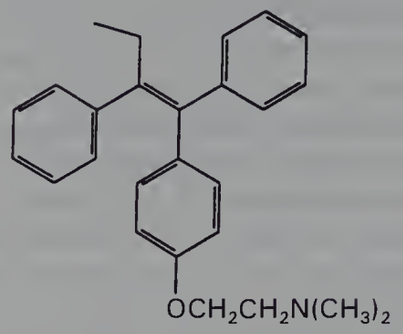
Absorption and clearance of filgrastim follows first-order kinetics with a positive linear correlation between the parenteral dose and both the serum concentration and area under the concentration-time curves. The elimination half-life is ~3.5 hr.

HORMONES

Steroid hormones, including estrogen, androgens, progestins, and glucocorticoids, act on the appropriate target tissues at the level of transcription. Generally, the effect is derepression of genetic template operation, which stimulates the cellular process. However, glucocorticoids act in lymphatic tissues to impair glucose uptake and protein synthesis. Target cells contain in their cytoplasm specific protein receptors with very high affinities for the hormones. Binding of the hormone to the receptor causes a transformation in the receptor structure, which is followed by migration of the resulting complex into the nucleus. In the nucleus, the complex interacts with an acceptor site to influence transcription.[352]

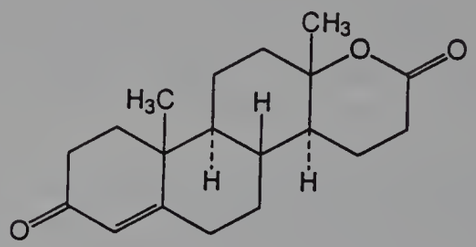
Normal and well-differentiated neoplastic target cells have a number of hormone receptors, and they are dependent upon the hormones for stimulation.[353–356] Less differentiated neoplastic cells become independent of hormonal control and lose their specific receptors. Thus, some neoplasms are hormone dependent and responsive to hormone-based therapy, whereas others are independent and unresponsive. Assays of the number of hormone receptors present in the neoplastic cells should be valuable in predicting the probability of a favorable response.

Hormonal effects in breast cancer are complex and not completely understood. The hormone dependency of breast cancer has been known since 1889,[357] and removal of the ovaries of premenopausal women, which results in decreased estrogens, is an established treatment. Some patients who do not respond to this procedure do respond to adrenalectomy, which suggests that the hormone dependence is not simply related to estrogens.[358] It has been shown that remission after adrenalectomy occurs more often in patients with estrogen receptors than in those lacking receptors. Administration of estrogens to postmenopausal women with metastatic breast cancer resulted in objective remissions in ~30% of the cases.[359] This response appears paradoxical, but the estrogen levels resulting from drug treatment are much greater than physiological levels. A recent suggestion is that high estrogen levels interfere with the peripheral action of prolactin, a pituitary hormone that also stimulates breast tissue.[360] Ethinyl estradiol is given orally in the treatment of breast cancer in postmenopausal women, and estradiol dipropionate or benzoate is used parenterally. Tamoxifen is an antiestrogen that has been used successfully in the treatment of postmenopausal women. It has very low toxicity.[361]



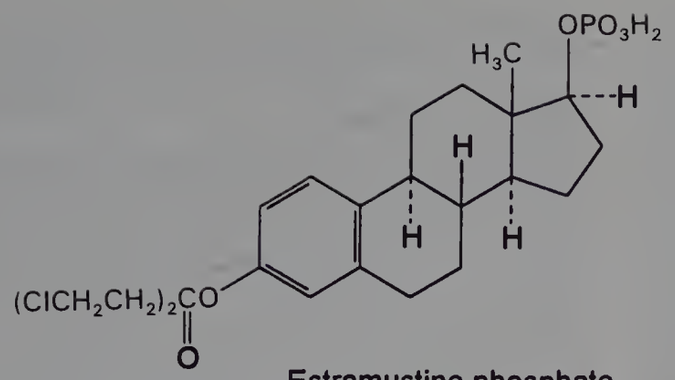
Tamoxifen

Androgens are active against metastatic breast cancer in ~20% of postmenopausal women. Their mode of action is not completely understood. Inhibition of the release of pituitary gonadotrophins has been suggested, but the situation must be more complicated than this because certain androgens are active in hypophysectomized patients.[362] Other useful effects of androgens in advanced breast cancer are stimulation of the hematopoietic system and reversal of bone demineralization. Testosterone propionate is the androgen most frequently used against breast cancer. Other compounds are 2α-methyltestosterone, fluoxymesterone, and 19-nor-17α-methyltestosterone. Testolactone is preferred in some cases because it has no androgenic side effects.

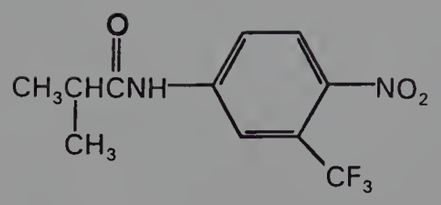


Testolactone

Estrogens can be used to induce remissions of disseminated prostatic cancer. It is not certain whether their effect is due to direct interference with peripheral androgens, inhibition of pituitary gonadotrophin, or both.[363] Diethylstilbestrol is the compound most widely used for advanced prostatic cancer, and it benefits >60% of patients. Chlorotrianisene also is used. Estramustine phosphate was designed to carry the nitrogen mustard group selectively into cells with estrogen receptors; however, it does not alkylate them.[364] It appears to act as an anti-androgen, and it promotes microtubule disassembly. The main therapeutic use is in prostate carcinoma.[365] Flutamide is a nitroaniline amide that acts as an androgen receptor antagonist. It is used in combination chemotherapy of prostate cancer.



Estramustine phosphate

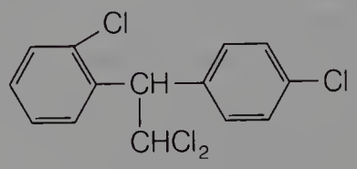


Flutamide

Progesterone and its analogs are active against certain neoplasms that are stimulated by estrogens. They appear to exert anti-estrogenic effects of uncertain mechanism. The neoplasms treated by progestins are metastatic endometrial carcinoma and advanced renal cell carcinoma.[366] Progesterone suspension in oil, megestrol acetate, and medroxyprogesterone acetate are used against endometrial cancer. They provide regressions of several months to 3 years in ~30% of women.[366] Medroxyprogesterone acetate causes regression of renal cell carcinoma in <10% of men and women.

Glucocorticoids cause pronounced acute changes in lymphoid tissues. Lymphocytes in the thymus and lymph nodes are dissolved, and lymphopenia occurs in peripheral blood.[367] In lymphocytic tissues, glucocorticoids promote programmed cell death (apoptosis) by a receptor-mediated active process that induces endonucleolytic cleavage of DNA. This property is used to advantage in the treatment of leukemia and Hodgkin's disease, wherein profound temporary regressions are observed following the administration of cortisone derivatives or ACTH.[368] Prednisone is usually the corticoid chosen for this purpose, and it is almost always used in combination with other chemotherapeutic agents such as mechlorethamine, vincristine, and procarbazine. Such combinations are effective in maintaining the remissions in many cases. Glucocorticoids also are useful in treating metastatic prostate cancer of patients who have relapsed after castration. The rationale for this use is that they inhibit release of ACTH from the pituitary, which leads to adrenal atrophy and decreased adrenal production of androgens.[369] Prednisone and cortisone acetate are used in the treatment of metastatic breast cancer. Their value in this condition derives not from an antineoplastic effect, but in alleviating specific complications such as hypercalcemia and anemia.[370]

Mitotane is unique among antitumor agents in its highly selective effect on one gland, the adrenal cortex. It has a direct cytotoxic action on adrenal cortical cells, in which it extensively damages the mitochondria.[371] This leads to cell death and atrophy of the gland. Mitotane is used specifically against adrenocortical carcinoma.[372]



Mitotane

Another way in which the proliferation of hormone-dependent tumors can be limited is to inhibit the release of gonadotropins from the anterior pituitary gland. This release is controlled by gonadotropin-releasing hormone (LH-RH), a nonapeptide, and it can be blocked effectively by continuous administration of certain analogs of this hormone. Leuprolide is a nonapeptide that is identical in structure with LH-RH, except that it has D-leucine replacing the natural L-leucine as the sixth amino acid. It is used for palliative treatment of advanced prostatic cancer.

A thorough discussion of the structures, nomenclature, properties, and dose forms of the steroid hormones is presented in Chap. 23. Only the products not included in detail in that chapter are described below.

PRODUCTS

Mitotane, USP. Lysodren, o,p-DDD, CB133, 1,1-dichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl)ethane. This compound is obtained as a constituent of commercial DDD, which is prepared from 2,2-dichloro-1-(o-chlorophenyl)ethanol, chlorobenzene, and sulfuric acid.[373] Isolation from commercial DDD gives mitotane as crystals that are soluble in alcohol and other organic solvents.[374] Scored 500-mg tablets are supplied.

About 40% of a single oral dose of mitotane is absorbed. Only 10% to 25% is excreted in urine as an unidentified metabolite, and 60% is excreted unchanged in feces. Most of the remainder is stored in fatty tissues of the body.

Mitotane is indicated only for treating inoperable adrenal cortical carcinoma. Frequently occurring side effects include gastrointestinal disturbances, CNS depression, and skin toxicity.

The usual regimen is 8 to 10 g daily, divided into three or four doses.

Dromostanolone Propionate, USP. Drolban, 17β-hydroxy-2α-methyl-5α-androstan-3-one propionate, 2α-methyldihydrotestosterone propionate. This semisynthetic androgen is prepared from dihydrotestosterone in a route involving condensation with ethyl formate followed by hydrogenation to give the 2α-methyl derivative and then reaction with propionic anhydride.[375] The compound is supplied in rubber-stoppered vials containing 500 mg of dromostanolone propionate in 10 ml of sesame oil, with 0.5% phenol as a preservative.

Dromostanolone propionate is used in the palliative treatment of metastatic breast carcinoma in postmenopausal women. It is contraindicated in premenopausal women and in carcinoma of the male breast. The most usual side effect is virilism, although this is less intense than that afforded by testosterone propionate. Edema occurs occasionally.

Testolactone, USP. Teslac, D-homo-17α-oxa-androsta-1,4-dien-3,17-dione, 1-dehydrotestolactone. This compound is prepared by microbial transformation of progesterone.[376] It is soluble in alcohol and slightly soluble in water. The compound is supplied as a sterile aqueous suspension providing 100 mg/ml of testolactone in multiple-dose vials of 5 ml. Tablets containing 50 mg or 250 mg of testolactone also are supplied.

Testolactone is used in the palliative treatment of advanced or disseminated breast cancer in postmenopausal women. It is contraindicated in breast cancer in men. Testolactone is devoid of androgenic activity in the commonly used doses.

Megestrol Acetate. Megace, 17α-acetoxy-6-methylpregna-4,6-dien-3,20-dione. This compound is prepared by a multistep synthesis from 17α-hydroxy pregnadienolone.[377] It is supplied as light blue scored tablets containing 20 or 40 mg of megestrol acetate.

Megestrol acetate is indicated for the palliative treatment of advanced breast or endometrial carcinoma when other methods of treatment are inappropriate. No serious side effects or adverse reactions have been reported. However, there is an increased risk of birth defects in children whose mothers take the drug during the first 4 months of pregnancy. In high doses, it can cause weight gain without inducing fluid accumulation. The usual doses are 160 mg/day in four equal doses for breast cancer and 40 to 320 mg/day in divided doses for endometrial cancer.

Tamoxifen Citrate. Nolvadex, (Z)-2-[4-(1,2-diphenyl-1-butenyl)phenoxy]-N,N-dimethylethanamine citrate. This compound is prepared by treating 2-ethyldeoxybenzoin with 4-[(2-N,N-dimethylamino)ethoxy]phenylmagnesium bromide,[378] followed by dehydration and separation of the E and Z isomers.[379] The citrate salt of the Z isomer is soluble in water. Tablets containing 15.2 mg of tamoxifen citrate, which is equivalent to 10 mg of tamoxifen, are supplied. They should be protected from heat and light.

The majority of a dose of tamoxifen is excreted in bile as conjugates of metabolites. N-Demethyltamoxifen is the primary metabolite, and its chronic levels exceed those of tamoxifen. It is thought to account for a large portion of the antitumor activity.[380]

Tamoxifen is a nonsteroidal agent that has shown potent antiestrogenic properties in animals. In the rat model, it appears to exert its antitumor effects by binding to estrogen receptors.[381] This binding causes a conformational change that leads to decreased DNA transcription. It is cell cycle–specific for the mid-G_2 phase. Tamoxifen is useful in the palliative treatment of advanced breast cancer in postmenopausal women. There are no known contraindications. The most frequent side effects are hot flashes, nausea, and vomiting. They are rarely severe enough to require dose reduction. The usual dose is one or two 10-mg tablets twice daily.

Flutamide. SCF-13521, Groganil, Eulexin, Euflex, Flucinom, Flugeril, Sebatrol, 2-methyl-*N*-[4-nitro-3-(trifluoromethyl)phenyl]propanamide. This compound is prepared from 3-trifluoromethyl-4-nitroaniline and isobutyryl chloride.[382] It is supplied as 125-mg capsules that are stable for 5 years when stored at or below 30°C. Administration is oral.

Flutamide is extensively and rapidly metabolized. One hour after dosing, only 2.5% of the drug in plasma is unchanged. The major metabolite in plasma is α-hydroxyflutamide; however, the major metabolite found in urine is 2-amino-5-nitro-4-(trifluoromethyl)phenol, which results from cleavage of the side chain.

Flutamide acts as an androgen receptor antagonist, inhibiting the uptake and binding of testosterone and dihydrotestosterone.[383] It is approved for treatment of advanced prostate cancer when combined with an inhibitor of gonadotropin-releasing hormone such as leuprolide. The most common side effects are gynecomastia and nipple pain.

Estramustine Phosphate. Estrocyte, Emcyt, NSC-89199, estra-1, 3, 5(10)-triene-3, 17β-diol-3-[bis(2-chloroethyl) carbamate] 17-disodium phosphate. Estramustine is prepared by treating estradiol with sodium hydroxide followed by nitrogen mustard chloroformate.[384] The water-soluble disodium phosphate derivative is made using phosphorus oxychloride followed by sodium hydroxide. It is available commercially in 140-mg capsules that are orally active. One to 10 capsules are used daily, and they may be taken with meals to lessen gastrointestinal upset. About 75% of the oral dose is absorbed.[385] The biologic half-life is long, and the drug undergoes dephosphorylation to estramustine followed by glucuronide conjugation and elimination in bile and urine. There also is some metabolism of the alkylating functionality.

Estramustine was designed to have an estrogenic molecule carry the alkylating nitrogen mustard functionality selectively into cells with estradiol hormone receptors; however, it may not act as an alkylating agent.[364] It is active in prostate cancer because of its estrogenic (anti-androgenic) effects. Another possible mode of action is binding to microtubule-

associated proteins to promote microtubule disassembly.[365] The dose-limiting toxicity is gastrointestinal upset. Gynecomastia also occurs.

Leuprolide Acetate. Lupron is a synthetic nonapeptide analog of naturally occurring gonadotropin-releasing hormone (LM-RM). It is supplied in 2.8-ml multiple dose vials containing 5 mg/ml of the drug and benzyl alcohol. These vials should be refrigerated until dispensed.

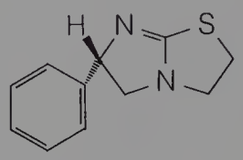
Leuprolide is used for palliative treatment of advanced prostatic cancer. There are no known contraindications. Worsening of symptoms may occur during the first few weeks of treatment, with increase in bone pain as the usual manifestation. Hot flashes and irritation at the injection site also occur.

IMMUNOTHERAPY

It is now generally accepted that cells of neoplastic potential are continually produced in the human body and that our immune surveillance system destroys them. The development of tumors implies that this system is not functioning properly. Evidence for this factor in carcinogenesis includes a high rate of cancer in organ-transplant patients whose immune systems are suppressed by drugs such as azathioprine, and a high correlation between cancer and immunodeficiency diseases such as bacterial and viral infections.[386] Stimulation of the body's immune system should provide a valuable method of cancer treatment, because it is capable of eradicating the neoplastic cells completely. Research in this area is expanding rapidly, and some promising leads are emerging.

The first attempt at immunotherapy was made in the 1890s by Coley, who injected bacterial toxins in cancer patients. His results were generally unaccepted because of rather extravagant claims. However, his techniques have been revived in recent years. Most oncologists now use a live bacteria tuberculosis vaccine named bacillus Calmette-Guerin (BCG).[387] This vaccine is given to certain patients who show a functioning immune system as determined by sensitivity toward dinitrochlorobenzene.[387] Remissions have been obtained in malignant melanoma, breast cancer, and leukemia. Unfortunately, BCG causes a number of undesirable effects, including fever, hypersensitivity, and liver disorders. Other immunostimulants currently under investigation as anticancer agents are the methanol-extracted residue (MER) of BCG, *Corynebacterium parvulum, Bordetella pertussis* vaccine, and synthetic polynucleotides.[388] The activity of these bacterial products is thought to be mediated by a protein known as tumor necrosis factor (TNF). This factor produces hemorrhagic necrosis of sensitive transplanted tumor cells, and it is synergistic with interferons.[389] Unfortunately, TNF does not show activity against primary tumors when used alone. However, clinical trials based on expected synergism with interferons are in progress. Potentiation of TNF by agents such as mitomycin C and vinblastine suggest that it might have a role in combination chemotherapy.

One approach to overcoming the difficulties of BCG therapy is to develop simpler chemical structures with immunostimulant properties. One such compound, levamisole, an anthelmintic agent found to be an immunostimulant by Renoux in 1972, is presently under clinical investigation as a potential anticancer drug. It appears to be most effective in patients with small-tumor burdens, and it acts by stimulating the responsiveness of lymphocytes to tumor antigens. Advantages of levamisole include oral activity and few adverse reactions. Levamisol may mediate the potentiation of interleukin-2–induced T-lymphocyte proliferation.[390] It is used in combination with 5-fluorouracil in treating colon cancer.



Levamisole

The induction phase of the immune response of both B and T lymphocytes is regulated by interactions between macrophages and subpopulations of T lymphocytes known as helper T cells. This interaction induces the production of soluble glycoproteins, lymphokines, which include interferons, interleukins, and B cell growth and differentiation factors. Lymphokines cause profound enhancing or suppressing effects on responding precursor cells of the immune system at nanomolar to picomolar concentrations.[391]

Interferons are secreted by cells in response to viral infections or other chemical or biological inducers. Three major classes of interferons—alpha, beta, and gamma—have been identified. They bind to specific high-affinity receptors on cell surfaces, which induces a sequence of intracellular events, including the induction of enzymes. This process produces effects, including release of other cytokines such as interleukin-2 and tumor necrosis factor, enhancement of natural killer cell activity, inhibition of certain oncogenes, and increase in the specific cytotoxic activity of lymphocytes for target cells.[391] Interferons alfa-2a, alfa-2b, and alfa-n3 promote the immunological response to neoplastic cells, which results in significant cytotoxicity in some instances. They are the drugs of choice for treating hairy cell leukemia.[392] They also are showing responses against renal cell cancer, multiple myeloma, melanoma, and Kaposi's sarcoma in clinical trials.

Another important lymphokine is interleukin-2 (IL-2). This glycoprotein interacts with specific receptors on T effector cells to activate their cytotoxicity. It also stimulates the activation and proliferation of antigen-nonspecific natural killer cells, which are involved in immune functions associated with tumor surveillance. These effects are thought to be mediated through the induction of gamma interferon. Human IL-2 is now produced by recombinant gene technology, which has permitted extensive clinical trials against a variety of tumors. In some of these trials, the IL-2 is given in combination with lymphokine-activated killer cells.

PRODUCTS

Interferon Alfa-2a. Roferon-A, rIFN, IFLrA. This is a highly purified protein containing 165 amino acids. It is manufactured from a strain of *Escherichia coli* bearing a genetically engineered plasmid containing an interferon alfa-2a gene from human leukocytes.[393] Vials containing 3 million IU with 5 mg of human serum albumin and 3 mg of phenol are supplied. A preparation of 18 million IU also is available. These preparations should be stored at 36°F to 48°F.

Interferon alfa-2a is used in patients 18 years old or older for treatment of hairy cell leukemia and for chronic myelogenous leukemia. It is contraindicated in persons who develop hypersensitivity. A majority of patients develop flu-like syndromes consisting of fever, fatigue, myalgias, headache, and chills. Gastrointestinal and CNS symptoms also occur. Caution must be used in administering the drug to patients with renal or hepatic disease, seizure disorders, or cardiac disease. Metabolism occurs by rapid proteolytic degradation during reabsorption in the kidney. The elimination half-life is 3.7 to 8.5 hr.

Interferon Alfa-2b. Interon, IFN-alfa 2, rIFN-α2, α-2-interferon. This is a highly purified protein produced by *E. coli* containing a plasmid with an alfa-2b gene. This plasmid is obtained from recombinant DNA technology using human leukocytes.[393] The drug is supplied in vials containing 3, 5, 10, or 25 IU. Sterile water diluent also is supplied. Reconstituted solutions are stable for 1 month at 36°F to 48°F.

Hairy cell leukemia is the present indication for interferon alfa-2b. It is also useful in treating malignant melanoma and renal cell carcinoma. Hypersensitivity to this protein has not been observed. Patients develop a flu-like syndrome, CNS effects, and cardiovascular effects, including hypotension, arrhythmia, or tachycardia.

Interferon Alfa-n3. This is a glycoprotein produced from cultures of human leucocytes treated with Sendai virus. It is purified initially by chromatography using a mouse monoclonal antibody that binds to multiple species of human interferon. Subsequent purification involves incubation at 4°C and pH 2 to kill viruses and gel filtration chromatography. It then has a specific activity of ~2 $\times$ 10^8 IU.[393] The drug is supplied in 1-ml vials containing 1 ml of phosphate buffered saline solution, phenol as a preservative, and 1 mg of human serum albumin as a stabilizer. This solution should be kept at 2°C to 8°C. Therapeutic indications and side effects of interferon alfa-n3 are similar to those described for interferons alfa-2a and alfa-2b.

Aldesleukin. Proleukin, Teceleukin, Interleukin-2, IL-2, T-cell growth factor. Human interleukin-2 is produced in mature T lymphocytes. Cleavage of a 20–amino acid signal sequence then results in an active protein containing 133 amino acids. It is glycosylated and has one disulfide bond, which is essential for activity. Recombinant IL-2 is produced from *E. coli* into which modified human IL-2 genes have been inserted. Teceleukin is nonglycosylated, but conforms to natural IL-2 in amino acid sequence, except for an additional *N*-terminal methionine. Proleukin is not glycosylated and differs from the natural protein in lacking the terminal alanine and having serine rather than cysteine for residue 125. Interleukin-2 activity is standardized in IU in an assay based on stimulation of T-cell growth in vitro. Proleukin is available in glass vials containing 1.2 mg of lyophilized powder (22 million IU) plus sodium decyl sulfate. It is reconstituted with 1.2 ml of solution supplied to give 18 million IU/ml. Teceleukin is supplied in vials containing 100 mg of IL-2, 25 mg of human albumin, and 5 mg of mannitol per million IU. It is reconstituted with Sodium Chloride for Injection, USP. All formulations of IL-2 require storage at 4°C to 8°C and protection from light.

Interleukin-2 usually is administered intravenously, although the subcutaneous and intramuscular routes are used. The infusion can produce severe hypotension and life-threatening cardiovascular toxicity when given at the maximally tolerated dose. Patients receiving such a dose must be closely monitored, and facilities must be available to treat them for hypotension, tachycardia, pulmonary edema, and, occasionally, delirium. The drug is rapidly cleared from the bloodstream following parenteral administration. The elimination is biphasic for an intravenous bolus injection. The primary route of elimination is renal, with catabolism occurring in the renal tubules.

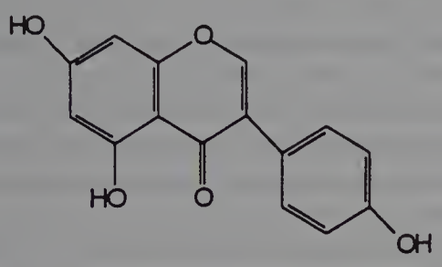
Interleukin-2 interacts with specific receptors on activated T lymphocytes. The resulting complex is internalized, and signal transduction, possibly involving tyrosine kinase activity, occurs.[394] Effects of IL-2 include stimulation of T-cell growth and regulation, proliferation and immunoglobulin production in B lymphocytes, macrophage activity enhancement, and especially generation of lymphokine-activated killer (LAK) cells. Those LAK cells generated within tumors, known as tumor-infiltrating lymphocytes (TIL), are thought to be the ultimate mediators of IL-2 toxicity. Therapy with IL-2 involves in vitro generation of large quantities of LAK cells, which are then infused with IL-2 to mediate tumor cell lysis.[395] This procedure is known as adoptive immunotherapy. Antitumor activity has been observed in metastatic renal cell cancer, chronic lymphocytic leukemia, malignant melanoma, malignant lymphoma, and colon cancer. All patients receiving IL-2 experience a flu-like syndrome. The major dose-limiting side effect is pulmonary edema resulting from an increase in capillary permeability.

Bacillus Calmette-Guerin (BCG). TheraCys, BCG live, NSC-116341 (Tice BCG). Two BCG preparations are approved for intravesical treatment of carcinoma in situ of the urinary bladder.[396] Connaught BCG (TheraCys) is a freeze-dried suspension of an attenuated strain of *Mycobacterium bovis* that has been grown on a potato- and glycerin-based medium. It contains 27 mg (~3.4 $\times$ 10^8 CFU) and 5% monosodium glutamate per vial. The Tice BCG is supplied in glass-sealed ampules that contain 8 $\times$ 10^8 CFU equivalent to ~50 mg of the drug. The vials should be stored at 2°C to 8°C and protected from light. Persons handling BCG preparations should be protected by masks and gloves. After ad-

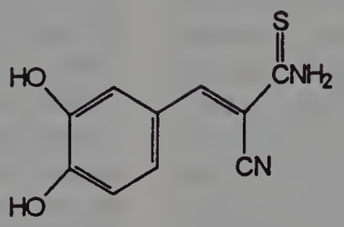
ministration, all equipment and material should be considered biohazards.

Intravesicle BCG promotes an inflammatory reaction in the urinary bladder that is associated with reduction in carcinoma in situ lesions. The mechanism of action is not known in detail, although a variety of processes that stimulate the immune response have been considered. Toxic effects of BCG include hematurea, dysurea, and bacterial urinary tract infections.

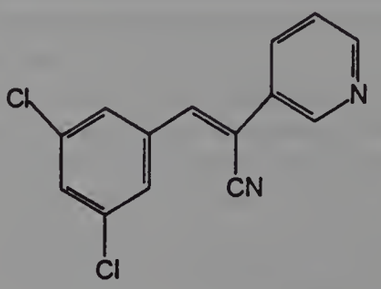
enzyme is present in normal cells. There also are serine protein kinases and other nucleotide-dependent enzymes. Nevertheless, a naturally occurring isoflavone, genestein, inhibits the protein tyrosine kinase of EGFR without significantly affecting serine and threonine kinases. A synthetic compound of the tyrphostin class inhibits the EGFR over insulin receptor tyrosine kinase. Second generation tyrphostins that lack hydroxyl groups are metabolically stable and active against human tumors in immunodeficient mice.[399]



Genestein



First generation tyrphostin



Second generation tyrphostin

FUTURE ANTINEOPLASTIC AGENTS

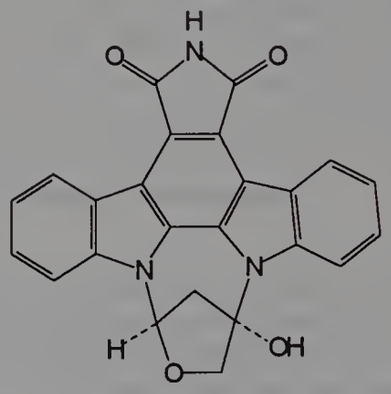
Most of the earlier research in antineoplastic drug discovery was related to inhibiting the synthesis and function of DNA. Today, a variety of other targets are under intensive investigation, and they should provide oncologists with significant new approaches to therapy. Although this research has not yet produced an approved agent, many new compounds are in clinical trials. The following new approaches to cancer chemotherapy are of special interest: signal transduction inhibitors, inhibition of proteases involved in metastasis, angiogenesis inhibitors, antisense technology, and monoclonal antibodies.

Signal Transduction Inhibitors

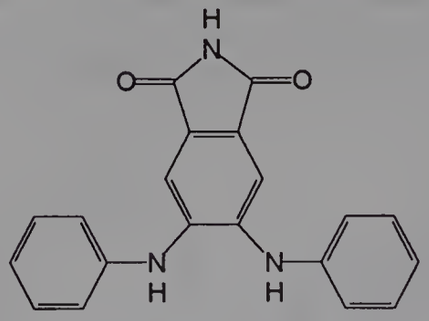
Protein tyrosine kinases are important in the transduction of signals initiating cellular replication and transformation. Cellular receptors that normally transduce growth factor signals frequently phosphorylate protein tyrosines as well. Many oncogene products are protein tyrosine kinases.[397] For example, a hybrid gene formed from the abl protooncogene from chromosome 9 with the bcr gene on chromosome 22 encodes a fusion protein with tyrosine kinase activity. This protein maintains the leukemic phenotype in human chronic myelogenous leukemia.[398] In another example, the erb-2 protooncogene encodes a 185-kD protein that is very similar to the epidermal growth factor receptor (EGFR) and has an extracellular, transmembrane, and cytoplasmic domain. A single point mutation in this gene causes overexpression of the 185-kD protein, which becomes a factor in a variety of solid tumors.[398]

Development of nontoxic inhibitors of protein tyrosine kinases in tumor cells is a problem because this type of

The microbial product staurosporine is a nonselective protein tyrosine kinase inhibitor of EGFR kinase. A simpler dianilinophthalimide, analog I, is a potent and selective inhibitor of EGFR kinase.[400]



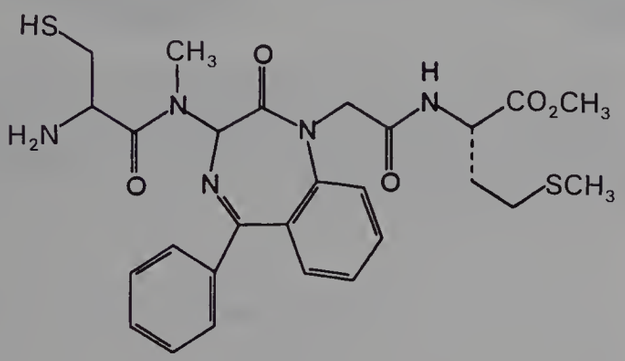
Staurosporine



Staurosporine analog

Ras protein is central among the many oncogene- or protooncogene-encoded proteins that serve as signal transducers in the pathway from the outer membrane to the nucleus of cells. It acts as a common relay point for signals from various growth factors. Single base mutations in the gene encoding the 21-kD ras protein are found in tumors, especially colon and pancreatic carcinomas.[401]

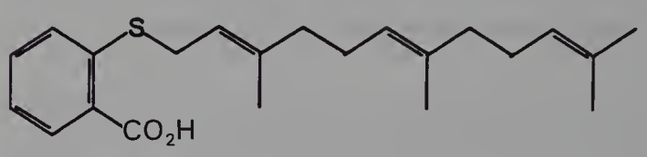
Ras protein is localized at the inner surface of the plasma membrane. It strongly binds guanine nucleotides and hydrolyzes GTP to GDP. The process of cycling between the active GTP-bound form and the inactive GDP-bound form serves as a switch for normal cellular growth and differentiation. Oncogenic ras proteins do not hydrolyze GTP and are, therefore, permanently in the active state.[402] Protein tyrosine kinases at the EGFR initiate a sequence of events that promote GDP release from ras, allowing it to bind ATP and assume an active conformation. Posttranslational modifications of ras, especially the addition of a farnesyl substituent, provide the lipophilicity required for its membrane binding.



benzodiazepine derivative

Farnesylation of ras is a complex process involving initial reaction between farnesyl pyrophosphate and a cysteine residue of ras to form a thioether linkage. The cysteine is located three residues from the end of the CO_2H terminal residue. These three amino acid residues are cleaved from ras, and the resulting terminal carboxylic acid group on the cysteine is methylated to the corresponding ester.[403]

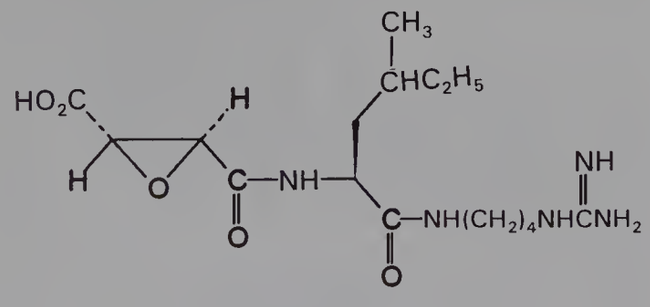
Current research is focused on inhibiting farnesyl transferase. Certain tetrapeptides such as CVFM-NH_2 show potent inhibition of this enzyme. Further modification of the tetrapeptide structure into a benzodiazepine derivative provided restoration of a normal growth pattern in ras-transformed cells.[404] Another research objective is inhibition of methyltransferases that catalyze the esterification of cysteine residues on farnesylated ras. The most potent inhibitor at this time is a farnesylthiosalicyclic acid derivative.[402]



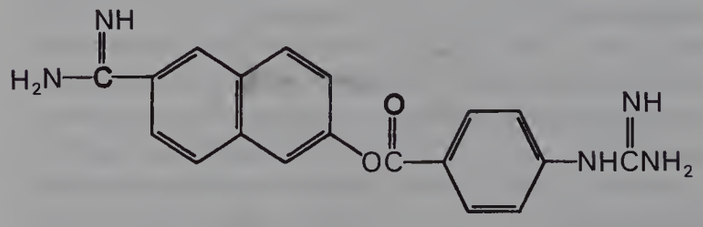
farnesylthiosalicylic acid

PROTEASES AND METASTASIS

The ability of cells from primary tumors to colonize secondary sites (metastasis) is the major cause of cancer mortality. Metastasis involves tumor cells entering and leaving the circulation and invading adjacent tissue. It requires degradation of the extracellular matrix by the concerted action of proteases. This process occurs normally, but it is controlled by the elaboration of protease inhibitors. The balance between proteases and inhibitors appears to be abnormally regulated in malignant cells.[405] Matrix protein is degraded by a variety of metalloproteinases, including collagenases, gelatinases, stromelysins, and matrilysins. These metaloproteinases can be activated by a cascade induced by tumor-secreted serine proteases, cysteine proteases, and aspartyl proteinases.[406] Among the synthetic compounds currently under investigation are E-64, an inhibitor of the cysteine proteinase cathepsin B,[407] and nafamostat, a serine protease inhibitor. Suramin blocks melanoma and mammary tumor invasiveness, possibly by inhibiting heparinase, cathepsin D secretion, and urinary plasminogen activator receptor.[406,408]



E-64



Nafamostat

ANGIOGENESIS INHIBITORS

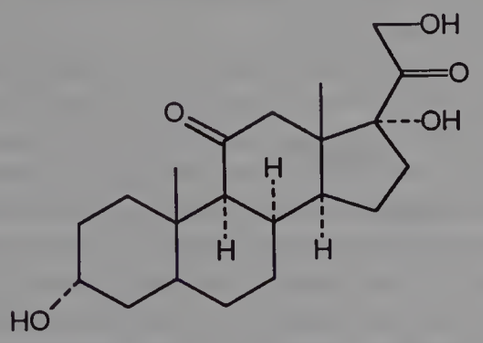
Angiogenesis is the formation of new blood vessels. It is a necessary but carefully regulated component of normal growth and wound healing. Uncontrolled angiogenesis is a driving factor in solid tumor growth. The process of angiogenesis is complex and requires the coordinated interaction of multiple cell types. Multiple sites for drug intervention are expected.[409] Endogenous angiogenesis inhibitors were sought in tissues such as cartilage, which lack blood vessels. This search afforded a protein named cartilage-derived inhibitor.[410] Laminin is a major component of basement membrane. Peptides based on its structure, such as CDPGYIGSR-NH_2, inhibit angiogenesis and solid tumor growth.[411] Platelet factor 4, a heparin-binding polypeptide, inhibits human colon cancer cells in mice. Heparin preparations alone promote angiogenesis, but they strongly promote the anti-angiogenic activity of small molecules. Synthetic heparin substitutes, including sulfated cyclodextrins[412] and suramin,[413] inhibit angiogenesis, and they are promoted by angiostatic corticosteroids. These corticosteroids are important agents, but their glucocorticoid and mineralocorticoid properties cause serious side effects. Related steroids that lack these side effects include 11α-hydrocortisone, tetrahydrocortisone, and medroxyprogesterone acetate.[414] The antibiotic fumagillin inhibits angiogenesis in tumors, and a more potent analog, AGM-1470, reduces the growth rate of lung cancer and melanoma in mice.[415]

Tumor necrosis factor α is a powerful stimulant to angiogenesis. The secretion of this factor by macrophages in prostatic cancers is decreased significantly by linomide, a quinoline-3-carboxamide derivative.[416]

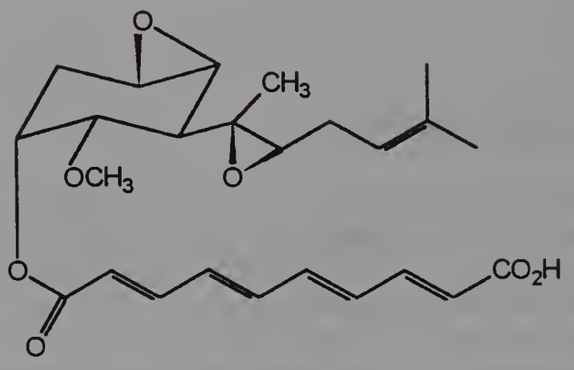
ANTISENSE TECHNOLOGY

Single-stranded messenger RNA (mRNA) can undergo sequence-specific, high-affinity binding to a complementary oligonucleotide sequence by Watson-Crick hydrogen bonding. Such a complementary agent is called an antisense oligomer. By binding with mRNA, the antisense oligomer can interfere with its translation into protein by ribosomal blockade. Complexes between mRNA and DNA-like antisense oligonucleotides also can activate RNAse H, an enzyme that specifically cleaves the RNA strand of an RNA/DNA duplex.[417]

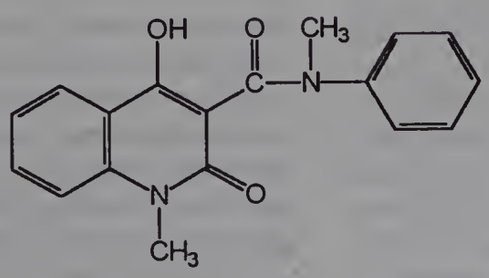
Limitations on the use of antisense oligomers include poor uptake into cells and instability toward degradation by nucleases. Cellular uptake has been increased by microinjection and by the addition of lipofectin, a cationic lipid that increases cell permeability.[418] Stability to nucleases is improved by replacement of one phosphate oxygen with sulfur to give phosphorothioate oligonucleotides. The phosphorothioate group is chiral, but racemates are generally used.[419] Other modifications to the oligonucleotide backbone include replacing the phosphate by various amides and acetals, and by using 2′-fluoro-2′-deoxysugars.[420] Peptide nucleic acids in which the sugar-phosphate backbone is replaced by N-(2-aminoethyl) glycine units have shown stability in vitro to degradation by nucleases.[420] Novel antisense oligomers have also been prepared with modified pyrimidines such as 5-(propyn-1-yl)uracil and 6-azathymidine[421] and modified purines such as 3-(1H-imidazolyl)propyl guanine.[422] These oligomers also have enhanced stability to nucleases.[423]



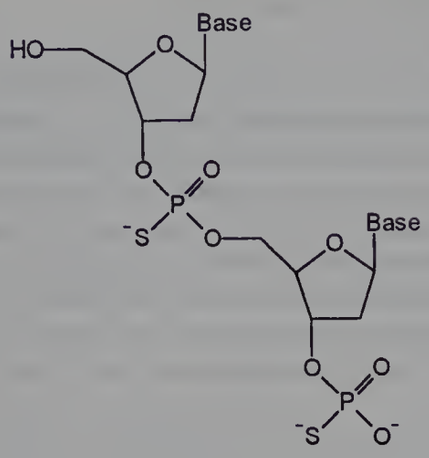
Tetrahydrocortisone



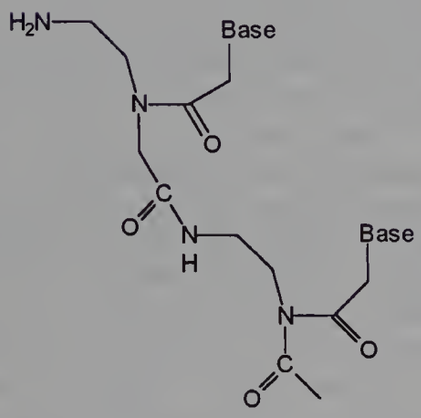
Fumagillin



Linomide



Phosphorothioate



Peptide nucleic acid

MONOCLONAL ANTIBODIES

The concept of using antibodies for the selective destruction of cancer cells was proposed first by Ehrlich in 1908; however, it could not be realized until Köbler and Milstein demonstrated the practical production of monoclonal antibodies from hybridoma cell lines in 1975.[424] Since then, numerous diagnostic and therapeutic monoclonals have been prepared, although establishing them as clinical agents has been difficult. The first monoclonal antibodies to human cancer cells were developed in mice.[425] They are easy to prepare, but their use in humans results in an immune response leading to human anti-mouse antibodies (HAMA), which inactivates the monoclonals.[426] Human monoclonals are difficult to prepare and not internalized into tumor cells. The HAMA problem has been partly solved by the development of chimeric antibodies that contain the variable region of mouse antibodies (binds with antigens) and the constant region of human antibodies (the effector part of the molecule).[427] A more recent development is the humanized antibody, in which only the complementarily determining regions of the variable domains are retained from the mouse monoclonals.[428] These antibodies contain only ~5% to 10% of mouse residues and are unlikely to produce the HAMA reaction. The first humanized antibody used in a clinical trial, CAMPATH-IH, induced remission in two non-Hodgkin's lymphoma patients and showed no detectable HAMA.[429]

As shown in Fig. 12-7, antibodies consist of two identical light chains and two identical heavy chains which form a Y shape. Antigen binding occurs at the ends of the arms of the Y, and each arm is an antigen binding fragment (Fab). Thus each antibody molecule can bind two antigens. The ends of the arms vary extensively in sequence and provide the binding specificity for the antigen. Each variable domain contains three complementary-determining regions (CDR) to give a total of six for binding each antigen molecule.[430]

Because monoclonal antibodies by themselves may not be sufficiently toxic to kill cancer cells, extensive efforts have been made to conjugate them with highly toxic substances such as radionuclides, toxins, and anticancer drugs. The radionuclide conjugates also provide diagnostic agents for tumors. Structures of the conjugates typically consist of the monoclonal antibody joined to a linker molecule, which is in turn joined to the cytotoxic agent.[431] Linkers are attached to the ϵ-amino groups of lysine residues of the monoclonal or to aldehyde groups formed by periodate oxidation of carbohydrate residues on the constant regions of the monoclonal.[432] For radionuclides, the other end of the linker has a chelating group, such as ethylenediamine tetraacetic acid (EDTA), which can bind radioactive metals such as ^{99m}Tc, ^{111}In, and ^{90}Y.[431]

Immunotoxins such as ricin, abrin, and diphtheria toxin are so potent that one molecule can kill a cell. Ricin consists of two subunits, the enzymatically active A chain and the targeting B chain, which are joined by a disulfide bond. Therapeutic agents are designed to have the B chain replaced by a monoclonal antibody targeted to tumor cells, taking advantage of the lability of the disulfide bond.[433] For anticancer drugs, the strategy is to join the drug to the linker molecule through a functional group capable of being hydrolyzed. It is expected that the conjugate will be internalized in the cancer cell and then the drug will be released. As an example of this strategy, the methyl ester in the vindoline moiety of vinblastine was converted into a hydrazide, which was then covalently bonded to an aldehyde group formed by periodate oxidation of the monoclonal. The resulting conjugate was relatively stable in serum, but it released vinblastine hydrazide over a 24-hour period.[434] An alternative strategy for chemotherapy is the use of monoclonal antibodies as carriers for enzymes to tumor cell surfaces. The enzymes convert relatively nontoxic drug precursors (prodrugs) into active anticancer drugs.[435]

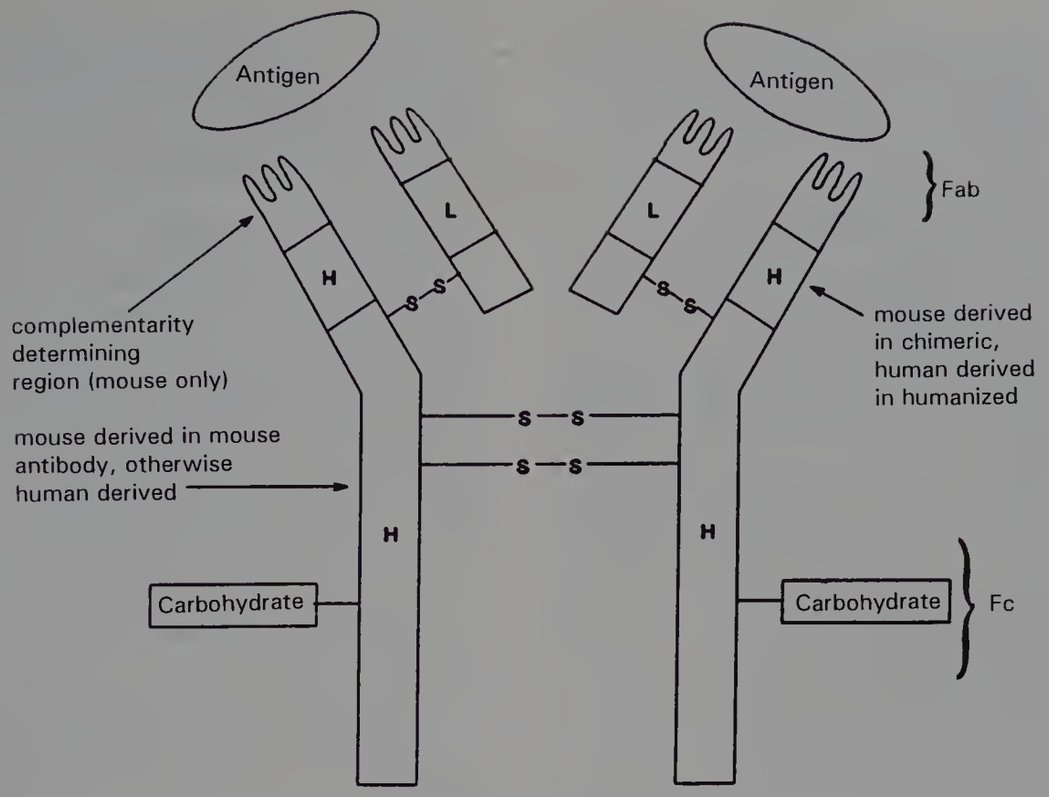
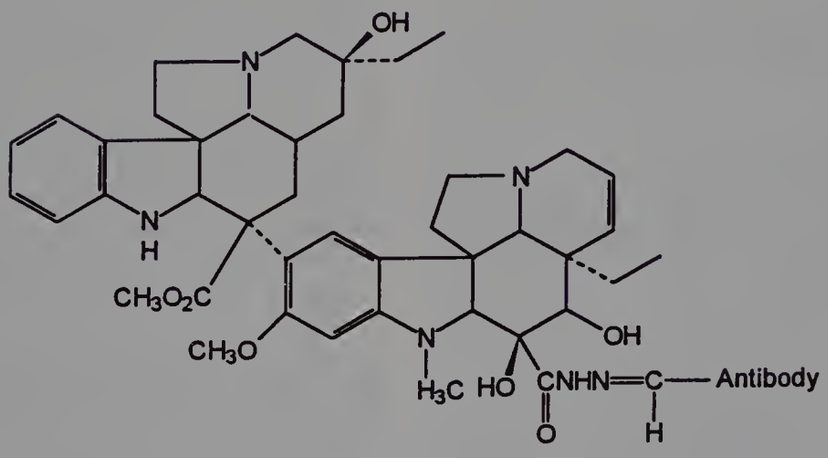


FIG. 12-7. Monoclonal antibody.



vinblastine-monoclonal antibody conjugate

Limitations encountered in cancer chemotherapy with monoclonal antibodies and their conjugates include lack of selectivity for tumor cells, heterogeneity of tumor cell antigens, insufficient drug density to kill tumor cells, loss of immunogenicity because drug conjugates block recognition sites, the HAMA effect, and lack of internalization into tumor cells. Internalization is essential with toxins, but less important with radionuclides.[436]

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CHAPTER 13

Agents for Diagnostic Imaging

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Tim B. Hunter

Medical imaging includes those techniques that allow noninvasive (nonsurgical) visualization of internal organs and processes. It began with Roentgen's discovery of x-rays in 1895, and it has been the domain of diagnostic radiology since then. In its earliest days, the specialty of radiology used x-rays to produce images of the chest and skeleton. At the present time, diagnostic radiology uses ionizing radiation (x-rays), magnetic resonance imaging (MRI) techniques, radionuclides (nuclear medicine), and high-frequency sound waves (ultrasound) to produce diagnostic images of the body. Today, radiologists and other physicians also use diagnostic imaging techniques to guide themselves in the performance of interventional procedures, such as organ biopsy or abscess drainage.

Nuclear medicine involves the use of radioisotopes to produce anatomic and physiologic images of the body for diagnostic purposes. The first artificial radionuclide (phosphorus-30) was produced by the French radiochemists, Frederic Joliet and Irene Curie. Nuclear medicine became a specialty in 1946 when radionuclides became available from cyclotrons and nuclear reactors. In many medical centers, nuclear medicine is considered part of diagnostic radiology, although in some locales it may be a freestanding discipline or reside in a pathology or internal medicine department.

The present chapter examines those pharmaceutical agents used in diagnostic radiology and nuclear medicine for medical imaging.

INTRODUCTION TO RADIATION

In chemical reactions, only the valence electrons of an atom are affected and the nucleus remains unchanged. Nuclear reactions may result from bombardment of a stable nucleus with high-energy particles or decomposition of an unstable nucleus. The nuclei of atoms are of two kinds: stable and radioactive. Radioactive nuclei have excess internal energy compared to nuclei with a stable arrangement of protons and neutrons. They obtain stability by emission of energy in the form of particulate and electromagnetic radiation. Radiation is the propagation of energy through space or matter. Ionizing radiation is radiation that, when interacting with matter, can cause changes in the atomic or nuclear structure of matter. The first type of ionizing radiation is particulate, which includes alpha (α), beta (β^-), positron (β^+), proton (p), and neutron (n) particles. Radiation is energy in the form of kinetic energy and on the atomic scale is usually measured in electron volts (eV). By simple definition, an electron volt is the energy needed to accelerate an electron across a potential difference of 1 volt. The second type of ionizing radiation is called electromagnetic radiation. Electromagnetic radiation is an electric and magnetic disturbance that is propagated through space at the speed of light. This type of radiation has no mass and is unaffected by either an electrical or magnetic field because it has no charge. There is no difference between radio (10^{-10} to 10^{-6} eV), microwave (10^{-6} to 10^{-2} eV), infrared (10^{-2} to 1 eV), visible light (1 to 2 eV), ultraviolet (2 to 100 eV), or x-rays and gamma (γ) rays (100 to 10^{+7} eV) except in their frequency and therefore their energy. The energy of electromagnetic radiation can be calculated in electron volts from the following equation:

$$E = h\upsilon = \frac{hc}{\lambda}$$

where h is Planck's constant (4.13×10^{-15} ev-sec), ν is the frequency (hertz), c is the speed of light (cm/sec), and λ is the wavelength (cm). The difference between x-rays and γ-rays is based on where they originate; x-rays come from outside the nucleus while γ-rays originate in the nucleus of an atom. The gamma-ray can exhibit some particulate properties, so it is sometimes called a photon.

X-rays used in diagnostic radiology are produced by applying a very high voltage (125,000 volts) to a glass vacuum tube that contains a cathode and a rotating anode (Fig. 13-1). The cathode is a filament that is heated to a very high

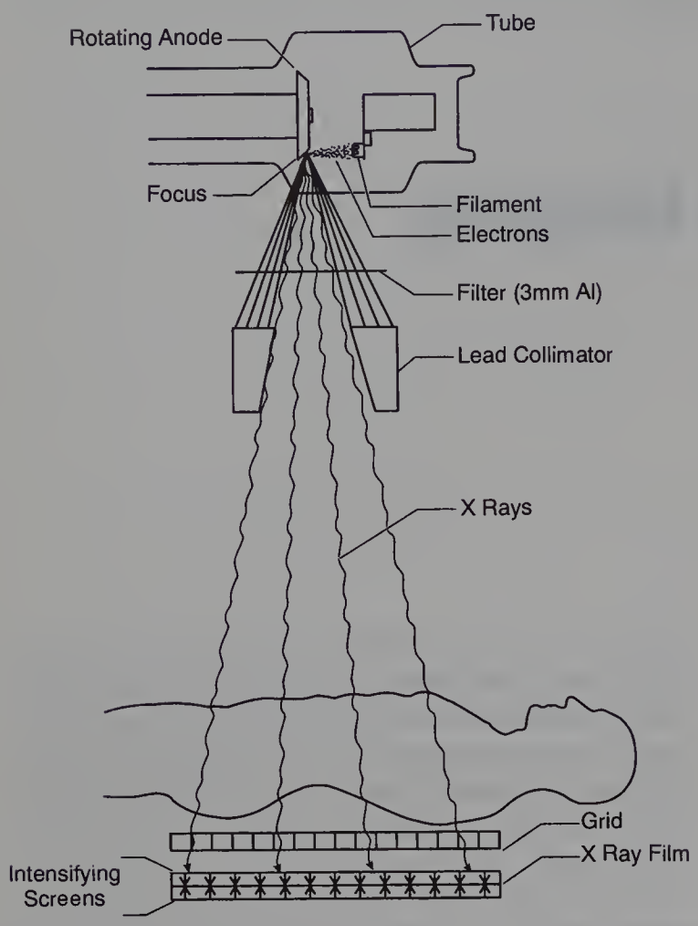


FIG. 13-1. *A schematic diagram of an x-ray tube producing x-rays that pass through the patient and expose the photographic film. The photographic film will not stop the x-rays, so a plastic screen coated with fluorescent particles that are activated by the x-rays emits light to expose the film within a light-tight film cassette. As x-rays pass through the body, some of them are scattered, so a moving grid device composed of alternating strips of lead and plastic decreases the scattered x-rays that degrade the image.*

temperature, which provides a copious source of electrons. The electrons are accelerated toward the positively charged anode (tungsten). When the accelerated electrons strike the anode (called the target), x-rays are produced. The distribution of x-rays is a continuous spectrum, and the low-energy x-rays, which will not travel through the body to the x-ray film, are absorbed by a filter (aluminum). An invaluable modification of the x-ray system is fluoroscopy. This modality permits the visualization of organs in motion, positioning of the patient for spot film exposures, instillation of contrast media into hollow cavities, and, most importantly, insertion of catheters into arteries. Figure 13-2 represents a schematic of a fluoroscopic system.

With conventional radiography and also with computed tomography (CT), sometimes called computed axial tomography (CAT) scanning, organs and tissues are made visible according to how well they attenuate x-rays. The attenuation of x-rays by tissues is a very complex process and depends on many factors, including the energy of the x-ray beam and the density of the tissue. Bone has an average density of ~1.16g/cm^3, which accounts for its ability to absorb most of the radiation it encounters. CT scanning (Fig. 13-3) uses ordinary x-ray energies for imaging but uses complex mathematical reconstructions to produce images of the body in the axial and other planes. In the process, it is able to increase the visibility of small differences in the radiographic densities between tissues to a far greater extent than ordinary radiographic film.

Radionuclides undergoing a transformation process is called radioactive decay, and most cases involve a transmutation of one element into another. A nucleus may undergo several decays before a stable configuration is achieved. A nuclear particle, either a proton or a neutron, is called a nucleon. The designation of a species of atom having a specified number of neutrons and protons in its nucleus is called a nuclide. Nuclides with the same number of protons and a different number of neutrons are called isotopes. Nuclides with the same atomic mass are called isobars. Nuclides with the same number of protons and atomic mass, but at two energy levels, are called isomers. The nucleus has energy levels analogous to the orbital electron shells, but at a higher energy. The lower energy level is called the ground (g) state, and the highest energy level is called the metastable (m) state. Nuclides are all species of elements, of which there are ~265 stable nuclides, 330 naturally occurring radionuclides, and >2,500 artificially produced radionuclides. In accordance with a recommendation of the International

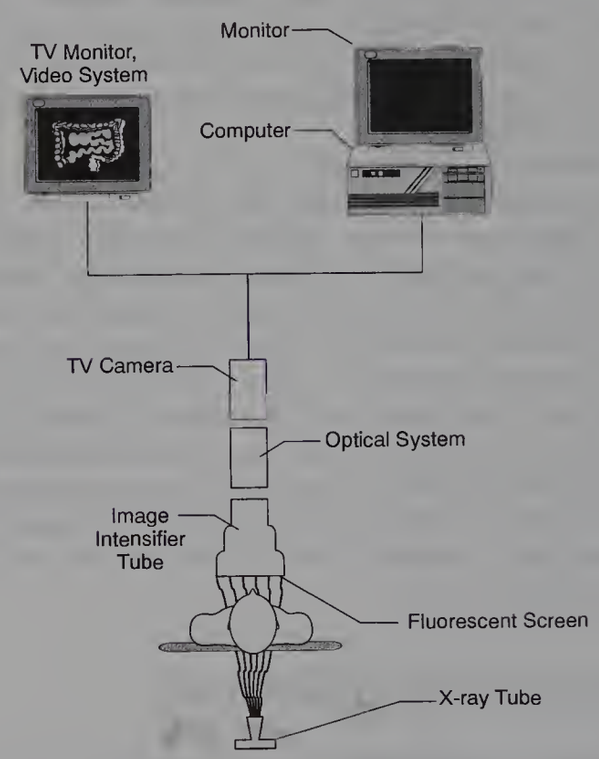


FIG. 13-2. *A schematic diagram of a fluoroscopic unit with the x-ray tube located behind the patient with a fluorescent screen-image intensifier system positioned on the opposite side. Amplification of the faint fluorescing image by the image intensifier increases brightness level and contrast. The real-time fluoroscopic images can be shown on a television camera for convenient viewing during the examination and stored on videotape, video disk, or computer for later viewing without distortion or destruction of the images.*

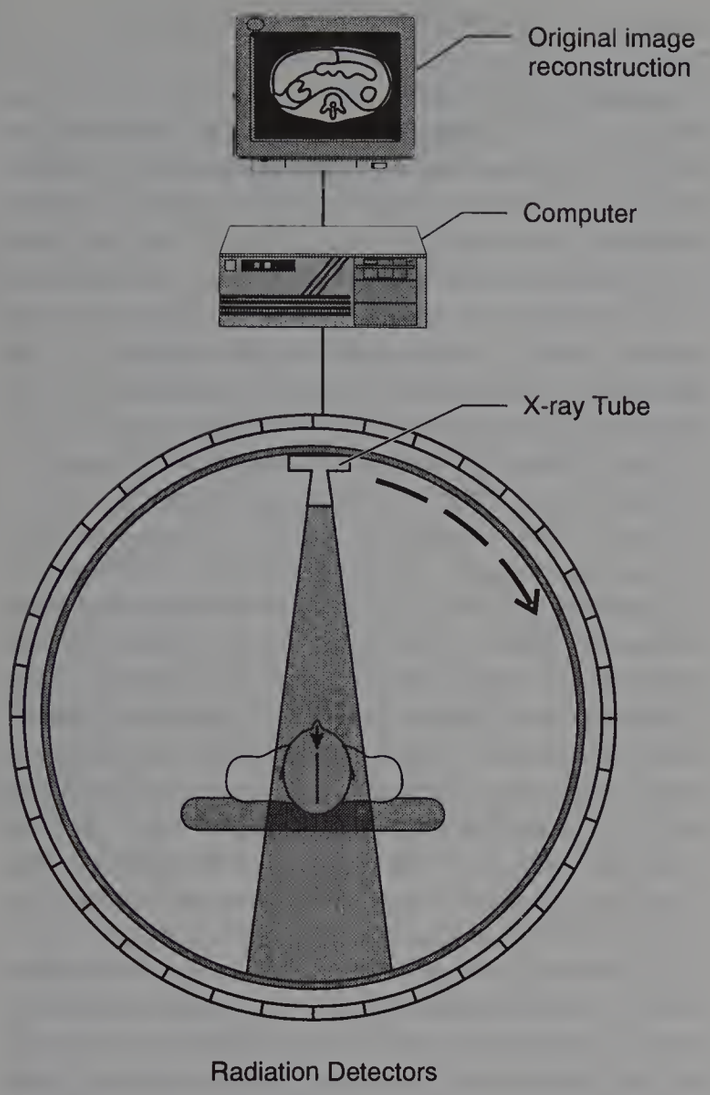


FIG. 13-3. *A schematic diagram of a computerized axial tomography (CAT) system, which produces thin cross-sectional images of the body. An x-ray tube rotates around the patient, and the transmitted x-rays are detected by a circle of moving radiation detectors. The absorptions of x-rays by tissues of different densities are assigned numerical values (CT numbers). The computer uses complex algorithms to reconstruct an anatomic cross-sectional image on a television monitor.*

Union of Pure and Applied Chemistry, the following notation should be used for the identification of a nuclide:

$${}^{A}_{Z}X^{(\text{valence})}_{N} \qquad \text{Example: } {}^{131}_{53}I^{-}_{78}$$

X is the symbol of the chemical element to which the nuclide belongs. The A represents the atomic mass (number of neutrons + number of protons), and Z represents the atomic number (number of protons). The right side of the element is reserved for the oxidation state, and N represents the number of neutrons. For most medical applications, it is sufficient to indicate the element chemical symbol and the mass number (i.e., ^{131}I, I-131, or Iodine-131).

The radionuclide at the beginning of the decay sequence is referred to as the parent, and the radionuclide produced by the decay is referred to as the daughter, which may be stable or radioactive. There are five types of radioactive decay. These are distinguished according to the nature of the primary radiation event. A radioactive nucleus may decay according to one of the following methods:

1. Alpha emission (α). The nucleus emits an α particle, which consists of a helium nucleus without the electrons. If the emission of the alpha particle leaves the nucleus in an excited energy state, the excess energy is liberated in the form of a γ-ray.

$$\text{Example:} \quad {}^{226}_{88}\text{Ra} \rightarrow {}^{222}_{86}\text{Rn} + {}^{4}_{2}\text{He} + \gamma\text{-ray}$$

2. Beta emission (β^-). The nucleus emits a negative electron when a neutron changes to a proton. A γ-ray may or may not accompany the emission of a beta particle.

$$\text{Example:} \quad {}^{131}_{53}\text{I} \rightarrow {}^{131}_{54}\text{Xe} + \beta^- + \gamma\text{-ray}$$

3. Positron emission (β^+). The nucleus emits a positive electron when a proton changes to a neutron. A γ-ray may or may not accompany the emission of the positron. However, a positron (particle of antimatter) emitted from the nucleus loses its kinetic energy by interacting with surrounding atoms. It finally combines with a free electron from one of the surrounding atoms in an interaction in which the rest masses of both particles are given up as 2 γ-rays of 0.511 MeV emitted at 180° to each other. Einstein's theory of relativity states that mass and energy are equivalent and is represented by the following equation:

$$E = mc^2$$

where, in the case of an electron, E represents energy equivalent to mass ($m = 9.109 \times 10^{-31}$kg) at rest and c is the speed of light (3×10^8 m/sec). It can be shown using the proper units that the mass of an electron is equivalent to 0.511 MeV. This is called annihilation radiation. It is utilized in a specialized organ-imaging technique called positron emission tomography (PET).

$$\text{Example:} \quad {}^{18}_{9}\text{F} \rightarrow {}^{18}_{8}\text{O} + \beta^+ + \gamma\text{-ray}$$
$$\beta^+ + e^- \rightarrow 2\ \gamma\text{-rays (0.511 MeV)}$$

4. Electron capture decay (EC). The nucleus captures an electron from the electron cloud of the atom (mainly the K shell), and a proton becomes a neutron:

$$\text{Example:} \quad {}^{123}_{53}\text{I} \xrightarrow{EC} {}^{123}_{52}\text{Te} + \gamma\text{-ray}$$

5. Isomeric transition (IT). Isomeric transition is a decay process involving neither the emission nor the capture of a particle. The nucleus simply changes from a higher to a lower energy level by emitting a γ-ray. Therefore, both mass number and atomic number remained unchanged. The daughter nucleus is the same chemical element as the origi-

nal nucleus. The original nucleus before the transition is said to be in a metastable (m) state.

Example: $^{99m}_{43}\text{Tc} \rightarrow {}^{99}_{43}\text{Tc} + \gamma\text{-ray}$

CHARACTERISTIC OF DECAY

It is impossible to predict when an individual atom of a radionuclide will decay. However, in quantitative terms, this transformation occurs at a rate that is characteristic of that specific radionuclide only and is expressed as its physical half-life. This is the time for one-half of the original number of atoms to disintegrate. The activity of radionuclides can be expressed in three ways: (a) as curies (Ci), millicuries (mCi), or microcuries (μCi); (b) as disintegrations per second (dps); and (c) as becquerels (Bq), 1 becquerel = 1 dps. A curie is the quantity of any radionuclide having a decay rate of 3.7×10^{10} dps. This number was chosen for historical reasons as this is the number of disintegrations occurring per second in 1 g of radium. The international system of units has adopted the becquerel as the official unit of radioactivity, but the curie is still widely used and we will use this unit in addition to the official unit. A relevant conversion factor to remember is the following:

1 millicurie (mCi) = 37 megabecquerels

The basic equation for radioactive decay is expressed as follows in terms of atoms:

$$N_t = N_0\, e^{-\lambda t}$$

However, the N_t (number of atoms at time t) and N_0 (number of atoms at time 0) can be replaced with activity.

$$A_t = A_0\, e^{-\lambda t}$$

where A_0 = original activity in (Ci, mCi, μCi)

A_t = activity (at time t)

λ = decay constant = $\dfrac{0.693}{T_{\frac{1}{2}}^{l}}$ (physical half-life)

$e^{-\lambda t}$ = decay factor

An example of a radioactive decay calculation follows: A sample of ^{123}I-sodium iodide was known to have an activity of 200 μCi on May 14 at 12 noon C.S.T. What is the activity on May 15 at 3 pm E.S.T.? (Note: Calculations of elapsed time must also indicate variations in time zones–elapsed time in this case is 26 hr.)

$$(T_{\frac{1}{2}}^{l} = 13.2 \text{ hr})$$

$$A = (200\ \mu\text{Ci})\ e^{-\left[\frac{0.693 \times 26 \text{ hr}}{13.2 \text{ hr}}\right]}$$

$$A = (200\ \mu\text{Ci})\ (e^{-1.36})$$

$$A = (200\ \mu\text{Ci})\ (0.255)$$

$$A = 51.0\ \mu\text{Ci}$$

BIOLOGICAL EFFECTS OF RADIATION

The absorption of ionizing radiation by living cells always produces effects potentially harmful to the irradiated organism. The amount of radiation energy absorbed by tissue is called radiation absorbed dose and is specified in rads or millirads. A dose of one rad implies 100 ergs of energy absorbed per gram of any tissue. The unit of exposure for x and γ radiation *in air*, the roentgen, is used to specify radiation levels in the environment. (One roentgen = the amount of radiation that will produce one electrostatic unit of charge of either sign per 0.001293 grams of air at STP.) The international system of units has adopted the gray to replace the rad (1 gray = 100 rads), but again we will use the more traditional units. In the case of x or γ radiation for medical diagnosis, the roentgen and rad turn out to be numerically equivalent. The major difference between electromagnetic radiation (x-rays or γ-rays) and particulate type radiation (beta and alpha particles) lies in the ability of electromagnetic rays to penetrate matter. Whereas beta particles travel only a few millimeters before expending all their energy, x- and γ-rays distribute their energy more diffusely and travel through several centimeters of tissue. Therefore, particles deliver highly localized radiation doses, whereas x- and γ-rays deliver more uniform doses and in a less concentrated way throughout the irradiated volume of tissue. The radiation dose of particles is more useful for a therapeutic dose of a radionuclide but not for a diagnostic dose. When cells are irradiated, damage is produced primarily by ionization and free radicals. Particles produce damage by ionization, whereas x-rays and gamma-rays produce damage by free radicals. Free radicals are atoms or molecules having an unpaired electron.

The effects of large doses of radiation were derived from epidemiological studies of the atomic bomb survivors at Hiroshima and Nagasaki. Radiobiological damage by large doses of ionizing radiation can be caused by two different mechanisms. One mechanism is the direct radiation effect, where damage is obtained by the absorption of radiation energy directly in a critical biological site or target. The other is called the indirect effect, which involves aqueous free radicals as intermediaries in the transfer of radiation energy to the biological molecules. All biological systems contain water as the most abundant molecule (70% to 90%), and radiolysis of water is the most likely event in the initiation of biological damage. The absorption of energy by a water molecule results in the ejection of an electron with the formation of a free radical ion ($H_2O\cdot_+$). The free radical ion dissociates to yield a hydrogen ion (H^+) and a hydroxyl free radical ($HO\cdot$). The hydroxyl free radicals combine to form hydrogen peroxide (H_2O_2), which is an oxidizing agent. In addition, hydrogen-free radicals ($H\cdot$) can form, which can combine with oxygen (O_2) and form a hydroperoxy-free radical ($HO\cdot_2$). These two reaction intermediates

are very reactive chemically, and can attack and alter chemical bonds. It is well known that the only significant "target" molecule for biologic damage is DNA. Types of DNA damage include single and double breakage, and inter- or intramolecular cross-linking in the double-stranded DNA molecule. With the direct effect of radiation, the damage makes cell replication impossible and cell death occurs. In the indirect effect of radiation, if the damage is not lethal, but changes the genetic sequence or structure, mutations occur that may lead to cancer or birth of genetically damaged offspring. Some effects of radiation may develop within a few hours, whereas others may take years to become apparent. Consequently, the effects of ionizing radiation on human beings may be classified as somatic (affecting the irradiated person) or genetic (affecting progeny).

Radiation dose can only be estimated and is called radiation dosimetry. In the case of x-ray exposure, most radiation "doses" in the literature are described as the entrance exposure (in roentgens/minute) to the patient. In diagnostic nuclear medicine procedures, patients are irradiated by radiopharmaceuticals localized in certain organs or distributed throughout their bodies. Since the radionuclides are taken internally, there are many variables, and the absorbed dose (RADS) to individual patients cannot be measured but only estimated by calculation. The methods to calculate the absorbed dose to patients from radiopharmaceuticals were changed in 1964 by the Medical Internal Radiation Dose (MIRD) Committee under the auspices of the Society of Nuclear Medicine. The method of calculation is illustrated in the MIRD primer for absorbed dose calculations that can be purchased from the Society of Nuclear Medicine (Reston, VA).

Although the knowledge of the effects of radiation are not totally understood, the benefits associated with low doses of radiation almost always outweigh any potential risks to individual patients. A large number of scientific and advisory groups have published risk estimates for ionizing radiation, but the most widely quoted is report number 5 of the National Academy of Sciences committee on the biological effects of ionizing radiation (BEIR-V). Under normal circumstances, no radiation worker or patient undergoing diagnostic investigation by radiopharmaceutical or radiographic procedures should ever suffer from any acute or long-term injury. Typical radiation doses to patients from radiopharmaceuticals are similar or less than that from radiographic procedures.

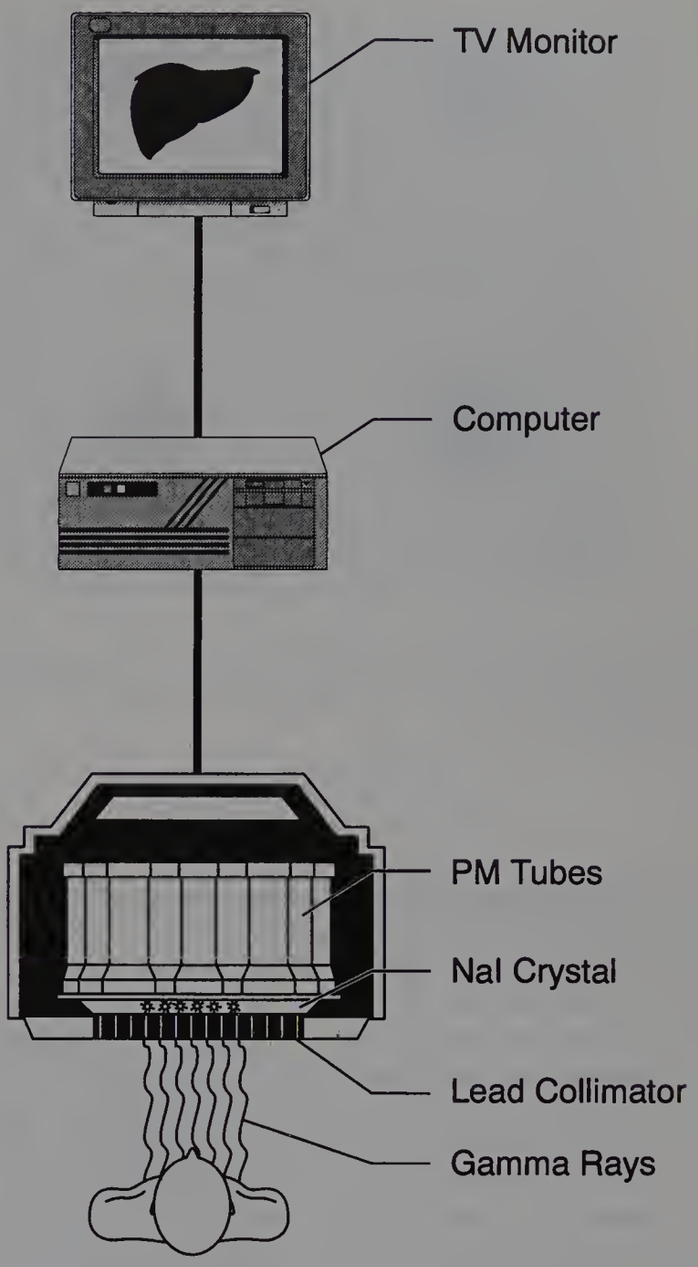


FIG. 13-4. *A schematic diagram of scintillation camera (Anger) system with a multihole lead collimator attached (to eliminate scattered gamma-rays), which is used to visualize tissues and organs after a diagnostic dose of a radiopharmaceutical is administered.*

RADIONUCLIDES AND RADIOPHARMACEUTICALS FOR ORGAN IMAGING

Nuclear medicine is a specialized branch of medicine devoted to the diagnostic and therapeutic use of radioactive compounds. The most common use of nuclear medicine is to image the distribution of a radiopharmaceutical in a specific organ system with a scintillation (Anger) camera for diagnostic purposes. Other uses of nuclear medicine include in vitro studies and therapeutic uses, which will not be discussed in this chapter. The specialty of nuclear medicine did not become available to the private hospital until the 1960s, after the introduction of the molybdenum-99/technetium-99m generator and the gamma (scintillation) camera developed by Hal Anger (Fig. 13-4). The scintillation camera consists of a radiation detector made of a large sodium iodide (thallium impurity) crystal that absorbs gamma rays. A lead shield (called a collimator) with multiple apertures (holes) like a honeycomb is placed in front of the crystal to decrease

FIG. 13-5. *Dynamic liver function study with scintillation camera. Normal hepatobiliary study after intravenous injection of Tc-99m disofenin (each sequential image is a 4-min time exposure). The radiopharmaceutical is excreted through the liver by the anionic transport system. Radioactivity is seen within the common bile duct (*small arrow*) and small bowel within 10 min, and the gallbladder (*large arrow*) is clearly identified after 24 min.*

scattered radiation and increase the overall resolution of the system. The absorbed energy in the crystal is emitted as a flash of light (called a scintillation) proportional to the energy of the gamma-ray. Coupled to the NaI(Tl) crystal are photomultiplier (PM) tubes that convert the light flashes to electrical pulses proportional to the amount of light. A computer assigns an x-y spatial coordinate to the various gamma-rays coming from the patient and stores this information in a matrix. After collection, the digital image is converted into an analog video signal for display on a video monitor. These images obtained with the scintillation camera are called scintigrams, scintigraphs, or scans. Nuclear medicine imaging studies involve the generation of images that represent the functional status of various organs in the body. Especially when interfaced with computer systems, information regarding dynamic physiological parameters such as organ perfusion, metabolism, excretion, and the presence or absence of obstruction can be obtained. Figure 13-5 demonstrates a normal dynamic function study of the liver using Tc-99m disofenin and the scintillation camera. Newer gamma cameras are mobile (can be brought to the patient's bedside) and can image one organ or the whole body by moving from head to toe.

Cross-sectional images of organs can be obtained by rotating a position-sensitive scintillation camera detector about the patient. This type of procedure is called single photon emission computed tomography (SPECT), which is the counterpart of CAT in x-ray. Figure 13-6 represents a schematic of a SPECT system. The vast majority of SPECT systems use one to three scintillation camera detectors that rotate about the patient. SPECT is routinely used when imaging the brain or heart to demonstrate three-dimensional distribution of radioactivity in these organs of the body. Figure 13-7 illustrates a patient study after injection of Tc-99m exametazime which is a brain-imaging agent.

A newer imaging modality uses multiple detector heads to image positron emitting radiopharmaceuticals by positron emission tomography (Fig. 13-8). Many biologically important molecules that are physiologically identical to the non-radioactive compound can be radiolabeled with positron-emitting radionuclides such as carbon-11 ($T\frac{1}{2}$ = 20 min), oxygen-15 ($T\frac{1}{2}$ = 2 min), nitrogen-13 ($T\frac{1}{2}$ = 10 min), and fluorine-18 (110 min). Figure 13-9 illustrates PET whole-body images performed on patients as a cancer management modality.

RADIONUCLIDE PRODUCTION

The radionuclides used in nuclear medicine are artificially produced. This is accomplished when neutrons, protons, alpha particles, or other particles impinge on atomic nuclei and initiate a process of nuclear change. The artificial production of a radionuclide requires preparation of a target system, irradiation of the target, and chemical separation of the radionuclide produced as a radiochemical from the target material. The radiochemical is converted to the desired radiopharmaceutical and quality assurance of the physical, chemical, and pharmaceutical qualities (i.e., sterility and apyrogenicity) of the final product is obtained. The systems used for practical production of radionuclides are a nuclear reactor, cyclotron, or radioisotope generator.

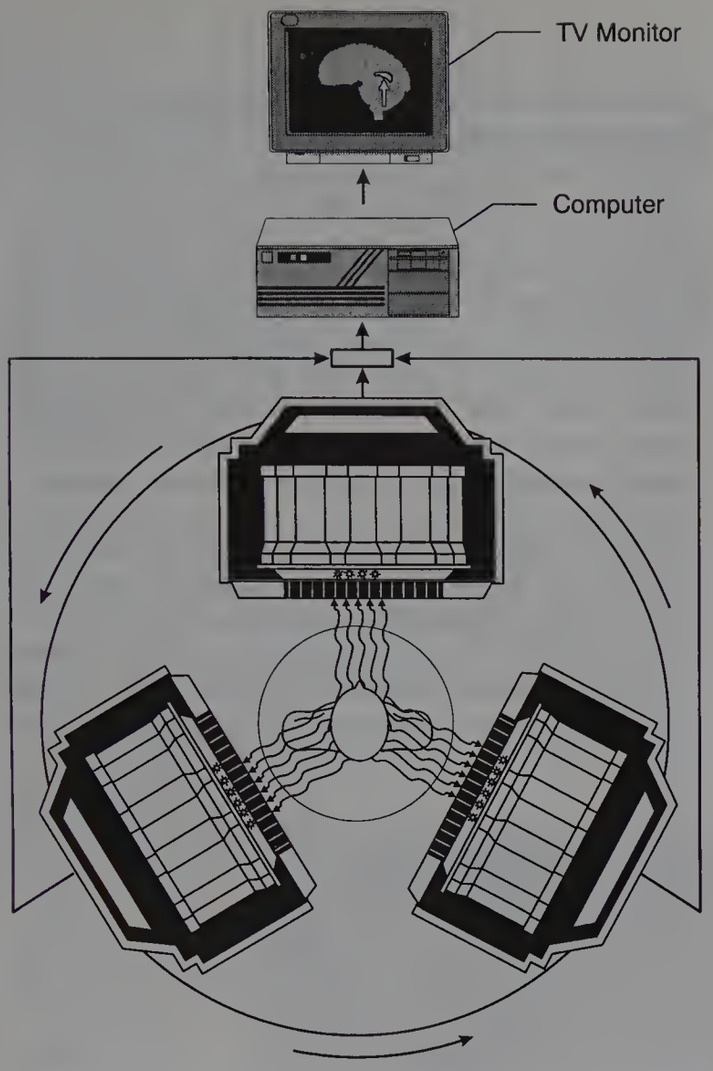


FIG. 13-6. *A schematic diagram of a rotating triple detector scintillation camera system for single photon emission computed tomography (SPECT) demonstrating a "cold" spot lesion in the brain on the sagittal view (*arrow*).*

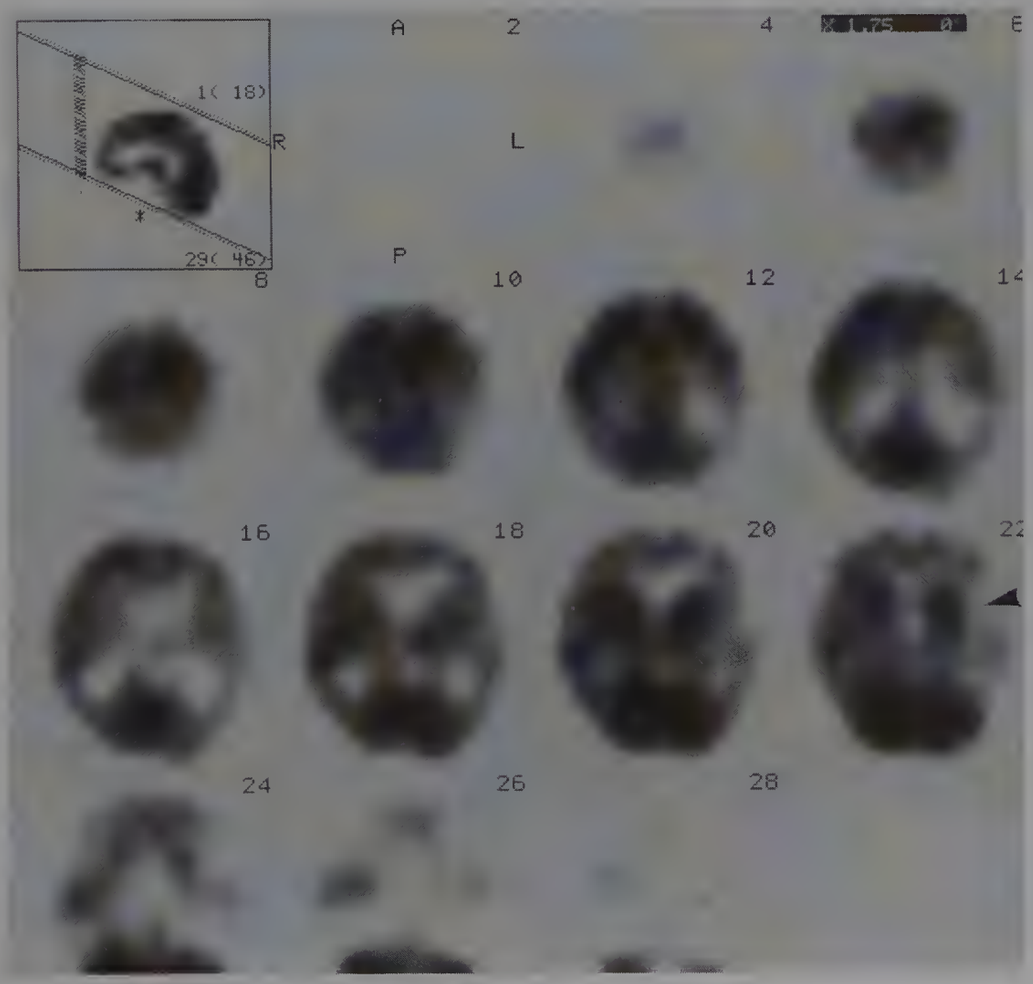


FIG. 13-7. *Abnormal SPECT brain study on a 70-year-old man with dementia and aphasia. The patient was placed in a quiet and dark environment for 20 min. Then the patient received an intravenous injection of Tc-99m exametazime, and SPECT images were obtained of the brain 15 min later. The data was processed, and images were displayed in the transaxial, coronal, and sagittal orientations. The transaxial images are displayed in this figure, and a decreased distribution of the radiopharmaceutical is noted in the left temporoparietal cortex (*arrow*). Note that the image of the left hemisphere is not as darkened as the right side. Although unilateral decreased perfusion to the temporoparietal cortex may be seen in some patients with Alzheimer's disease, the pattern is more specific for a cerebral vascular accident (CVA).*

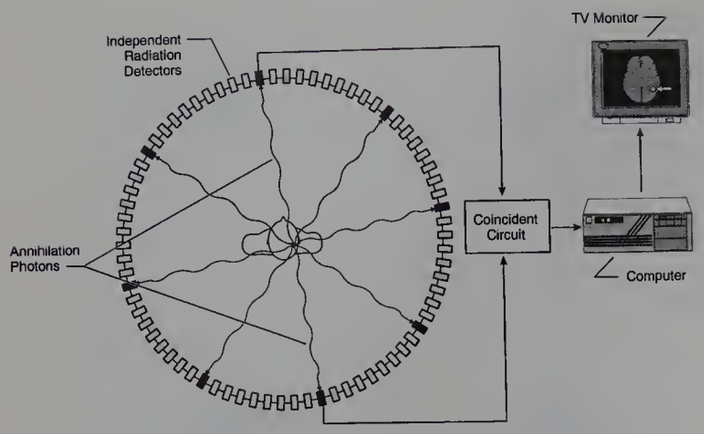


FIG. 13-8. *A schematic diagram of a PET imaging system with multiple scintillation detectors that localize the positron decay along a line. By using multiple position-sensitive detectors around the patient, the annihilation gamma-rays are acquired along many parallel lines and many angles simultaneously with four rings of detectors (only showing one ring). After using reconstruction algorithms, the internal distribution of the radioactivity can be determined and displayed on a cathode ray tube.*

The shorthand nuclear physics notation of a cyclotron production reaction is written as follows:

$$^{112}_{48}\text{Cd (p, 2n) } ^{111}_{49}\text{In}$$

where Cd-112 is the stable target material; a proton is the bombarding particle; two neutrons are emitted from the nucleus; and In-111 is the radionuclide produced.

The introduction of radionuclide generators into nuclear medicine arose from the need to administer large doses of a short half-life radionuclide to obtain better statistics in imaging. In consideration of radioactive (parent and daughter) pairs, we can distinguish two general cases, depending on which of two radionuclides has the longer half-life. If the parent has a longer half-life than the daughter, a state of so-called radioactive equilibrium is reached. That is, after a certain time, the ratio of the disintegration rates of parent and daughter become constant. In the second case, if the parent half-life is shorter than that of the daughter, it is evi-

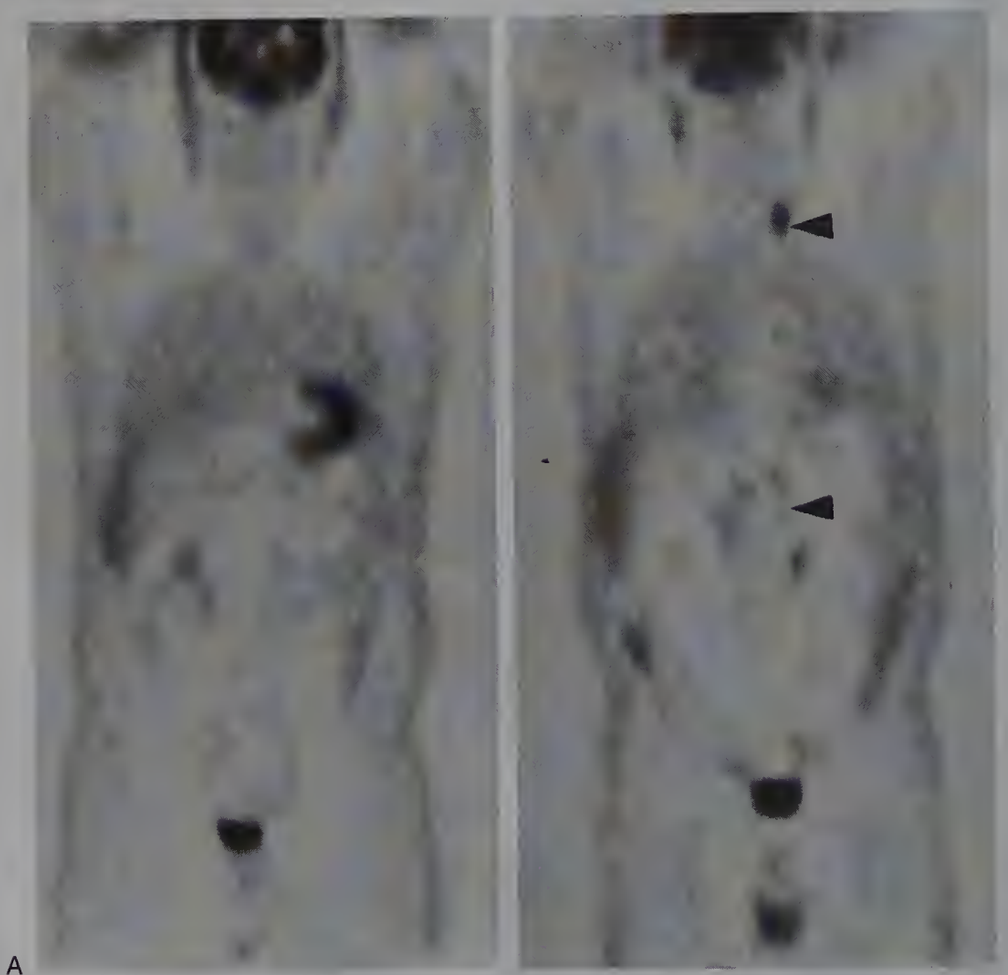


FIG. 13-9. *PET whole-body images were performed for metastases after injection of 4 mCi [148 MBq] of fluorine [^{18}F]-2-fluoro-2-deoxy-D-glucose (F-18 FDG).* ***A:*** *A normal whole-body PET image (coronal view) was obtained on a patient with lymphoma after treatment with chemotherapy and radiotherapy. The patient is fasting for 12 hr to maintain the blood glucose level between 80 and 140 mg%. The F-18 FDG uptake is decreased in the tumors if the blood glucose level is not in that range since the mechanism of uptake is an increased rate of glycolysis. (Note increased brain and cardiac uptake because of high glycolytic rates in these organs.)* ***B:*** *PET whole-body image obtained with the same technique on a patient with pancreatic carcinoma. Abnormal sites of F-18 FDG uptake are seen in the upper abdomen, posterior mediastinum, and left lower neck (*arrows*) consistent with neoplastic involvement. (Note increased brain, but not cardiac uptake of F-18 FDG, in this patient who had a desirable blood glucose level for tumor imaging.)*

dent that no equilibrium is reached at any time. Therefore, the general principle of the radionuclide generator is that the longer lived parent is bound to some adsorbent material in a chromatographic ion exchange column and the daughter is eluted from the column with some solvent or gas. There are >100 possible generator systems for clinical use, but there is only one in routine use in nuclear medicine (the molybdenum-99/technetium-99m system). All of the molybdenum-99 at the present time is obtained as a fission product of uranium-235 in a nuclear reactor.

$$^{235}_{92}\text{U (n, fission)} \rightarrow {}^{99}_{42}\text{Mo} + \text{other radionuclides}$$

Using elegant inorganic radiochemistry, the molybdenum-99 is separated from the other radionuclides. Molybdenum-99 ($T\frac{1}{2} = 66$ hr) decays by beta particle emission to technetium-99m ($T\frac{1}{2} = 6$ hr), which decays by isomeric transition (IT) to technetium-99 by emission of a gamma-ray (140 keV). The anionic molybdate ($^{99}MoO_4^{-2}$) is then loaded on a column of alumina (Fig. 13-10). The molybdate ions firmly adsorb to the alumina, and the generator column is autoclaved to sterilize the system. Then the rest of the generator is assembled under aseptic conditions into its final form in a lead-shielded container. Each generator is eluted with sterile normal saline (0.9% sodium chloride). The column is an inorganic ion exchange column, and the eluate contains sodium pertechnetate, so the chloride ions (Cl^-) are exchanging for the pertechnetate ions ($^{99m}TcO_4^-$) but not molybdate ions (MoO_4^{-2}). The method for calculating how much daughter is present on the column at any given time is more complex since it has to take into consideration the decay rates of the parent and daughter (Fig. 13-11). The simplified equation for any case where both the parent and the daughter are radioactive and in equilibrium is as follows:

$$A_d = (A_p)\lambda_d \frac{(e^{-\lambda_p t} - e^{-\lambda_d t})}{(\lambda_d - \lambda_p)}$$

where A_p is the activity (mCi) of the parent, A_d is the activity of the daughter, λ_p and λ_d are their respective decay constants, and t the time since the last elution of the generator. In the case of Mo-99 ($T\frac{1}{2} = 66$ hr), only 87.2% of the atoms decay to Tc-99m ($T\frac{1}{2} = 6$ hr) and 12.8% of the atoms decay directly to Tc-99. The generator system can be eluted several times per day to obtain more activity (mCi) per day because the ingrowth of Tc-99m is a logarithmic function.

TECHNETIUM RADIOCHEMISTRY

The element 43 in the periodic table, technetium, is a transition state metal and is the only "artificial" element below

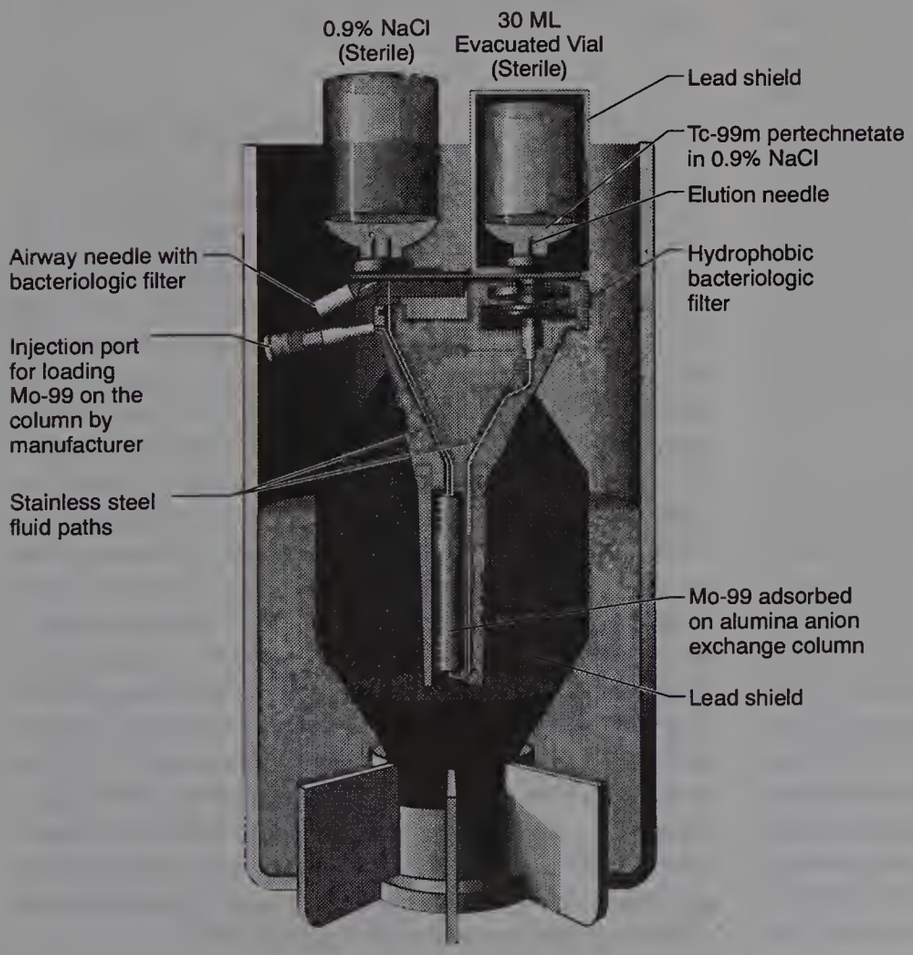


FIG. 13-10. *Cross section of a radionuclide generator for the production of technetium-99m (Tc-99m) by elution of a sterile alumina (Al_2O_3) column that has molybdenum-99 (Mo-99) adsorbed on it with sterile 0.9% sodium chloride. (Courtesy of Dupont–Pharma, Billerica, MA.)*

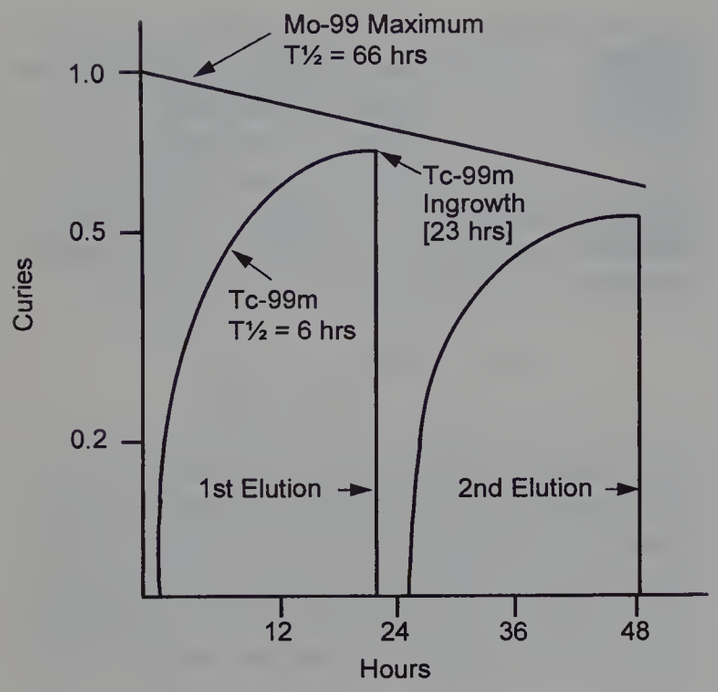


FIG. 13-11. *Elution graph of radioactivity (exponential) versus time (linear) of the Mo-99m/Tc-99m radionuclide generator with sterile 0.9% sodium chloride for 2 days (actual generator is useful for 12 days postcalibration). The* upper straight line *represents the radioactive decay of the parent (Mo-99) to the daughter (Tc-99m), which reaches equilibrium at four half-lives of the daughter (6 hr × 4 = 24 hr).*

uranium. All 22 known isotopes of technetium are radioactive, and there are eight nuclear isomers. Since no stable isotopes of technetium exist, the chemistry has been poorly developed; however, milligram quantities of Tc-99 (a weak beta particle emitter; $T\frac{1}{2} = 2.1 \times 10^5$ years) are now available for determination of the structures of the technetium complexes, and >150 structures have been characterized. The chemistry of technetium is similar to rhenium and is dominated by forming compounds by bonding between the electron-deficient metal and electronegative groups, which are capable of donating electron pairs. Some examples of these electronegative groups are sulfhydryl, carboxylic acid, amine, phosphate, oxime, hydroxyl, phosphine, and isonitrile.

Basically, all technetium radiopharmaceuticals are metal-electron donor complexes. Compounds that contain two or more electron donor groups and bind to a metal are called chelating agents. Technetium as a transition state element can have oxidation states from −1 to +7. As a pertechnetate (TcO^-_4) ion, technetium will not form many metal-donor complexes. However, it can be reduced to species that will complex with a variety of monodentate, bidentate, or polydentate ligands. The oxidation state of technetium in various complexes and the actual structure is unknown for several of the compounds. Deutsch et al.[1] claim the oxidation states that are most common in the chemistry of technetium are +1, +3, and +5. Technetium (TcO^-_4) can be reduced by a stannous salt, ascorbic acid, sodium borohydride, and electrolysis. The most common reducing agent is the stannous ion because of water-solubility, stability, low toxicity, and effectiveness at room temperature (Nowotnik).[2] Reviews of the chemistry of technetium are presented by Hjelstuen[3] and Schwochau,[4] but the stereochemistry of the technetium coordination complexes is not shown. An excellent review by Jurisson et al.[5] covers all coordination compounds used in nuclear medicine with a special emphasis on Tc-99m complexes.

Tc-99m radiopharmaceuticals are prepared at the hospital or local nuclear pharmacy by nonradioactive components in a sterile serum reaction vial. The primary chemical substances in the vial are the complexing agent (ligand) and reducing agent, usually some stannous salt (stannous chloride, stannous fluoride, or stannous tartrate). After preparation of the radiopharmaceutical, tests for radiochemical purity should be carried out to assure that the radiotracer is in the right chemical form. The analytical methods used include paper and thin layer chromatography, column chromatography, and solvent extraction. Likely radiochemical impurities include sodium pertechnetate ($Na^{99m}TcO_4$); some insoluble compound, i.e., reduced hydrolyzed technetium (TcO_2) or technetium tin colloid; and a different complex other than the one expected (i.e., ^{99m}Tc-monodentate rather than ^{99m}Tc-bidentate ligand). The sterile serum vials containing the stannous salt and the ligand are lyophilized under a sterile inert gas atmosphere (i.e., nitrogen or argon). The ligand in the reaction vial determines the final chemical structure of the ^{99m}Tc complex and the biological fate after the intravenous injection of the radiopharmaceutical.

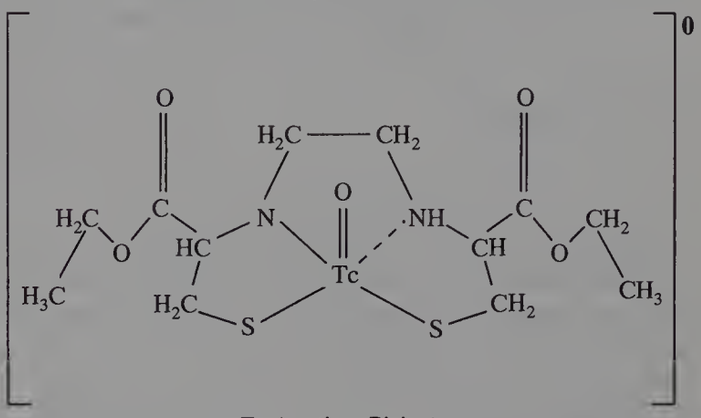
TECHNETIUM RADIOPHARMACEUTICALS

Technetium (^{99m}Tc) Albumin Injection. A sterile colorless to pale yellow solution containing human albumin (m.w. ~60,000 daltons) radiolabeled wtih Tc-99m pertechnetate. The reducing agent is stannous tartrate, which reduces the $^{99m}TcO^-_4$ to an unknown oxidation state and is weakly chelated by the tartrate and possibly forms a complex with sulfhydryl groups on the albumin by ligand exchange. The precise structure of the stannous technetium albumin complex is unknown at this time. The patient receives an intravenous injection of 25 mCi (925 MBq) of Tc-99m albumin. Multiple images of the blood in the heart are taken by electrocardiographic gating (R-R interval). The rising portion of the R wave coincides with end-diastole. These images are stored in a computer to reconstruct a movie of the beating heart. This procedure is sometimes called a multigated acquisition (MUGA). Information obtained by this technique includes cardiac chamber wall motion and calculation of ejection fraction. Indications for the procedure include evaluation of effects of coronary artery disease, follow-up of coronary artery bypass graft patients, heart failure, heart transplant evaluation (pre- and postoperative), cardiomyopathies, and effects of cardiotoxic drugs (i.e., doxorubicin).

Technetium (^{99m}Tc) Albumin Aggregated. This product consists of a sterile white suspension of human albumin aggregates formed by the denaturation of human albumin by heating at 80°C at pH 4.8 (isoelectric point of albumin). The precise structure of the stannous technetium albumin aggregated complex is unknown at this time. The particle size and number can be estimated with a hemacytomer grid. The particle size of the suspension should be between 10 and 100 μm with no particles greater than 150 μm. The clinical use of this agent is for imaging the pulmonary microcirculation for pulmonary embolus and to assess regional pulmonary function for surgery (i.e., lung transplants). The patient receives an intravenous injection of 2 to 4 mCi (74 to 148 MBq) of the Tc-99m albumin aggregates, which lodge in some of the small pulmonary capillaries, and the distribution can be imaged. The suggested number of aggregates recommended for good image quality and safety is 100,000 to 500,000 particles; thus, only a small fraction of the 280 billion capillaries are occluded. Multiple images of the lung are obtained to assess lung perfusion. The distribution of the particles in the lung is a function of regional blood flow; consequently, in the normal lung, the particles are distributed uniformly throughout the lung. When blood flow is occluded because of emboli, multiple segmental "cold" (decreased radioactivity) defects are seen. This procedure is almost always combined with a xenon-133 gas lung ventilation scan (should be normal) and same-day chest x-ray (should be normal).

Technetium (^{99m}Tc) Albumin Colloid Injection. This product consists of a sterile opalescent colorless dispersion of colloidal human albumin labeled with Tc-99m pertechnetate after it is reduced with a stannous salt. The precise structure of the stannous technetium albumin colloid complex is unknown at this time. The particle size may be examined with a hemacytomer grid. The particle size range of the colloid is 0.1 to 5.0 μm. After the patient receives an intravenous injection of 5 mCi (185 MBq) of Tc-99m albumin colloid, the agent is cleared from the blood by the reticuloendothelial (RE) cells. These RE cells are located principally in the liver (85%) and spleen (10%), and the remainder are in the bone marrow, kidney, and lung. About 15 min after injection, multiple images of the liver and spleen are obtained. An initial dynamic flow study may be obtained to determine liver and spleen perfusion in cases of abdominal trauma. Liver and spleen imaging is useful to determine organ size, the presence of hepatic metastases, and the degree of hepatocellular dysfunction in diffuse liver disease (i.e., cirrhosis).

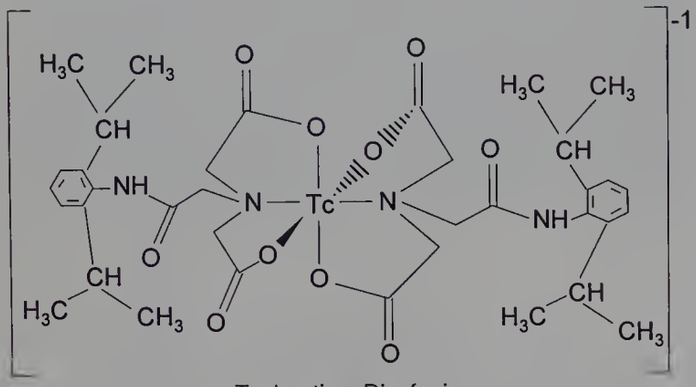
Technetium (^{99m}Tc) Bicisate Injection. A sterile colorless solution of bicisate is complexed with Tc-99m pertechnetate after reduction with a stannous salt. The precise structure of the technetium complex is [*N*, N′-ethylenedi-L-cysteinato(3-)]oxo [^{99m}Tc]technetium(V), diethylester. This radiopharmaceutical is a neutral and lipophilic complex that crosses the blood-brain barrier and exhibits selective retention in the brain. Therefore, this radiotracer is used as a brain-perfusion imaging agent. After intravenous injection of 20 mCi (740 MBq) of Tc-99m bicisate, ~5% of the injected dose is localized within the brain cells at 5 min after injection and demonstrates rapid renal excretion (74% in 24 hr). Images of the brain can be obtained for up to 6 hr after injection. The patient should be in a controlled environment for at least 20 min prior to injection (i.e., covered eyes, quiet room with indirect lighting). The indications for this study are for evaluation of stroke and other brain lesions. The normal study demonstrates a homogenous and symmetric distribution of radioactivity throughout the brain.



Technetium Bicisate

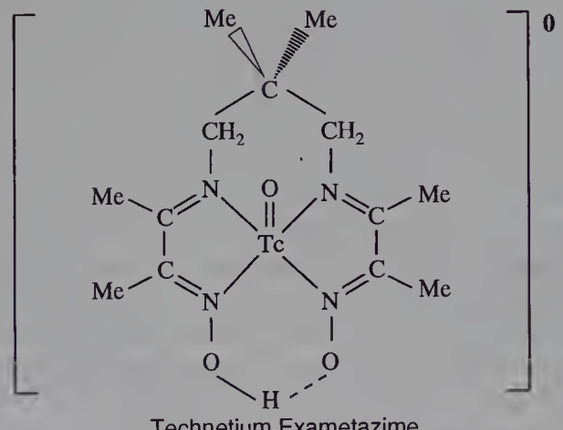
Technetium (^{99m}Tc) Disofenin Injection. A sterile colorless solution of disofenin is complexed with Tc-99m pertechnetate after reduction with a stannous salt. The precise structure of the technetium complex is unknown at this time. However, Costello et al.[6] specify that an analog of this complex Tc-99m lidofenin provides a single technetium (III) distorted octahedral (1:2) complex with a coordination number of 6. The patient receives an intravenous injection of 5 mCi (185 MBq) of Tc-99m disofenin, which is taken up by the functional cells (hepatocytes) in the liver by active anionic transport. Then the radiopharmaceutical is excreted in bile, via the biliary canaliculus, into the bile ducts with accumulation in the gallbladder and finally excretion via the common bile duct into the small bowel (duodenum) within a certain period of time. In the normal patient, there is an early accumulation of the radiopharmaceutical within the liver and visualization of the gallbladder and small bowel within 1 to 2 hr after injection. The primary clinical indication for this study is to separate acute from chronic cholecystitis. In acute cholecystitis, there is obstruction of the cystic duct, with nonvisualization of the radiopharmaceutical in the gallbladder. This is the procedure of choice to prevent the development of gangrenous cholecystitis and perforation with its associated morbidity and mortality by drug and surgical intervention. Patients must be fasting (2 to 3 hr) before the procedure. Inpatients that have fasted for a long period of time, because of illness, are given an intravenous injection of sincalide (a synthetic cholecystokinin derivative) prior to the injection of Tc-99m disofenin to prevent a false-positive study. Some other clinical conditions that can be diagnosed by biliary images are common bile duct obstruction, biliary leak from surgery, biliary atresia, and a choledochal cyst. A newer biliary imaging agent is Tc-99m mebrofenin, which is more lipophilic because it has bromine on the benzene ring. In the presence of elevated serum bilirubin levels, there

is less renal excretion due to higher lipid solubility. In addition, the product is more stable, which makes it more cost-effective for a centralized nuclear pharmacy.



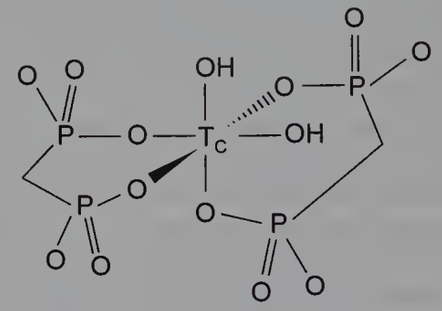
Technetium Disofenin

Technetium (^{99m}Tc) Exametazime Injection. A sterile colorless solution of exametazime is complexed with Tc-99m pertechnetate after reduction with a stannous salt. The precise structure of the technetium complex is unknown at this time. However, Jurisson et al.[7] specify that analogs of this complex Tc-99m propylene amine oxime provides a technetium (V) square pyramidal complex with a coordination number of five. This radiopharmaceutical is lipid-soluble and, therefore, crosses the blood-brain barrier and is trapped within the brain. The possible mechanisms proposed for localization includes binding to glutathione, change in ionic state, and chemical degradation. The patient receives an intravenous injection of 20 mCi (740 MBq) of Tc-99m exametazime in a controlled environmental state. The patient is supine, with covered eyes (20 min), in a quiet room and with indirect lighting prior to injection. The radiopharmaceutical is irreversibly bound to the brain after 10 min. Some of the indications for this study are localization of seizure foci, evaluation of dementia, identification of drug abuse induced brain defects (i.e., cocaine), and evaluation of stroke. The normal study is represented by a homogenous and symmetric distribution of radioactivity throughout the brain. Cerebellar activity is usually greater than the rest of the brain. This is the agent of choice to determine brain death in patients on life support systems. The major use of this radiopharmaceutical at this time is for the radiolabeling of autologous leukocytes as an adjunct in the localization of intra-abdominal infection and inflammatory bowel disease.

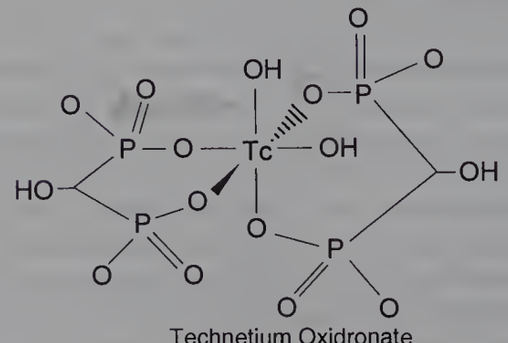


Technetium Exametazime

Technetium (^{99m}Tc) Medronate Injection. A sterile, colorless solution containing sodium medronate (methylene diphosphonate) and a stannous salt complexed with Tc-99m pertechnetate. A structure proposed by Libson et al.[8] of the technetium medronate complex is shown below. However, de Ligny et al.[9] specify that Tc-99m bone imaging agents are mixtures of many components (polymers and oligomers) that can be separated by high performance anion-exchange chromatography. The clinical use of this agent for investigation of skeletal problems such as metastatic disease to the bones, osteomyelitis, Paget's disease, fractures, primary bone tumors, avascular necrosis, metabolic bone disease, and loose or infected hip prostheses. The patient receives an intravenous injection of 15 to 20 mCi (555 to 740 MBq) of Tc-99m medronate, which localizes in bone according to the degree of metabolic activity. Tc-99m medronate is absorbed onto hydroxyapatite crystals at sites of new bone formation with ~50% to 60% of the injected dose distributed throughout the skeleton within 3 hr, and the rest is excreted by the kidneys. The patient waits 3 to 4 hr after injection, while drinking lots of fluids and urinating frequently to minimize the radiation dose to the bladder, before images of the whole skeleton are obtained. Abnormalities on the bone image appear as "hot" spots as a result of increased bone formation in the diseased site, and "cold" spots are observed in avascular necrosis because of decreased blood perfusion. Stress fractures can be diagnosed by bone imaging when x-rays are completely normal. The bone image is probably the most commonly performed nuclear medicine diagnostic procedure (i.e., especially for metastatic breast and prostate cancer). A newer bone imaging agent, Tc-99m oxidronate (a hydroxyl group on the carbon of medronate), demonstrates a higher binding affinity for hydroxyapatite crystals in bone; however, subjective criteria indicate no advantage of this agent.

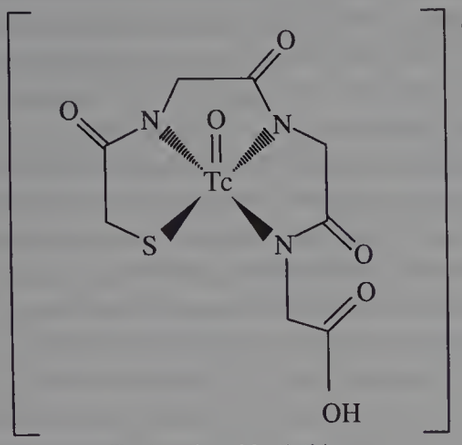


Technetium Medronate



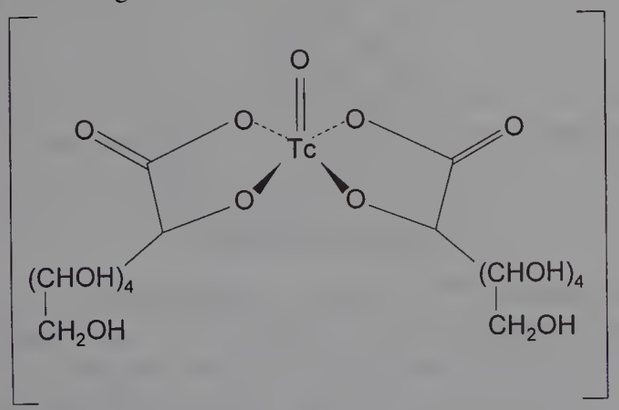
Technetium Oxidronate

Technetium (^{99m}Tc) Mertiatide Injection. A sterile, colorless solution of mertiatide complexed with Tc-99m pertechnetate after reduction with a stannous salt. The precise structure of the technetium mertiatide complex is shown below.



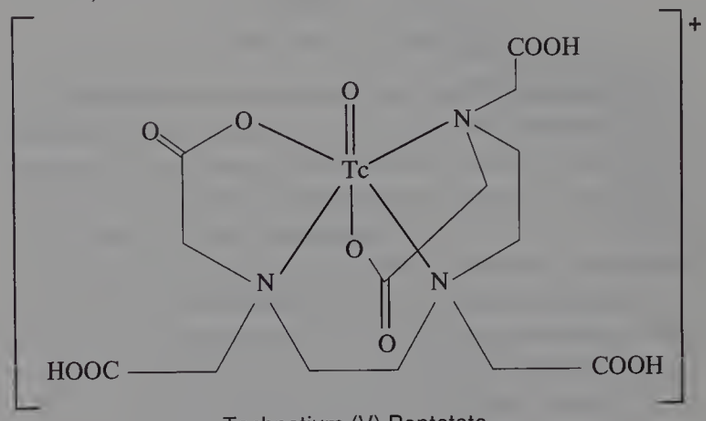
Technetium Mertiatide

This radiopharmaceutical is the agent of choice to provide information about relative function of the kidneys and urine outflow because it has a higher extraction efficiency than Tc-99m pentetate. Indications include renal artery stenosis in nonperfused kidneys, renal transplant assessment, and outflow obstruction. The patient receives a bolus intravenous injection of 10 mCi (370 MBq) Tc-99m mertiatide, and dynamic images are obtained every 3 to 5 sec to study blood flow to the kidneys. Sequential static images are then obtained for 20 to 30 min to evaluate renal cortical uptake, excretion, and tubular clearance. Delayed images may be required to evaluate patients with obstruction or renal failure. Normally, there is prompt symmetric bilateral perfusion, good cortical accumulation bilaterally with visualization of the collecting systems by 3 to 5 min postinjection, and rapid excretion into the bladder with no delay to indicate partial or complete obstruction. An older renal imaging agent, Tc-99m gluceptate (a Tc-99m hydroxy acid complex; see below for the ligand), is now used as a transchelation agent for radiolabeling monoclonal antibodies.



Technetium Gluceptate

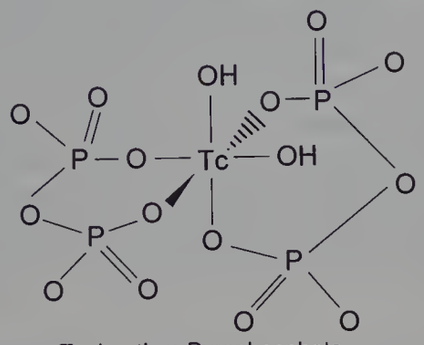
Technetium (^{99m}Tc) Pentetate Injection. A sterile, colorless or slightly yellow solution of sodium pentetate or calcium trisodium pentetate is complexed with Tc-99m pertechnetate after reduction with a stannous salt. The precise structure of Tc-99m pentetate is unknown; however, Jurisson et al.[5] suggested the possible structure below. The primary clinical use of this agent is for renal studies and glomerular filtration rate (GFR), but it is occasionally used for brain death and brain tumor localization. The patient receives an intravenous injection of 3 to 20 mCi (111 to 740 MBq), and the kidneys are imaged for 20 to 30 min. The GFR is calculated by a quantitative method using a combination of imaging and counting the radioactivity in serum and urine samples. Normal extraction efficiency is 20% (80 to 140 ml/min).



Technetium (V) Pentetate

Technetium (^{99m}Tc) Pyrophosphate Injection. A sterile, colorless solution of pyrophosphate is complexed with Tc-99m pertechnetate after reduction with a stannous salt. The precise structure of technetium (^{99m}Tc) pyrophosphate is not known at this time; however, a suggested structure is shown below. Originally, technetium complexed with pyrophosphate was used as a skeletal imaging agent. The in vivo instability of the Tc-99m inorganic phosphate complex led to development of a Tc-99m organic phosphate complex (Tc-99m medronate). The present clinical use for this agent is for the diagnosis of recent myocardial infarction.[10] The study is limited to a time interval of 24 to 72 hr after infarction and can become normal after 5 days. This time delay has limited the usefulness of ^{99m}Tc pyrophosphate in assessing acute interventional studies or drugs designed to reduce infarct size. The patient receives an intravenous injection of 15 to 20 mCi (555 to 740 MBq) of Tc-99m pyrophosphate, and images are obtained at 1 to 3 hr after injection. Tc-99m pyrophosphate clears rapidly from the blood, and by 3 hr, $<$10% is in the blood and the rest is in the bone (40% to 50%) and urine (45% to 50%). The mechanism of the localization of the radiotracer in the infarct is not well understood, but the maximum concentration occurs in peripheral zones of the infarct where there is adequate blood perfusion. The distribution pattern is similar to where there is increased calcium deposition. In the normal study, there is no uptake above blood background in the myocardium. In the positive study, there is a "hot" spot in the myocardium that is usually graded (1 + to 4 +) relative to rib uptake of the radiopharmaceutical. In a multicentre investigation, the specificity of Tc-99m pyrophosphate was only 64%. Thus, its use is limited to patients in whom the diagnosis by electrocardiography

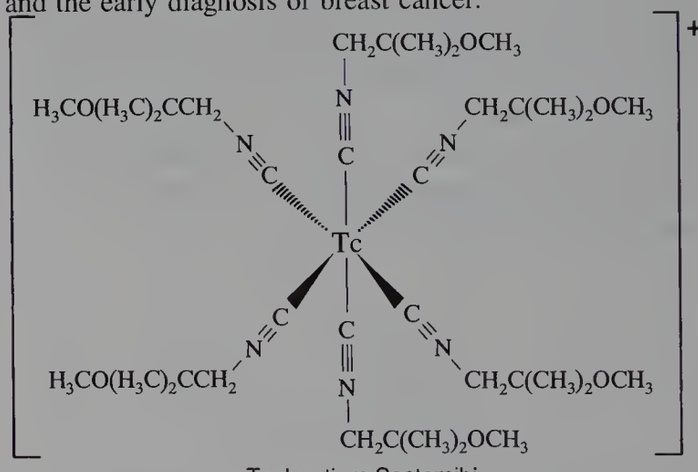
and enzymes are normal or who have intraoperative infarctions.



Technetium Pyrophosphate

Technetium (^{99m}Tc) Red Blood Cells (Autologous). A sterile reaction vial containing stannous citrate (Ultratag RBC kit) is used for radiolabeling a patient's red blood cells (RBC) with Tc-99m pertechnetate. Briefly, the patient's blood (1 to 3 ml) is drawn using acid citrate dextrose (ACD) or heparin (100 units) as an anticoagulant. The blood is labeled with the patient's name and hospital number and added to the sterile reaction vial. After mixing and incubation for 5 min, sodium hypochlorite (6 mg) is added to the vial to oxidize excess stannous ions (+2) to stannic ions (+4). A citrate buffer is added to adjust the pH to ~7.4. Then 30 mCi (1,110 MBq) of Tc-99m pertechnetate is added to the blood in the vial and mixed and incubated for 20 min. Without further preparation, the patient receives an intravenous injection of 25 mCi (925 MBq) of their own radiolabeled red blood cells. Three different studies can be performed after injection of the Tc-RBC. First, the MUGA study for evaluation of cardiac function using Tc-99m albumin, can be obtained using the same techniques. This is the agent of choice for cardiac function studies because the Tc-99m red blood cells remain in the circulating blood volume, whereas the Tc-99m albumin leaks into the extracellular spaces. This increases the background radioactivity around the heart and contributes to degradation of the blood pool image. Second, the Tc-99m red blood cells are used to noninvasively localize the preoperative site of active lower gastrointestinal bleeding. The patient should be studied during the clinical period of maximal blood loss. The patient is injected with his own Tc-99m-red blood cells, which remain within the circulating blood for sufficient time to extravasate and accumulate within the bowel lumen at the site of bleeding. Images are obtained at various times, and delayed images can be obtained even up to 24 hr after injection. The positive study demonstrates an abnormal "hot" spot of the radiotracer that appears where the bowel is expected, and the radioactivity persists and may increase with time. In addition, the radioactivity should move with peristalsis of the intestine. Third, Tc-99m red blood cells can be crenated (wrinkled) by heating at 49.5°C for a half-hour in the sterile reaction vial with a water bath. These damaged radiolabeled red blood cells are preferentially extracted from the circulating blood volume by splenic tissue. Indications for this study include trauma, confirmation of accessory spleen, and successful surgical splenic transplants.

Technetium (^{99m}Tc) Sestamibi Injection. A sterile, colorless solution of sestamibi is synthesized by a reaction with Tc-99m pertechnetate after reduction with a stannous salt. The precise structure of the technetium complex is Tc-99m $(MIBI)^{+}{}_{6}$, where MIBI is 2-methoxyisobutyl isonitrile. This was the first Tc-99m–labeled agent introduced to replace thallous (^{201}Tl) chloride for myocardial perfusion imaging. The shorter half-life of Tc-99m (6 hr) compared with Tl-201 (73 hr) allows administration of a larger dose, which provides clearer images of the heart. There is relatively stable tracer uptake and little redistribution after stress, allowing a longer imaging time. There is a good correlation between thallous (^{201}Tl) chloride and Tc-99m sestamibi uptake and myocardial blood flow. The patient receives an intravenous injection of 8 to 10 mCi (296 to 370 MBq) of Tc-99m sestamibi for a resting imaging study performed ~1 hr after injection. During that time, the patient is encouraged to eat a fatty meal to wash out the liver accumulation of the radiopharmaceutical, which can interfere with the interpretation of defects in perfusion of the inferior wall of the heart. Then, 4 hr after the rest injection, a second injection of 25 to 30 mCi (925 to 1,110 MBq) is given after "physical" stress (treadmill exercise) or "pharmacological" stress with an intravenous infusion of a vasodilator (dipyridamole or adenosine). Ischemic myocardium may appear normal at rest, but will appear abnormal at stress because if there is narrowing of a coronary artery, then blood flow cannot be increased sufficiently during stress and the ischemic area shows less radiopharmaceutical uptake. New investigational indications for the use of the Tc-99m sestamibi complex appear to include the preoperative localization of parathyroid adenoma and the early diagnosis of breast cancer.

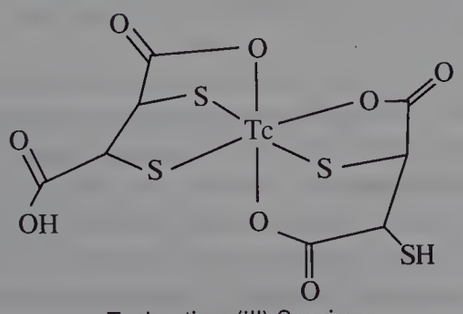


Technetium Sestamibi

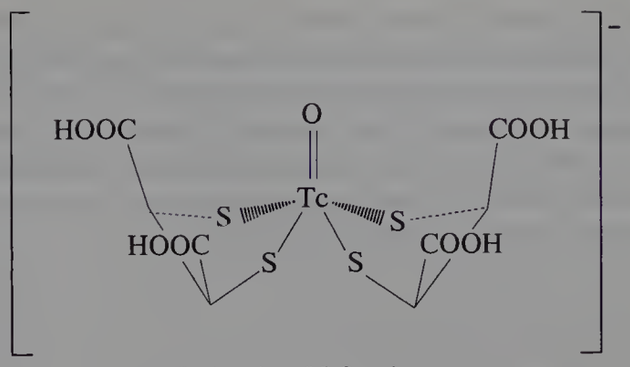
Technetium (^{99m}Tc) Sodium Pertechnetate. This is a sterile, colorless solution containing sodium pertechnetate ($Na^{99m}TcO_4$) in normal saline (0.9% NaCl) obtained by elution of the sterile Mo-99/Tc-99m generator. The pertechnetate ion ($^{99m}TcO_4^-$), which has a similar ionic radius and charge as the iodide ion (I^-), is concentrated in the thyroid, salivary glands, kidneys, stomach, and choroid plexus in the brain. It can be used directly from the Mo-99/Tc-99m generator to image the thyroid, Meckel's diverticulum (stomach

tissue in the intestine), salivary glands for tumors, and processes that disrupt the blood-brain barrier (i.e., tumors, abscesses, strokes). Unlike the iodide ion, the pertechnetate ion is not converted to thyroid hormone, but only trapped (1% to 2%) by the thyroid. Tc-99m pertechnetate is usually the preferred agent for thyroid imaging unless there is a special indication for a radioactive iodide scan. Tc-99m pertechnetate is always available, less expensive, has ideal physical characteristics for imaging, and results in a lower radiation dose to the patient. The primary indication for thyroid imaging is for evaluating the functional status of thyroid nodules. In the normal thyroid, there is symmetrical radiotracer uptake throughout both lobes of the thyroid. Thyroid nodules can appear nonfunctional (decreased uptake—''cold'' defect) or functional (increased radiotracer uptake—''hot'' defect). The thyroid nodule that is suspicious for concern is the single ''cold'' nodule, which is not trapping the pertechnetate ion. This ''cold'' nodule could be a tumor or cyst, so a fine-needle biopsy is performed after the thyroid scan if the nodule is ''cold.'' Other indications are multinodular goiter, and functional assessment and weight determination prior to sodium iodide (^{131}I) treatment of thyrotoxicosis. The patient receives an intravenous injection of 5 to 10 mCi (185 to 370 MBq) of Tc-99m pertechnetate, and images are obtained of the thyroid 0 to 20 min after injection. The usual dose for the other imaging procedures is the same for Meckel's diverticulum and salivary glands, and 20 mCi (740 MBq) for brain tumor imaging.

Technetium (^{99m}Tc) Succimer Injection. A sterile, colorless solution of succimer (2,3-dimercaptosuccinic acid) is complexed with Tc-99m pertechnetate after reduction with a stannous salt at acid pH. The precise structure of Tc-99m (III) succimer is unknown; however, Moretti[11] suggested the possible structure below. Tc-99m succimer is very useful for demonstrating the functioning renal parenchyma, because ~40% of the dose is bound to the renal cortex at 1 hr after injection. The patient is injected with 5 mCi (185 MBq) of Tc-99m succimer, and multiple images are taken at 2 to 4 hr after injection. This study can be useful for evaluating renal trauma, renal masses (e.g., tumors, cysts), and renal scarring. Tc-99m succimer is the diagnostic agent of choice in children who have chronic urinary tract infections causing renal scarring. If the pH is adjusted to 8.0 to 8.5, a technetium (V) succimer complex is formed, which is useful for imaging tumors (Ohta et al.).[12] Blower et al.[13] have proposed the following structure for Tc-99m (V) succimer.



Technetium (III) Succimer

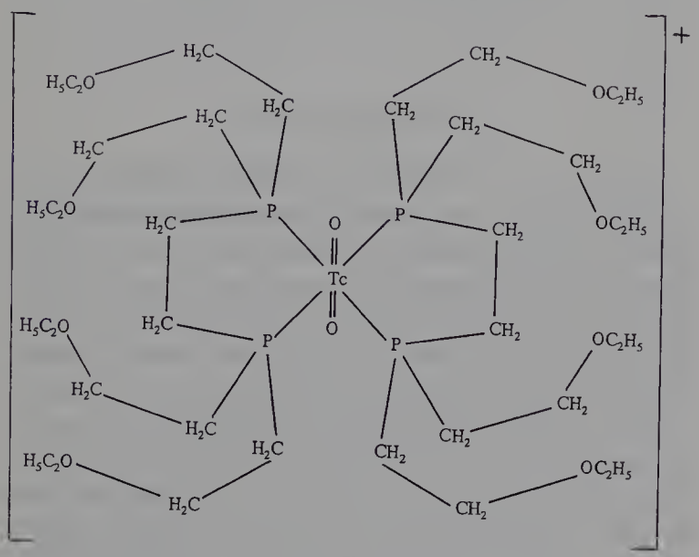


Technetium (V) Succimer

Technetium (^{99m}Tc) Sulfur Colloid Injection. This product is a sterile, opalescent colloidal dispersion of sulfur, a unit of structure built up from polymeric molecules and ions (micelles) radiolabeled with Tc-99m pertechnetate formed by heating in dilute hydrochloric acid. The radiocolloid should be stabilized with gelatin A to inhibit clumping of the negatively charged colloidal particles. The particle size of the colloid is 0.1 to 3 μm. After intravenous injection of 5 to 10 mCi (185 to 370 MBq) of Tc-99m sulfur colloid, the radiopharmaceutical is rapidly cleared from the blood by the reticuloendothelial (RE) cells of the liver, spleen, and bone marrow. Uptake of the Tc-99m sulfur colloid is dependent upon the relative blood perfusion rate and the functional capacity of RE cells. In the normal patient, 85% of the radiocolloid is phagocytized by Kupffer cells in the liver, 7.5% by the spleen, and the remainder by the bone marrow, lungs, and kidneys. In bone marrow imaging, the study is performed 1 hr after injection of 10 mCi (370 MBq) of Tc-99m sulfur colloid. Normal bone marrow will take up the radiocolloid, but diseased bone marrow will appear as ''cold'' defects in patients with tumor deposits in the bone marrow. Tc-99m sulfur colloid is used as a secondary agent in liver and spleen imaging if Tc-99m albumin colloid is not available. However, it is the primary agent used for gastrointestinal studies such as gastroesophageal reflux (GER) and gastric emptying of solid food. Gastroesophageal reflux imaging is performed after having the patient swallow acidified orange juice mixed with Tc-99m sulfur colloid. Normal patients have no GER. This study is reported to have a 90% sensitivity in detecting GER. Gastric emptying imaging is performed after the patient swallows solid food (i.e., scrambled eggs or pancakes) radiolabeled with Tc-99m sulfur colloid. In general, the normal gastric emptying half-time is <90 min for solid food.

Technetium (^{99m}Tc) Tetrofosmin Injection. A sterile, colorless solution of tetrofosmin is complexed with Tc-99m pertechnetate after reduction with a stannous salt. The precise structure of the technetium complex is shown below (Forster).[14] The formulation contains gluconate to form a weak technetium (V) chelate to keep the technetium in the (V) oxidation state for transchelation to form the technetium (V) tetrofosmin complex. Technetium (V) tetrofosmin is another cationic Tc-99m complex that has been found to accumulate in viable myocardium similar to thallous (^{201}Tl) chlo-

ride. Myocardial uptake of this agent in humans is ~1.2% at 5 min after intravenous injection and decreases to 1.0% at 2 hr. This agent appears to be less specific for detecting ischemia (66%) than Tl-201 chloride (77%) in a small study (252 patients). However, the radiopharmaceutical appears to have a fast clearance through nontarget organs (liver), which presents less high background imaging problems.

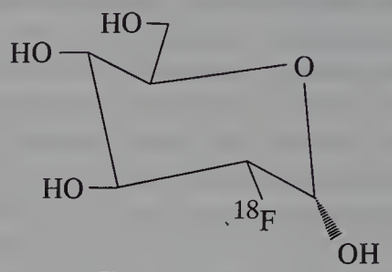


Technetium Tetrofosmin

FLUORINE RADIOCHEMISTRY

The useful radioisotope of this element for organ imaging is fluorine-18. Fluorine-18 is produced in a cyclotron by the $^{18}O(p,n)^{18}F$ nuclear reaction. Fluorine-18 ($T\frac{1}{2} = 109$ min) decays by electron capture and positron emission to oxygen-18 with gamma-ray emissions of 511 keV (194%). Fluorine-18 can be attached to a number of physiologically active molecules and with the great strength of the C—F bond appears to be a very useful label for radiopharmaceuticals.[15] However, radiotracer production involves relatively complicated synthetic pathways and the preparation of high specific activity compounds presents many problems. The short half-life of fluorine-18 makes it necessary to complete the synthetic and purification procedure within 3 hr. Consequently, a separate chemistry system (black box type) is needed for each compound. The chemistry of fluorine is complicated, but some compounds can be fluorinated by $^{18}F^-$ exchange reactions and direct fluorination with the elemental fluorine ($^{18}F_2$); as well, compounds with an aromatic ring may be fluorinated by several synthetic reactions. For example, partially fluorinated heteroaromatics can readily be obtained by the conversion of an amino group on the aromatic ring to fluoride using the Balz-Schiemman and several related reactions.

Fluorine (^{18}F)-2-Fluoro-2-Deoxy-D-Glucose. The only F-18 radiopharmaceutical presently available is fluorine (^{18}F)-2-fluoro-2-deoxy-D-glucose (F-18 FDG). The precise structure of fluorine (^{18}F)-2-fluoro-2-deoxy-D-glucose is shown below and is the only PET agent approved by the FDA. The current method of synthesis of F-18 FDG was introduced by Hamacher[16] by nucleophilic fluorination. This radiopharmaceutical has been used to study metabolism in the brain and heart, but appears to be most useful in cancer management.[17] The high glycolytic rate of many neoplasms facilitates tumor imaging with this glucose analog. Because of the widespread anatomic distribution of metastases, a whole-body imaging technique using a tumor-specific radiopharmaceutical is very useful for tumor detection and mapping to evaluate the extent and relative metabolic activity of the disease.



Fluorodeoxyglucose

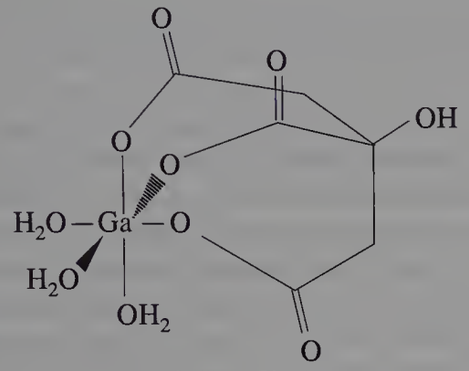
GALLIUM RADIOCHEMISTRY

The only radioisotope of this element that is presently used is gallium-67, which is produced in a cyclotron by proton bombardment of a zinc metal target by a $^{68}Zn(p,2n)^{67}Ga$ nuclear reaction. Gallium-67 ($T\frac{1}{2} = 78.2$ hr) decays by electron capture to stable zinc-67 with principal gamma-ray emissions of 93 keV (38%), 185 keV (24%), and 300 keV (16%). The radiotracer is isolated by dissolution of the target in hydrochloric acid and extracting the gallium-67 with isopropyl ether from the zinc and other impurities. The gallium-67 is back-extracted from the isopropyl ether into 0.2 M hydrochloric acid, evaporated to dryness, and dissolved in sterile, pyrogen-free 0.05 M hydrochloric acid. Gallium is an amphoteric element that acts as a metal at low pH but forms insoluble hydroxides when the pH is raised above 2.0 in the absence of chelating agents. At high pH, gallium hydroxide acts as a nonmetal and dissolves in ammonia to form gallates. Gallium forms compounds of oxidation states +1, +2, and +3; however, only the Ga^{+3} state is stable in aqueous solutions.

GALLIUM RADIOPHARMACEUTICALS

Gallium (^{67}Ga) Citrate. The gallium (III) citrate complex is formed by adding the required amount of sodium citrate (0.15 M) to gallium (III) chloride and adjusting the pH to 4.5 to 8.0 with sodium hydroxide. The proposed structure of gallium (^{67}Ga) citrate is shown below.[5] The patient receives an intravenous injection of 5 to 10 mCi (185 to 370 MBq) of gallium (^{67}Ga) citrate, and whole-body images are then obtained at 24, 48, and 72 hr after injection. Gallium

will localize normally in the liver and spleen, bone, nasopharynx, lacrimal glands, and breast tissue. There is also some secretion in the bowel; consequently, the patient should receive a laxative and/or enemas to evacuate this radioactivity prior to the 48-hr image. Gallium localizes at sites of inflammation or infection as well as some tumors such as Hodgkin's disease, lymphoma, bronchogenic carcinoma, and so forth. The nonspecific localization of gallium radioactivity requires correlation with other radiographic studies and clinical findings.



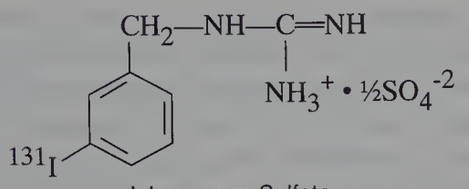
Gallium Citrate

IODINE RADIOCHEMISTRY

The useful radioisotopes of iodine for organ imaging are iodine-131 and iodine-123 because of their desirable physical characteristics. Iodine-131 is obtained from a reactor by production of tellurium-131. It is obtained by the nuclear reaction $^{235}U(n,fission)^{131}Te$ or $^{130}Te(n,gamma)^{131}Te$. The tellurium-131 ($T\frac{1}{2}$ = 25 min) decays by beta emission to iodine-131. Iodine-131 ($T\frac{1}{2}$ = 8.04 days) transforms by beta decay to stable xenon-131 with five significant gamma-ray emissions of 80 to 723 keV, with the major gamma-ray of 364 keV (82%) providing good tissue penetration for organ imaging. Undesirable properties of iodine-131 are the high radiation dose from the beta particles, the long half-life, and the poor image produced by the high-energy gamma-rays. Iodine-123 ($T\frac{1}{2}$ = 13.3 hr) decays by electron capture to tellurium-123 with a principal gamma-ray emission of 159 keV (83%), which makes it the ideal radioisotope of iodine for organ imaging because of increased detection efficiency and reduced radiation to the patient. Iodine-123 is produced in a cyclotron bombarding an antimony metal target with alpha (α) particles according to the $^{121}_{51}Sb(\alpha,2n)^{123}_{53}I$ reaction or an iodine target with high-energy protons by the $^{127}_{53}I(p,5n)^{123}Xe$ nuclear reaction. The xenon-123 decays by electron capture to iodine-123. However, it is relatively expensive to produce and, at the present time, has limited availability for radiolabeling compounds. Iodine is in Group VIIB with the other halogens (fluorine, chlorine, bromine, and astatine). In aqueous solution, compounds of iodine are known with at least five different oxidation states; however, in nuclear medicine, the -1 and $+1$ oxidation states are the most significant. The -1 oxidation state represented as sodium iodide (NaI) is important for thyroid studies and, when obtained in a reductant-free solution (no sodium thiosulfate), is the starting compound for the radiolabeling of most iodinated radiopharmaceuticals. The common methods for introducing radioiodine into organic compounds can be classified as isotope exchange reactions, electrophilic substitution of hydrogen in activated aromatic systems, nucleophilic substitution, and addition to double bonds.[18] The replacement of aromatic hydrogen in activated aromatic systems is used for protein labeling, and the electrophilic iodine (I^+) can be generated by a variety of oxidizing agents, including (a) chloramine-T (N-chloro-p-toluene sulfonamide) sodium, (b) enzyme oxidation of I^- (lactoperoxidase), and (c) iodogen (1,3,4,6-tetrachlora-3a-6a-diphenylglycoluril). The actual iodinating molecule depends on the oxidizing agent but is probably HOI or H_2OI^+.

IODINE RADIOPHARMACEUTICALS

Iobenguane Sulfate (^{131}I) Injection (I-131-Metaiodobenzylguanidine Sulfate). This radiopharmaceutical is radiolabeled by a Cu^{+1} catalyzed isotopic nucleophilic exchange reaction. It is a radioiodinated arylalkylguanidine and is similar to the antihypertensive drug (guanethidine) and to the neurotransmitter norepinephrine. The proposed structure of iobenguane (^{131}I) sulfate is shown below. Functional tumors of the adrenal medulla (pheochromocytomas) and tumors of neuroendocrine origin (neuroblastoma) can be localized on I-131 meta-iodobenzyl-guanidine (^{131}I-MIBG) images, as abnormal tissue that takes up the radiopharmaceutical and appears as increased activity on the image.[19] Drug intervention studies in animals using reserpine have demonstrated that the ^{131}I-MIBG enters adrenergic neurons and chromaffin cells by an active transport mechanism of catecholamine uptake into adrenergic storage granules. Neuroblastoma is a malignant tumor of the sympathetic nervous system occurring mostly in children. The tumor is of neural crest origin, consisting of cells that form the sympathetic nervous system called sympathogonia. They migrate to the adrenal medulla and many other parts of the body. Metastases may be found in the liver (stage IV) and in the regional lymph nodes, bone, bone marrow, and soft tissues. After an initial report by Kimmig et al.[20] of ^{131}I-MIBG uptake in neuroblastoma, successful use of this tracer was described by others. The increased uptake of ^{131}I-MIBG is so tissue-specific that, in a child with a tumor of unknown origin, it can establish the diagnosis of neuroblastoma. Prior to administration of the radiopharmaceutical, the patient is pretreated with Lugol's solution (up to 40 mg/day) 24 hr before and 4 to 7 days after to block thyroid uptake of free $^{131}I^-$. The ^{131}I-MIBG is administered by slow intravenous injection 0.3 to 0.5 mCi (11–18.5 MBq), and patients are imaged at 24, 48, and 72 hr later. Occasionally, the patient receives a renal imaging agent for better localization of the adrenal tumor.



Iobenguane Sulfate

Sodium Iodine (^{123}I) Capsules. The major indications for thyroid imaging with sodium iodide (^{123}I) are for ectopic thyroid tissue (e.g., lingual or mediastinal), for substernal thyroid, and in a small number of patients with thyroid cancer (1% to 3%) that retains the trapping function and therefore appears as a warm nodule on the Tc-99m pertechnetate scan. The patient is fasting for 4 hr prior to receiving the oral dose of 0.4 mCi (15 MBq) of sodium iodine (^{123}I). Images are obtained of the thyroid and surrounding area at 4 to 24 hr.

Sodium Iodine (^{131}I) Oral (Solution or Capsule). The thyroid cancer patient receives an oral dose of 5 to 10 mCi (185 to 370 MBq) of sodium iodide (^{131}I), which localizes in residual thyroid tissue after ''total'' thyroidectomy and functioning thyroid metastasis from thyroid carcinoma. Images of the whole body are obtained 48 to 72 hr later. These metastatic radioiodide surveys are used to detect regional or distant metastases for large-dose 150 mCi (5,550 MBq) inpatient therapy for thyroid carcinoma. Any thyroid hormone medication should be discontinued for 2 weeks (T_3) or 4 weeks (T_4). In addition, the patient should have blood drawn for a thyroid stimulating hormone (TSH) test to ensure that it is elevated prior to administration of the therapy dose in order to permit maximum stimulation of thyroid tissue. The patient should be fasting for 4 hr prior to receiving the oral dose of radiotracer.

INDIUM RADIOCHEMISTRY

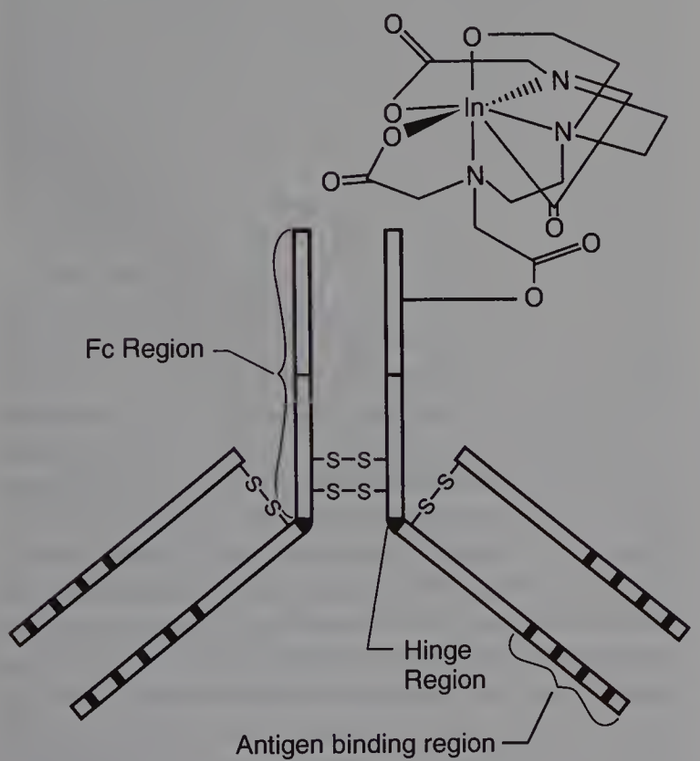
The most useful radioisotope of this element is indium-111, which is produced in a cyclotron by proton bombardment of a cadmium metal target by a ^{112}Cd(p,2n)^{111}In nuclear reaction. Indium-111 ($T\frac{1}{2}$ = 67.4 hr) decays by electron capture to stable cadmium-111 with principal gamma-ray emissions of 172 keV (91%) and 247 keV (94%). The radiotracer is isolated by dissolution in hydrochloric acid to form ^{111}In-chloride and separated from cadmium and other impurities by several dissolution and extraction steps. The last extraction is done using isopropyl ether, evaporating to dryness, and dissolving in sterile, pyrogen-free 0.05 M hydrochloric acid. In aqueous solution, lower valence states of indium have been described, but they are unstable and are rapidly oxidized to the trivalent state. In acid solution, indium salts are stable at low pH, but are hydrolyzed (above pH 3.5) to form a precipitate of indium hydroxide or trioxide. However, indium will remain in solution above pH 3.5 if it is complexed with a weak chelating agent such as sodium citrate and stronger chelating agents such as 8-hydroxy quinoline (oxine) or diethylenetriaminepentaacetic acid (DTPA). The radiolabeling of monoclonal antibodies or peptides by indium is accomplished by using compounds called bifunctional chelating agents. Bifunctional chelating agents are molecules that can both bind metal ions and be attached to other molecules. An example would be the cyclic anhydride of diethylenetriaminepentaacetic acid.

INDIUM RADIOPHARMACEUTICALS

Indium (^{111}In) Chloride Injection. Indium (III) chloride is a sterile, colorless solution that is radiolabeled with indium-111 in a hydrochloric acid solution (0.05 M) and has a pH of 1.5. This radiopharmaceutical can be used as a tumor, bone marrow, and abscess imaging agent under a physician's IND. It is primarily used for radiolabeling other compounds for use in cisternography and white blood cell labeling studies, and is particularly recommended for radiolabeling monoclonal antibodies for metastatic cancer imaging. If this agent is injected intravenously for clinical use, the patient's blood must be drawn into the syringe containing the radiopharmaceutical to buffer the agent to a higher pH to eliminate the burning sensation upon injection. When the acidic compound is mixed with blood, the indium-111 chloride binds quickly to transferrin, the iron-binding protein in the plasma. The mechanism of localization of the indium (III) chloride in bone marrow is probably explained by its ability to behave metabolically like iron and yet not get incorporated into hemoglobin in the red blood cell in the bone marrow. The mechanism of localization of the radiotracer in tumors and abscesses is probably due to increased blood flow and capillary permeability in the area of the tissue damage. Transferrin receptors have been suggested as a mechanism for localization, but not proven at this time.

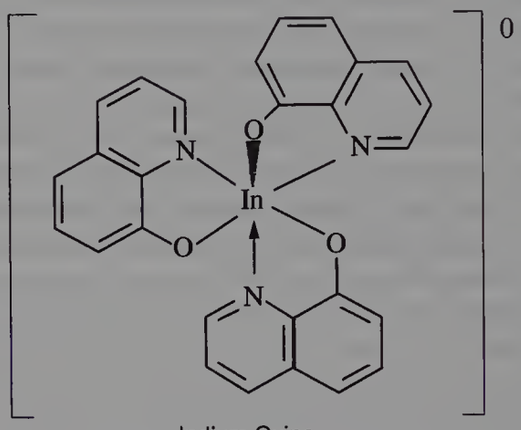
Indium (^{111}In) Oncoscint CR/OV (Satumomabpendetide). The simplified structure of indium (^{111}In) satumomabpendetide is shown below. Antibodies are a heterogeneous group of proteins isolated from human and animal serum and are called immunoglobulins. They are divided into classes arising from differences in structure and biological properties. They are assigned to major classes called IgG (80%), IgM (10%), and IgA, IgD, IgE (<10%). All antibodies have a general structure consisting of two heavy chains (m.w. ~55,000 daltons) and two light chains (m.w. ~20,000 daltons) of glycoproteins held together by disulfide bonds. Many tumors express antigenic markers on their surface that permit detection with radiolabeled antibodies. Antibodies are glycoproteins produced by B lymphocytes and plasma cells sensitized to an antigen. Hybridoma technology permits the manufacture of large quantities of antibody directed against specific antigens. Diagnostic antibodies are of two

types: polyclonal and monoclonal. Each chain has a variable region for antigenic binding and a constant region for complement fixation. Polyclonal antibodies include numerous antibody species of varying affinity for the antigen-binding surfaces. Monoclonal antibodies are generated from a single clone of antibody-producing cell and have a uniform affinity for their antigenic determinant.[21] Monoclonal antibodies are produced by immunizing a mouse with purified material from the surface of the human tumor cell. The antigen used in Oncoscint CR/OV is a tumor associated glycoprotein–72 (TAG-72), a high molecular weight glycoprotein expressed by colorectal and ovarian carcinomas.[22] The radiolabeling of the Oncoscint CR/OV monoclonal antibody was developed by Rodwell[23] as a site-specific method using a bifunctional chelate. Briefly, carbohydrate moieties on the monoclonal antibody (F constant region) are oxidized with periodate, and the aldehyde groups on the antibody are reacted with alpha amino groups of glycyl-tyrosyl-lysine-N-diethylene-triamine pentacetic acid. The Schiff's base form (imine) is stabilized by reduction with sodium cyanoborohydride. In-111 is chelated to a DTPA-carbohydrate molecule attached to the constant region of the monoclonal antibody. The specificity of radiolabeled antibody imaging for tumors is greater than that of gallium (^{67}Ga) citrate studies. However, sites of nonspecific uptake have been reported such as recent surgical wounds, colitis, bone fracture, and normal colostomy stoma.

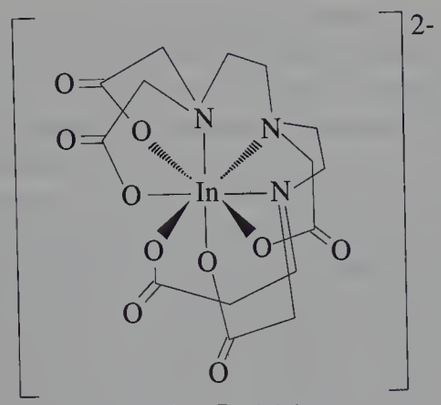


Indium Satumomabpendetide

Indium (^{111}In) Oxine (8-Hydroxyquinoline). The indium (III) oxine complex can be formed by adding the required amount of 8-hydroxyquinoline sulfate to indium (III) chloride and adjusting the pH to 6.5 to 7.5 with HEPES buffer. The precise structure of indium (III) oxine as determined by Green[24] is shown below. The patient has 45 to 90 ml of blood drawn for a 1.5- to 2-hr in vitro process of separating and labeling cellular elements such as leukocytes or platelets. Images are acquired at 2 to 24 hr after reinjection of the labeled cells. In the case of indium-111 leukocytes, the procedure is performed to confirm the presence or absence of infection at an unknown site. This technique has replaced gallium (^{67}Ga) citrate imaging for acute infection because of greater specificity and better image quality. Some chronic infections such as osteomyelitis may be better imaged with gallium (^{67}Ga) citrate after a ^{99m}Tc-phosphate bone scan. Indium (^{111}In) platelets are used to detect thromboses, measure platelet life span, and monitor the success of kidney transplants.

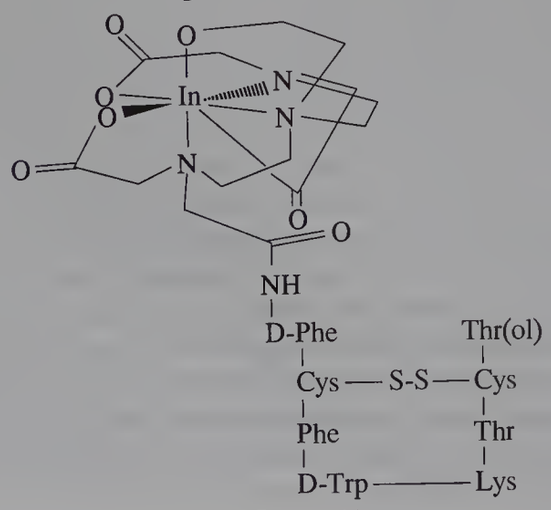

Indium Oxine

Indium (^{111}In) Pentetate (^{111}In-DTPA). The indium (III) pentetate complex is formed by adding the required amount of calcium or sodium pentetate (diethylenetriaminepentaacetate) to the indium (III) chloride and adjusting the pH to 7.0 to 8.0 with sodium hydroxide and/or hydrochloric acid. A proposed structure of indium (III) pentetate is shown below.[5] The patient undergoes a lumbar puncture under sterile conditions and receives an intrathecal injection of 0.5 to 1.0 mCi (18.5 to 37.0 MBq) of indium (^{111}In) pentetate, which distributes into the cerebral spinal fluid (CSF). Initial images are performed to ensure a good intrathecal injection. Images of the spinal canal and CSF spaces of the brain are acquired at 0 to 72 hr to assess CSF patency or leaks. The normal CSF pattern demonstrates the radiopharmaceutical ascends to the basilar cisterns in 2 to 4 hr and flows over the cerebral convexities in 24 hr. From 24 to 72 hr, there should be a gradual clearance from the CSF via the choroid plexus. Cisternography is helpful in the differential diagnosis of hydrocephalus, which is a form of communicating hydrocephalus associated with ataxia, dementia, and urinary incontinence. A CSF leak can be determined by putting cotton pledgets in the nostrils for 24 hr and checking for radioactivity on the pledgets. Additional indications would be to evaluate brain shunt patency to the peritoneum or atrium by injecting the radiopharmaceutical directly into the shunt reservoir.



Indium Pentetate

Indium (^{111}In) Pentreotide Injection. A proposed structure of indium (^{111}In) pentreotide is shown below.[25] Pentreotide has a bifunctional chelating agent, DTPA, linked to octreotide, which is a long-acting analog of the human hormone somatostatin. Somatostatin is a peptide hormone consisting of 14 amino acids. It is present in the gastrointestinal tract, pancreas, cerebral cortex, brain stem, and hypothalamus. Somatostatin receptors have been identified on many cells of neuroendocrine origin. Neuroendocrine tumors are small and slow-growing in nature, which makes them hard to detect by CT or MRI. Somatostatin receptors are expressed in nearly all tumors of neuroendocrine origin and can be imaged with the DTPA-octreotide analog, which chelates indium (III) chloride. Indium (^{111}In) pentreotide binds to somatostatin receptors on many cell surfaces throughout the body. The patient receives an intravenous injection of 5 mCi (185 MBq) of ^{111}In-pentreotide, and, within an hour, the radiopharmaceutical diffuses into the extracellular fluid space and concentrates in tumors containing a high number of somatostatin receptors. Whole body images are obtained at 4 to 48 hr after injection to localize the primary tumor and sites of metastases. Normally, the pituitary gland, thyroid gland, liver, spleen, kidneys, urinary bladder, and, in most patients, the bowel are visualized on the image. Receptor-bearing tumors can be visualized as "hot" spots on the image when compared to normal surrounding tissue.



Indium Pentreotide

THALLIUM RADIOCHEMISTRY

The only useful radioisotope of this element is thallium-201, which is produced in a cyclotron by proton bombardment of a thallium metal target by a ^{203}Tl(p,3n)^{201}Pb nuclear reaction. The lead-201 ($T_{1/2}$ = 9.4 hr) is allowed to decay by electron capture to Tl-201. Thallium-201 ($T_{1/2}$ = 73.0 hr) decays by electron capture to stable mercury-201, with principal gamma-ray emissions of 135 keV (2%) and 167 keV (8%), and mercury-201 daughter x-rays of 68 to 80 keV (94.5%), all of which can be used for organ imaging. The thallium target is dissolved in hydrochloric acid, and the Pb-201 is isolated from the thallium-203 by an ion-exchange chromatographic column. The lead-201 (^{201}Pb) is allowed to decay on the column to thallium-201. The thallium-201 (^{201}Tl) is removed from the column by ion exchange, and the chloride salt is formed by adding hydrochloric acid and evaporating to dryness. Then the pH is adjusted to 4.5 to 7.0 with sodium hydroxide and the salt is sterilized. The solution is made isotonic with sodium chloride containing benzyl alcohol as a preservative.

THALLIUM RADIOPHARMACEUTICALS

Thallium (^{201}Tl) Chloride. This is the only radiopharmaceutical of thallium-201 in use at the present time. Thallium (^{201}Tl) chloride has been found to accumulate in viable myocardium in a physiologic mechanism analogous to potassium (i.e., active transport). Research using human volunteers using radiolabeled microspheres has demonstrated that the myocardial distribution of thallous (I) chloride correlates with regional blood perfusion. In clinical imaging studies, the areas of myocardial infarction are visualized as nonperfused ("cold") areas. The patient submits to either a physiologic "stress" (treadmill exercise) or pharmacologic "stress" with an intravenous infusion of a vasodilator (dipyridamole or adenosine) depending on physical condition. At maximum stress, the patient is injected with 2.0 to 4.0 mCi (74 to 148 MBq) of thallous (I) chloride, which localizes in the heart muscle (myocardium) in proportion to regional blood flow and cell viability. The "stress test" accentuates the myocardial perfusion abnormality. Images of the heart are obtained immediately after stress and the damaged myocardium shows less Tl-201 chloride uptake than surrounding normal heart muscle. At 4 hr poststress, the patient is injected with 1.5 to 2.0 mCi (44 to 74 MBq) of Tl-201 chloride in a resting state to provide information under normal conditions. Ischemic muscle, which still has some blood supply, appears normal on the resting state image, whereas infarcted heart muscle continues to show less radiotracer uptake than surrounding normal heart muscle. Medical applications are in the differential diagnosis of chest pain, the follow-up of patients who have had myocardial infarctions, and patients who have undergone interventions such as coronary artery bypass surgery or balloon angioplasty.

XENON RADIOCHEMISTRY

The useful radioisotope of this element for organ imaging is xenon-133. Xenon-133 is produced in a nuclear reactor as a byproduct of uranium fission by the nuclear reaction $^{235}U(n,fission)^{133}Xe$. Xenon-133 ($T\frac{1}{2} = 5.3$ days) decays by beta-particle emission to cesium-133, with gamma-ray emissions of 81 keV (36%). A requirement for gases used in lung ventilation studies is that they are chemically inert and, at the concentrations used, are physiologically inert. Xenon-133 is chemically inert and insoluble in water, which makes it insoluble in body fluids. Unfavorable physical characteristics of xenon-133 include poor image quality because of low tissue penetration of the low-energy gamma-ray, increased patient dose due to beta-particle emission, and the low gamma-ray emission (36 gamma-rays/100 disintegrations). An alternative would be xenon-127, which is unavailable and not cost-effective.

XENON RADIOPHARMACEUTICALS

Xenon (^{133}Xe) Gas. This radioactive gas is supplied at standard pressure and room temperature in a septum sealed glass vial (2 ml) in doses of 10 to 20 mCi (370 to 740 MBq). The glass vial can contain atmospheric air or a mixture of 5% xenon and 95% carbon dioxide, and is suitable for inhalation by the patient for the diagnostic evaluation of pulmonary function and imaging. The general procedure involves mixing the xenon-133 gas in air or oxygen in a closed-circuit spirometer system, which delivers the radioactive gas and rebreathing of the gas mixture. The inhalation study consists of equilibrium and washout phases with the patient sitting or supine. In the washout study, the patient exhales the xenon-133 gas into an activated charcoal trap to prevent exposure to the technologist. Dynamic posterior images are obtained with the gamma-ray camera during the entire procedure. This study is always combined with a lung blood perfusion study with Tc-99m aggregated albumin. In the normal equilibrium study, there is a homogeneous distribution of the radioactivity throughout the lungs, and, in the washout phase, the xenon-133 gas will clear readily from the lungs. In the abnormal study, the xenon-133 gas will be delayed in the obstructed area (''cold'' spots) but will diffuse into obstructed areas at equilibrium. In the washout phase, normal lung will lose radioactivity quickly, but a poorly ventilated area will clear slowly and appear as ''hot'' spots.

RADIOLOGIC CONTRAST AGENTS

A photographic film containing a radiographic image is properly called a ''radiograph,'' although it is commonly referred to as an ''x-ray'' or a ''film.'' The relative difference between the light and dark areas on a radiographic image reflects what is called radiographic contrast. On traditional radiographic images of the body, such as skeletal, abdominal, and chest x-rays, there are five radiographic densities: air (gas) density, fat density, fluid (soft tissue) density, bone (calcium) density, and metallic density.

Whereas traditional radiologic ''film'' studies have been used since 1895 and continue to be a mainstay of diagnostic medical imaging, they have their limitations. Many organs and tissues of the body do not show up well on traditional radiographic images. For example, the liver, spleen, kidneys, intestines, bladder, and abdominal musculature all have very similar radiographic densities and are difficult, if not impossible, to distinguish from each other.

From the earliest days of radiology, much effort has been devoted to the development of compounds that, if swallowed or injected, would increase the radiographic contrast between various tissues and organs. Injection of air or other gases into a gastrointestinal tube in the esophagus, stomach, duodenum, or into a rectal tube in the colon will provide increased radiographic contrast for evaluating the gut; however, the information obtained by this technique is limited, and more opaque substances have been developed.

Any agent or compound administered to a patient to improve the visualization of an organ or tissue is called a contrast agent. Contrast agents can be classified as either negative or positive. Air and other gases are negative contrast agents, because they render a structure, such as the gut, more translucent. Any agent that increases the radiographic opacity of an organ or tissue is a positive contrast agent. The vast majority of contrast agents used in diagnostic radiology are positive contrast agents.

An ideal contrast agent should have the following properties: (a) ready availability and low cost; (b) excellent x-ray absorption characteristics at the x-ray energies used in diagnostic radiology; (c) minimal toxicity and ready patient acceptance; (d) chemical stability; (e) high water solubility with low viscosity and no significant osmotic effects; and (f) the ability to be administered for selective tissue uptake and excretion.

No compound has all these characteristics. However, barium sulfate and a variety of iodine compounds have been found to produce excellent radiologic contrast with low patient toxicity and relatively low cost. The use of barium and iodine compounds as radiologic contrast agents is based on their radiographic appearance and their distribution and elimination from the body. Contrast media are used in very large quantities and are usually administered over a short time period.

Barium sulfate is a nearly ideal contrast agent for oral and rectal studies of the gastrointestinal tract. It produces a metal-like density on radiologic studies, is readily available at low cost, and, when used properly, has minimal patient morbidity and mortality. Many water-soluble barium compounds are quite toxic, but barium sulfate is an insoluble

TABLE 13-1

COMMON CONTRAST AGENTS

High osmolality (1,400–2,938 mosm/kg) ("ionic") agents
- Conray 60 (60% meglumine iothalamate)
- Conray 400 (66.8% sodium iothalamate)
- Hypaque 50 (50% sodium diatrizoate)
- Hypaque 60 (60% sodium diatrizoate)
- Hypaque 76 (76% sodium diatrizoate)
- Hypaque M 90 (60% meglumine diatrizoate, 30% sodium diatrizoate)
- Renografin 60 (52% meglumine diatrizoate, 8% sodium diatrizoate)
- Renografin 76 (66% meglumine diatrizoate, 10% sodium diatrizoate)
- Reno-M-60 (60% meglumine diatrizoate)

Low osmolality ("nonionic") agents (290–862 mosm/kg)
- Omnipaque (38.8% to 75.5% iohexol)
- Isovue (40.8% to 61% iopamidol)
- Hexabrix (58.9% ioxaglate): This is an ionic hexaiodinated dimer
- Optiray (34% to 68% Ioversol)
- Amipaque (metrizamide)
- Cistobil, Colepax, Telepaque, Teletrast (iopanoic acid)
- Cholebrine, Cholimil (iocetamic acid)
- Bilopac, Bilopaque, Lumopaque, Tyropaque (tyropanoate sodium)
- Bilimiro, Bilimiron, Oravue, Videobil (iopronic acid)

Oily and fat-soluble contrast agents (water-insoluble agents)
- Ethiodol (ethyl diiodostearate//ethyl monoiodostearate)
- Dionosil (propyliodone)
- Lipiodol (iodinated poppyseed oil)

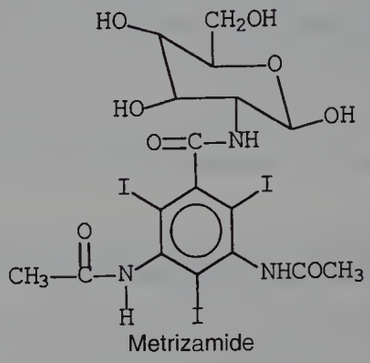
white power that is formulated in water as a colloidal suspension.

The vast majority of contrast agents used to opacify blood vessels and to increase contrast in solid organs, such as the liver, are water-soluble organic iodides. Iodine absorbs x-rays effectively at many energy levels and produces a type of "calcific" or "bone" density on radiographic studies. Its density is somewhat less than that of barium sulfate but quite acceptable.

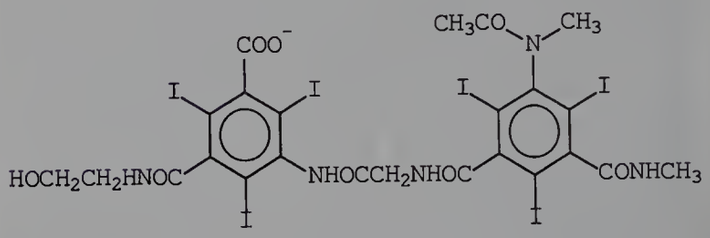
Until the 1980s, most water-soluble contrast agents consisted of triiodinated benzoic acid salts. Upon solution, they dissociate into two particles, a triiodinated anion and a cation, which consists either of a sodium ion or methylglucamine (meglumine) ion. These compounds (Table 13-1), known as "high osmolar contrast media" (HOCM), have in effect three iodine atoms for every two ions in solution, a 3:2 ratio. They are often called ionic ratio 1.5 contrast agents or tri-iodinated monomers.[1] They are mainly represented by diatrizoate and iothalamate compounds, and find frequent use for urography and contrast-enhanced CT studies.

It is necessary to administer the water-soluble iodinated organic compounds in fairly high concentrations in order to achieve satisfactory radiologic contrast. It is not unusual to administer ≥100 ml intravenously of a 60% solution of one of these compounds for urography. A typical concentration used for intravascular studies has an osmolality of five to seven times greater than that of normal plasma. Therefore, administration of these agents can be associated with osmotoxic effects, such as local pain, flushing, nausea, and vomiting. The common triiodinated contrast agents listed in Table 13-1 represent what are commonly called "ionic" or "high osmolar" agents. They are relatively inexpensive and have been in use since the 1960s.

A substantial amount of research has gone into the development of water-soluble compounds with a higher iodine to osmotic particle ratio. The first commercially available nonionic contrast agent was metrizamide, which dissolves in water in a nondissociated form, giving three iodine atoms for every molecule in solution, and is referred to as a ratio-3 contrast agent. Metrizamide was mainly used for myleographic studies of the spinal canal and has been largely replaced by newer agents. This has led to the development of ionic hexaiodinated dimers, such as ioxaglate (Hexabrix) and nonionic water-soluble contrast agents (Table 13-1).

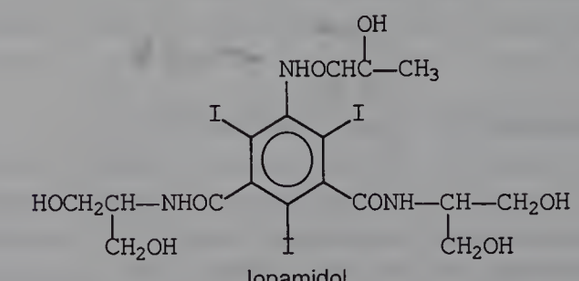


Metrizamide

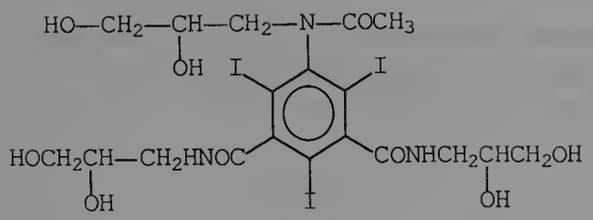


Ioxaglate

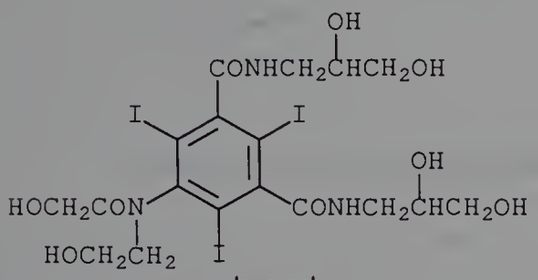
Iopamidol, iohexol, and ioversol are newer "low-osmolar, nonionic" contrast agents (Table 13-1) and are heavily used around the world for many types of radiologic studies. These types of agents, known as "low osmolar contrast media" (LOCM), produce far fewer osmotoxic effects, such as local arm pain, flushing, and nausea and vomiting. They are generally considered to be safer than the common ionic triiodinated contrast agents, and in many locales they have replaced the former in daily practice. However, they are very expensive in comparison with the ionic agents. In the United States, they may cost up to 10 to 20 times that of the ionic agents.[27,28]



Iopamidol



Iohexol

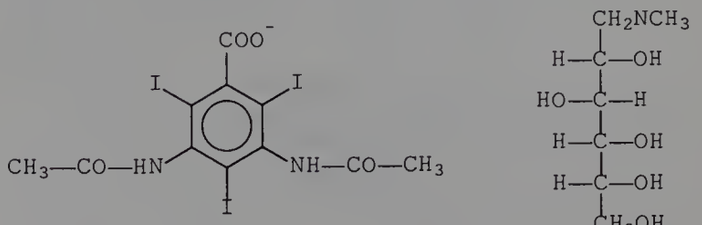


Ioversol

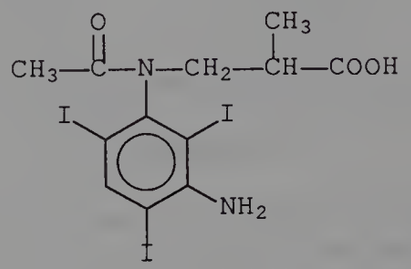
In general, high or moderate osmolality and high viscosity are the hallmarks of all iodinated contrast media. Because of this, there are considerable hemodynamic and subjective effects caused by administration of these agents. There is a rapid initial shift of water from the interstitial and cellular spaces into the plasma after injection of an iodinated contrast agent. This is typically accompanied by vasodilatation, local pain and warmth, a metallic taste in the mouth, and flushing. Later, there is an osmotic diuresis as these agents are excreted by the kidneys.

All the water-soluble iodinated contrast media are clear, colorless liquids with no visible precipitates. They are viscous and when spilled produce a somewhat sticky mess. Even though they are clear liquids, they are often called "dyes" when their administration is being explained to patients. The sodium salts have slightly less viscosity than the meglumine salts. Contrast media viscosity can also be reduced by heating them to body temperature prior to administration.

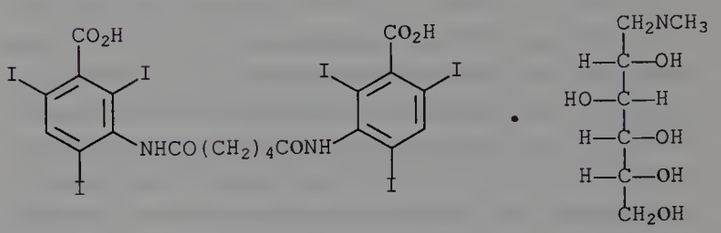
The water-soluble iodinated contrast media have relatively small molecular sizes and low chemical reactivity with body fluids and tissues. Agents of this class include diatrizoate meglumine, diatrizoate sodium, iocetamic acid, iodipamide meglumine, iopanoic acid, iopronic acid, iothalamate sodium, ipodate sodium, and tyropanoate sodium. They have pharmaceutical characteristics similar to extracellular tracers. They have low lipid solubility and distribute throughout the extracellular space. There is no significant penetration of them into the intracellular space. The water-soluble iodinated contrast agents are cleared from the body by glomerular filtration. They are not reabsorbed or secreted by the renal tubules. When there is a compromise in renal function, these contrast agents are eliminated in part or totally through the liver and gut. This vicarious excretion occurs at a much slower rate than elimination by normal glomerular filtration in a healthy person. The half-time for the renal clearance portion of a water-soluble contrast agent is 1 to 2 hr in a patient with normal renal function. The water-soluble organic iodides comprise the largest group of radiologic contrast agents, and the importance of their clinical use is only approached by that of barium sulfate.



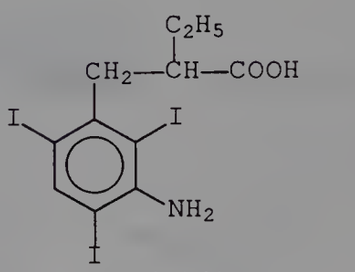
Diatrizoate Meglumine



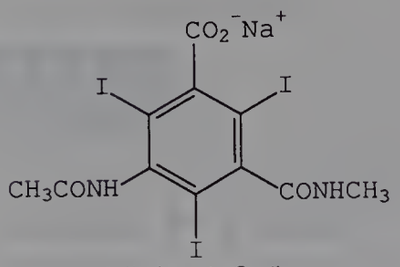
Iocetamic Acid



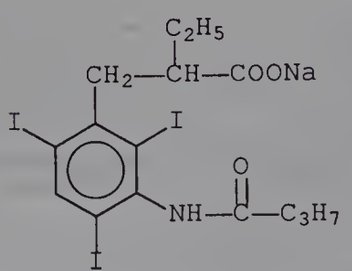
Iodipamide Meglumine



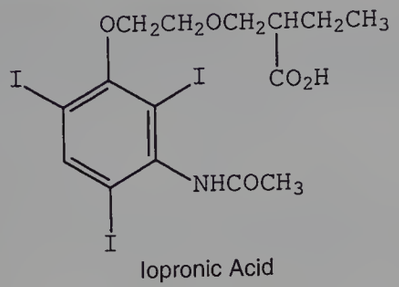
Iopanoic Acid



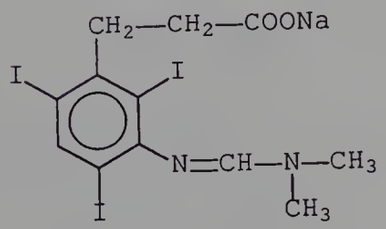
Iothalamate Sodium



Tyropanoate Sodium

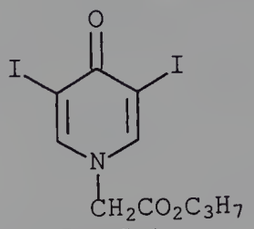


Iopronic Acid



Ipodate Sodium

On the other hand, there is a heterogenous group of water-insoluble compounds of iodine that only rarely find use as radiologic contrast agents. These compounds consist of ester derivatives of iodinated vegetable (poppyseed) oils and iodinated pyridones such as propyliodone (Table 13-1). In the case of iodized oils, unsaturated vegetable fatty acid moieties are iodinated by addition across double bonds and then converted into various esters. Some water-insoluble aromatic iodides have also been occasionally used as radiologic contrast agents. These substances are more resistant to breakdown from exposure to light and air, but, like the iodized oils, they cannot be used intravascularly.



Propyliodone

PARAMAGNETIC COMPOUNDS

MRI is a unique method of medical imaging (Fig. 13-12). When a patient is placed in a strong, uniform magnetic field, it is possible to use smaller, well-directed gradient fields to selectively excite hydrogen nuclei (protons) in a selected small volume of the patient's body. The excitation is done using radiofrequency fields, and once the excitation fields are removed, the excited protons lose the energy they gained. They emit a weak, but detectable radio wave, whose strength and manner of decay can be used to generate diagnostic medical images. The Tesla has been adopted as the official unit of magnetic field strength for the international system of units. The conventional unit, the Gauss, is 10,000 times smaller. MRI is normally performed at 0.5 to 1.0 Tesla.

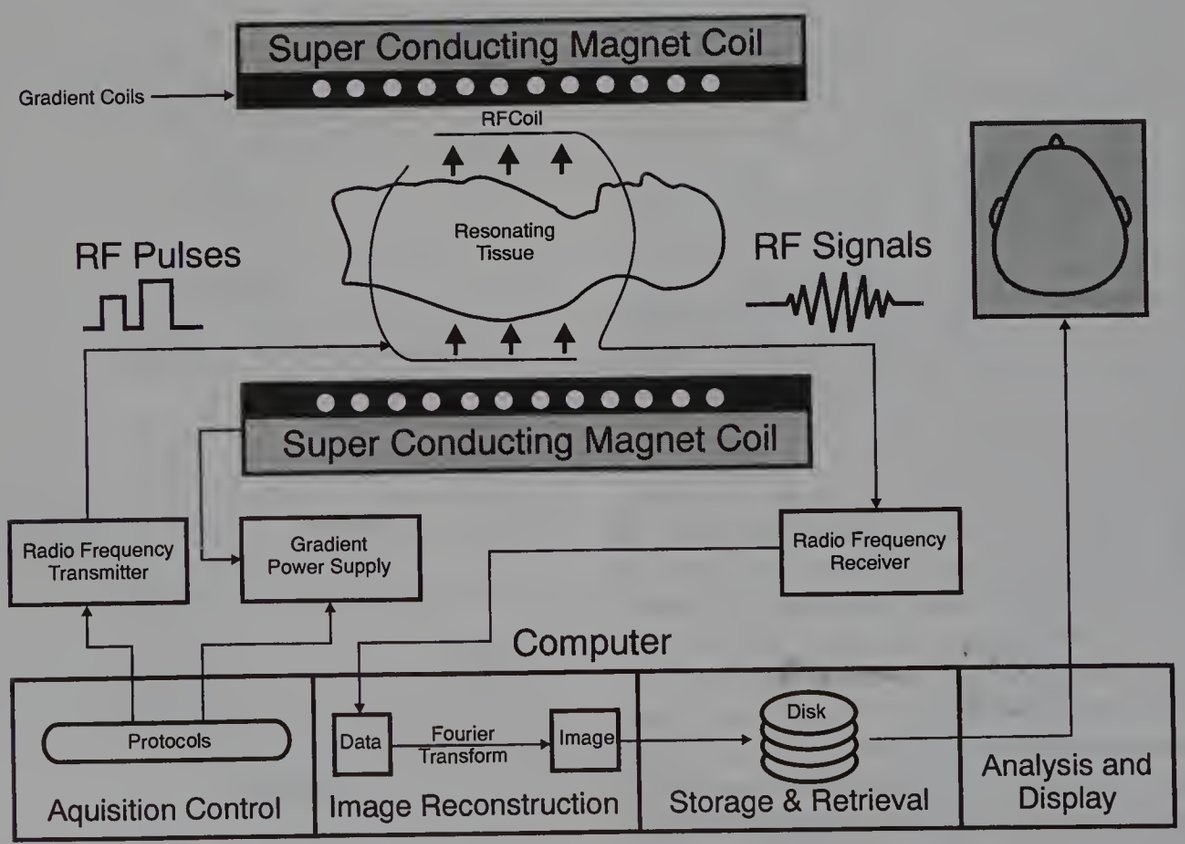


FIG. 13-12. *Schematic diagram of a magnetic resonance imaging (MRI) system. MRI uses radiofrequency waves to image a patient in a strong magnetic field. Subsequent image information is reconstructed with similar computer techniques to those for CT. MRI can produce images in any desired plane (axial, coronal, sagittal, or oblique).*

The images produced by MRI are superficially similar to the cross-sectional images of the body produced by computed tomography (CT). However, MRI has the added advantage of not utilizing ionizing radiation, such as x-rays or gamma-rays, and it is easy to produce exquisite soft tissue images of the body in any desired plane (coronal, axial, sagittal, oblique, et cetera). With MRI, image contrast is determined by many physical parameters, whereas with conventional radiography and CT scanning, there is only a single tissue parameter, x-ray beam attenuation, that is important. The three most important parameters in MRI include water content (proton density), blood flow, and relaxation times (T_1 and T_2). T_1 is a measure of the proton's ability to exchange energy with its environment, and T_2 portrays how quickly a tissue loses its magnetism. When a compound is placed in a magnetic field, it will display a certain susceptibility to become magnetized. There are four main categories of magnetic susceptibility of materials: (a) diamagnetic: small, negative, paired electrons; (b) paramagnetic: positive, unpaired electrons; (c) superparamagnetic: large, positive; and (d) ferromagnetic: large, positive, with magnetization remaining when the magnetic field has been removed.

There are two ways to categorize MR contrast agents. One classification system groups them into paramagnetic agents and iron oxides. The paramagnetic agents predominantly decrease tissue T_1 relaxation time and increase tissue intensity on T_1-weighted images. Iron oxides in various formulations are used primarily to decrease tissue signal intensity, because they primarily decrease T_2 relaxation times. Contrast agents developed for MRI thus far have relied on the use of paramagnetic ions. Another way to classify magnetic resonance imaging agents is to categorize them as either extracellular or intracellular agents.

The main category of MR contrast agents finding general clinical use are the extracellular paramagnetic agents. However, a large amount of research is taking place in all phases of magnetic resonance imaging, including development of new contrast agents. In the near future, there will probably be several MR contrast agents introduced to provide selective imaging of various organs and tissues, such as the liver, the reticuloendothelial system (RES), and the lymphatic system.

The first paramagnetic metal ion used for MRI is gadolinium, which is a member of the rare earth elements. Gadolinium (Gd) forms the Gd(III) ion when prepared in 0.05 M hydrochloric acid, and it is extremely effective for enhancing water proton relaxation rates. It is, however, toxic for human use because of its biological half-life of several weeks. When the acidic Gd(III) ion is used intravenously, it quickly binds to Fe(III) protein binding sites, most notably plasma transferrin. In addition, the Gd(III) ions readily form insoluble compounds by interacting with endogenous ions including phosphate, carbonate, and hydroxide.[29] Consequently, the development of Gd(III) contrast agents has required chelated compounds that clear rapidly from the body through the kidneys and exhibit minimal toxicity. The three MRI contrast agents approved by the U.S. FDA are gadoteridol (Prohance), gadopentetate dimeglumine (Magnevist), and gadodiamide (Omniscan).

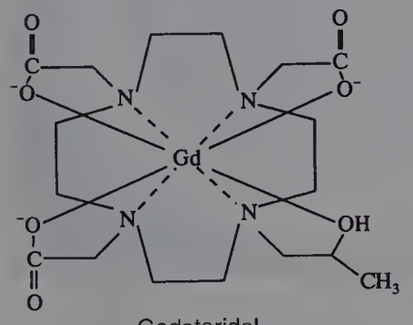
TABLE 13-2

RADIOLOGIC PROCEDURES

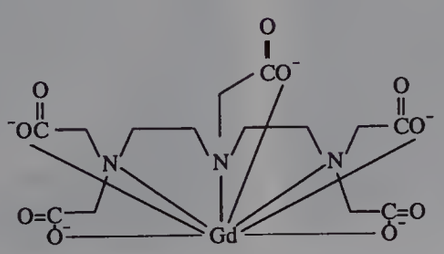
- Plain film radiography
  - Routine chest, abdomen, and skeletal studies
- Contrast studies
  - Barium studies
    - Esophogram, upper gastrointestinal tract (UGI) exam, small bowel follow-through (SBFT), barium enema (BE), and other specialized gastrointestinal tract exams
  - Water-insoluble contrast studies
    - Lymphangiography, and formerly bronchography, and myelography
  - Gallbladder studies
    - Abdominal ultrasound, oral cholecystography, intravenous cholangiography
  - Studies using water-soluble contrast agents
    - Excretory urography (intravenous urography [IVP]), venography, arteriography, contrast-enhanced computed tomography (CT) and magnetic resonance imaging (MRI), arthrography, hysterosalpingography, and myelography
- Cross-sectional imaging studies
  - Ultrasound examinations
  - CT studies
  - MRI studies

RADIOLOGIC PROCEDURES

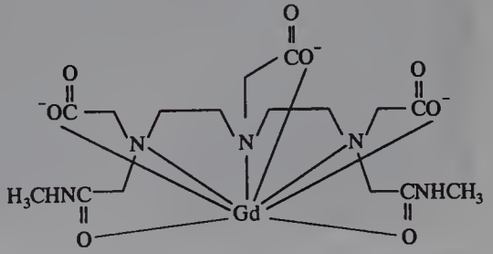
Modern radiology has a multitude of imaging procedures that use contrast agents (Table 13-2). Some of the more common procedures are discussed below and illustrated with examples to show the usefulness of contrast agents.



Gadoteridol



Gadopentetate Dimeglumine



Gadodiamide

INTRAVENOUS PYELOGRAPHY, INTRAVENOUS UROGRAPHY, EXCRETORY UROGRAPHY, AND COMPUTED TOMOGRAPHY

The intravenous pyelogram (IVP) is one of the oldest and most fundamental diagnostic radiologic studies using contrast material. It is a mainstay of genitourinary (GU) radiology and delineates the kidneys and the urinary tracts (renal calices, pelvis, and ureters) as well as the bladder (Fig. 13-13). A more modern name and one that better describes the relevant physiology involved in the study is "excretory urography." Nevertheless, the term "IVP" has been in common use for so long that it still is the name used most frequently. Excretory urography is based on the rapid renal clearance of water-soluble iodinated benzoic acid compounds (whether they are a low-osmolar or a high osmolar agent) after they are injected intravenously. In normal individuals, the ionic and nonionic water-soluble iodinated contrast agents are all excreted by glomerular filtration.

Many body and head CT studies (Figs. 13-14 and 13-15) use intravenous contrast material to improve the quality of the study. The type of contrast material and the doses used are similar to excretory urography except that higher volumes of contrast and a more rapid injection system are often employed. The contrast material increases the relative contrast between space occupying lesions (tumors, cysts, and

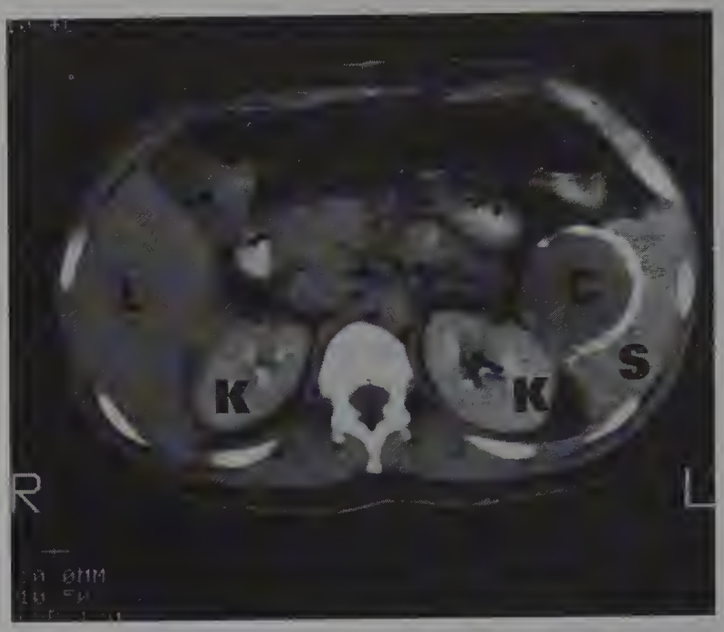


FIG. 13-14. *CT scan of the upper abdomen in a middle-aged adult. The image is an axial view of the patient's upper abdomen viewed as if looking up from the patient's feet. The patient's right side is on the left side of the image. The liver (L), kidneys (K), and spleen (S) are partially visualized. There is a large cyst (C) in the spleen with a calcified rim.*

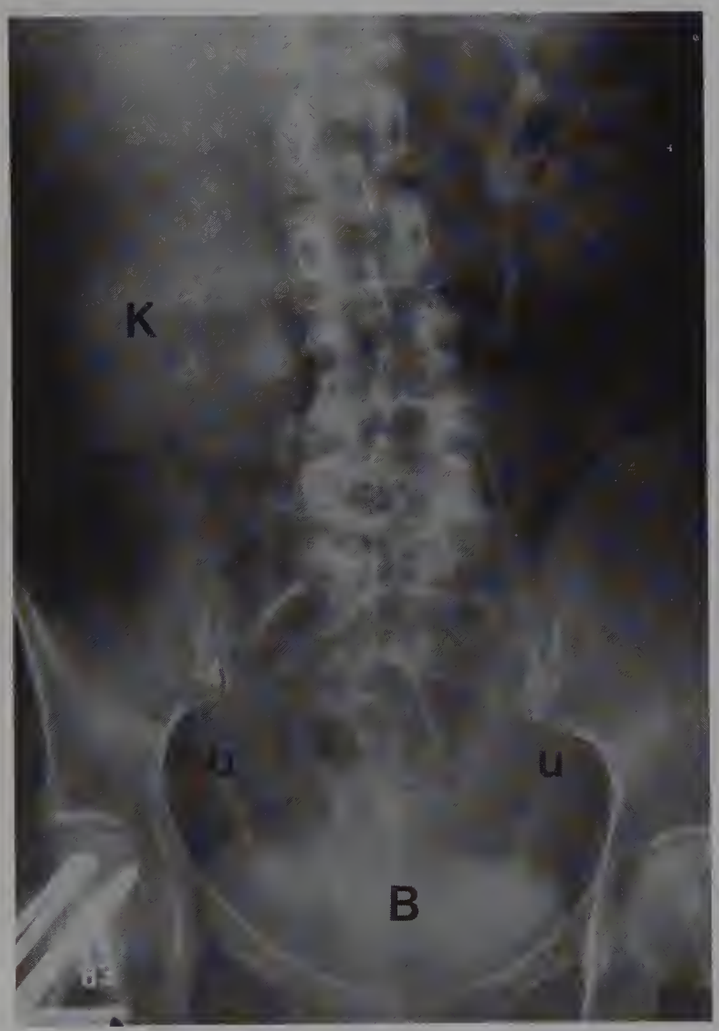


FIG. 13-13. *IVP in an elderly patient. Note the contrast visualization of the kidneys (K), ureters (U), and the bladder (B). The patient also has fixation screws in her right hip from past surgery to stabilize a hip fracture.*

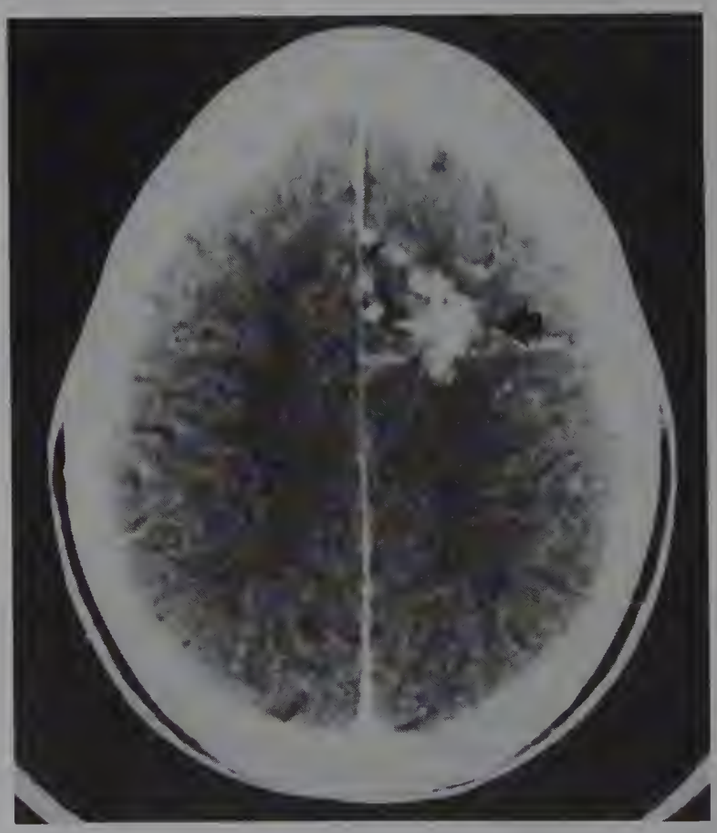


FIG. 13-15. *Axial CT scan of the brain of a patient with an arteriovenous malformation (AVM) after injection of an intravenous iodinated contrast agent (*arrow*).*

abscesses) and normal body tissues. It also frequently shows blood vessels to better advantage.

ARTERIOGRAPHY AND VENOGRAPHY

Angiography refers to the radiographic visualization of blood vessels by contrast injection directly into an artery (arteriography) (Fig. 13-16) or a vein (venography). There are many types of arteriograms, including cerebral angiography (visualization of head and neck vessels), coronary angiography (visualization of the coronary arteries), aortography (visualization of the abdominal or thoracic aorta), and peripheral arteriography (visualization of the major arteries of the upper and lower extremities).

Arteriography is widely practiced for diagnosing vessel narrowing or blockage, aneurysm formation, and sites of bleeding. The type of contrast used depends on the vessel being injected and the preferences of the physician performing the study. Venography, the contrast opacification of venous structures, is performed less commonly than arteriography. The main indication for venography is to diagnose deep venous thrombosis in the lower extremities, based on changes in ultrasound by a moving reflector (blood).

High-quality modern diagnostic ultrasonography coupled with Doppler imaging techniques has largely replaced venography in the workup of suspected deep venous thromboses. Sonography is easy to perform, is much safer than venography, and causes much less patient discomfort. Venography is still used in selected cases, particularly when sonography is equivocal or the patient has a past history of documented venous thrombosis and requires detailed evaluation of his or her venous anatomy.

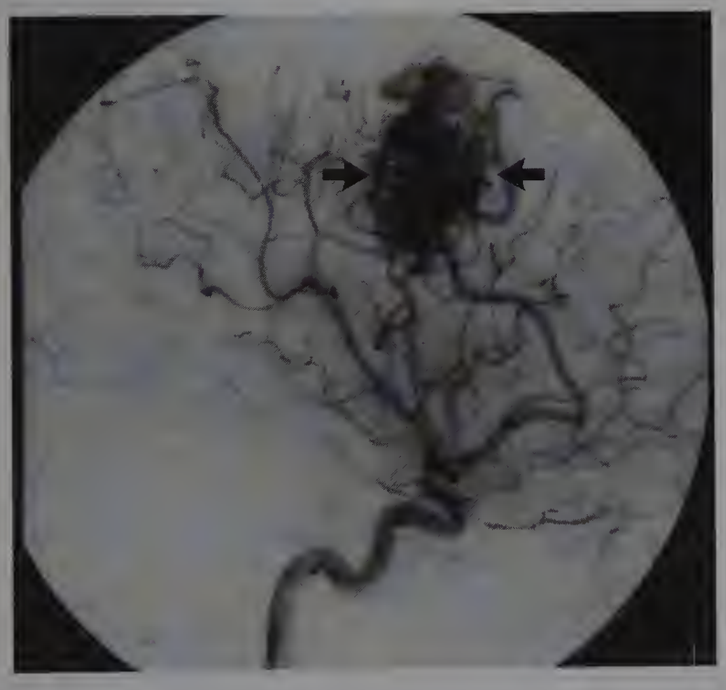


FIG. 13-16. *Same patient as shown in Fig. 13-15. A cerebral arteriogram has been performed to delineate the vascular anatomy of the patient's AVM. In this case, a digital subtraction technique was used, whereby digital images were obtained prior to injection of the contrast agent and subtracted from the contrast agent images using a computer.*

CHOLECYSTOGRAPHY AND CHOLANGIOGRAPHY

Cholecystography refers to contrast visualization of the gallbladder. Modern gallbladder visualization and diagnosis rely mainly on abdominal ultrasonography or nuclear medicine and rarely use traditional radiologic techniques.

Oral cholecystographic agents consist of analogs of 2,4,6-triiodinated alkylbenzoic acids. They have various substituents in the 1 and 3 positions and are absorbed orally, followed by hepatic excretion. Oral cholecystographic agents include iopanoic acid, iocetamic acid, sodium tyropanoate, and sodium ipodate. In general, these agents are bound to serum albumin and converted by the liver into water-soluble glucuronide conjugates. These conjugates are excreted in the bile and stored in the gallbladder, thus facilitating gallbladder visualization.

Iodipamide meglumine (Cholografin) is the main agent used for intravenous cholangiography. Under ideal circumstances, iodipamide meglumine produces excellent visualization of the intrahepatic and extrahepatic bile ducts as well as the gallbladder. Oral cholecystography generally produces better visualization of the gallbladder itself, but rather poor visualization of the bile ducts in comparison to intravenous cholangiography.

MYELOGRAPHY

Myelography involves injection of contrast material into the subarachnoid space, usually in the lower lumbar region, for visualization of the spinal cord, nerve roots, and subarachnoid space. It has been somewhat superseded by modern MRI and CT imaging of the spinal canal, and it can be performed by itself or in conjunction with a subsequent CT study of the spinal canal. Until the advent of the low-osmolar contrast agents, iophendylate (Pantopaque) was the standard agent used for myelography. It was first replaced by the use of metrizamide, which has now been largely supplanted by iohexol and similar agents. The latter give improved image detail and diminished patient toxicity.

It is critical that great care be taken for performance of contrast injection into the subarachnoid space. Improper technique could lead to a devastating infection or spinal injury, and the use of the wrong contrast agent can also have devastating results, such as convulsions and subsequent, severe arachnoid inflammation.

HYSTEROSALPINGOGRAPHY

Hysterosalpingography refers to visualization of the uterine cavity and fallopian tubes (Fig. 13-17). Contrast material is

FIG. 13-17. *Normal hysterosalpingogram performed in a young woman as part of an infertility workup. The contrast material outlines the uterus (U) and has traveled through patent fallopian tubes to spill into her peritoneum (*arrows*).*

injected into the uterus through the cervical canal in order to assess uterine anatomy and the patency of the fallopian tubes. Most practitioners use some type of water-soluble contrast material for hysterosalpingography, though in the past the use of oily agents, such as ethiodol, was popular. One popular agent (Salpix) consists of a water-soluble gel, which is a 2:1 mixture of diatrizoate meglumine and iodipamide meglumine. Iohexol and other low-osmolar agents are also used for hysterosalpingography, and they may produce slightly lower incidence of abdominal pain and discomfort after the procedure.

GASTROINTESTINAL STUDIES

Traditional gastrointestinal tract studies have used various preparations of barium sulfate to opacify the hypopharynx and esophagus (barium swallow); the lower esophagus, stomach, and duodenum (UGI); the small bowel, i.e., small bowel followthrough (SBFT); and the colon, i.e., barium enema (BE) (Fig. 13-18). In the case of the barium enema, the barium is administered per rectum as an enema, usually with the intention of visualizing the entirety of the colon and the terminal most portion of the ileum. Gastrointestinal tract studies are used to diagnose peptic acid disease (ulcers), benign and malignant tumors, and such conditions as gastroesophageal reflux and inflammatory bowel conditions.

If there is a possibility of a gastrointestinal tract perforation, a water-soluble agent is used in place of a barium sulfate preparation. If there is leakage of contrast from the gastrointestinal tract into the peritoneum, retroperitoneum, or mediastinum, water-soluble agents are generally rapidly absorbed by these tissues with no untoward patient effects. Barium

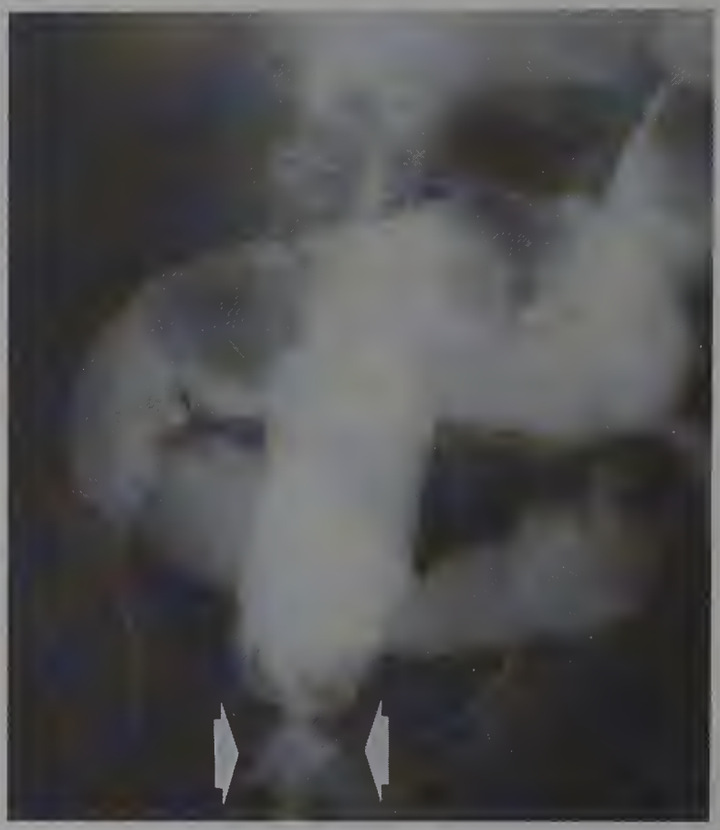


FIG. 13-18. *Barium enema performed on an elderly man. The barium absorbs x-rays very well and has a white appearance, thereby showing the rectum and sigmoid portions of the colon. In the rectum, there is a large tumor (*arrows*), which encircles the rectum and narrows the barium column.*

preparations are particulate and will not be easily cleared from these spaces. Barium mixed with feces may produce a severe peritonitis and be life-threatening.

Oral contrast material is often used as a part of an abdominal CT study to opacify the bowel. A relatively dilute barium sulfate mixture or a diluted solution of ionic contrast material mixed with a flavoring agent are commonly used.

ARTHROGRAPHY

Arthrography represents radiographic visualization of the internal structure of a joint. The shoulder, hip, knee, and wrist are joints commonly visualized by arthrography, although the procedure may be applied to other joints, such as the elbow or ankle. Some forms of arthrography, especially knee arthrography, have been completely replaced by magnetic resonance imaging studies. An arthrogram is performed by the injection of a small amount (1 to 10 cc) of water-soluble, usually low-osmolar, contrast material into the joint space. The contrast agent may be mixed with a local anesthetic to reduce patient discomfort, and air or carbon dioxide may also be injected to produce a double-contrast effect. In the latter case, the water-soluble contrast material outlines the surface of the joint, including the joint capsule and cartilage

surfaces, whereas the gas produces a ''negative contrast'' effect as it distends the joint space.

ADVERSE REACTIONS

Radiologic contrast agents can be taken orally in large amounts and some of them are injected intravascularly in gram doses with no ill effects. Nevertheless, any radiologic contrast material may produce an untoward patient reaction, even sudden death.[30–32] Untoward patient events occur when contrast material is aspirated or when there is leakage of contrast material from the gastrointestinal tract. Hypertonic ionic water-soluble contrast agents are potentially dangerous if aspirated into the tracheobronchial tree. They are very irritating and have been reported to cause pulmonary edema. Any contrast material that leaks out of the bowel into the abdomen, pelvis, or chest is potentially quite dangerous, especially if it is barium sulfate. Barium sulfate is insoluble; its particulate nature means it is poorly cleared from the mediastinum, peritoneum, and retroperitoneum. Water-soluble agents, on the other hand, are rapidly absorbed from the mediastinum, peritoneum, and retroperitoneum, almost as quickly as if they had been injected intravenously. In general, water-soluble agents that leak from the gastrointestinal tract cause no significant problems other than a transient inflammation.

The intravenous or intraarterial injection of iodinated contrast material for pyelography, contrast-enhanced CT studies, and angiographic studies opens the door to a diverse assortment of contrast reactions, most of which are minor and easily treated. Minor reactions include arm pain, a feeling of general body warmth and discomfort, mild nausea and vomiting, a strong metallic taste in the mouth, and mild urticaria (hives). Minor reactions dissipate in a few minutes with patient reassurance and observation.

Intermediate or moderate reactions are those that require some form of therapy but are not life-threatening. They include difficulty breathing, severe hives, severe nausea and vomiting, mild hypotension, wheezing, and other similar reactions. The treatment ranges from the administration of intravenous fluids to the use of intravenous diphenhydramine for hives. Epinephrine may be administered, and atropine is used if there is a vasovagal reaction with hypotension and bradycardia.

Severe reactions are those that are life-threatening. They include sudden cardiovascular collapse and death, as well as severe hypotension, severe shortness of breath, wheezing, or laryngoedema, loss of consciousness, massive hives and angioneurotic edema, ventricular cardiac arrhythmias, angina, and myocardial infarction. Their treatment depends on the patient's signs and symptoms and includes intravenous fluids, oxygen, various drugs, including epinephrine, diphenhydramine, and atropine, and possible cardiopulmonary resuscitation (CPR).

The incidence of adverse reactions to radiopharmaceuticals is estimated to be <0.006%. The majority of reactions are allergic in nature and occur within minutes after intravenous injection. In the case of radiolabeled murine antibodies, an anaphylactic reaction may occur, although serious reactions of this type have not been reported.

PRODUCTS

Barium Sulfate. The multiple commercial preparations of barium sulfate differ in their density and their ability to coat the bowel wall. These characteristics are determined by the particle size of the barium suspension, its viscosity, and its pH, which in turn are determined by the addition of small amounts of flavoring agents, suspending agents, and so forth. These additives are the proprietary secret of the manufacturer and do improve the diagnostic properties of the barium colloidal suspension for gastrointestinal radiologic studies.

Barium sulfate preparations are used to study the esophagus, stomach, and duodenum as part of an esophogram or upper intestinal tract series (UGI) and are given orally. Most patients find the taste of these dense mixtures acceptable (they are usually flavored with a strawberry or lemon flavor), but they dislike the heavy texture of the barium. Barium sulfate suspensions are also given orally to study the entire small bowel (SBFT) or rectally to examine the colon (BE) (Fig. 13-18).

Typical barium suspensions range from 30% to >120% weight/volume, and because they are colloidal suspensions, they cannot be given intravascularly; the colloidal particles would produce fatal pulmonary embolism. The barium suspensions used for UGI or BE studies are too dense to be used for gut opacification during CT studies of the abdomen, because they produce disturbing radiographic artifacts. Instead, commercial barium preparations are diluted to the 1% to 4% weight/volume range.

Diatrizoate. This is classified as an ionic monomeric contrast agent and is available in the meglumine, sodium, or combination of meglumine and sodium salts of the fully substituted triiodobenzoic acid. It has a molecular weight of 614, and the organically bound iodine content is 62%. Its salts are used mainly for angiography and excretory urography radiographic procedures. The diatrizoate meglumine (66%) and diatrizoate sodium (10%) combination is used orally or rectally to delineate the GI tract. This preparation is indicated where the use of barium sulfate is potentially dangerous (i.e., whenever a suspected perforation of the GI tract exists), because water-soluble contrast agents are quickly absorbed through the peritoneal surface. The high osmolality prevents water absorption and leads to rapid transit through the GI tract. The meglumine salt dilute solution (18% w/v) is used for cystography after sterile catherization.

Ethiodol. This is classified as a sterile preparation containing ethyl esters of the iodinated fatty acids of poppyseed oil and it is used for lymphangiography. It contains 35% to

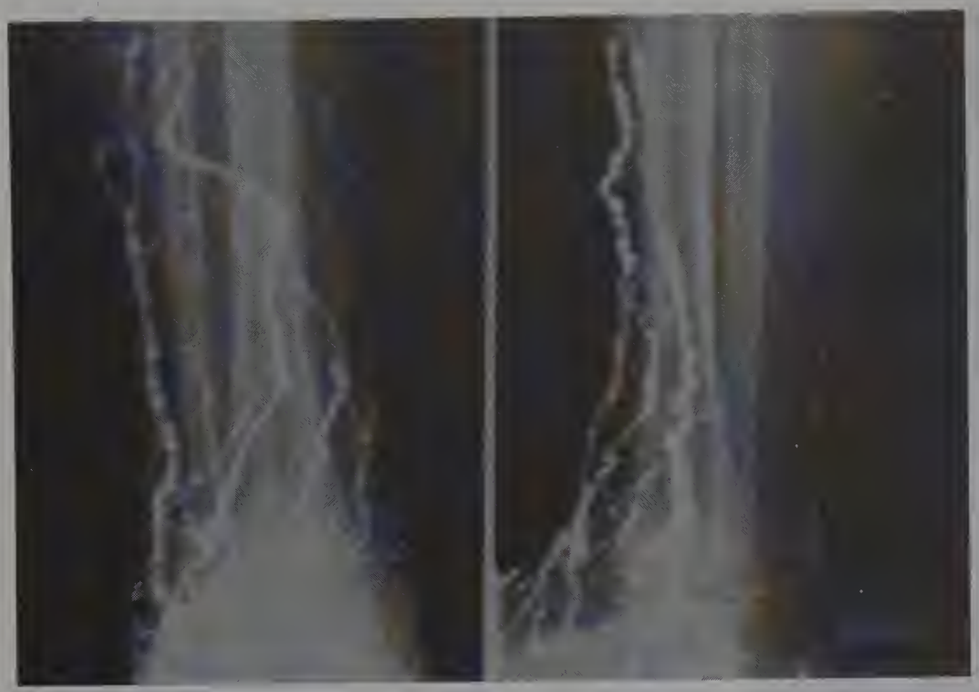


FIG. 13-19. *Lymphangiogram of a normal leg using ethiodol.*

39% of organically bound iodine that has been added to the double bonds of the fatty acids. Because it is an oily substance and not miscible with plasma, it cannot be administered intravascularly. For lymphangiography, it is injected into tiny lymphatics of the web space of the hand or foot after subcutaneous injection of a dye such as pontamine sky blue, mixed with a local anesthetic, to facilitate isolation and cannulation. The lymphatics carry tiny globules of ethiodol to regional lymph nodes, where they are partially filtered by the nodes and localized in the nodes. The iodine in the ethiodol makes the nodes sufficiently radiopaque to be visualized on plain film radiographs. A lymphangiogram typically is used to evaluate lymph nodes in the groin, pelvis, and abdomen in patients with Hodgkin's disease and sometimes, other malignancies. A typical normal lymphangiogram of the lower leg is illustrated in Fig. 13-19.

The oily nature of ethiodol precludes its more generalized use. Injection of this agent into the vascular system in any significant amount is very dangerous; it ultimately embolizes to the pulmonary arterial circulation. In addition, iodized oils are unstable chemically, and they tend to decompose on exposure to light.

Iocetamic Acid. This is classified as a high-osmolar ionic monomeric contrast agent and is used as an oral cholecystographic agent for the radiographic visualization of the biliary tract and gallbladder. It has a molecular weight of 614 and an organically bound iodine content of ~62%. A dose of 3.0 to 4.5 g is given orally 10 to 15 hr prior to radiographic examination, and ~60% of this dose is excreted in the urine within 48 hr. A typical radiograph of a gallbladder is illustrated in Fig. 13-20.

Iodipamide Meglumine. This is classified as an ionic dimeric contrast agent and is given as the meglumine salt, which is highly water-soluble. It has a molecular weight of

FIG. 13-20. *A radiograph of a normal gallbladder after administration of iopanoic acid.*

1,530 and an organically bound iodine content of 49.9%. Iodipamide meglumine is very strongly bound to serum albumin and is excreted by the liver into the biliary system unmodified. Unfortunately, iodipamide meglumine produces a high incidence of adverse patient reactions, such as urticaria and hypotension, and intravenous cholangiography has generally been discontinued. Modern ultrafast helical (spiral) CT scanners, however, can produce exquisite high-contrast studies of the liver and biliary system, and sophisticated CT scanning techniques have renewed interest in the use of intravenous cholangiography.

Iopamidol. This is classified as a nonionic monomeric contrast agent. It has a molecular weight of 777 and an organically bound iodine content of 49%. While the osmolality is much lower than that of the ionic contrast agents, the viscosity is very similar. It is used for a variety of angiographic procedures including myelography, excretory urography, arthrography, and visualization of the GI tract. It is available in solutions containing 30.6% to 75.5% of iopamidol. The dose and strength varies with the procedure and route of administration.

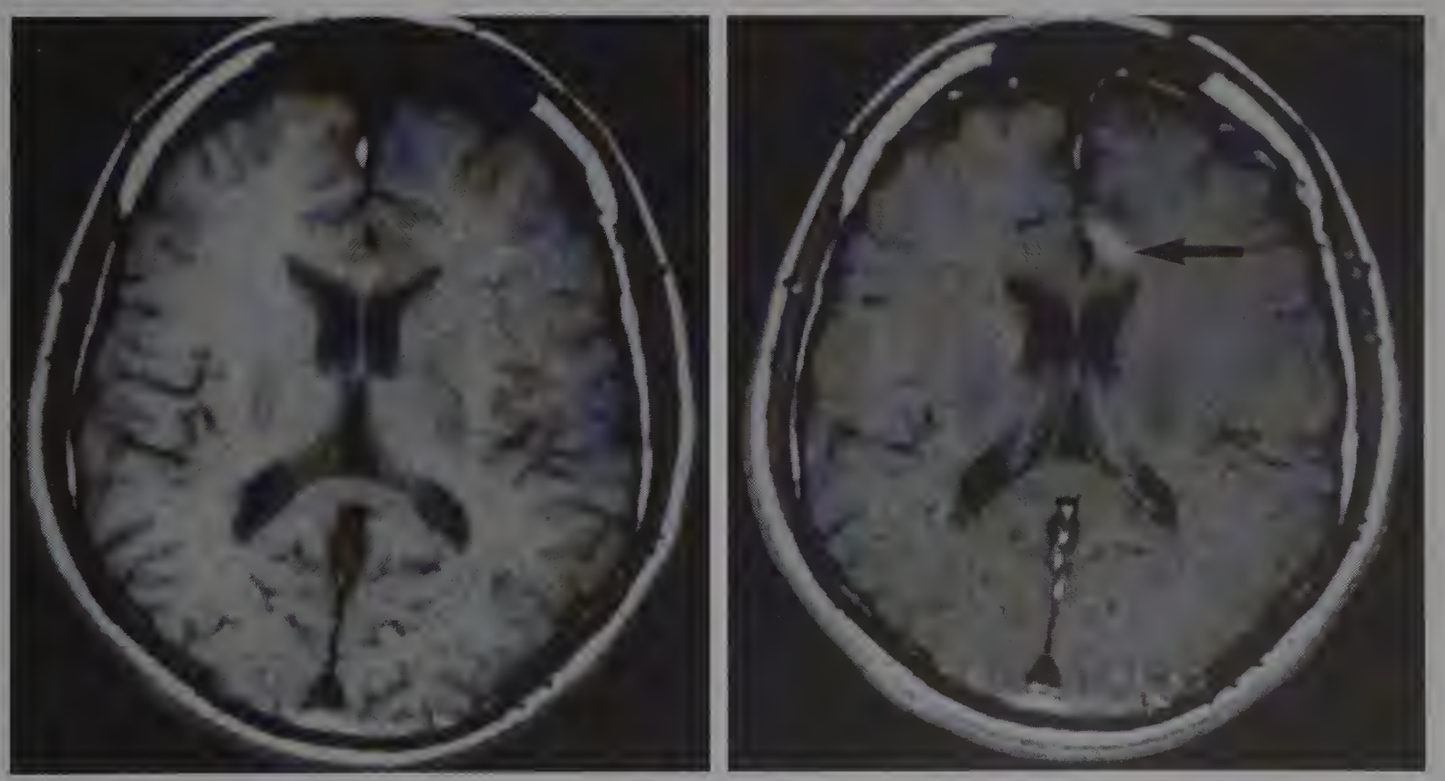
Tyropanoate Sodium. This is classified as a high-osmolal ionic monomeric contrast agent that is given by mouth for the radiographic visualization of the biliary tract and gallbladder. It has a molecular weight of 633 and an organically bound iodine content of 57.4%. A dose of 3.0 g is given with water 10 to 12 hr prior to radiographic examination. This agent is not recommended for children under 12 years of age.

Ipodate Sodium. This is classified as a high-osmolal ionic monomeric contrast agent and given by mouth for radiographic visualization of the biliary tract and gallbladder. It has a molecular weight of 620 and an organically bound iodine content of 61.4%. A dose of 3 to 6 g is given 10 to 12 hr prior to radiographic examination. The sodium salt is less readily absorbed from the GI tract than the calcium salt. The calcium salt may be used for intravenous cholangiography, but it is not the agent of choice.

Iopanoic Acid. This is classified as an ionic monomeric contrast agent. It has a molecular weight of 571 and an organically bound iodine content of 66.7%. It is used as an oral cholecystographic agent for the radiographic visualization of the biliary tract and gallbladder. A dose of 3 g is given by mouth with ample water ~10 to 14 hr before radiographic examination. It frequently produces mild gastrointestinal effects.

Ioversol. This is classified as a low-osmolal nonionic monomeric contrast agent. It has a molecular weight of 807 and an organically bound iodine content of 47.2%. Its main uses are angiography and excretory urography. Ioversol is associated with fewer physiological problems than other ratio-1.5 contrast media.

Metrizamide. This is classified as a low-osmolal nonionic monomeric contrast agent. It has a molecular weight of 789 and an organically bound iodine content of 48.2%. It is used mainly in the radiographic examination of the spinal cord and central nervous system (i.e., myelography, cisternography, and ventriculography). The most frequent adverse ef-



A B

FIG. 13-21. *MRI of the brain in a 36-year-old patient with a single episode of acute weakness.* ***A:*** *T_1-weighted axial image without contrast agent taken 3 days later.* ***B:*** *T_1-weighted image after intravenous injection of gadopentetate dimeglumine, which has concentrated in the left frontal lobe (*arrow*). The patient was diagnosed as having a cerebrovascular accident. (Courtesy of Berlex Laboratories, Inc.)*

fects are headache, nausea, and vomiting. Metrizamide was the first commercially available nonionic contrast agent. On-site reconstitution of the lyophilized powder is necessary because of instability in solution.

Ioxaglate. This is classified as a low-osmolal ionic dimeric contrast agent that is formulated as a combined solution of its meglumine and sodium salts. It has a molecular weight of 1,269 and an organically bound iodine content of 60%. Ioxaglate is used for angiography, arthrography, urography, venography, and hysterosalpingography. This agent should never be injected by the intrathecal route; thus, it is not suitable for myelography.

Propyliodone. Dionosil, a commercial preparation of propyliodone, a pyridone ester, was once popular for the performance of bronchography. It consists of a white powder suspended in an oily medium and was used for bronchography and laryngography (examination of the larynx). Other methods of imaging, such as CT, ultrasonography, and MRI, as well as modern fiber optic bronchoscopy have largely replaced bronchography.

MRI Agents. The three MRI contrast agents are similar in that they are highly soluble in water and can be administered by intravenous injection. They are classified as ionic (gadopentetate dimeglumine) or nonionic (gadodiamide and gadoteridol) and they are either linear (gadopentetate and gadodiamide) or macrocyclic (gadoteridol) chelates. Gadolinium-based contrast agents are administered in a typical dose of 0.1 mmol/kg, which can be increased to a total dose of 0.3 mmol/kg for the nonionic agent gadoteridol in patients who have poorly enhancing tumors or equivocal scans. Figure 13-21 illustrates a MRI study of a brain before and after injection of gadopentetate dimeglumine.

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CHAPTER 14

Central Nervous System Depressants

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The therapeutic classes of drugs reviewed in this chapter are the general anesthetics, sedative-hypnotics, anxiolytics, anticonvulsants, and antipsychotics. It is useful to remember that there is considerable overlap among them. The first four groups, for example, have much in common. Thus, most sedative-hypnotics also possess anxiolytic and anticonvulsant properties and at higher concentrations are general anesthetics. Antipsychotics are unique among the classes listed in that they are able to ameliorate the thought disorder that underlies schizophrenia. Additionally, their mechanism of action at the molecular level is quite distinct from the other four classes. However, although mechanisms of action are different, antipsychotic drugs have important anxiolytic properties, and many of them are sedative as well. Among the first four classes, the shared mechanisms of action appear to be a consequence of structural similarities of the compounds comprising the groups. Many of the compounds have structures that one associates with allosteric or nonspecific binding to receptor proteins. A hydrophobic group attached to a semipolar group capable of acting as a protein donor or acceptor is a common structural arrangement.

Nearly all antipsychotic drugs have structures that one would logically associate with an ability to act as dopamine receptor antagonists. They have a dopamine-like grouping that is hydrophobically substituted. Of the dopamine receptors (D_1 to D_5), the receptor most relevant to typical antipsychotics is the postsynaptic D_2 receptor on dopaminergic neurons in the mesolimbic system. Other dopamine receptors, especially D_3 and D_4, may be significant among atypical antipsychotics. Additionally, if a drug can compete with dopamine for receptors, then there is a possibility that it can compete for receptors for other β-arylamines such as serotonin (5HT) as well. Accordingly, combination D_2 and $5HT_2$ antagonists are reaching the clinic and are reported to relieve negative as well as positive symptoms of schizophrenia.

SOME MECHANISMS OF ACTION: ANESTHETICS, SEDATIVE-HYPNOTICS, ANXIOLYTICS, AND ANTICONVULSANTS

ANESTHETICS

The vaporizable inhalation anesthetics,[1] alcohols,[1] the 2,6-disopropyl phenol, propofol,[2] the 3α-hydroxy-5α-pregnane steroids, including the neurosteroids and alphaxalone,[2] the general anesthetic barbiturates,[3,4] and the anesthetic benzodiazepines[5] all bind allosterically to $GABA_A$ receptors to potentiate the effect of GABA in opening chloride channels. The data for alcohols and volatile anesthetics indicate that they potentiate GABA on $GABA_A$ receptors. This may be by allosteric binding to the $GABA_A$ receptor. Or the action of these agents on $GABA_A$ receptors could be an indirect one.[5]

A number of anesthetic compounds appear to inhibit glutamic acid binding to glutamic acid receptors. Volatile anesthetics and alcohols experimentally inhibit the CNS exciting effects of glutamic acid at the N-methyl-D-aspartate (NMDA), kainate, and quisqualate subtypes of glutamic acid ionotropic receptors. Pentobarbital inhibits the kainate and ionotropic quisqualate receptors, but not the NMDA receptor, at anesthetic concentrations.[6] These results were extrapolated to indicate that general anesthetics (volatile anesthetics, alcohols, and barbiturate anesthetics) may act in part by kainate and quisqualate inhibition, since channels regulated by these glutamate receptors mediate fast excitatory CNS transmission: inhibition would produce general CNS depression.[6] Inhibition of the NMDA receptor might be involved in the intoxicating effects of some of these agents, for example, ethanol, since glutamate activation of NMDA receptors is thought to mediate complex neutral phenomena and cognitive function.[6] Anxiolytic effects of these agents could be associated largely with allosteric binding to $GABA_A$ receptors.[7]

Longoni and Olsen[5] observed that a unified theory of general anesthesia can be built around facilitation of $GABA_A$ receptor function to promote chloride conductance. Benzodiazepines,[8–10] barbiturates,[3,4,7] and 3α-hydroxy-5α-pregnane–derived anesthetics[11] all bind allosterically. The evidence is that $GABA_A$ is involved in the anesthetic action of volatile anesthetic and alcohols whether by allosterically binding to $GABA_A$ or by an indirect action. In either case, they can be encompassed by the unifying theory.[5] The low potential of benzodiazepines to produce anesthesia might be explained by their receptor selectivity. Their binding requires either a γ_{2S} or γ_{2L} receptor subunit (in combination with α and β subunits). Ethanol has a poor reputation as an anesthetic and its effect on $GABA_A$ receptors, whether by allosteric binding or by an indirect effect is very selective, requiring a γ_{2L} subunit in combination with α and β subunits. Barbiturates, propofol, and neurosteroids all bind allosterically to $GABA_A$ receptors regardless of subunit composition to facilitate the effect of GABA. Additionally, it has been shown that barbiturates can function at their allosteric binding site even in the absence of GABA from its binding site to promote chloride flux; i.e., they have, in addition to a GABA facilitating effect, a GABA mimetic effect. This last effect is especially true of the more sedative-hypnotic and anesthetic barbiturates.[3]

SEDATIVE-HYPNOTICS

From the foregoing descriptions of drug action on $GABA_A$ receptors, it is apparent that the sedative-hypnotic action of barbiturates, benzodiazepines, and related compounds can be equated with positive modulation of GABA, especially on a broad range of $GABA_A$ receptors or by acting in lieu of GABA as in the case of the sedative barbiturates, implicitly across a broad range of receptors. Involvement of other receptors, for example, appropriate glutamic acid receptor subtypes and adenosine receptor subtypes, could be significant for some compounds. Finally, inhibition of entry of CA^{2+} into presynaptic neurons with inhibition of transmitter release is important.[7]

ANXIOLYTICS

The anxiolytic component of action of the drugs in our present area of purview: benzodiazepines, barbiturates, and the like appears to be closely associated with action at select $GABA_A$ receptors to positively reinforce or to mimic GABA activity. It is of course likely, analogously to the case of sedative-hypnotics, that other mechanisms for these compounds could be important. It is pertinent to reiterate that among other structural classes of compounds other mechanisms are involved in anxiolysis: agonism at $5HT_{1A}$ receptors, antagonism of DA at D_2, D_3, and D_4 receptors, possibly antagonism of serotonin at central $5HT_2$ receptors, possibly central adrenergic antagonism, possibly central antihistaminic H_1 activity, action at central cholecystokinin-B receptors, and more.

ANTICONVULSANTS

It is apparent that drug action on $GABA_A$ receptors to stimulate chloride flux into the neuron is an important component of action of many antiepileptic drugs. It may be especially relevant to anti-absence activity. In addition to GABAergic effects on chloride ion flux, decrease of neuronal excitability by inhibition of neuronal flux of Na^+ and Ca^{2+} (block of sustained repetitive firing and decreased neurotransmitter release) is considered to be especially relevant in antigeneralized tonic-clonic seizure drugs.[12] Additional important antiepileptic actions can be achieved by blocking glutamic acid receptors (e.g., block kindling by NMDA receptor block) and by agonism of adenosine receptors (e.g., block spreading by agonism at A_1 receptors).

GENERAL ANESTHETICS

Today, general anesthesia for surgery employs multiple drug regimens.[13] Some drugs are intended to potentiate or otherwise augment the effect of the general anesthetic agent, for example, neuroleptics and narcotic analgesics. Other drugs add an action, for example, the skeletal muscle relaxants, and finally drugs to control side effects, such as anticholinergics, may be employed. For the ways in which these agents may be orchestrated into an effective harmonious whole, the interested reader should consult a pharmacology or medical textbook. The ensuing discussion will examine only those agents clearly used as general anesthetic agents.

These anesthetic agents can be broadly categorized as those useful by the inhalation route and those useful by the intravenous (IV) route.

INHALATION ANESTHETICS

The inhalation anesthetics in use today are halothane, enflurane, isoflurane, methoxyflurane, sevoflurane, desflurane, and nitrous oxide. Older agents, such as diethyl ether and cyclopropane, became obsolete because of a fundamental chemical property. They are explosive and flammable and add an unacceptable level of danger to the production of anesthesia.

Halothane, USP. 2-Bromo-2-chloro-1,1,1-trifluoroethane (Fluothane); $CH(Br)C1CF_3$. Halothane, a volatile halogenated hydrocarbon (bp, 50°C), has been the standard inhalation anesthetic agent. It was introduced in 1956 and gained rapid acceptance. One major factor in its acceptance was its nonflammability. Additionally, the drug has high potency and a relatively low blood/gas partition coefficient. Accord-

ingly, induction of and recovery from anesthesia are relatively rapid. In actual practice, intravenous sodium thiopental is usually used to induce anesthesia.

Most of halothane is eliminated intact in the expired air. There is sufficient reactivity to oxidative processes, however, to allow up to 20% of the administered compound to undergo metabolism. The trifluoromethyl group is quite stable, in itself, because the high electronegativity of fluorine stabilizes the C-F bonds. The C-Cl, C-Br, and C-H bonds, however, are destabilized. The latter is the probable initial site of metabolic entry. Metabolites are chloride and bromide ions and trifluoroacetic acid. Additionally, loss of HF yields the olefin 1,1-difluoro-2-chloro-2-bromoethylene, which reacts with the SH group of glutathione.

There has been a low incidence of hepatic necrosis associated with halothane. This has reduced its use. It is suggested that reactive metabolites formed, as outlined in the foregoing, might produce an immunoreactive response that is responsible.[14,15]

Hypotension is a common side effect of the drug and sometimes may be used to advantage.

Halothane does not have a wide margin of safety. Respiratory depression is notable, and mechanical ventilation and increased oxygen concentrations are often required. Opioids or nitrous oxide are often needed to obtain adequate surgical analgesia.

Methoxyflurane, USP. 2,2-Dichloro-1,1-difluoroethyl methyl ether; $CHCl_2CF_2$-O-CH_3 (Penthrane). Methoxyflurane is a volatile liquid (bp, 105°C). The agent does not have a high vapor pressure at room temperature; thus, concentrations in the inspired air are low. This, together with a large blood/gas partition coefficient, produces a slow induction of anesthesia featuring an excitatory phase. Accordingly, induction may be made with intravenous sodium thiopental. The compound is very soluble in lipids; consequently, recovery is slow. The agent produces excellent analgesia and good muscle relaxation.

Methoxyflurane is as much as 70% metabolized. Apparently, all labile sites are attacked. Metabolites include dichloroacetic acid, difluoromethoxyacetic acid, oxalic acid, and fluoride ions. Fluoride ion especially and oxalic acid also are responsible for the renal damage the agent produces when used to produce deep anesthesia over prolonged periods. Accordingly, it has restricted application as an anesthetic.[16] It is said to be safe when used by intermittent inhalation as an analgesic during labor.

Enflurane, USP. 2-Chloro-1,1,2-trifluoroethyl difluoromethyl ether; HF_2COCF_2CHFCl (Ethrane). Enflurane is a volatile liquid with a vapor pressure at room temperature about three-fourths that of halothane and a blood/gas partition coefficient also about three-fourths that of halothane. Consequently, induction is relatively easy, although an ultrashort-acting barbiturate is usually employed for this purpose. The agent is said to be a relatively easy agent with which to work and to have a relatively low frequency of adverse cardiovascular effects. Respiration is depressed, and thus mechanical ventilation and oxygen supplementation are employed. At high doses in a small percentage of patients, tonic-clonic convulsive activity is seen. Accordingly, enflurane should not be used in patients with epileptic foci.

As much as 5% of administered drug is metabolized. Difluoromethoxydifluoroacetic acid and fluoride ion have been reported as metabolites. The fluoride concentration, however, is generally thought to lie within safe limits.[17]

Isoflurane, USP. 1-Chloro-,2,2,2-trifluoroethyl difluoromethyl ether; $F_3CC(H)ClOCF_3$ (Forane). Isoflurane is a close structural relative of enflurane and shares many properties with it. Changes, however, in the electroencephalogram (EEG) and tonic-clonic activity are not reported. It does differ considerably in extent of metabolism; only ~0.2% is metabolized.[18] Metabolites are fluoride ion and trifluoroacetic acid. Neither kidney nor liver damage has been reported for the drug.

Desflurane. (Suprane); $FC(H_2)$-O-C(H)F-$C(H)F_2$. Desflurane has an oil/gas partition coefficient about one-fifth and a blood/gas partition coefficient one-third that of isoflurane. Its physical properties confer pharmacokinetic properties claimed to be superior to isoflurane. It and isoflurane are metabolized to about the same extent.

Sevoflurane. $FC(H)_2$-O-$CH(CF_3)_2$. Reportedly, sevoflurane will be introduced into use in the United States. The oil/gas partition coefficient is about one-half that of isoflurane and the blood/gas partition coefficient about one-third that of isoflurane. Pharmacokinetically, it is said to have the advantages of rapid uptake and rapid elimination. It is metabolized to about the same extent as enflurane.

Nitrous Oxide, USP. Nitrogen monoxide; N_2O. Nitrous oxide is a gas at room temperature and is supplied as a liquid under pressure in metal cylinders. It is a good analgesic, but requires such high concentrations in the inspired mixture (up to 80%) to achieve anesthesia that there are attendant dangers of hypoxia. Accordingly, it is rarely used as the sole anesthetic agent. It is often useful in combination with other agents, permitting their use at decreased concentrations. Some studies have suggested that nitrous oxide analgesia is mediated through opioids.[19] Other studies have failed to substantiate this and suggest that analgesia may be a consequence of a general depressant effect on synaptic transmission of pain messages.[20]

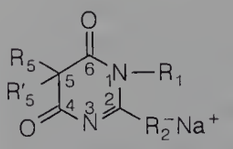
INTRAVENOUS ANESTHETICS

The sodium salts of the ultrashort-acting barbiturates may be administered intravenously in aqueous solutions for the induction of anesthesia. Thereafter, the maintaining volatile anesthetic with or without nitrous oxide is employed. Respiratory depression is marked with the barbiturates at anesthetic doses; consequently, these agents are not used to maintain surgical anesthesia. Unconsciousness is produced within

TABLE 14-1

ULTRASHORT-ACTING BARBITURATES USED TO PRODUCE GENERAL ANESTHESIA

General Structure



Generic Name Proprietary Name	*Substituents* R_5	R'_5	R_1	R_2
Methohexital sodium *Brevital Sodium*	$CH_2{=}CH{-}CH_2{-}$	$CH_3CH_2C{\equiv}C{-}\underset{\displaystyle CH_3}{\underset{\vert}{CH}}{-}$	CH_3	O
Thiamylal sodium *Surital Sodium*	$CH_2{=}CH{-}CH_2{-}$	$CH_3CH_2CH_2\underset{\displaystyle CH_3}{\underset{\vert}{CH}}{-}$	H	S
Thiopental sodium *Pentothal Sodium*	$CH_3CH_2{-}$	$CH_3CH_2CH_2\underset{\displaystyle CH_3}{\underset{\vert}{CH}}{-}$	H	S

seconds of intravenous injection, and the duration of action is ~30 min. The rapid onset of action is believed to be caused by the agents quickly partitioning from the blood across the blood-brain barrier into the sites of action in the brain. Thiopental, for example, has an exceptionally high lipid/water partition coefficient. The very short duration of action is attributed to partitioning from the brain into peripheral tissues—initially to well-perfused tissues and, subsequently, to body fat. Additionally, for methohexital, rapid metabolism may be involved as well. The structures of the compounds can be seen in Table 14-1.

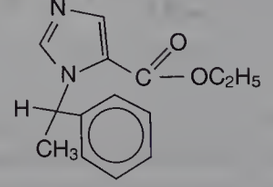
Methohexital Sodium. Sodium-(±)-1-methyl-5-allyl-5-(1-methyl-2-pentynyl) barbiturate (Brevital Sodium). Methohexital is an N-methyl barbiturate and has a pK_a of 8.4 versus ~7.6 for the non-N-methylated compounds. This pK_a change increases the concentration of the lipid-soluble free acid form at physiologic hydrogen ion concentrations. The compound also has extensive hydrophobic character (total nine hydrocarbon carbons); consequently, the lipid/water partition coefficient of the free acid form is high. Finally, it has an accessible site of metabolism, the CH_2 α to the triple bond. Overall, the compound has the properties to rapidly penetrate the CNS after intravenous injection and, then, rapidly redistribute to other body sites and also undergo rapid metabolic inactivation.

Thiamylal Sodium. Sodium 5-allyl-5-(1-methylbutyl)-2-thiobarbiturate (Surital Sodium). Thiamylal is a highly hydrophobic thiobarbiturate and has structural features closely related to thiopental. It has biologic properties similar to thiopental. After intravenous administration, unconsciousness is produced within seconds, with recovery of consciousness within 30 min.

Thiopental Sodium, USP. Sodium 5-ethyl-5-(1-methylbutyl)-2-thiobarbiturate (Pentothal Sodium). Thiopental sodium is the most widely used ultrashort-acting anesthetic barbiturate. Additionally, the compound is the prototype for the ultrashort-acting barbiturates. Most discussions of how structure influences duration of action relate specifically to it. The compound's onset of action is about equal to the time required for it to travel to the brain from the site of administration. Consciousness is regained within 30 min.

Benzodiazepines. These alone are unable to produce surgical anesthesia. However, some of the more CNS depressant benzodiazepines, for example, diazepam and midazolam, are used intravenously to induce anesthesia. Diazepam has a very high lipid/water partition coefficient and consequently is highly depotized and very long acting. So, it is usually not chosen for induction for short-term anesthetic procedures. Midazolam (structure on p. 444) has a lower lipid/water partition coefficient. This improves pharmacokinetic properties. It has a marked amnesiac effect that is valued in this use. Its overall physical and biologic properties make it a frequent choice for induction anesthesia. The sedative effect of these compounds can be reversed by flumenazil.

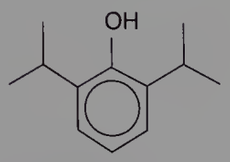
Etomidate. (Amidate).



Etomidate can be seen to contain a 4-carboxylic acid ester-substituted imidazole moiety, which is also present in a num-

ber of compounds that are structural variations off of the triazolo—and imidazolo—benzodiazepines. Not unexpectedly it is a positive modulator of $GABA_A$ receptors. Since it is a hydrophobically substituted imidazole, a side effect of the drug that can have serious clinical consequences is depression of steroidogenesis. The compound is a base, and water-soluble salts can be made for intravenous administration.

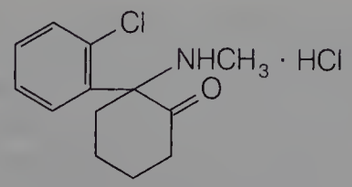
Propofol. (Diprivan).



Simple phenols are rarely seen among useful CNS depressants, possibly because of tissue destructive effects and general toxicity largely due to the phenolic hydroxy group. It is likely that the 2,6-isopropyl groups favorably influence the biological properties of the hydroxyl group. It is useful for induction and maintenance of anesthesia. It is not water soluble and is given intravenously as an emulsion. Penetration into the brain is rapid, as is redistribution to other tissues, characteristics of compounds with a high lipid/water partition coefficient. The drug bends allosterically to $GABA_A$ receptors at a site different from the benzodiazepine site.[2]

Alphaxalone. 5α-pregnane-3α-ol,11,20-dione. This is now discontinued as an induction anesthetic agent; it was originally introduced into the clinic after the anesthetic properties of first cholesterol, and then a number of 3-hydroxy metabolites of progesterone and desoxycortisone were noted. The field was rediscovered after the discontinuation of alphaxalone. It was found that a number of such compounds are positive allosteric modulators at $GABA_A$ receptors. It is thought that they have normal physiological CNS functions, and they are called neurosteroids. There is also optimism that research in the area may open a new field of CNS active drugs.

Ketamine Hydrochloride, USP. (±)-2-(o-Chloropenyl)-2-methylaminocyclohexanone hydrochloride (Ketalar). Ketamine was designed as a structural relative of the medically discontinued agent phencyclidine (PCP) (see Chap. 11), with which it shares a number of biologic properties. Block of glutamic acid NMDA receptors explains many of its actions. The incidence of hallucinations, however, is much less with ketamine. Ketamine produces a sense of dissociation from events being experienced, followed by anesthesia, analgesia, and sometimes amnesia. The anesthetic state produced by ketamine has also been described as cataleptic anesthesia.[2] The prevalence of hallucinations and excitement is higher in adults than in children. The drug may be used as the sole agent (mainly for minor surgical procedures in children), it can be used to induce anesthesia that is then maintained by one of the potent inhalation agents, or it and nitrous oxide may be used together for general anesthesia.



Ketamine Hydrochloride

ANXIOLYTIC, SEDATIVE, AND HYPNOTIC AGENTS

Agents of the anxiolytic, sedative, and hypnotic group can encompass a wide variety of drugs. For example, most antipsychotic agents reduce anxiety and are sedative, possibly related to block of DA and norepinephrine (NE) receptors. They no longer are often used for minor tranquilizing effects (e.g., in the psychoneuroses) because of the danger of inducing tardive dyskinesia. Additionally, many H_1-antihistamines have antianxiety and sedative properties, possibly related to central H_1 receptor block and, at times, may be employed in these capacities. Many opioid analgesics produce sedation and reduction of anxiety, and these actions are important and often valuable components of their use in pain control. Also many antihypertensive drugs that act on noradrenergic systems have sedative side effects. The role of partial agonists and agonists of serotonin at $5\text{-}HT_{1A}$ receptors in antianxiety therapy is clearly established. Among agents acting at peptidergic receptors, cholecystokinin (CCK) agonists at central CCK-B receptors are in development. Finally, most anticonvulsant drugs also possess sedative or anxiolytic effects, or both.

The compounds considered here are used more or less exclusively for one or more of the following actions: anxiolytic, sedative, and hypnotic. Many agents in this group also have anticonvulsant properties. There are indications that, at least occasionally, the neuronal effects related to anxiolytic, sedative, and hypnotic effects also relate to the anticonvulsant effects. In particular, the two numerically largest groups, the benzodiazepines and the barbiturates, have received much study.

BENZODIAZEPINES AND RELATED COMPOUNDS

Benzodiazepines and benzodiazepine-like drugs bind to a benzodiazepine recognition site or benzodiazepine receptor, one of several discrete allosteric sites that modulate the effect of γ-aminobutyric acid (GABA) when it binds to type A GABA ($GABA_A$) receptors. The $GABA_A$ receptor is a ligand-gated chloride ion channel. It is a protein anchored in the cell membrane and is probably pentomeric as are other ligand gated ion channels. The five polypeptide subunits that together make up its structure come from four possible subunit families: α, β, γ, and δ. There are six possible forms of the α-polypeptide (α_1 to α_6), three of the β (β_1 to β_3), four of the γ (γ_1 to γ_3, with two forms of γ_2, γ_{2S} and an

alternatively spliced form γ_{2L}, containing a consensus phosphorylation sequence), and one form of the δ.

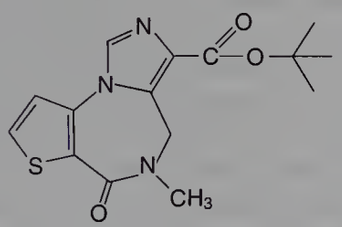
About 13 different $GABA_A$ receptor subtypes have so far been tentatively identified in rat brain. Different receptor subtypes are localized in different brain areas. The subunit composition of the $GABA_A$ receptors has great bearing on the pharmacological response to benzodiazepines and other ligands. In $\alpha\beta\gamma$ combinations, the α and γ subunits largely determine the pharmacologic response. Both affinity and efficacy are affected. The most commonly expressed combination in rat brain is $\alpha_1\beta_2\gamma_2$. (Stoichiometry of the various combinations is not known at present.) Others that are highly expressed are $\alpha_2\beta_2\gamma_2$ and $\alpha_2\beta_3\gamma_2$. Combinations with δ are mainly found in the spinal cord. The δ subunit markedly decreases affinity for benzodiazepines. It has been suggested that the ataxic effect of some of the least selective of the benzodiazepines is due to an ability to bind with spinal cord $GABA_A$ receptors containing the δ subunit.

β-Carbolines can also bind to the benzodiazepine binding site. However, because of the heterogeneity of $GABA_A$ receptors, there appear to be receptors that recognize benzodiazepines and not β-carbolines and vice versa.

There are other modulatory sites that recognize neurosteroids and barbiturates, respectively. Other binding sites exist for picrotoxin and pentylenetetrazol, respectively.

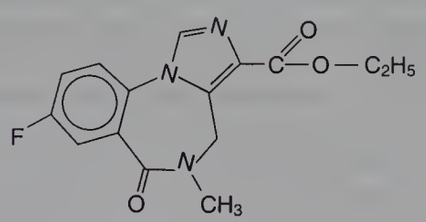
Most classical 1,4-benzodiazepines are positive modulators, are also known as agonists, and enhance the effect of GABA binding to $GABA_A$ to increase chloride ion flux into the neuron.

Most β-carbolines (it is thought that these may be biologically derived from indoleamines) and certain imidazolobenzodiazepines, for example, RO19-4603,

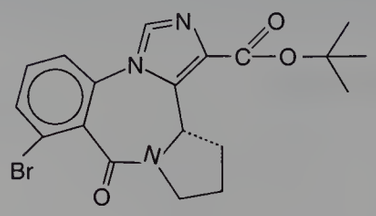


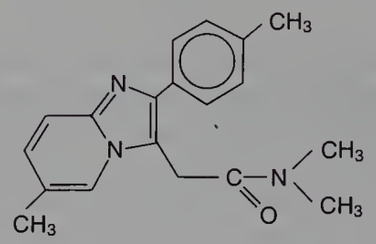
are negative modulators (inverse agonists). They diminish the positive effect of GABA on chloride flux. In whole animal tests, they appear to increase anxiety, produce panic attacks, and improve memory.

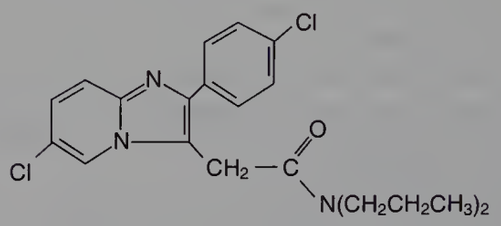
Antagonists (zero modulators) block the effect of either positive modulators (agonists) or negative modulators (inverse agonists) by occupying the recognition site and having no effect themselves on chloride ion flux. Flumenizil,



one such compound, has employment as an agent to counteract the sedative effect of benzodiazepines. Another possibility, arising from the foregoing, is for a mixed drug action such as partial agonism. Clonazepam (for structure see the anticonvulsant section) is considered a prime example of a partial agonist with decreased sedative action relative to full agonists at full anti-absence doses. Also regarded as representing partial agonists are bretazenil (antiepileptic, low sedation), and zolpidem (sedative-hypnotic) and alpidem (anxiolytic) both with reduced side effects.







Experts in the area have considered that intrinsic activity and affinity differences, i.e., type of drug action and potency, among these various drugs are due entirely to differences in subunit makeup among the various GABA receptors. More recent opinion appears to be that intrinsic activity and affinity variations among benzodiazepines and related compounds are due in part to the variations in the subunit makeup of receptors and in part to variations in structural features of the drugs. For example, most classical 1,4-benzodiazepines tend to positively modulate $GABA_A$ receptors to which they bind, and most β-carbolines tend to negatively modulate $GABA_A$ receptors to which they bind.

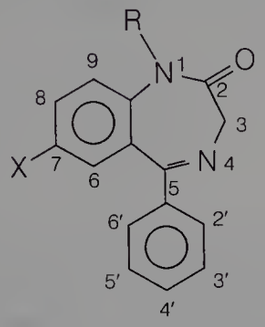
Another possible contribution to benzodiazepine action may stem from binding to peripheral benzodiazepine receptors that occur on mitochondrial glial cells as well as peripherally. They were once not considered relevant to benzodiazepine action. Today, some investigators believe that binding of benzodiazepines to these receptors is associated with the development of tolerance. Experimentally, tolerance can be correlated with increases in peripheral benzodiazepine receptor binding capacity. An opposing finding is that benzodiazepine binding to the peripheral (and central) receptor increases the production of 3α-hydroxyprogesterone-derived neurosteroids. An increase in these positive modulators would increase the overall CNS depressant effects of benzodiazepines. This is considered a possibility at this time. To potentially complicate the situation further, it is known that certain close congeners of the positive modulatory neurosteroids can act as negative modulators. Were there an increase in these (this has not been shown metaboli-

cally), they could produce a decrease in effectiveness of the positive modulating benzodiazepines.

At this writing, the structure-activity relationships (SARs) for the classical 1,4-benzodiazepines appear to be still valid and are given below. SARs for partial agonists, antagonists, and inverse agonists appear to be still formative. They will doubtless be delineated as the structural diversity of these agents expand and more is learned about differences in binding sites among the various $GABA_A$ receptor subtypes.

The field of benzodiazepines was opened with the synthesis of chlordiazepoxide by Sternbach and the discovery of its unique pharmacologic properties by Randall.[22]

Chlordiazepoxide (refer to the discussion of individual compounds) is a 2-amino benzodiazepine, and other amino compounds have been synthesized. However, when it was discovered that chlordiazepoxide is rapidly metabolized to a series of benzodiazepin-2-ones (see the general scheme of metabolic relationships) that are active, emphasis shifted to the synthesis and testing of the later group. Empirical SARs for antianxiety activity have been tabulated for this group (analogous statements apply for the older 2-amino group).[22,23] Reference can be made to the following general structure to visualize them.

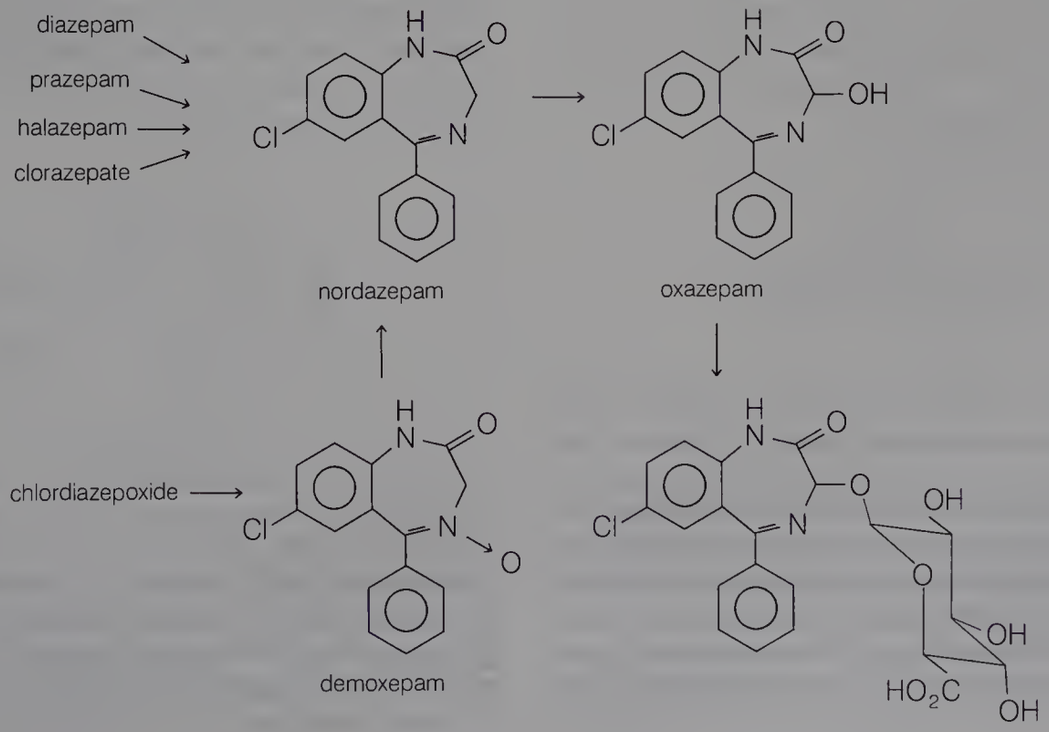


The presence of an electron-attracting substituent at position 7 is required for activity, and the more electron attracting it is, the higher is the activity. Positions 6, 8, and 9 should not be substituted. A phenyl at the 5-position promotes activity. If this group is *ortho* (2′) or di*ortho* (2′,6′) substituted with electron-attracting substituents, activity is increased. On the other hand, *para* substitution decreases activity greatly. Saturation of the 4,5 double bond or a shift to the 3-4–position decreases activity. Alkyl substitution at the 3-position decreases activity, whereas substitution with a hydroxy does not. The presence or absence of the 3-hydroxyl is important pharmacokinetically. Compounds without the hydroxyl are nonpolar, have long half-lives and undergo hepatic oxidation. Compounds with the hydroxyl are much more polar and are readily converted to the excreted glucuronide (see the overall metabolic relationship scheme). The 2-carbonyl function is optimal for activity, as is the nitrogen atom at position 1. The N-substituent should be small.

Additional research yielded compounds with a fused triazolo ring represented by triazolam and alprazolam midazolam, with a fused imidazolo ring, also followed. These compounds are metabolized mainly by hydroxylation of the methyl substituent on the triazolo or imidazolo ring. The resulting hydroxy compound is active, but is quickly conjugated. The compounds are also metabolized by 3-hydroxylation of the benzodiazepine ring. Interestingly, the presence of an electron-attracting group at position 7 is not required for activity among these compounds.

The metabolism of benzodiazepines has received much study.[24,25] Some of the major metabolic relationships are shown in the scheme in the scheme below.

The benzodiazepines are well absorbed from the gastrointestinal tract, although the more polar compounds (e.g., those



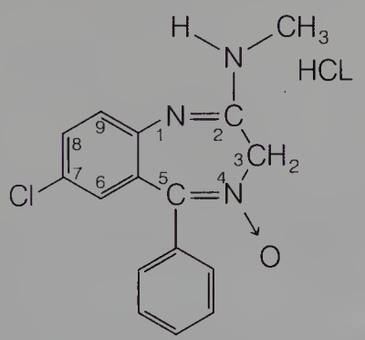
with a hydroxyl at the 3-position) tend to be absorbed more slowly than the more nonpolar compounds.

The drugs tend to be highly bound to plasma proteins; in general, the more nonpolar the drug, the greater the extent of binding. They are also very effectively distributed to the brain. Again, generally, the more nonpolar the compound, the greater the extent of distribution to the brain, at least initially. When diazepam is used as an anesthetic, it redistributes to sites outside the brain.

Compounds without the 3-hydroxyl group usually have long half-lives and undergo conversion to the 3-hydroxy compounds by hepatic oxidation. Compounds with the 3-hydroxyl group have short half-lives because of rapid conjugation to the 3-glucuronide, which undergoes urinary excretion. In patients with impaired liver function, the 3-hydroxy compounds, which do not require hepatic oxidation, tend to present fewer hazards than the more nonpolar compounds.

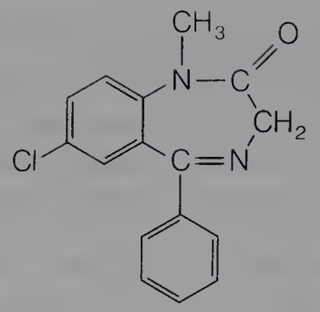
In addition to lower abuse potential, and a much greater margin of safety than the barbiturates, the drugs have fewer drug interactions. Especially noteworthy is that they do not promote the metabolism of other drugs.

Chlordiazepoxide Hydrochloride, USP. 7-Chloro-2-(methylamino)-5-phenyl-3*H*-1,4-benzodiazepine 4-oxide monohydrochloride (Librium). Absorption from the gastrointestinal tract is good. Peak plasma levels are reached in 2 to 4 hr. *N*-Demethylation and hydrolysis of the condensed amidino group is rapid and extensive, producing demoxapam as a major metabolite. Demoxapam, in turn, is converted principally to nordazepam. Nordazepam, in turn, is converted principally to oxepam which undergoes conjugation to the excreted glucuronide. Of course, other routes of metabolism can occur, for example, opening of the seven-membered ring by hydrolysis of the lactam group.



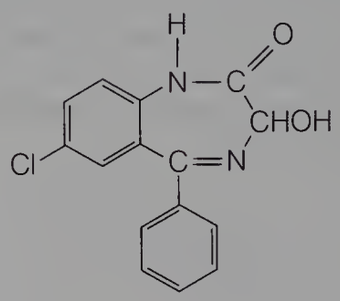
Chlordiazepoxide Hydrochloride

Diazepam, USP. 7-Chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one (Valium). Diazepam was the first member of the benzodiazepin-2-one group to be introduced. It is very nonpolar and is rapidly absorbed. Diazepam is metabolized by *N*-demethylation to nordazepam, which is then metabolized according to the general scheme. It is widely used for several anxiety states and has an additional wide range of uses (e.g., as an anticonvulsant, a premedication in anesthesiology, and in various spastic disorders).



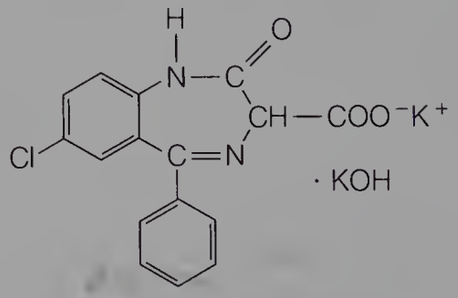
Diazepam

Oxazepam, USP. 7-Chloro-1,3-dihydro-3-hydroxy-5-phenyl-2*H*-1,4-benzodiazepin-2-one (Serax). Oxazepam can be considered a prototype for the 3-hydroxy compounds. It is much more polar than diazepam, for example. Metabolism is relatively uncomplicated, and the duration of action is short.



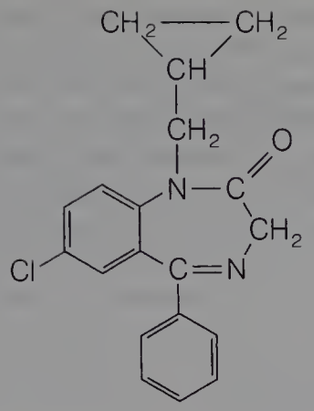
Oxazepam

Clorazepate Dipotassium. 7-Chloro-2,3-dihydro-2-oxo-5-phenyl-1*H*-1,4-benzodiazepine-3-carboxylic acid dipotassium salt monohydrate (Tranxene). Clorazepate can be considered to be a prodrug. Itself inactive, it undergoes rapid loss of water and decarboxylation to nordazepam, which has a long half-life and undergoes hepatic conversion to oxazepam. Despite the polar character of the drug as administered, because it is quickly converted to a nonpolar compound, it has an overall long half-life.



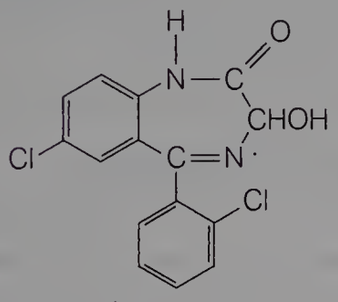
Clorazepate Dipotassium

Prazepam, USP. 7-Chloro-1-(cyclopropylmethyl)-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one (Verstran). The overall half-life is long. Extensive *N*-dealkylation occurs to yield nordazepam. 3-Hydroxylation of prazepam and of nordazepam occur.



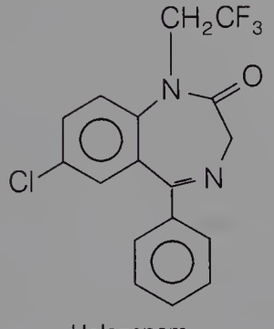
Prazepam

Lorazepam, USP. 7-Chloro-5-(2-chlorophenyl)-3-dihydro-3-hydroxy-2*H*-1,4-benzodiazepin-2-one (Ativan). Lorazepam can be recognized as the 2′-chloro substituted analogue of oxazepam. In keeping with overall SARs, the 2′-chloro substituent increases activity. Metabolism is relatively rapid and uncomplicated because of the presence of the 3-hydroxyl group.



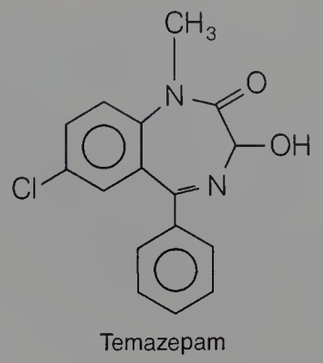
Lorazepam

Halazepam, USP. 7-Chloro-1,3-dihydro-5-phenyl-1(2,2,2-trifluoroethyl)-2*H*-1,4-benzodiazpin-2-one (Paxipam). Halazepam is well absorbed. It is active and present in plasma, but much of its activity is due to the major metabolite (as well as oxazepam). The drug is marketed as an anxiolytic.



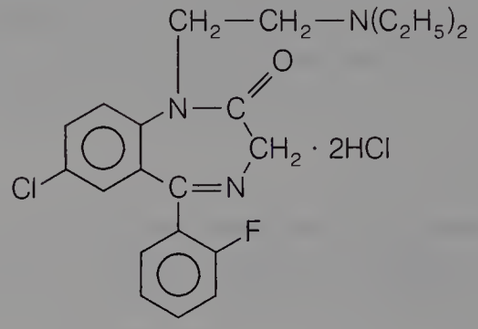
Halazepam

Temazepam. 7-Chloro-1,3-dihydro-3-hydroxy-1-methyl-5-phenyl -2*H*-1,4-benzodiazepin-2-one (Restoril). The compound is a minor metabolite of diazepam. It can also be visualized as *N*-methyl oxazepam. There is a small amount of *N*-demethylation. However, metabolism proceeds mainly through conjugation of the 3-hydroxyl group. The duration of action is short. It is marketed as a hypnotic with little or no residual effects.



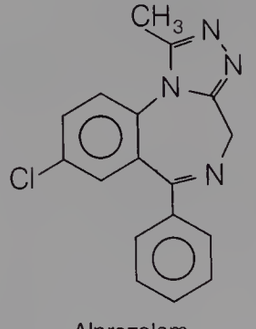
Temazepam

Flurazepam Hydrochloride, USP. 7-Chloro-1-[2-(diethylamino)ethyl]-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one dihydrochloride (Dalmane). Flurazepam is notable as a benzodiazepine indicated almost exclusively in insomnia. Metabolism of the dialkyl aminoalkyl side chain is extensive. A major metabolite is N^1-dealkyl flurazepam, with a very long half-life, persisting for several days after the drug is administered.



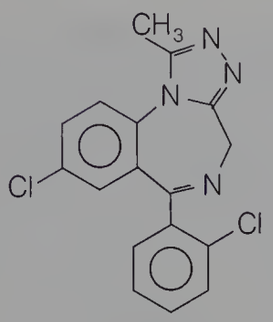
Flurazepam Hydrochloride

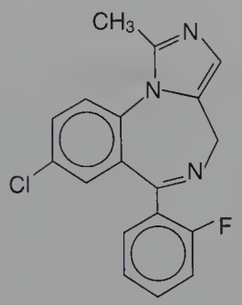
Alprazolam, USP. 8-Chloro-1-methyl-6-phenyl-4*H*-*s*-triazolo[4,3-*a*][1,4]benzodiazepine (Xanax) is rapidly absorbed. Protein binding is lower (~70%) than for most benzodiazepines. Oxidative metabolism of the methyl group to the methyl alcohol followed by conjugation is rapid and the duration of action is short. The drug is highly potent as an anxiolytic on a milligram basis.



Alprazolam

Triazolam, USP. 8-Chloro-6-(*o*-chlorophenyl)-1-methyl-4*H*-s-triazolo[4,3-a][1,4]benzodiazepine (Halcion). Triazolam has all of the characteristic benzodiazepine actions. It is marketed as a sedative-hypnotic drug said to produce little, if any, daytime impairment of function. It is rapidly metabolized to the 1-methyl alcohol, which is then excreted.





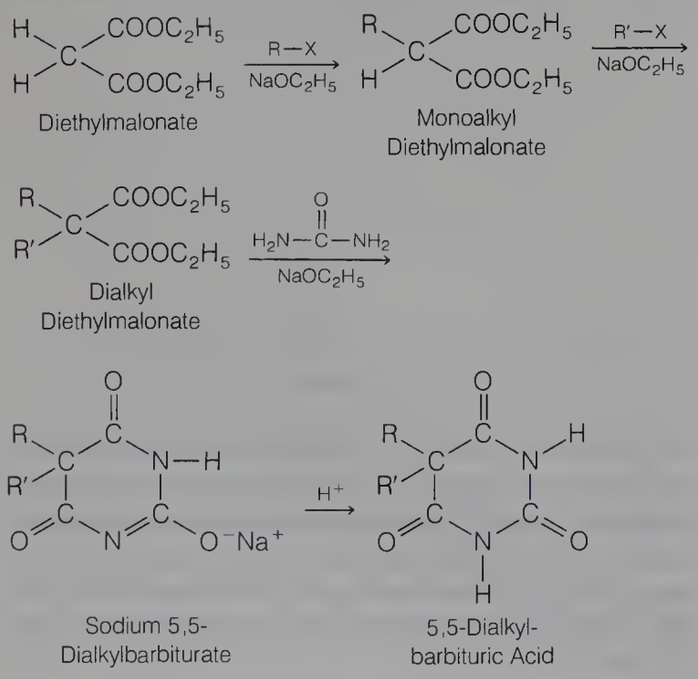
Midazolam

This drug is a potent full agonist and is used intravenously as a sedative-hypnotic and induction anesthetic. Further information can be found in the section on anesthetics.

BARBITURATES

The first sedative-hypnotic barbiturate, 5,5-diethylbarbituric acid, was introduced in 1903. With time, many members were added, and the barbiturates dominated the sedative-hypnotic field until the advent of the benzodiazepines, which for reasons outlined earlier, but most notably a much greater margin of safety, displaced the barbiturates as the most broadly useful agents in sedative-hypnotic applications.

The barbiturates are 5,5-disubstituted barbituric acids. The following scheme shows how the 5,5-dialkyl compounds are synthesized. Substitution of thiourea for urea produces the 2-thiobarbiturates, useful as induction anesthetics.



Diethylmalonate → Monoalkyl Diethylmalonate → Dialkyl Diethylmalonate → Sodium 5,5-Dialkylbarbiturate → 5,5-Dialkylbarbituric Acid

A consideration of the structure of 5,5-disubstituted barbituric acids reveals their acidic character. Those without methyl substituents on the nitrogen have pK_as of ~7.6, those with a methyl substituent have pK_as of ~8.4. The free acids have poor water solubility and good lipid solubility (the latter largely a function of the two hydrocarbon substituents on the 5-position, although in the 2-thiobarbiturates the sulfur atom increases lipid solubility).

Sodium salts of the barbiturates are readily prepared and are water-soluble. Their solutions generate an alkaline pH. A classic incompatibility is the addition of an agent with an acidic pH in solution, which results in formation and precipitation of the free water-insoluble disubstituted barbituric acid.

Sodium salts of barbiturates in aqueous solution decompose at varying rates by base-catalyzed hydrolysis, generating ring-opened salts of carboxylic acids.

That the names of many barbiturates end in *al* (e.g., phenobarbital), appearing to denote an aldehydic compound, derives from the fact that chloral hydrate was widely known as a sedative-hypnotic when the first barbiturates were introduced. Accordingly, the suffix was an attempt to denote a therapeutic, not a chemical, class.

Structure-Activity Relationships

Extensive synthesis and testing of the barbiturates over a long time span has produced well-defined structure-activity relationships, which have been summarized.[26]

Both hydrogen atoms at the 5-position of barbituric acid must be replaced. If one hydrogen is available at position 5, tautomerization to a highly acidic trihydroxypyrimidine (pK_a ~ 4) can occur. Consequently, the compound is largely in the anionic form at physiologic pHs, with little nonionic lipid-soluble compound available to cross the blood-brain barrier.

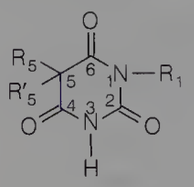
Beginning with lower alkyls, there is an increase in onset and a decrease in duration of action with increasing hydrocarbon content up to about seven to nine total carbon atoms substituted on the 5-position. Lipophilicity and an ability to penetrate the brain in the first case and an ability to penetrate liver microsomes in the second may be involved. Also, for more hydrophobic compounds, partitioning out of the brain to other sites can be involved in the second instance. There is an inverse correlation between the total number of carbon atoms substituted on the 5-position and the duration of action, which is even better when the character of these substituents is taken into account, for example, the relatively polar character of a phenyl substituent (approximates a three- to four-carbon aliphatic chain), branching of alkyls, presence of an isolated double or triple bond, and so on. Additionally, these groups can influence the ease of oxidative metabolism by effects on bond strengths as well as by influencing partitioning.

Metabolism of the barbiturates is discussed in Chap. 3. Suffice it to say that increasing the lipid/water partition coefficient generally increases the rate of metabolism, except for compounds with an extremely high lipid/water partition coefficient (e.g., thiopental), which tend to depotize and are thus relatively unavailable for metabolism. Metabolism generally follows an ultimate (Ω) or penultimate (Ω-1) oxidation pattern. Ring-opening reactions are usually minor. *N*-methylation decreases duration of action, in large part, probably by increasing the concentration of the lipid-soluble free barbituric acid. 2-Thiobarbiturates have a very short duration of action because the lipid/water partition coefficient is extremely high, promoting depotization. Barbiturates find employment as sedatives, as hypnotics, for induction of anesthesia, and as anticonvulsants. Absorption from the gastrointestinal tract is good. Binding to blood proteins is substantial. Compounds with low lipid/water partition coefficients may be excreted intact in the urine. Those with

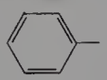
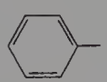
TABLE 14-2

BARBITURATES USED AS SEDATIVES AND HYPNOTICS

General Structure



A. LONG DURATION OF ACTION (6 OR MORE HR)

Generic Name Proprietary Name	*Substituents* R_5	R_5'	R_1	*Sedative Dose (mg)*	*Hypnotic Dose (mg)*	*Usual Onset of Action (min)*
Mephobarbital USP *Mebaral*	C_2H_5		CH_3	30–100*	100	30–60
Metharbital USP *Gemonil*	C_2H_5	C_2H_5	CH_3	50–100*		30–60
Phenobarbital USP *Luminal*	C_2H_5		H	15–30*	100	20–40

B. INTERMEDIATE DURATION OF ACTION (3–6 HR)

Butalbital USP *Sandoptal*	$CH_2{=}CHCH_2-$	$(CH_3)_2CHCH_2-$	H		200–600	20–30
Amobarbital USP *Amytal*	CH_3CH_2-	$(CH_3)_2CHCH_2CH_2-$	H	20–40	100	20–30
Aprobarbital *Alurate*	$CH_2{=}CHCH_2-$	$(CH_3)_2CH-$	H	20–40	40–160	
Butabarbital Sodium USP *Butisol Sodium*	CH_3CH_2-	$CH_3CH_2\underset{\displaystyle CH_3}{\underset{\vert}{C}}H-$	H	15–30	100	20–30
Talbutal USP *Lotusate*	$CH_2{=}CHCH_2-$	$CH_3CH_2\underset{\displaystyle CH_3}{\underset{\vert}{C}}H-$	H	50	120	20–30

C. SHORT DURATION OF ACTION (LESS THAN 3 HR)

Pentobarbital Sodium USP *Nembutal Sodium*	CH_3CH_2-	$CH_3CH_2CH_2\underset{\displaystyle CH_3}{\underset{\vert}{C}}H-$	H	30	100	20–30
Secobarbital USP *Seconal*	$CH_2{=}CHCH_2-$	$CH_3CH_2CH_2\underset{\displaystyle CH_3}{\underset{\vert}{C}}H-$	H	15–30	100	20–30

*Daytime sedative and anticonvulsant.

higher lipid/water partition coefficients are excreted after metabolism to polar metabolites.

Some of the more frequently used barbiturates are described briefly in the following sections. For the structures, the usual dosages required to produce sedation and hypnosis, the times of onset, and the duration of action, see Table 14-2.

Barbiturates with a Long Duration of Action (≥6 hr)

Barbital. 5,5-Diethylbarbituric acid. Barbital, although discontinued as a sedative-hypnotic, is interesting because of the biologic consequence of its low lipid/water partition coefficient. It is slowly eliminated, mostly in the intact form, by the kidney.

Metharbital. 5,5-Diethyl-1-methylbarbituric acid (Gemonil). This methyl derivative of barbital finds a limited employment in a variety of epilepsies.

Phenobarbital, USP. 5-Ethyl-5-phenylbarbituric acid (Luminal). The compound is a long-acting sedative and hypnotic. It is also a valuable anticonvulsant, especially in generalized tonic-clonic and partial seizures (see the discussion on anticonvulsants). Metabolism to the *p*-hydroxy compound followed by glucuronidation accounts for ~90% of a dose.

Barbiturates with an Intermediate Duration of Action (3 to 6 hr)

These compounds are used principally as sedative-hypnotics. They include **Amobarbital, USP,** 5-Ethyl-5-isopentylbarbituric acid (Amytal), and its water-soluble sodium salt, **Amobarbital Sodium, USP,** 5-Allyl-5-isopropylbarbituric acid (Aprobarbital, Alurate); **Butabarbital Sodium, USP,** the water-soluble sodium salt of 5-*sec*-butyl-5-ethylbarbituric acid (Butisol Sodium), and **Talbutal, USP,** 5-Allyl-5-*sec*-butylbarbituric acid (Lotusate).

Barbiturates with a Short Duration of Action (<3 hr)

Included in this group, which have substituents in the 5-position promoting more rapid metabolism (e.g., by increasing the lipid/water partition coefficient) than the intermediate group, are **Pentobarbital Sodium, USP,** Sodium 5-ethyl-5-(1-methylbutyl)barbiturate (Nembutal); **Secobarbital, USP,** 5-Allyl-5-(1-methylbutyl)barbituric acid (Seconal); and the sodium salt, sodium secobarbital.

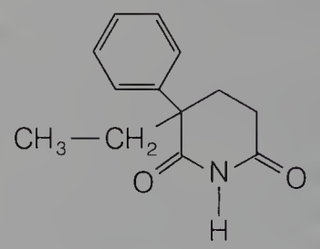
Barbiturates with an ultrashort duration of action are discussed under anesthetic agents.

MISCELLANEOUS SEDATIVE-HYPNOTICS

A wide range of chemical structures (e.g., imides, amides, alcohols) can produce sedation and hypnosis resembling that produced by the barbiturates. Despite this apparent structural diversity, the compounds have generally similar structural characteristics and chemical properties: a hydrophobic portion and a semipolar portion that can participate in H-bonding. In many cases, modes of action are undetermined. As a working hypothesis, some of the agents could be envisioned to act by mechanisms similar to those proposed for barbiturates and alcohols.

Amides and Imides

Glutethimide, USP. 2-Ethyl-2-phenylglutarimide (Doriden). This imide has many structural relationships with the barbiturates and resembles them in many respects biologically. It is an effective sedative-hypnotic. It is very hydrophobic, and absorption from the gastrointestinal tract is somewhat erratic. Metabolism is extensive, and the drug is an enzyme inducer. In the therapeutic dosage range, adverse effects tend to be infrequent. Toxic effects in overdose are as severe as and possibly more troublesome than those of the barbiturates.

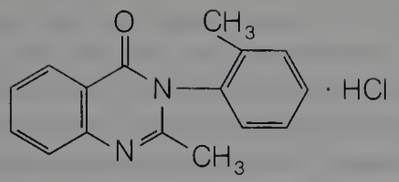


Glutethimide

Methyprylon, USP. 3,3-Diethyl-5-methyl-2,4-piperidinedione (Noludar). The drug is an effective sedative-hypnotic. Its effects resemble those of the barbiturates. Metabolism is extensive, and, similar to the barbiturates and glutethimide, it is an enzyme inducer. Some of the effects of overdose and of withdrawal resemble those of the barbiturates.

Methyprylon

Methaqualone Hydrochloride. 2-Methyl-3-*o*-tolyl-4(3*H*)-quinazolinone monohydrochloride. The drug is an effective sedative-hypnotic, but has been withdrawn because of abuse potential.



Methaqualone Hydrochloride

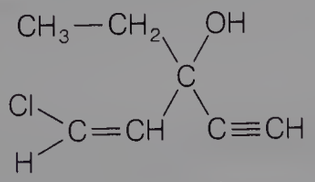
Metabolites include the *N*-oxide and oxidative metabolites of the tolyl methyl group. Some of the central effects have been reported to resemble those of the opioids. The effects of overdosage are complex, with some of them resembling those of the barbiturates.

Alcohols and Their Carbamate Derivatives

A very simple alcohol, ethanol, has a long history of use as a sedative and hypnotic. Studies attempting to determine its modes of action were described in the introduction to this chapter. Because its use in these capacities is associated with so many potential hazards (e.g., production of alcoholism and its ability to exert toxic effects on many organ systems), it is seldom a preferred agent medically, and other drugs have been developed.

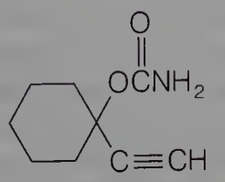
As the homologous series of normal alcohols is ascended from ethanol, CNS depressant potency increases up to eight carbon atoms, with activity decreasing thereafter (the Meyer–Overton parabola, Chap. 2). Branching of the alkyl chain increases depressant activity and, in an isometric series, the order of potency is tertiary > secondary > primary. In part, this may be because tertiary and secondary alcohols are not metabolized by oxidation to corresponding carboxylic acids. Replacement of a hydrogen atom in the alkyl group by a halogen has the effect of increasing the alkyl portion and, accordingly, for the lower molecular weight compounds, increases potency. Carbamylation of alcohols generally increases depressant potency. Carbamate groups are generally much more resistant to metabolism than hydroxyl functions. Hydroxylic compounds and their carbamate derivatives used as sedative and hypnotics are described in the following paragraphs.

Ethchlorvynol, USP. 1-Chloro-3-ethyl-1-penten-4-yn-3-ol (Placidyl). Ethchlorvynol is an effective sedative-hypnotic with a rapid onset and short duration of action. Metabolism, probably involving the hydroxyl group, accounts for ~90% of a dose. Acute overdose has several features in common with barbiturate overdose.



Ethchlorvynol

Ethinamate, USP. 1-Ethynyl-cyclohexanol carbamate (Valmid). The compound is a modestly active sedative and hypnotic agent. The onset of action is rapid and the duration of action is short. Metabolism involves hydroxylation of the cyclohexane ring.

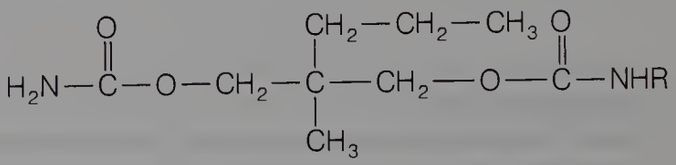


Ethinamate

Meprobamate, USP. 2-Methyl-2-propyltrimethylene dicarbamate; 2-methyl-2-propyl-1,3-propanediol dicarbamate (Equanil, Miltown). Meprobamate is discussed here because its official indication is as an antianxiety agent, and it is also extensively used as a sedative-hypnotic. Whether or not its antianxiety properties are separate from sedative effects is uncertain. Another reason for considering the compound here is that it has many overall properties resembling benzodiazepines and barbiturates. The mechanism of action underlying anxiolytic effects is unknown but may involve effects on conductivity in specific brain areas.[27] It does not appear to act through effects on GABAergic systems.

The drug is effective against absence seizures and may worsen generalized tonic-clonic seizures.

Meprobamate could also be grouped with the centrally acting skeletal muscle relaxants, which are discussed later. Its relative, carisoprodol, is discussed there. This muscle relaxant action, in part, may be due to blockade of the polysynaptic reflexes in the spinal cord and appears to be associated with



Meprobamate: R = H
Carisoprodol: R = —CH(CH3)2

appropriately substituted glycols, other dihydric compounds, and their carbamate derivatives.[27] The major route of metabolism of mephenesin involves oxidative hydroxylation of the carbon next to the terminal atom of the *n*-propyl radical.

Aldehydes and Their Derivatives

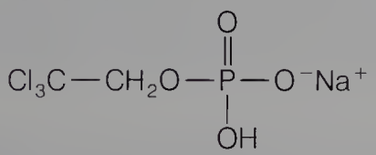
This heading has the potential to mislead. Actually, few aldehydes are valuable hypnotic drugs. The one aldehyde in use, chloral (as the hydrate), is thought to act principally through a metabolite, trichloroethanol, and the derivative of acetaldehyde is a cyclic trimer, which could also be grouped as an ether.

Chloral Hydrate, USP. Trichloroacetaldehyde monohydrate; $CCl_3CH(OH)_2$ (Noctec). Chloral hydrate is an aldehyde hydrate sufficiently stable to be isolated. The relative stability of this *gem*-diol is largely due to an unfavorable dipole-dipole repulsion between the trichloromethyl carbon

and the carbonyl carbon present in the parent carbonyl compound.[28]

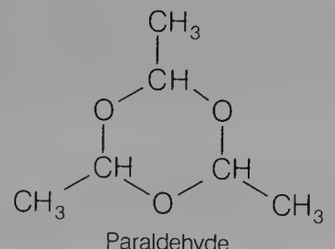
The compound is unstable under alkaline conditions, undergoing the last step of the haloform reaction to yield chloroform and formate ion. In combination with ethanol, it forms the hemiacetal. Whether or not this compound is the basis for the notorious and potentially lethal effect of the combination of ethanol and chloral hydrate can be argued. Synergism between two different CNS depressants also could be involved. Additionally, ethanol, by increasing the concentration of NADH, enhances the reduction of chloral to trichloroethanol, and, finally, chloral can inhibit the metabolism of alcohol because it inhibits alcohol dehydrogenase. Although it is suggested that chloral hydrate per se may act as a hypnotic,[29] it is also recognized that chloral hydrate is very quickly converted to trichloroethanol and the latter compound is generally assumed to account for almost all of the hypnotic effect. It appears to have potent barbiturate-like binding to $GABA_A$ receptors.

Triclofos Sodium. 2,2,2,-Trichloroethanol dihydrogen phosphate monosodium salt (Triclos). Chloral hydrate is irritating to the gastrointestinal mucosa. Its active metabolite, trichloroethanol, also has unpleasant properties when given orally. Triclofos is the nonirritating sodium salt of the phosphate ester of trichloroethanol. Accordingly, triclofos sodium has biologic properties similar to chloral hydrate.



Triclofos Sodium

Paraldehyde, USP. 2,4,6-Trimethyl-*s*-trioxane; paracetaldehyde. Paraldehyde is recognizable as the cyclic trimer of acetaldehyde. It has a strong characteristic odor detectable in the expired air and an unpleasant taste. These properties limit its use almost exclusively to an institutional setting (e.g., in the treatment of delirium tremens).



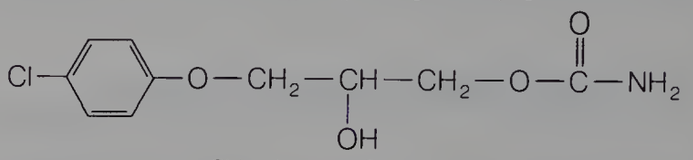
Paraldehyde

CENTRAL NERVOUS SYSTEM DEPRESSANTS WITH SKELETAL MUSCLE RELAXANT PROPERTIES

AGENTS USED IN ACUTE MUSCLE SPASMS

The agents in this group find use in a number of conditions, such as strains and sprains, that may produce acute muscle spasm. They have interneuronal-blocking properties at the level of the spinal cord, and these are said to be partly responsible for skeletal muscle relaxation.[27] Also, they have general CNS depressant properties that may contribute to, or be mainly responsible for, the skeletal muscle relaxant activity. Dihydric compounds and their carbamate (urethane) derivatives, as described earlier in the discussion of meprobamate, are prominent members of the group.

Chlorphenesin Carbamate. 3-(*p*-Chlorophenoxy)-1,2-propanediol 1-carbamate (Maolate). This is the *p*-chloro substituted and 1-carbamate derivative of the lead compound in the development of this group of agents, mephenesin.

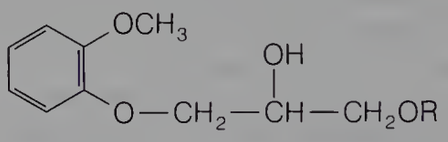


Chlorphenesin Carbamate

Mephenesin is weakly active and short-lived largely because of facile metabolism of the primary hydroxyl group. Carbamylation of this group increases activity. *p*-Chlorination increases the lipid/water partition coefficient and seals off the *p*-position from hydroxylation.

Metabolism, still fairly rapid, involves glucuronidation of the secondary hydroxyl group. The biologic half-life in humans is 3.5 hr.

Methocarbamol, USP. 3-(*o*-Methoxyphenoxy)-1,2-propanediol 1-carbamate (Robaxin). Methocarbamol is said to be more sustained in effect than mephenesin. Likely sites for metabolic attack include the secondary hydroxyl group and the two ring positions opposite the ether functions. The dihydric parent compound, guaifenesin, is used as an expectorant.



Guaifenesin: R = H

Methocarbamol: R = —C(=O)—NH2

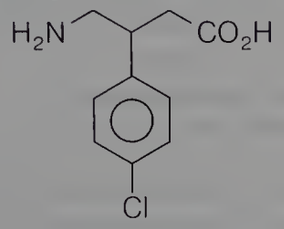
Carisoprodol, USP. *N*-Isopropyl-2-methyl-2-propyl-1,3-propanediol dicarbamate; 2-methyl-2-propyl-1,3-propanediol carbamate isopropylcarbamate (Soma). The compound is the mono-*N*-isopropyl substituted relative of meprobamate. The structure is given in the discussion of meprobamate. It is indicated in acute skeletomuscular conditions characterized by pain, stiffness, and spasm. As can be expected, a major side effect of the drug is drowsiness.

DRUGS USED IN SPASTICITY

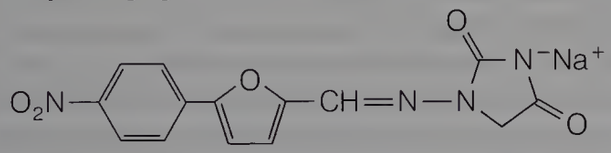
Several drugs benefit the spasticity associated with diseases such as multiple sclerosis and cerebral palsy. Notable compounds are the centrally acting agents diazepam (discussed

earlier) and baclofen, and the peripherally (acting directly on skeletal muscle) active agent dantrolene.

Baclofen, USP. 3-(*p*-Chlorophenyl)-γ-aminobutyric acid (Lioresal). Baclofen, a substituted GABA analogue, is useful in spasticity involving diseases of the spinal cord. It depresses monosynaptic and polysynaptic transmission. The exact mechanisms of action were for some time unclear. Some effects resembled those of GABA,[30] and others did not.[31] It is now understood that (−)-baclofen is active as an agonist at $GABA_B$ receptors. (+)-Baclofen is inactive. $GABA_B$ receptors are heterogeneous, but, broadly, $GABA_B$ agonism at $GABA_B$ receptors regulates neurotransmitter (various, but including GABA and glutamate) release.[32]



Dantrolene Sodium. Dantrium. Dantrolene decreases the release of calcium ion by the sarcoplasmic reticulum and thereby blocks contraction of skeletal muscle.[33] It is useful in cerebral palsy and multiple sclerosis. Its effect on calcium ion release is also the basis for its usefulness in malignant hyperthermia. Peripherally acting, it is grouped here with centrally acting agents because of its muscle relaxant effects.

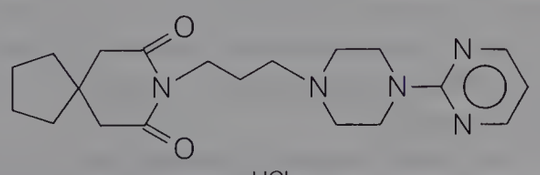


Dantrolene Sodium

The most common side effect is muscle weakness. Another serious problem that limits the use of the drug is hepatic toxicity.

$5HT_{1A}$ AGONISTS AND PARTIAL AGONISTS

Busiprone. 8-[4-[4-(Pyrimidinyl)-1-piperazinyl]-butyl]azaspiro[4,5]decane-7,9-dione monohydrochloride (Buspar). Buspirone is the first clinically introduced anxiolytic and antidepressant representative of a group of compounds under study that affect serotoninergic systems. It is reported to act as a partial agonist of serotonin at $5\text{-}HT_{1A}$ receptors. The compound also has antidopaminergic activity. There is concern that the latter activity could lead to extrapyramidal effects.



HCl

Reportedly, relatives such as gepirone, ipsapirone, and tandospirone are more receptor selective than buspirone. At least one full $5\text{-}HT_{1A}$ agonist appears to be antidepressant.[35,51]

ANTIPSYCHOTICS

Antipsychotics are drugs that ameliorate mental aberrations that are characteristic of the psychoses. The psychoses differ from the milder behavioral disorders, the neuroses, in that thinking tends to be illogical, bizarre, and loosely organized. Importantly, patients have difficulty understanding reality and their own conditions. There are often hallucinations (usually auditory) and delusions.

Psychoses can be organic and related to a specific toxic chemical, as in delirium produced by some central anticholinergic agents, or to a definite disease process, such as dementia, or they can be idiopathic. Idiopathic psychoses may be acute or chronic. Idiopathic acute psychotic reactions have been reported to follow extremely severe acute stress. Schizophrenia is a group of chronic idiopathic psychotic disorders with the overall symptomology described earlier.

The term "antipsychotic" was slow in gaining acceptance. Now it is widely acknowledged that antipsychotics actually diminish the underlying thought disorder that is the chief characteristic of the schizophrenias.[35] The agents often have a calming effect in agitated psychotic patients; hence, they also have been referred to as "major tranquilizers." Finally, because they induce a lessening of reactivity to emotional stimuli, with little effect on consciousness, they are referred to as "neuroleptics."

The most frequent uses of the agents are in manic disorders and the schizophrenias. In the manic disorders, it could be conjectured that the agents block dopamine (DA) at D-2 receptors, reducing euphoria and hyperactivity. In the chronic idiopathic psychoses, the clinically active conventional agents appear to act largely by blocking DA at D-2 receptors in the mesolimbic area of the brain.[36,37] Other DA receptor blocks that contribute to antipsychotic action among compounds are D_3 and D_4. The rauwolfia alkaloids, seldom used today as antipsychotics, are considered to act as antipsychotics by depleting neuronal DA, rather than acting by a postsynaptic receptor block. Also, selective presynaptic DA receptor agonists, the net effect of which is to reduce the release of DA into the synapse, where it is available for interaction with postsynaptic receptors, are being studied as antipsychotic agents.

A useful paradigm for the schizophrenias is that there is excessive DA activity in the limbic system. Consequently, more information is supplied than can be interpreted, and an involuntary picking and choosing of the informational overload begins (i.e., the brain begins to receive in code, and perceptions of reality are altered). Finally, the person responds in code, and, because we do not have the key to the code, we perceive bizarre and illogical behavior. It has ironically been observed that if the key were found and the

underlying pattern discerned, then there could be seen a certain logic or sanity to psychotic behavior.[35]

Extrapyramidal side effects (EPS) of the clinically useful antipsychotics include a parkinsonism that resembles the symptomology of Parkinson's disease: acute dystonic reactions, characterized by spasm of the tongue, face, and neck; and akathesia, characterized by a restlessness and a need to be in constant motion. The effects are thought to arise from a block of D-2 receptors in the striatum.

The tardive dyskinesia that is sometimes seen with use of these agents is probably related to prolonged or potent block of D-2 striatal receptors. It appears to arise from biologic compensation for D-2 striatal block by antipsychotic agents and is characterized by excessive striatal DA activity. Symptoms resemble those of Huntington's chorea and include involuntary movements of the lips, tongue, and mouth, and purposeless choreiform motions of the extremities. It is not clear that the condition is reversible. Since tardive dyskinesia has been recognized as a potential effect of therapy, there has been increased care in dosing; that is, the smallest effective dose for the shortest possible time is given. Also, it has led to a reluctance to use the agents as tranquilizers or sedatives in the minor psychiatric disorders such as the neuroses.

Many, but not all, of the agents are strongly anticholinergic. This can produce typical peripheral anticholinergic effects. Centrally, this action can work to advantage. It may minimize drug-induced parkinsonism by a block of striatal muscarinic receptors that partly compensates for DA striatal block.

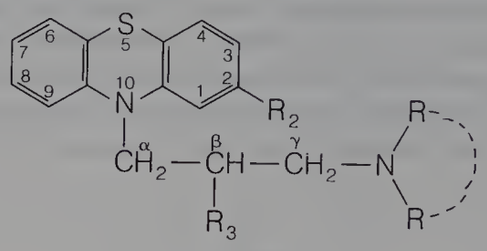
The drugs increase prolactin levels by a block of DA receptors in the hypothalamus and have an antiemetic effect by a block of DA receptors in the chemoreceptor trigger zone.

Postural hypotension, possibly related to peripheral α_1-adrenoreceptor block, is a fairly common side effect, especially among the N,N-dimethylamino group. Skin reactions, including urticaria, contact dermatitis, and photosensitivity, are reported for the phenothiazine group of antipsychotics. Agranulocytosis is always a possibility in patients treated with phenothiazines. If oxidation, rather than conjugation of the 7-hydroxymetabolite, occurred, some potential protein reactive metabolites would be formed.

PHENOTHIAZINES

Many potentially useful phenothiazine derivatives have been synthesized and pharmacologically evaluated. Consequently, there is a large body of information permitting accurate statements about the structural features associated with activity. Many of the features were summarized and interpreted by Gordon et al.[38] The best position for substitution is the 2-position. Activity increases (with some exceptions) as electron-withdrawing ability of the substituent increases.

Another possibly important structural feature in the more potent compounds is the presence of an unshared electron pair on an atom or atoms of the 2-substituent. Substitution at the 3-position can improve activity over nonsubstituted compounds, but not as significantly as substitution at the 2-position. Substitution at position 1 has a deleterious effect on antipsychotic activity, as, to a lesser extent, does substitution at the 4-position.



Phenothiazine Antipsychotic Agents–General Structure

The significance of these substituent effects could be that the hydrogen atom of the protonated amino group of the side chain H-bonds with an electron pair of an atom of the 2-substituent to develop a DA-like arrangement. Horn and Snyder, from x-ray crystallography, proposed that the chlorine-substituted ring of chlorpromazine base could be superimposed on the aromatic ring of dopamine base with the sulfur atom aligned with the *p*-hydroxyl of dopamine and the aliphatic amino groups of the two compounds also aligned.[39] The model used here is based on the interpretation of the SARs by Gordon et al.[38] and on the Horn and Snyder proposal,[39] but involves the protonated species rather than the free base. The effect of the substituent at the 1-position might be to interfere with the side chain's ability to bring the protonated amino group into proximity with the 2-substituent. In the Horn and Snyder scheme,[39] the sulfur atom at position 5 is in a position analogous to the *p*-hydroxyl of dopamine, and it was also assigned a receptor-binding function by Gordon et al.[38] A substituent at position 4 might interfere with receptor binding by the sulfur atom.

The three-atom chain between position 10 and the amino nitrogen is required. Shortening or lengthening the chain at this position drastically decreases activity. The three-atom chain length may be necessary to bring the protonated amino nitrogen into proximity with the 2-substituent.

As expected, branching with large groups, such as phenyl, decreases activity, as does branching with polar groups. Methyl branching on the β-position has a variable effect on activity. More importantly, there is a high separation of antipsychotic potency between *levo*-(the more active) and *dextro*-isomers. This has long been taken to suggest that a precise fit, that is, receptor binding, is involved in the action of these compounds.

Decreases in size from a dimethylamino group, as in going to a monomethylamino, greatly decrease activity, as do effective size increases, such as the one that occurs with *N,N*-diethylamino. Once the fundamental requirement of an *effec-*

tive size of about that equivalent to a dimethylamino is maintained, as in fusing *N,N*-diethyl substituents to generate a pyrrolidino group, activity can be enhanced with increasing chain length, as in N_2-substituted piperizino compounds.

The criticalness of the size about the nitrogen suggests the importance of the amino group (here protonated) for receptor attachment. The effect of the added chain length, once the size requirement is met, could be to add receptor-binding forces. It appears to have been reasonably proved that the protonated species of the phenothiazines can bind to DA receptors.[40]

Metabolism of the phenothiazines is complex in detail, but simple overall. A major route is hydroxylation of the tricyclic system. The usual pattern, for which there are good chemical reasons, is hydroxylation para to the 10-nitrogen atom of the ring other than the ring bearing the electron-attracting substituent at the 2-position. Thus, the major initial metabolite is frequently the 7-hydroxy compound. This compound is further metabolized by conjugation with glucuronic acid, and the conjugate is excreted. Detailed reviews of the metabolites of phenothiazines (as well as SARs and pharmacokinetic factors) are available.[41]

Products

The structures of the phenothiazine derivatives described in the following section are given in Table 14-3.

Chlorpromazine Hydrochloride, USP. 2-Chloro-10-[3-(dimethylamino)propyl]phenothiazine monohydrochloride (Thorazine). Chlorpromazine is the earliest phenothiazine compound introduced into therapy. It is still useful as an antipsychotic. Other uses are in nausea and vomiting and hiccough. In activity comparisons, it is the reference compound, that is, the compound to which others are compared. The drug has significant sedative and hypotensive properties, possibly reflective of central and peripheral α_1-noradrenergic-blocking activity, respectively. Effects of peripheral anticholinergic activity are common. As with the other phenothiazines, the effects of other CNS depressant drugs, such as sedatives and anesthetics, can be potentiated.

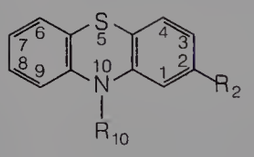
Trifluprom azine Hydrochloride, USP. 10-[3-(Dimethylamino)propyl]-2-(trifluoromethyl)phenothiazine monohydrochloride (Vesprin). Trifluprom azine has decreased sedative and hypotensive effects relative to chlorpromazine and a greater milligram potency as an antipsychotic. EPS are higher. The 2-CF_3 versus the 2-Cl is associated with these changes. Overall, the drug has uses analogous to those of chlorpromazine.

Thioridazine Hydrochloride, USP. 10-[2-(1-Methyl-2-piperidyl)ethyl]-2-(methylthio)phenothiazine monohydrochloride (Mellaril). Thioridazine is a member of the piperidine subgroup of the phenothiazines. The drug has a relatively low tendency to produce EPS. The drug has high anticholinergic activity, and this activity in the striatum, counterbalancing a striatal DA block, has been said to be responsible for the low EPS. The drug has sedative and hypotensive activity in common with chlorpromazine. Antiemetic activity is decreased. At high doses, pigmentary retinopathy has been observed. A metabolite of the drug is mesoridazine (discussed next).

Mesoridazine Besylate, USP. 10-[2-(Methyl-2-piperidyl)ethyl]-2-(methylsulfinyl)phenothiazine monobenzenesulfonate (Serentil). This drug shares many properties with thioridazine. Pigmentary retinopathy, however, has not been reported for the drug.

TABLE 14-3

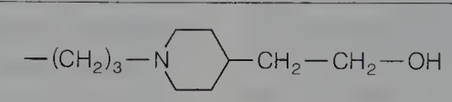
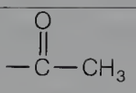
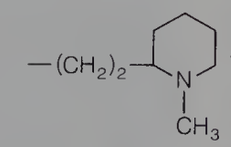
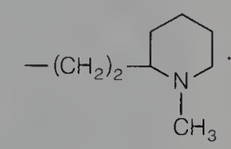
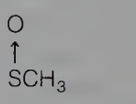
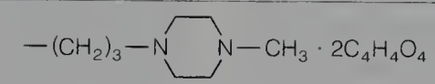
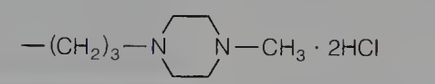
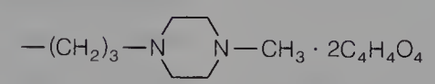
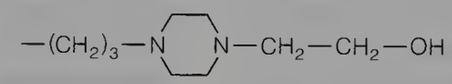
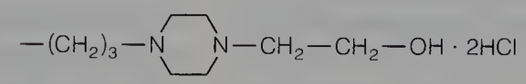
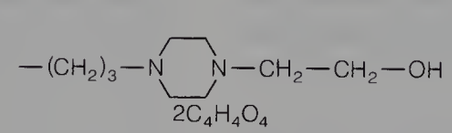
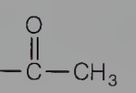
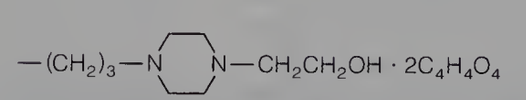
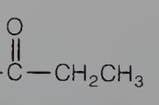
PHENOTHIAZINE DERIVATIVES (AMINOPROPYL SIDE CHAIN)



Generic Name Proprietary Name	R_{10}	R_2
PROPYL DIALKYLAMINO SIDE CHAIN		
Promazine hydrochloride USP *Sparine*	$-(CH_2)_3N(CH_3)_2 \cdot HCl$	H
Chlorpromazine hydrochloride USP *Thorazine*	$-(CH_2)_3N(CH_3)_2 \cdot HCl$	Cl
Trifluprom azine hydrochloride USP *Vesprin*	$-(CH_2)_3N(CH_3)_2 \cdot HCl$	CF_3

TABLE 14-3 *Continued*

PHENOTHIAZINE DERIVATIVES (AMINOPROPYL SIDE CHAIN)

Generic Name Proprietary Name	R_{10}	R_2
ALKYL PIPERIDYL SIDE CHAIN		
Piperacetazine USP *Quide*		
Thioridazine hydrochloride USP *Mellaril*		SCH_3
Mesoridazine besylate USP *Serentil*		
PROPYL PIPERAZINE SIDE CHAIN		
Prochlorperazine maleate USP *Compazine*		Cl
Trifluoperazine hydrochloride USP *Stelazine*		CF_3
Thiethylperazine maleate USP *Torecan*		SCH_2CH_3
Perphenazine USP *Trilafon*		Cl
Fluphenazine hydrochloride USP *Permitil, Prolixin*		CF_3
Acetophenazine maleate USP *Tindal*		
Carphenazine maleate USP *Proketazine*		

Piperacetazine, USP. 10-[3-[4-(2-Hydroxyethyl)piperidino]propyl]phenothiazin-2-yl methyl ketone; 2-acetyl-10-{-3-[4-(β-hydroxyethyl)piperidino]propyl}phenothiazine (Quide). Piperacetazine has a piperidino moiety in common with thioridazine and mesoridazine. However, the molecular arrangement resembles the substituted piperazino compounds. Consequently, overall pharmacologic properties lie between the piperidine group and the piperazine group.

Prochlorperazine Maleate, USP. 2-Chloro-10-[3-(4-methyl-1-piperazinyl)propyl]phenothiazine maleate (Compazine). The piperazine subgroup of the phenothiazines is characterized by high milligram antipsychotic potency, a high prevalence of EPS, and low sedative and autonomic effects. Prochlorperazine is more potent on a milligram basis than its alkylamino counterpart, chlorpromazine. However, because of its high prevalence of EPS, it is used mainly for its antiemetic effect, rather than for its antipsychotic effect.

Trifluoperazine Hydrochloride, USP. (10-[3-(4-Methyl-1-piperazinyl)propyl]-2-trifluoromethyl)phenothiazine di hydrochloride (Stelazine). Because it has both 2-trifluoro-

methyl and piperazine groups, trifluoperazine is a potent antipsychotic agent. EPS are high, and sedative and hypotensive effects are low.

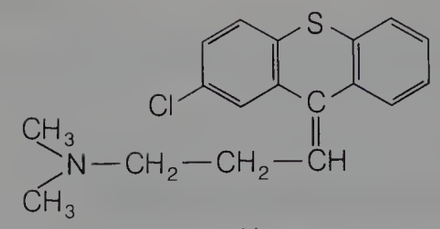
Other agents in the piperazine group that reportedly have overall pharmacologic profiles roughly similar to trifluoperazine are **Acetophenazine Maleate, USP,** 10-[3-[4-(2-Hydroxyethyl)-1-piperazinyl]propyl]phenothiazine-2-yl methyl ketone maleate (1:2) (Tindal); **Carphenazine Maleate, USP,** 1-[10-[3-[4-(2-Hydroxyethyl)-1-piperazinyl]propyl]-phenothiazin-2-yl]-1-propanone maleate (1:2) (Proketazine); and **Perphenazine, USP,** 4-[3-(2-Chlorophenothiazine-10-yl) prophyl]-piperazineethanol; 2-chloro-10-[3-[4-(2-hydroxyethyl)piperazinyl]propyl]phenothiazine (Trilafon).

Finally, the member of the piperazine subgroup with a trifluoromethyl group at the 2-position of the phenothiazine system and the most potent phenothiazine on a milligram basis is **Fluphenazine Hydrochloride, USP,** 4-[3-[2-(Trifluoromethyl)phenazin-10-yl] propyl]-1-piperazineethanol dihydrochloride; 10-[3-[4-(2-hydroxyethyl)piperazinyl]propyl]-2-trifluoromethylphenothiazine dihydrochloride (Permitil, Prolixin). It is available as two lipid-soluble esters for depot intramuscular injection, the enanthate (heptanoic acid ester) and the decanoate ester. These long-acting preparations can be crucial in treating psychotic patients who do not take their medication or who are subject to frequent relapse.

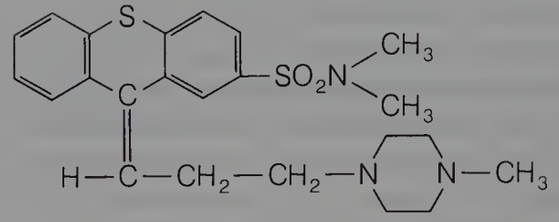
RING ANALOGUES OF PHENOTHIAZINES: THIOXANTHINES, DIBENZOXAZEPINES, AND DIBENZODIAZEPINES

This is a group of close structural relatives of the phenothiazine antipsychotics. They share many clinical properties with the phenothiazines. However, the dibenzodiazepine clozapine has some important differences, notably a low production of EPS.

Chlorprothixene, USP. 3-(2-Chloro-9*H*-thioxanthen-9-ylidene)-*N*, *N*-dimethyl-1-propanamine; 2-chloro-9-(3′-dimethylaminopropylidene)thioxanthene (Taractan) and **Thiothixene, USP,** *Z-N, N*-dimethyl-9-[3-(4-methyl-1-piperazinyl)propylidene]thioxanthene-2-sulfonamide (Navane). The thioxanthene system differs from the phenothiazine system by replacement of the N-H moiety with a carbon atom doubly bonded to the propylidene side chain. With the substituent in the 2-position, *Z*- and *E*-isomers are produced. In accordance with the concept that the presently useful antipsychotics can be superimposed on DA, the *Z*-isomers are the more active antipsychotic isomers. The compounds are very similar in pharmacologic properties to the corresponding phenothiazines. Thus, chlorprothixene displays properties similar to chlorpromazine, and thiothixene displays properties similar to the piperazine subgroup of the phenothiazines.

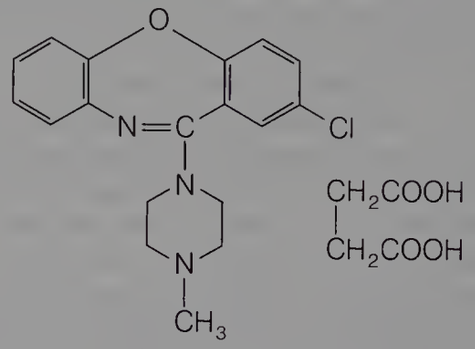


Chlorprothixene



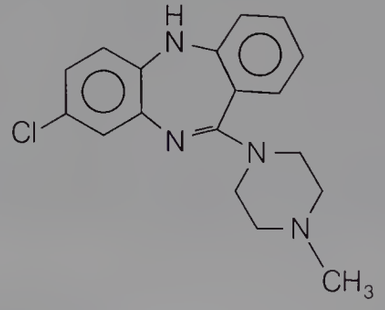
Thiothixene

A dibenzoxazepine derivative in use is **Loxapine Succinate,** 2-Chloro-11-(4-methyl-1-piperazinyl)dibenz[*b*, *f*][1,4]oxazepine succinate (Daxolin). The structural relationship to the phenothiazine antipsychotics is apparent. It is an effective antipsychotic and has side effects similar to those reported for the phenothiazines.



Loxapine Succinate

The dibenzodiazepine derivative is **Clozapine.** It is not a potent antipsychotic on a milligram basis (note the "wrong" orientation of the *N*-methyl piperazino group relative to the chlorine atom), but it is effective and has received much attention because the production of EPS is low. It has been used extensively in Europe. It has been introduced (Clozaril) into the United States, but with restrictions, because of a high frequency of agranulocytosis associated with its use.



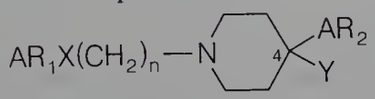
Clozapine

Several theories to account for the low EPS have been proposed. One is that it effectively blocks striatal cholinergic receptors, thus balancing out a striatal D-2 block.[42] It has also been proposed that it blocks limbic receptors more so

than striatal D-2 receptors.[43,44] The compound has usefulness as a model for the development of other compounds that have low EPS potential.

FLUOROBUTYROPHENONES

The fluorobutyrophenones belong to a much studied class of compounds, with many compounds possessing high antipsychotic activity. Only a few of these are used in the United States, which can be misleading about the importance of the group and its evolved relatives. The structural requirements for antipsychotic activity in the group are well worked out.[45] General features are expressed in the following structure.

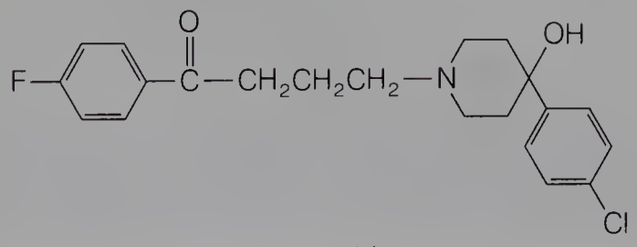


Optimal activity is seen when AR_1 is an aromatic system. A *p*-fluoro substituent aids activity. When X = C=O, optimal activity is seen, although other groups, C(H)OH and C(H)aryl, also give good activity. When n = 3, activity is optimal: longer or shorter chains decrease activity. The aliphatic amino nitrogen is required, and highest activity is seen when it is incorporated into a cyclic form. AR_2 is an aromatic ring and is needed. It should be attached directly to the 4-position or occasionally separated from it by one intervening atom. The Y group can vary and assist activity. An example is the hydroxyl group of haloperidol.

The empirical SARs could be construed to suggest that the 4-aryl piperidino moiety is superimposable on the 2-phenylethylamino moiety of dopamine and, accordingly, could promote affinity for D-2 receptors. The long *N*-alkyl substituent could help promote affinity and produce antagonistic activity.

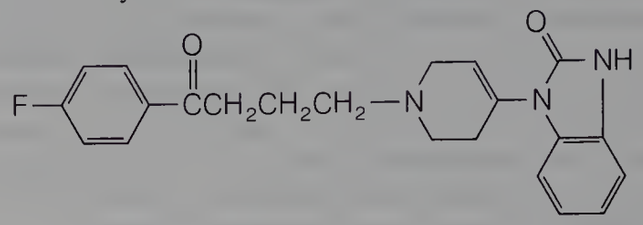
Some members of the class are extremely potent antipsychotic agents and D-2 receptor antagonists. The EPS are extremely marked in some members of this class, which may, in part, be due to a potent DA block in the striatum and almost no compensatory striatal anticholinergic block. Most of the compounds do not have the structural features associated with effective anticholinergic activity.

Haloperidol, USP. 4[4-(*p*-Chlorophenyl)-4-hydroxypiperidino]-4-n′-fluorobutyrophenone (Haldol). The compound is a potent antipsychotic useful in schizophrenia and in psychoses associated with brain damage. It is often chosen as the agent to terminate mania. Therapy for Gilles de la Tourette's syndrome often employs the drug.



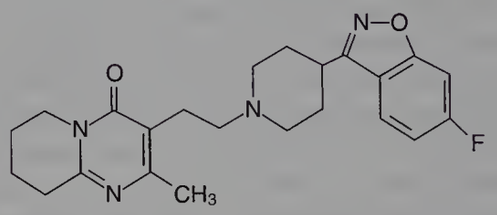
Haloperidol

Droperidol, USP. 1-{1-[3-(*p*-Fluorobenzoyl)propyl]-1,2,3,6-tetrahydro-4-pyridyl}-2-benzimidazolinone (Inapsine). The agent may be used alone as a preanesthetic neuroleptic or as an antiemetic. Its most frequent use is in combination (Innovar) with the narcotic agent fentanyl (Sublimaze) preanesthetically.



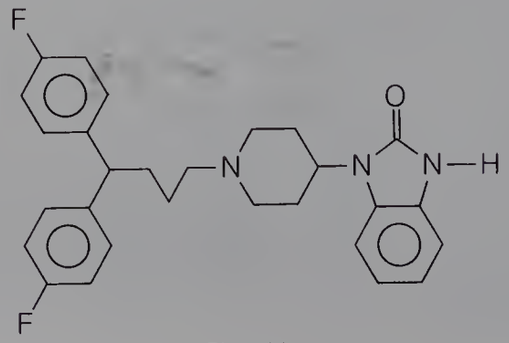
Droperidol

Risoperidone. (Risperdal).

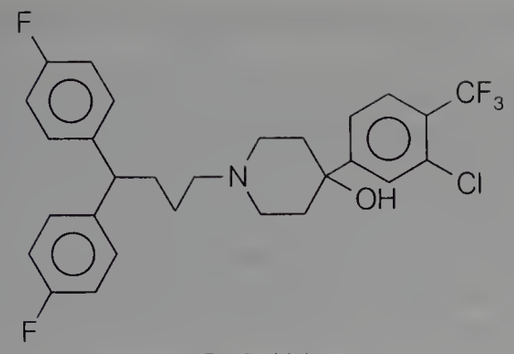


Risoperidone (Risperdal) is a structural hybrid of a butyrophenone antipsychotic and a trazodone-like antidepressant. It benefited refractory psychotic patients, with Parkinsonism controlled at one-tenth the dose of antiparkinsonian drugs used with haloperidol.[46] Coexisting anxiety and depressive syndromes were also benefited. It is reported to benefit the negative (e.g., withdrawal, apathy) as well as the positive (e.g., delusions, hallucinations) symptoms of schizophrenia. This is reported to be a consequence of the compound's combination $5HT_2$-D_2 receptor antagonistic (antidepressant and antipsychotic) properties.[47]

The diphenylbutylpiperidine class can be considered as a modification of the fluorobutyrophenone class. Because of their high hydrophobic character, the compounds are inherently long acting. Penfluridol has undergone clinical trials in the United States, and pimozide has been approved for antipsychotic use. Overall, side effects for the two compounds resemble those produced by the fluorobutyrophenones.



Pimozide

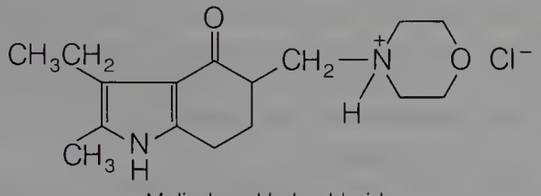


Penfluridol

β-AMINOKETONES

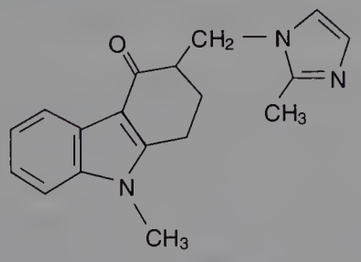
Several of these agents have been examined.[41] They evolved out of research on lobeline. The overall structural features associated with activity can be seen in the structure of the compound, molindone. In addition to the β-aminoketone, there must be an aryl group positioned as in molindone. It might be conjectured that the proton on the protonated amino group in these compounds H-bonds with the electrons of the carbonyl oxygen atom. This would produce a cationic center, two-atom distance, and an aryl group that could be superimposed on the analogous features of protonated dopamine.

Molindone Hydrochloride. 3-Ethyl-6,7-dihydro-2-methyl-5-morpholinomethyl)indole-4(5*H*)-one monohydrochloride (Moban). The compound is about as potent an antipsychotic as trifluoperazine. Overall, side effects resemble those of the phenothiazines. Hypotension may not be a problem, and the compound is said to produce less weight gain than other antipsychotics.



Molindone Hydrochloride

Ondansetron. (Zophran).

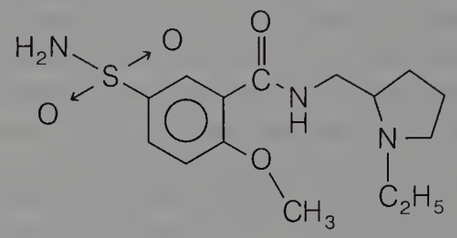


Ondansetron is primarily used for the nausea and vomiting of cancer chemotherapy. Reportedly, it is an antagonist of serotonin at $5HT_3$ receptors.[48] It is being tried as an antipsychotic and anxiolytic. From the structure, dopamine receptor blocking properties can be anticipated.

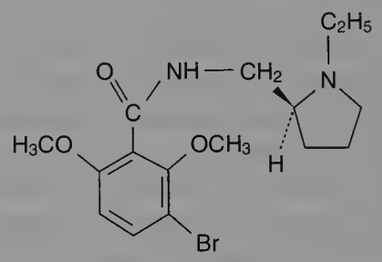
BENZAMIDES

The benzamides evolved from observations that the gastroprokinetic and antiemetic agent metoclopramide has antipsychotic activity apparently related to D-2 receptor block. It is hoped that the group might yield compounds with diminished EPS liability. It is said that an H-bond between the amido H and the unshared electrons of the methoxy group, to generate a pseudo ring, is important for antipsychotic activity in these compounds. Presumably, when the protonated amine is superimposed on that of protonated dopamine, this pseudo ring would superimpose on dopamine's aromatic ring.[49] These features can be visualized in sulperide and remoxipride.

Sulperide.



Remoxipride.



Remoxipride exemplifies ongoing research in the benzamide group of antipsychotics. It is a selective D_2 receptor blocker.[46] It is said to be as effective as haloperidol with fewer extrapyramidal side effects.[48]

Apomorphine at selected doses, can act principally as a presynaptic DA agonist rather than a postsynaptic agonist and has served to stimulate interest in presynaptic agonists as antipsychotic agents. Another approach to the DA system could be made through agonists of cholecystokinin.[50] It is now thought that agonists acting at central CCK-B receptors are more relevant than central CCK-A agonists.[51] Finally, although the block of dopamine receptors works well to explain the action of currently useful antipsychotic drugs, many of the agents have effects on other systems, including noradrenergic and serotoninergic systems. These effects have also been reported to be involved in antipsychotic actions. An approach to antipsychotics could be made through the glutaminergic (e.g., NMDA receptors) system.

ANTIMANIC AGENTS

Lithium Salts

The lithium salts used in the United States are the carbonate and the citrate. Lithium chloride is not employed because

of its hygroscopic nature and because it is more irritating than the carbonate or citrate to the gastrointestinal tract.

The active species in these salts is the lithium ion. The classic explanation for its antimanic activity is that it resembles the sodium ion (as well as potassium, magnesium, and calcium ions) and can occupy the sodium pump. However, it cannot, unlike the sodium ion, maintain membrane potentials. Accordingly, it might prevent excessive release of neurotransmitters (e.g., dopamine) that characterize the manic state. Many of the actions of lithium ion have been reviewed.[52] The indications for lithium salts are acute mania (often with a potent neuroleptic agent to immediately control the mania because lithium is slow to take effect), and as a prophylactic to prevent occurrence of the mania of bipolar manic-depressive illness. Lithium salts are also being used in severe recurrent unipolar depressions. Present explanations for the dual nature (antimanic and antidepressant) of lithium revolve around its ability to act on G-proteins and the second messenger, inositol triphosphate.

Because of its water solubility, the lithium ion is extensively distributed in body water. It tends to become involved in the many physiologic processes involving sodium and potassium ions, hence, the side effects and potential drug interactions are many. The margin of safety is low; therefore lithium should be used only when plasma levels can be routinely determined. In the desired dose range, side effects can be adequately controlled.

Because of the toxicity of lithium, there is substantial interest in design of safer compounds. As more and more is learned about lithium's specific actions, likelihood of successful design of compounds designed to act on specific targets is increased.[51]

Lithium Carbonate, USP. (Eskalith, Lithane). **Lithium Citrate.** (Cibalith-S). The carbohydrate and the citrate are the salts commercially available in the U.S.

ANTICONVULSANT OR ANTIEPILEPTIC DRUGS

As is customary, these two terms, ''anticonvulsant'' and ''antiepileptic,'' will be used interchangeably in this discussion. Strictly speaking, however, the term ''anticonvulsant'' designates an agent that blocks experimentally produced seizures in laboratory animals, and an antiepileptic drug is a drug used medically to control the epilepsies, not all of which are convulsive, in humans.

A classification of the epilepsies has been widely accepted because its accuracy facilitates diagnosis, drug selection, and a precise discussion of the epilepsies.[53,54] The major classification types are (a) generalized seizures, which essentially involve the entire brain and do not have an apparent local onset; (b) unilateral seizures (involve one entire side of the body); (c) partial (or focal) seizures that have a focus (i.e., begin locally); (d) erratic seizures of the newborn; and (e) unclassified seizures (severe seizures associated with a high mortality such that time does not permit a precise categorization).

Two major types of generalized seizures are the generalized tonic-clonic seizure (grand mal) and the nonconvulsive seizures or absence (petit mal) seizures. The typical generalized tonic-clonic seizure is often preceded by a series of bilateral muscular jerks; this is followed by loss of consciousness, which, in turn, is followed by a series of tonic and then clonic spasms. The typical absence seizure (classic petit mal) consists of a sudden brief loss of consciousness, sometimes with no motor activity, although there is often some minor clonic motor activity.

Major types of focal (partial) epilepsies are simple focal and complex focal seizures. A prototype simple partial seizure is jacksonian motor epilepsy in which the jacksonian march may be seen. As the abnormal discharge proceeds over the cortical site involved, the visible seizure progresses over the area of the body controlled by the cortical site. The complex partial seizure is represented by the psychomotor or temporal lobe seizure. There is an aura, then a confused or bizarre but purposefully appearing behavior lasting 2 to 3 min, often with no memory of the event. The seizure may be misdiagnosed as a psychotic episode.

For the purposes of broad considerations of how structure relates to antiepileptic activity, further condensation of the classification of the epilepsies is traditionally made (generalized tonic-clonic seizures, simple partial seizures, complex partial seizures, and absence seizures). The broad general pattern of structural features associated with antigeneralized tonic-clonic seizure activity is discernible for barbiturates, hydantoins, oxazolidine-diones, and succinimides. This SAR also applies to simple partial seizures. It applies with less certainty to complex partial seizures, which are relatively resistant to treatment. With fewer effective drug entities, overall structural conclusions are more tenuous. The other general seizure type for which a broad SAR pattern among the cited compounds can be seen is the absence seizure. These features are cited under the heading ''SARs Among Anticonvulsants.''

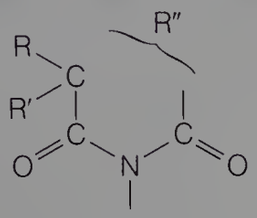
Likewise, animal models characteristically discern three types of activity: activity against electrically induced convulsions correlates with activity against generalized tonic-clonic and partial seizures, and activity against pentylenetetrazole (PTZ)–induced seizures correlates with antiabsence activity.

Each of the epilepsies is characterized by a typical abnormal pattern in the EEG. The EEG indicates that there is sudden and excessive electrical activity in the brain. The precise causes of these sudden and excessive electrical discharges may be many, and not all are understood. However, a working hypothesis is that there is a site or focus of damaged or abnormal, and consequently hyperexcitable, neurons in the brain. These can fire excessively and sometimes recruit adjacent neurons that, in turn, induce other neurons to fire. The location and extent of the abnormal firing determines the epilepsy. A refinement in this theory is the kindling model.[55] Experimentally, a brief and very localized

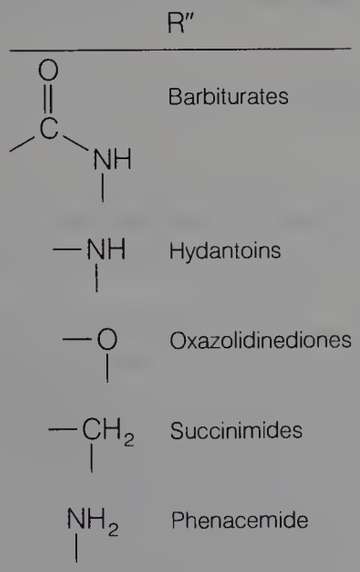
electrical stimulus is applied to a site in the brain, with long intervals between applications. As the process is repeated, neuronal afterdischarges grow both longer and more intense at the original site and at new sites far from the original site. It is thought that changes occur in neurons at the discharge site and these neurons, in turn, induce changes in neurons far from the site. Progressively more severe seizures can be induced, and these can arise from secondary foci that have been kindled far from the site of stimulation.

SARS AMONG ANTICONVULSANTS

Several major groups of drugs have the common structure shown below:



Structure common to anticonvulsant drugs.



An overall pattern in the foregoing is that R and R′ should both be hydrocarbon radicals. If both R and R′ are lower alkyls, the tendency is to be active against absence seizures (petit mal) and not active against generalized tonic-clonic (grand mal) or partial seizures. If one of the hydrocarbon substituents is an aryl group, activity tends to be directed toward generalized tonic-clonic and partial seizures, and not toward antiabsence activity.[56]

A conformational analysis of the aryl-containing antigeneralized tonic-clonic agents indicates that the conformational arrangement of the hydrophobic groups is important.[57]

BARBITURATES

Although sedative-hypnotic barbiturates commonly display anticonvulsant properties, only phenobarbital and mephobarbital (and, marginally, metharbital) display adequate anticonvulsant selectivity for use as antiepileptics.

For the structures of these agents, consult Table 14-2, and for discussion of chemical properties see the section on barbiturates under sedative-hypnotic-anxiolytic agents. The metabolism of phenobarbital involves *p*-hydroxylation, followed by conjugation.

Mephobarbital is extensively *N*-demethylated in vivo and is thought to owe most of its activity to the metabolite phenobarbital. In keeping with their structures, both agents are effective against generalized tonic-clonic and partial seizures. Metharbital has been said to act more as a sedative than as a specific antigeneralized tonic-clonic agent.

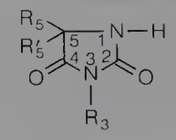
HYDANTOINS

The hydantoins are close structural relatives of the barbiturates, differing in lacking the 6-oxo moiety. They are cyclic monoacylureas rather than cyclic diacylureas. Consequently, they are weaker organic acids than the barbiturates (e.g., phenytoin pK_a = 8.3). Thus, aqueous solutions of sodium salts, such as of phenytoin sodium, generate strongly alkaline solutions.

The compounds have a trophism toward antigeneralized tonic-clonic rather than antiabsence activity. This is not an intrinsic activity of the hydantoin ring system. All of the clinically useful compounds (Table 14-4) possess an aryl substituent on the 5-position, corresponding to a branched atom of the general pharmacophore. Hydantoins with lower

TABLE 14-4

THE ANTICONVULSANT HYDANTOIN DERIVATIVES



Generic Name Proprietary Name	*Substituents* R_5	R'_5	R_3
Phenylethylhydantoin *Nirvanol*		$CH_3—CH_2—$	H
Phenytoin USP *Dilantin, Diphentoin*			H
Mephenytoin USP *Mesantoin*		$CH_3—CH_2—$	$CH_3—$
Ethotoin *Peganone*		H	$CH_3—CH_2—$

alkyl substituents have been reported to have antiabsence activity.

Phenytoin and Phenytoin Sodium, USP. 5,5-Diphenylhydantoin (Dilantin). Phenytoin is the first anticonvulsant in which it was clearly demonstrated that anticonvulsant activity could definitely be separated from sedative-hypnotic activity. It is often cited as the prime example of an anticonvulsant acting as a sodium channel blocker.[13,58] An effect of neuronal sodium channel block is to decrease glutamic acid release, giving anticonvulsant activity.[58,59] Another consequence is to reduce glutamate induced ischemia damage to neurons.[58,59] The drug is useful against all seizure types except absence. It is sometimes noted that the drug is incompletely or erratically absorbed from sites of administration. This is due to the very low water solubility of the drug.

Metabolism proceeds by *p*-hydroxylation of an aromatic ring, followed by conjugation.

Mephenytoin, USP. 5-Ethyl-3-methyl-5-phenyl-hydantoin (Mesantoin). Mephenytoin is metabolically *N*-dealkylated to 5-ethyl-5-phenylhydantoin, believed to be the active agent. Interestingly, 5-ethyl-5-phenylhydantoin, the hydantoin counterpart of phenobarbital, as one of the first hydantoins introduced into therapy. It was introduced as a sedative-hypnotic and anticonvulsant under the name Nirvanol, but was withdrawn because of toxicity. Presumably, mephenytoin may be considered a pro-drug, which ameliorates some of the toxicity—serious skin and blood disorders—of the delivered active drug.

Metabolic inactivation of mephenytoin and its demethyl metabolite is by, as expected, *p*-hydroxylation and conjugation of the hydroxyl group. The drug has a spectrum of activity similar to phenytoin. It may worsen absence seizures.

Ethotoin. 3-Ethyl-5-phenylhydantoin (Peganone). The compound is *N*-dealkylated and *p*-hydroxylated; the *N*-dealkyl metabolite, presumably the active compound, is likewise metabolized by *p*-hydroxylation. The hydroxyl group is then conjugated.

The compound is employed against generalized seizures, but usually on an adjunctive basis owing to its low potency. In general, agents that are not completely branched on the appropriate carbon are of lower potency than their more completely branched counterparts.

OXAZOLIDINEDIONES

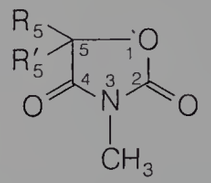
Replacement of the N-H group at position 1 of the hydantoin system with an oxygen atom yields the oxazolidine-2,4-dione system.

The oxazolidinedione system is sometimes equated with antiabsence activity, but this trophism probably is more dictated by the fact that the branched atom of these compounds is substituted with lower alkyls. Aryl-substituted oxazolidine-2,4-diones have shown activity against generalized tonic-clonic seizures.

Trimethadione, USP. 3,5,5,-Trimethyl-2,4-oxazolidinedione; 3,5,5-trimethadione (Tridione). Trimethadione was the first drug introduced specifically for treating absence seizures. It is important as a prototype structure for antiabsence compounds. Dermatologic and hematologic toxicities limit its clinical use.

The drug is metabolized by *N*-demethylation to the putative active metabolite dimethadione.[60] Dimethadione is a water-soluble and lowly lipophilic compound and, thus, is excreted as such without further metabolism.

Paramethadione, USP. 5-Ethyl-3,5-dimethyl-2,4,-oxazolidinedione (Paradione). Paramethadione is very closely related to trimethadione in structure and has similar actions, uses, and side effects, although it may be safer. The *N*-demethyl metabolite, which is excreted rather slowly, is thought to be the active drug.

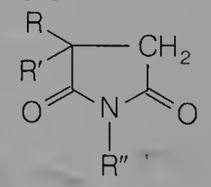


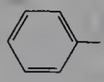
Trimethadione $R_5 = R'_5 = CH_3$
Paramethadione $R_5 = CH_3$; $R'_5 = C_2H_5$

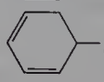
SUCCINIMIDES

In view of the activity of antiepileptic agents such as the oxazolidine-2,4-diones, substituted succinimides (CH_2 replaces O) were a logical choice for synthesis and evaluation. Three are now in clinical use.

Phensuximide, USP. *N*-Methyl-2-phenylsuccinimide (Milontin). Some trophism toward antiabsence activity is produced by the succinimide system. The —CH_2— could be viewed as an α-alkyl branch condensed into the ring. Phensuximide is used primarily against absence seizures, but it is of low potency and is relegated to a secondary status. The phenyl substituent confers some activity against generalized tonic-clonic and partial seizures. *N*-Demethylation occurs to yield the putative active metabolite. Both phensuximide and the *N*-demethyl metabolite are inactivated by *p*-hydroxylation and conjugation.



Phensuximide R =  , R′ = H, R″ = CH_3

Methsuximide R =  , R′ = CH_3, R″ = CH_3

Ethosuximide R = C_2H_5—, R′ = CH_3, R″ = H

Methsuximide. *N*,2-Dimethyl-2-phenylsuccinimide (Celontin). *N*-demethylation and *p*-hydroxylation of parent

and metabolite occur. The drug has some use against absence and complex partial seizures.

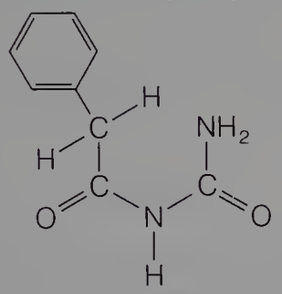
Ethosuximide, USP. 2-Ethyl-2-methylsuccinimide (Zarontin). Ethosuximide conforms very well to the general structural pattern for antiabsence activity. The drug is more active and less toxic than trimethadione; consequently, it has emerged as a drug of choice for typical absence seizures. Toxicity primarily involves the skin and blood.

Some of the drug is excreted intact. The major metabolite is produced by oxidation of the ethyl group.

UREAS AND MONOACYLUREAS

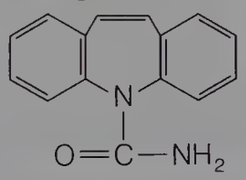
The two chemical classes, ureas and monoacylureas, have a long history of producing compounds with anticonvulsant activity. However, the numerical yield of clinically useful compounds has not been great.

Phenacemide, USP. Phenylacetylurea (Phenurone). Phenacemide, a rather broad-spectrum agent, finds some use in psychomotor epilepsy. Its principal problems are severe side effects, including personality changes and blood, renal, and skin disorders. Metabolism is by *p*-hydroxylation.



Phenacemide

Carbamazepine, USP. 5*H*-Dibenz[b,f]lazepine-5-carboxamide (Tegretol). For SAR discussion purposes, carbamazepine can be viewed either as an ethylene-bridged 1,1-diphenylurea or an amido-substituted tricyclic system. Either view fits a very general activity pattern for anticonvulsants, namely, a hydrophobic moiety joined to a rather simple nonionic polar H-bonding group. The two phenyls on the nitrogen fit the pattern of antigeneralized



Carbamazepine

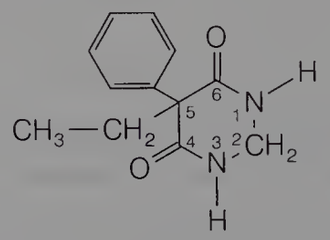
tonic-clonic activity. Carbamazepine is useful (a drug of choice) in generalized tonic-clonic and partial seizures.

The drug has the potential for serious hematologic toxicity, and it is used with caution.

Metabolism proceeds largely through the epoxide formed at the *cis*-stilbene double bond. In humans the epoxide reportedly is converted largely to the 10S, 11S *trans*-diol.[61] The epoxide is a suspect in the idiosyncratic reactions carbamazepine may produce, e.g., aplastic anemia. With this in mind, compounds designed to avoid the epoxide such as oxcarbazepine (Z-stilbene double bond reduced to $—CH_2—CH_2—$, then one CH_2 converted to the ketone) are in development.[48]

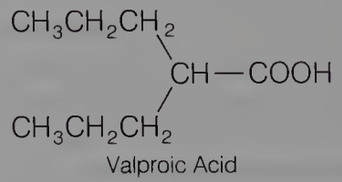
MISCELLANEOUS AGENTS

Primidone. 5-Ethyldihydro-5-phenyl-4,6-(1*H*,5*H*)-pyrimidinedione (Mysoline). Primidone is sometimes described as a 2-deoxybarbiturate. It appears to act as such, and through conversion to phenobarbital and to phenylethylmalonyldiamide (PEMA).[62] The efficacy is against all types of seizures except absence. The agent has good overall safety, but rare serious toxic effects do occur.



Primidone

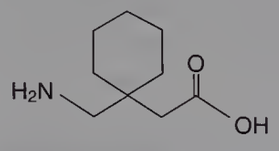
Valproic Acid. 2-Propylpentanoic acid (Depakene). Many carboxylic acids have anticonvulsant activity, although often of a low order of potency, possibly in part because extensive dissociation at physiologic pH produces poor partitioning across the blood-brain barrier. Valproic acid has good potency and is used against several seizure types. It is a drug of choice for typical and atypical absence seizures and in absence seizure with generalized tonic-clonic seizure. Metabolism is by conjugation of the carboxylic acid



Valproic Acid

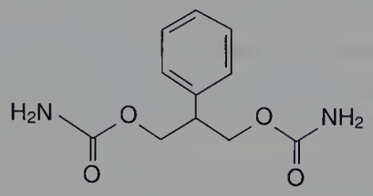
group and oxidation of one of the hydrocarbon chains. Many of the side effects are mild. However, a rare, but potentially fatal, fulminant hepatitis has caused concern.

Gabapentin. (Neurontin).



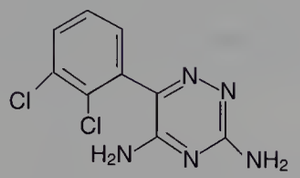
Despite the fact that gabapentin is a relative of GABA with increased hydrophobic character its mechanism of action appears not to involve an interaction with $GABA_A$ receptors. It may involve the L-system amino acid transporter protein. It is said to cross the blood-brain barrier well and to have a good pharmacokinetic profile. It was introduced for adjunctive therapy of refractory partial seizures and secondarily generalized tonic-clonic seizures. It is being studied as single-drug therapy for various seizures.[47]

Felbamate. (Felbatol).



Felbamate has been used successfully in refractory patients with generalized tonic-clonic seizures and complex partial seizures. The mechanism of action may involve an interaction with the strychnine insensitive receptor on the NMDA receptor.[47]

Lamotrigine. (Lamictal).



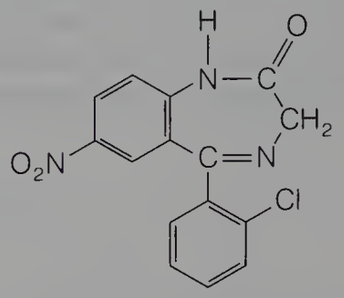
Lamotrigine has been found effective against refractory partial seizures.[48] Described as a glutamate antagonist, it is said to act by modulating sodium channels and preventing glutamate release.[58] Compounds of its type tend to reduce neuronal cell death in ischemia. Another sodium channel modulator and anticonvulsant, riluzole (2-amino-6-(trifluoroethoxy)benzothiazole) slowed the progression of amyotrophic lateral sclerosis (ALS). However, initial studies with lamotrigine did not detect a slowing of the disease's progression.[58]

The development of sodium channel modulators that inhibit glutamate release affords the opportunity to study anticonvulsant action arising from inhibiting metabotropic glutamate receptors. Until the present, investigations have centered on blocking at ionotropic (regulate ion flux, e.g., NMDA receptors) receptors. Metabotropic receptors gain access to the metabolism of the cell by activating a G-protein within the cell (rather than by activating ion flux). Eight subtypes of glutamate metabotropic receptors ($mGluR_{1-8}$) have so far been discovered. They modulate excitatory synaptic transmission by a variety of mechanisms, including modulation of K^+ and Ca^{2+} channels, enhancement of ionotropic glutamate receptor currents, and presynaptic inhibition of glutamate release. Drugs that suppress overactivity of mGluR are anticipated to yield drugs active against epilepsy (concisely, epilepsy has been said to be caused by excessive glutaminergic activity or to little GABAergic activity), cerebral ischemia, chronic pain, neurodegenerative diseases, and a variety of psychiatric disorders, including psychoses and anxiety disorders.[59]

BENZODIAZEPINES

For details of the chemistry and SARs for the benzodiazepines, see the discussion for anxiolytic-sedative-hypnotic drugs. The structural features associated with anticonvulsant activity are identical with those associated with anxiolytic-sedative-hypnotic activity.[22] Animal models predict benzodiazepines to be modestly effective against generalized tonic-clonic and partial seizures and very highly active against absence seizures. This difference in seizure control trophism is markedly different from the barbiturates, hydantoins, and most other chemical compounds when they are aryl- or diaryl-substituted. Despite the high effectiveness of benzodiazepines as a group in animal models, only three benzodiazepines have achieved established positions in anticonvulsant therapy.

Clonazepam, USP. 5-(2-Chlorophenyl)-3-dihydro-7-nitro-2*H*-1,4-benzodiazepin-2-one (Klonopin). Clonazepam, a partial agonist at benzodiazepine allosteric binding sites on $GABA_A$ receptors, is useful in absence seizures and in myoclonic seizures. Development of tolerance to the anticonvulsant effect often develops. This is a common problem with the benzodiazepines. Metabolism involves hydroxylation of the 3-position followed by glucuronidation and nitro group reduction, followed by acetylation.



Clonazepam

Diazepam. Valium. For details on the chemical entity, see its discussion under sedative-hypnotic-anxiolytics. The drug is mainly useful in treating generalized tonic-clonic status epilepticus, which is an ongoing and potentially fatal generalized tonic-clonic seizure.

Chlorazapate. Tranxene. See the detailed discussion of this agent in the sedative-hypnotic-anxiolytic section. Its principal anticonvulsant use is adjunctively in complex partial seizures.

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CHAPTER **15**

Central Nervous System Stimulants

Eugene I. Isaacson

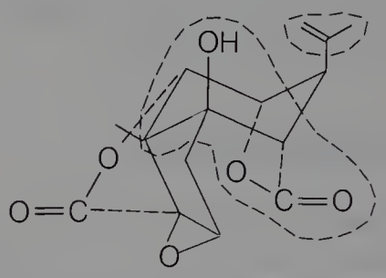
This chapter discusses a broad range of agents that produce stimulation of the central nervous system (CNS). The *analeptics* are a group of agents with a very limited range of use (as respiratory stimulants) because of the general nature of their effects. The *methylxanthines* have interesting stimulatory properties, and caffeine is much used informally as a cortical stimulant. The *central sympathomimetic agents* amphetamine and its close relatives have alerting and antidepressant properties but are now used more often as anorexiants. The *antidepressant drugs* are employed most frequently in serious depressive disorders and are broadly groupable into the monoamine oxidase inhibitors (MAOIs) and the tricyclic (and mechanistically related) agents. The so-called *psychedelic drugs* have a broad range of CNS effects, and because one effect of several of these agents is CNS stimulation, they are discussed in this chapter.

ANALEPTICS

The analeptics are a group of potent and relatively nonselective CNS stimulants. The convulsive dose lies near their analeptic dose. They once had some employment as respiratory stimulants in countering the effects of CNS-depressant drugs; however, they are now obsolete for that use. Some members of the group retain a very small therapeutic niche in the treatment of chronic obstructive pulmonary disease (COPD). Certain of the compounds have usefulness as pharmacologic tools and interesting mechanisms of action.

Picrotoxin

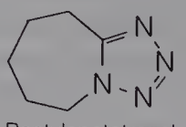
Picrotoxin is obtained from the seeds of *Anamirta cocculus*. The active ingredient is picrotoxinin, with the following structure:



According to Jarboe et al.,[1] the encircled hydroxylactonyl moiety is mandatory for activity, with the encircled 2-propenyl group assisting. Picrotoxin exerts its effects by interfering with the inhibitory effects of γ-aminobutyric acid (GABA) at the level of the $GABA_A$ receptor's chloride channel (i.e., it is said to jam chloride channels). The drug is obsolete medically. Pharmacologically it is used as an aid in determining how certain sedative–hypnotics and anticonvulsants act at the molecular level.

Pentylenetetrazol

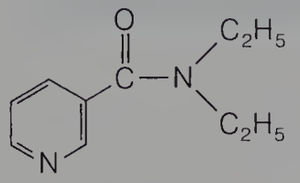
6,7,8,9-Tetrahydro-5*H*-tetrazoloazepine; 1,5-pentamethylenetetrazole (Metrazol). Pentylenetetrazol has been used in conjunction with the electroencephalograph to help locate epileptic foci. It is used routinely as a laboratory tool in determining potencies of potential anticonvulsant drugs in experimental animals. The drug acts as a convulsant by interfering with chloride conductance.[2] It binds to an allosteric site on the $GABA_A$ receptor and acts as a negative modulator. Overall, it appears to share similar effects on chloride conductance with several other convulsive drugs, including picrotoxin.



Pentylenetetrazol

Nikethamide

N,N-Diethylnicotinamide (Coramine). Nikethamide appears to act by facilitating excitatory processes rather than by depressing inhibitory ones. The overall effect resembles that of an amphetamine more than that of a drug such as picrotoxin.

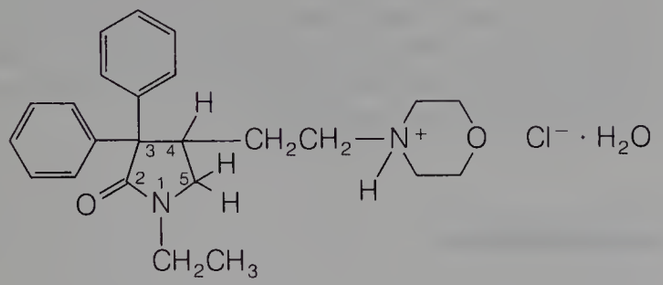


Nikethamide

It is possible to stimulate respiration with the drug without inducing generalized CNS stimulation. However, selectivity is still very low. The drug is obsolete in managing poisoning from sedative–hypnotic drugs. It may have a very limited place in treating acute respiratory insufficiency in COPD. It also may have value in correcting respiratory depression caused by oxygen therapy in COPD.

Doxapram Hydrochloride, USP

1-Ethyl-4-(2-morpholinoethyl)-3, 3-diphenyl-2-pyrrolidinone hydrochloride hydrate (Dopram). Doxapram has CNS-stimulant properties resembling those of nikethamide more than those of picrotoxin or pentylenetetrazol. It appears to have greater selectivity as a respiratory stimulant than nikethamide, but symptoms of generalized CNS stimulation are still frequent. Uses of doxapram are as described for nikethamide.



Doxapram Hydrochloride

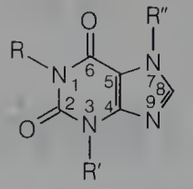
METHYLXANTHINES

The naturally occurring methylxanthines are caffeine, theophylline, and theobromine. Refer to Table 15-1 for their structures and occurrence and to Table 15-2 for their relative potencies.

Caffeine enjoys wide use as a CNS stimulant. Theophylline has some use as a CNS stimulant (as will be discussed later); its CNS-stimulant properties are encountered more often as side effects, sometimes severe and potentially life-threatening, of its use in bronchial asthma therapy. Theobromine has very little CNS activity and will not be discussed further as a CNS stimulant. Its 1-[5-oxohexyl] derivative pentoxifylline (Trental) is useful in intermittent claudication, presumably in part by improving red blood cell deformability. Its potential use in other occlusive disorders, such as acute stroke, is under investigation.

TABLE 15-1

XANTHINE ALKALOIDS



Xanthine
(R, R′ & R″ = H)

Compound	*R*	*R′*	*R″*	*Common Source*
Caffeine	CH_3	CH_3	CH_3	Coffee, Tea
Theophylline	CH_3	CH_3	H	Tea
Theobromine	H	CH_3	CH_3	Cocoa

Caffeine is used often as it occurs in brewed coffee (~ 85 mg/cup), brewed tea (~ 60 mg/cup), and cola beverages (~ 50 mg/12 fl oz). In most subjects 85 to 250 mg of caffeine acts as a cortical stimulant and facilitates clear thinking and wakefulness, promotes an ability to concentrate on the task at hand, and lessens fatigue. As the dose is increased, side effects indicative of excessive stimulation, such as restlessness, anxiety, nervousness, and tremulousness, become more marked. (They may be present in varying degrees at lower dose levels.) With further increases in dosage, convulsions can occur.

Theophylline's CNS effects at lower dose levels have been studied little. At high doses, the tendency to produce convulsions is greater for theophylline than for caffeine.

In addition to being cortical stimulants, theophylline and caffeine are medullary stimulants, and both are used in treating sleep apnea in preterm infants. Caffeine (as caffeine and sodium benzoate) may be used rarely in treating poisoning from CNS-depressant drugs, though it is not a preferred choice.

The important use of theophylline and its preparations in bronchial asthma is discussed elsewhere. Caffeine also is

TABLE 15-2

RELATIVE PHARMACOLOGIC POTENCIES OF THE XANTHINES

Xanthine	*CNS Stimulation*	*Respiratory Stimulation*	*Diuresis*	*Coronary Dilatation*	*Cardiac Stimulation*	*Skeletal Muscle Stimulation*
Caffeine	1*	1	3	3	3	1
Theophylline	2	2	1	1	1	2
Theobromine	3	3	2	2	2	3

*1 = most potent.

reported to have valuable bronchodilating properties. Finally, caffeine has value, presumably because of central vasoconstrictive effects, in treating migraine and tension headaches.

The basis for the CNS-stimulating effects of the methylxanthines often has been attributed to their phosphodiesterase-inhibiting ability. For example, they retard the metabolism of cyclic adenine monophosphate (cAMP). This action is probably irrelevant at therapeutic doses. The evidence indicates that the CNS-stimulant action is related more to the ability of these compounds to antagonize adenosine at A_1 receptors.[3–5] The adenosine receptor subtypes and their pharmacology have been reviewed.[6,7] Problems with the present compounds, such as caffeine and theophylline, are lack of receptor selectivity. Another problem, even more difficult, is the ubiquitous nature of the various receptor subtypes, for example, unwanted effects on the heart, concurrent with a desired action.

Caffeine and theophylline are chemically interesting. Both are weak Bronsted bases. The reported pK_a values are 0.8 and 0.6 for caffeine and 0.7 for theophylline. These values represent the basicity of the imino nitrogen at position 9. As acids, caffeine has a pK_a over 14 and theophylline, a pK_a of 8.8. In theophylline, a proton can be donated from position 7 (i.e., it can act as a Bronsted acid). Caffeine cannot donate a proton from position 7 and does not act as a Bronsted acid at *p*H values under 14. Caffeine does have electrophilic sites at positions 1, 3, and 7. In addition to its Bronsted acid site at 7, theophylline has electrophilic sites at 1 and 3. In condensed terms, both compounds are electron pair donors, but only theophylline is a proton donor in most pharmaceutic systems.

Although both compounds are quite soluble in hot water (e.g., caffeine 1:6 at 80°C), neither is very soluble in water at room temperature (caffeine about 1:40, theophylline about 1:120). Consequently, a variety of mixtures or complexes designed to increase solubility are available (e.g., citrated caffeine, caffeine and sodium benzoate, and theophylline ethylenediamine compound [aminophylline]).

Caffeine in blood is not highly protein-bound, whereas theophylline is about 50% bound. Differences in the substituent at the 7-position may be involved. Additionally, caffeine is more lipophilic than theophylline and reputedly achieves higher brain concentrations. The half-life for caffeine is 5 to 8 hours and for theophylline, about 3.5 hours. About 1% of each compound is excreted unchanged. The compounds are metabolized in the liver. The major metabolite of caffeine is 1-methyluric acid and that of theophylline, 1,3-dimethyluric acid.[8] Neither compound is metabolized to uric acid, and they are not contraindicated in gout. Caffeine and theophylline are marketed in a variety of tablet strengths. Citrated caffeine is available in tablet form. Theophylline ethylenediamine is available in a variety of enteral and parenteral forms. Other complexes and derivatives of theophylline are available.

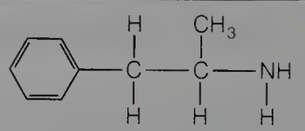
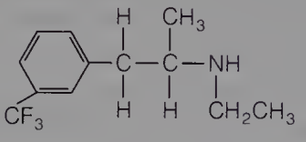
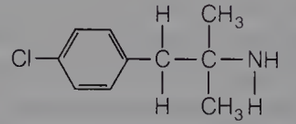
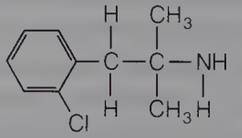
CENTRAL SYMPATHOMIMETIC AGENTS (PSYCHOMOTOR STIMULANTS)

Sympathomimetic agents, whose effects are manifested mainly in the periphery, are discussed in Chap. 16. A few simple structural changes in these peripheral agents produce compounds that are more resistant to metabolism, more nonpolar, and better able to cross the blood–brain barrier. These effects increase the proportion of central to peripheral activity, and the agents are designated, somewhat arbitrarily, as "central sympathomimetic agents."

In addition to CNS-stimulating effects, manifested as excitation and increased wakefulness, many central sympathomimetics exert an anorexiant effect. Central sympathomimetic (noradrenergic) action is often the basis for these effects. However, other central effects, notably dopaminergic and serotoninergic, can be operative.[9] In some agents, the proportion of excitation and increased wakefulness to anorexiant effects is decreased, and the agents are marketed as anorexiants. Actually, in one drug, fenfluramine, acting as an anorexiant by predominantly serotoninergic mechanisms, sedation and drowsiness, rather than excitation, typically are seen. Representative structures of this group of compounds are given in Table 15-3. Additionally, the structures of the

TABLE 15-3

SYMPATHOMIMETICS WITH SIGNIFICANT CENTRAL STIMULANT ACTIVITY

Generic Name	*Base Structure*
Amphetamine	
Methamphetamine	H H CH_3
Phentermine	H CH_3 H
Benzphetamine	H H CH_3 $CH_2C_6H_5$
Diethylpropion	O* H C_2H_5 C_2H_5
Fenfluramine	
Chlorphentermine	
Clortermine	

*Carbonyl.

anorexiants phenmetrazine, phendimetrazine, and mazindol, as well as of the alerting agents methylphenidate and pemoline, useful in attention-deficient disorders, are given in the text.

Structural features for many of the agents can be visualized easily by considering that within their structure they contain a β-phenethylamine moiety, and this grouping can give some selectivity for pre- or postsynaptic noradrenergic systems.

β-Phenethylamine, given peripherally, is without central activity. Facile metabolic inactivation by monoamine oxidases (MAOs) is responsible. Branching with lower alkyl groups on the carbon atom adjacent (α) to the amino nitrogen increases CNS, rather than peripheral, activity (e.g., amphetamine, presumably by retarding metabolism). The α-branching generates a chiral center. The *dextro(S)*-isomer of amphetamine is up to ten times as potent as the *levo(R)*-isomer for alerting activity and about twice as active as a psychotomimetic agent.[10] Hydroxylation of the ring or hydroxylation on the β-carbon (to the nitrogen) decreases activity, in large measure by decreasing ability to cross the blood–brain barrier. For example, phenylpropanolamine, with a β-OH, has about 0.01th the ability to cross the blood–brain barrier of its deoxy congener, amphetamine.

Halogenation (F, Cl, Br) of the aromatic ring decreases sympathomimetic activity. Other activities may increase. *p*-Chloroamphetamine has strong central serotoninergic activity (and is a neurotoxin, destroying serotoninergic neurons in experimental animals).[11,12]

Methoxyl substitution on the ring tends to produce psychotomimetic agents, suggesting trophism for dopaminergic (D-2) receptors.

N-Methylation increases activity, as with methamphetamine compared with dextroamphetamine. Di-*N*-methylation decreases activity. Mono-*N*-substituents larger than methyl decrease excitatory properties, but many compounds retain anorexiant properties. Consequently, some of these agents are useful anorexiants, with decreased abuse potential relative to amphetamine.

There can be some departure from the basic β-phenethylamine structure when compounds act by indirect noradrenergic mechanisms, such as block of norepinephrine (NE) uptake, as with cocaine, mazindol, and many tricyclic antidepressants. However, a concealed β-phenethylamine structure also can be present in these compounds.

The abuse potential of the more euphoriant and stimulatory of the amphetamines and amphetamine-like drugs, such as cocaine, is well documented. They produce an exceedingly destructive addiction. Apparently, both a euphoric "high" (possibly related to effects on dopaminergic systems) and a posteuphoric depression contribute to compulsive drug use with these agents.

Recognized medical indications for dextroamphetamine and some very close congeners include narcolepsy, Parkinson's disease, attention deficit disorders, and, although not the preferred agents for it, anorexia. In some conditions, such as Parkinson's disease, for which its main use is to decrease rigidity, the antidepressant effects of dextroamphetamine can be beneficial. It also has been reported to be an effective antidepressant in terminal malignancies. However, in almost all cases of depression, and especially in major depressive disorders of the unipolar type, dextroamphetamine has been superseded by other agents, notably the MAOIs and the tricyclic and mechanistically related antidepressants.

The compounds and certain of their metabolites can have complex, multiple actions. In a fundamental sense, however, the structural basis for action is quite simple. The compounds and their metabolites resemble NE and can participate in the various neuronal and postsynapatic processes involving NE, such as synthesis, release, uptake, and pre- and postsynaptic receptor activation. Also, because dopamine (DA) and, to a lesser extent, serotonin (5-HT) bear a structural resemblance to NE, processes in DA- and 5-HT-activated systems can be affected.

PRODUCTS

Amphetamine Sulfate, USP

α-Methylbenzene-ethanamine sulfate; (±)-1-phenyl-2-aminopropane (Benzedrine). The racemic mixture has a higher proportion of cardiovascular effects than the *dextro*-isomer. For most medical uses, the dextrorotatory isomer is preferred.

Dextroamphetamine Sulfate, USP, and Dextroamphetamine Phosphate

(+)-(*S*)-Methylphenethylamine salts with sulfuric acid (Dexedrine) and with phosphoric acids, respectively. The phosphate is the more water-soluble salt and is preferred if parenteral administration is required. The dextrorotatory isomer has the *(S)*-configuration and fewer cardiovascular effects than the levorotatory *(R)*-isomer. Additionally, it may be up to ten times as potent as the *(R)*-isomer as an alerting agent and about twice as potent as a psychotomimetic agent. Although it is more potent as a psychotomimetic agent than the *(R)*-isomer, it has a better ratio of alerting to psychotomimetic effects.

The alerting actions appear to relate to increased NE available to interact with postsynaptic receptors. The major mode of action of dextroamphetamine is release of NE from the nerve terminal.[9] Other mechanisms, such as inhibition of uptake, may make a small contribution to the overall effects. The psychotomimetic effects are said to be linked to release of DA. Effects on 5-HT systems also have been linked to some behavioral effects of dextroamphetamine.

Dextroamphetamine is a strongly basic amine with a pK_a of 9.77 to 9.94. Absorption from the gastrointestinal tract occurs as the lipid-soluble amine. The drug is not extensively protein-bound. Varying amounts of the drug are excreted intact under ordinary conditions. The amount is insignificant under conditions of alkaline urine. Under conditions producing systemic acidosis, 60% to 70% of the drug can be excreted unchanged. This fact can be used to advantage in treating drug overdose.

The α-methyl group retards, but does not terminate, metabolism by MAOs. Under most conditions, the bulk of a dose of dextroamphetamine is metabolized by *N*-dealkylation to phenylacetone and ammonia. Phenylacetone is degraded further to benzoic acid.

In experimental animals, about 5% of a dose accumulates in the brain, especially the cerebral cortex, the thalamus, and the corpus callosum. It is first *p*-hydroxylated and then β-hydroxylated to produce *p*-hydroxynorephedrine, which may be the major active metabolite involved in NE and DA release.[13]

Methamphetamine Hydrochloride

(+)-*N*,α-Dimethylphenethylamine hydrochloride; deoxyephedrine hydrochloride; (+)-1-phenyl-2-methylaminopropane hydrochloride (Desoxyn). Methamphetamine is the *N*-methyl analogue of dextroamphetamine. It has more marked central and decreased peripheral actions relative to dextroamphetamine. It has very high abuse potential, and by the intravenous route, its salts are known as "speed." Therapeutic uses of methamphetamine are analogous to those of dextroamphetamine.

Phentermine Ion-Exchange Resin (Ionamin); Phentermine Hydrochloride, USP (Wilpowr)

The free base is α,α-dimethylphenethylamine; 1-phenyl-2-methylaminopropane. In the resin preparation, the base is bound with an ion-exchange resin to afford a slow-release product; the hydrochloride is a water-soluble salt.

Interestingly, phentermine has a quaternary carbon atom with one methyl oriented analogously to the methyl of *(S)*-amphetamine and one methyl oriented analogously to the methyl of *(R)*-amphetamine, and it is reported to have pharmacologic properties partaking of both the *(R)*- and *(S)*-isomers of amphetamine. The compound is used as an appetite suppressant and is a Schedule IV agent, indicating less abuse potential than dextroamphetamine.

Chlorphentermine Hydrochloride

p-Chloro-α,α-dimethylphenethylamine hydrochloride; 1-(4-chlorophenyl)-2-methyl-2-aminopropane hydrochloride (Pre-Sate). Chlorphentermine is structurally interesting because of the *p*-chloro substituent on α,α-dimethylphenylethylamine. It is an effective anorexiant with less abuse potential than dextroamphetamine.

Clortermine Hydrochloride

o-Chloro-α,α-dimethylphenethylamine hydrochloride; 1-(2-chlorophenyl)-2-methyl-2-aminopropane hydrochloride (Voranil). Clortermine is the *o*-chloro isomer of chlorphentermine. It is an effective appetite suppressant and, like its *p*-isomer, has less abuse potential than dextroamphetamine.

Benzphetamine Hydrochloride

(+)-*N*-Benzyl-*N*, α-dimethylphenethylamine hydrochloride; (+)-1-phenyl-2-(*N*-methyl-*N*-benzylamine)propane hydrochloride (Didrex). This compound is *N*-benzyl-substituted methamphetamine. The large (benzyl) *N*-substituent decreases excitatory properties, in keeping with the general structure–activity relationship (SAR) for the group. The compound has been observed to share mechanism-of-action characteristics with methylphenidate. The agent reduces appetite with fewer CNS excitatory effects than dextroamphetamine.

Diethylpropion Hydrochloride, USP

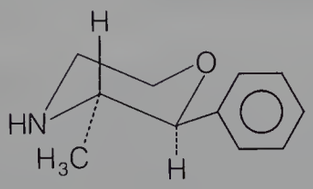
2-(Diethylamino)propiophenone hydrochloride; 1-phenyl-2-diethylaminopropan-1-one hydrochloride (Tenuate, Tepanil). With two large (relative to H or methyl) *N*-alkyl substituents, diethylpropion has fewer cardiovascular and CNS-stimulatory effects than amphetamine. It is said to be the anorexiant agent best suited for the treatment of obesity in patients with hypertension and cardiovascular disease.

Fenfluramine Hydrochloride

N-Ethyl-α-methyl-*m*-(trifluoromethyl)-phenylethylamine HCl (Pondimin). Fenfluramine is unique in this group of drugs in that it tends to produce sedation rather than excitation. Effects are said to be mediated principally by central serotoninergic, rather than central noradrenergic, mechanisms. In large doses in experimental animals, the drug has been reported to be a serotonin neurotoxin.[14] The drug is favored in patients in whom CNS stimulation is to be avoided, and in weight reduction in non-insulin-dependent diabetes mellitus (NIDDM). The (+) form is in clinical trials.

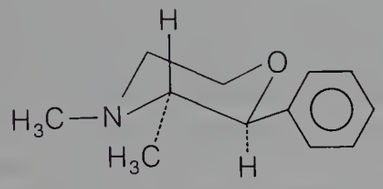
Phenmetrazine Hydrochloride, USP

3-Methyl-2-phenylmorpholine hydrochloride (Preludin). A β-phenethylamine moiety is present in this Schedule II anorexiant. The configuration was deduced by Clarke.[15] Although less excitatory than dextroamphetamine, it still possesses abuse potential.



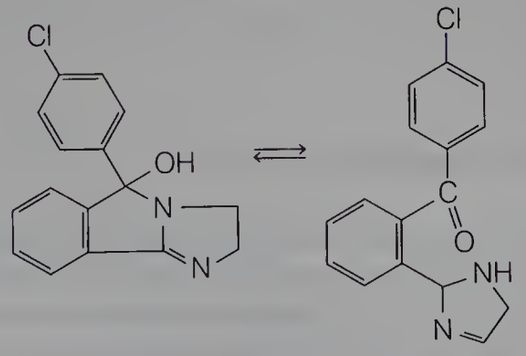
Phendimetrazine Tartrate, USP

(2*S*, 3*S*)-3, 4-Dimethyl-2-phenylmorpholine-L-(+)-tartrate (Plegine). This optically pure compound is considered an effective anorexiant and is classed as a Schedule III compound. The stereochemistry of (+)phendimetrazine is analogous to that of (+)phenmetrazine.[16]



Mazindol, USP

5-(*p*-Chlorophenyl)-2,5-dihydroxy-3*H*-imidazol [2,1-*a*]isoindole-5-ol (Sanorex). Mazindol can exist in two tautomeric forms. In acidic media, the imidazoisoindole structure is preferred. Seemingly, either structure is far removed from any resemblance to a protonated β-arylamine, but taking the isoindole structure and protonating the cyclic amidino group produces a cationic center, about a two-atom distance and two aryl groups. This fits the structural features associated with block of neuronal NE and DA uptake. A major mode of action is block of NE uptake.[17] Mazindol is an effective anorexint.



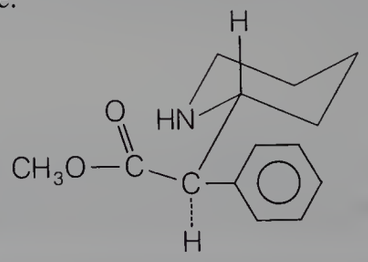
Mazindol

Methylphenidate Hydrochloride, USP (Ritalin)

There are two asymmetric centers in methylphenidate and four possible isomers. The *threo*-racemate is the marketed compound and is about 400 times as potent as the *erythro*-racemate.[18] The absolute configuration of each of the *threo*-methylphenidate isomers has been determined.[19] Considering that the structure is fairly complex, it is likely that one of the two components of the *threo*-racemate contains most of the activity. Evidence indicates that the *d*(2*R*,2′*R*) *threo*-isomer is involved principally in the behavioral and pressor effects of the racemate.[20] As is likely with many central psychomotor stimulants, the mode of action at the molecular level is multiple. Methylphenidate or its *p*-hydroxy metabolite, or both, blocks NE uptake, acts as a postsynaptic agonist, depletes the same NE pool as reserpine, and has effects on DA systems such as a block of DA uptake.

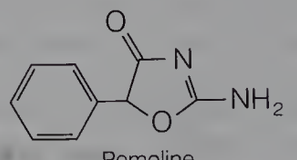
Methylphenidate is an ester drug with interesting pharmacokinetic properties arising from its structure. The pK_a values are 8.5 and 8.8. The protonated form in the stomach reportedly resists ester hydrolysis. Absorption of the intact drug is very good. However, 80% to 90% of the drug is hydrolyzed rapidly to inactive ritalinic acid after absorption from the gastrointestinal tract.[21] (The extent of hydrolysis may be about fivefold that for *d* versus *l*.[22]) Another 2% to 5% of the racemate is oxidized by liver microsomes to the inactive cyclic amide. About 4% of a dose of the racemate reaches the brain in experimental animals and, there, is *p*-hydroxylated to yield the putative active metabolite.

Methylphenidate is a potent CNS stimulant. Indications include narcolepsy and attention-deficit disorder. The structure of the (2*R*,2′*R*)-isomer of the *threo*-racemic mixture is shown here.



Pemoline

2-Amino-5-phenyl-4(5*H*)-oxazolone (Cylert). The structure of this compound is unique as can be seen from its depiction below.



Pemoline

The compound is described as having an overall effect on the CNS similar to that of methylphenidate. However, the agent requires 3 to 4 weeks of administration to take effect. A partial explanation for the delayed effect may be that one of the actions of the agent, as observed in rats, is to increase the rate of synthesis of DA.

MONOAMINE OXIDASE INHIBITORS

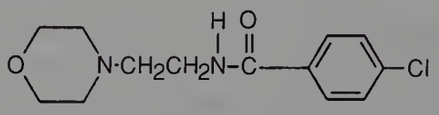
Antidepressant therapy usually implies therapy directed against major depressive disorders of the unipolar type and is centered around two groups of chemical agents, the MAOIs and the tricyclic and mechanistically related antidepressants, as well as electroshock therapy. The highest cure or remission rate is achieved with electroshock therapy. In some patients, especially those who are suicidal, this is the treatment of choice. MAOIs and tricyclic antidepressants and their mechanistically related compounds have about the same response rate (about 60% to 70%). In the United States, the latter group is usually chosen over MAOIs in most antidepressant therapy.

Side effects of tricyclics include troublesome anticholinergic effects and effects of noradrenergic stimulation. In overdose, the combination of effects can be lethal. Newer mechanistically related agents, such as the serotonin-selective reuptake inhibitors (SSRIs), typically have decreased anticholinergic and noradrenergic properties. A severe problem associated with the MAOIs, and one that is a major factor in relegating them to second-line drug status, originates in the fact that most available compounds inhibit liver MAOs in addition to brain MAOs, thereby allowing dietary pressor amines that normally would be inactivated to exert their effects. A number of severe hypertensive responses, sometimes fatal, have followed ingestion of foods high in pressor amines. It has been hoped that the development of agents, such as deprenyl, that presumably spare liver MAO might solve this problem. Another prominent side effect of the MAOI is orthostatic hypotension, said to arise from a bretylium-like action. Actually, one MAOI, pargyline, has been used clinically for its hypotensive action. Also, some of the first compounds produced serious hepatotoxicity. Compounds available today reportedly are much safer in this regard but suffer the stigma of association with the older compounds.

The history of MAOI development illustrates the role of serendipity. Isoniazid is an effective antitubercular agent but a very polar compound. To gain better penetration into the *Mycobacterium tuberculosis* organism, a more hydrophobic compound, isoniazid substituted with an isopropyl group on the basic nitrogen (iproniazide), was designed and synthesized. It was introduced into clinical practice as an effective antitubercular agent. However, it was noted that CNS stimulation occurred, and the drug was withdrawn. Later, it was determined in experimental animals, as well as in in vitro experiments with purified MAO, that MAO inhibition, resulting in higher levels of NE and 5-HT, could account for the CNS effects. Thereafter, the compound was reintroduced into therapy as an antidepressant agent. It stimulated an intensive interest in hydrazines and hydrazides as antidepressants and inaugurated effective drug treatment of depression.[23] It continued to be used in therapy for several years but eventually was withdrawn because of hepatotoxicity.

With one exception, moclobemide, available compounds can be considered mechanism-based inhibitors of MAO.[24] They are converted by MAO to agents that inhibit the enzyme. Inhibition by the agents is often irreversible. All appear to have the ability to form reactants that bond covalently with the enzyme or its cofactor. A consequence of irreversible inactivation is that the action of the agents may continue for up to 2 weeks after administration is discontinued. Consequently, many drugs degraded by MAO cannot be administered during that time.

It is possible to have agents that act exclusively by competitive enzyme inhibition. The harmala alkaloids harmine and harmaline are thought to act primarily as CNS stimulants by competitive inhibition of MAO. Reversible inhibitors selective for each of the two major MAO subtypes (A and B) are forthcoming.



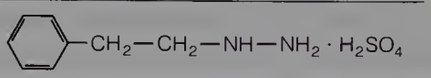
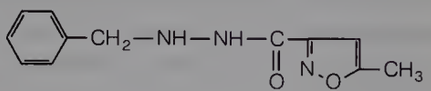
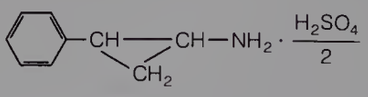
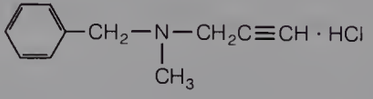
Moclobemide

Moclobemide has received considerable attention. A reversible inhibitor of MAO-A, it is considered to be an effective antidepressant and permits dietary tyramine to be metabolized.[25] It is thought that metabolites of the drug are involved in the activity. Reversible inhibitors of MAO-A (RIMA) reportedly are antidepressant without production of hypertensive crises. Presently, selective MAO-B inhibition has failed to correlate positively with antidepressant activity; however, selegiline has been of benefit in Parkinson's disease.

Most of the clinically useful MAOI antidepressants are nonselective between inhibiting metabolism of NE and 5-HT. Agents selective for an MAO that degrade 5-HT (e.g., clorgyline) have been under study for some time. The structures of phenelzine, isocarboxazid, tranylcypromine, and pargyline are given in Table 15-4.

TABLE 15-4

MONOAMINE OXIDASE INHIBITORS

Generic Name Proprietary Name	***Structure***
Phenelzine sulfate USP *Nardil*	
Isocarboxazid USP *Marplan*	
Tranylcypromine sulfate USP *Parnate*	
Pargyline hydrochloride USP *Eutonyl*	

PRODUCTS

Phenelzine Sulfate, USP

2-(Phenylethyl)hydrazine sulfate (Nardil). Phenelzine is an effective antidepressant agent. A mechanism-based inactivator, it irreversibly inactivates the enzyme or its cofactor, presumably after oxidation to the diazine, which can then break up into molecular nitrogen, a hydrogen atom, and a phenethyl free radical. The latter would be the active species in irreversible inhibition.[26]

Isocarboxazid, USP

5-Methyl-3-isoxazole-carboxylic acid 2-benzylhydrazide; 1-benzyl-2-(5-methyl-3-isoxazolylcarbonyl) hydrazine (Marplan). It is generally held that hydrazides such as isocarboxazide are prodrugs: hydrolysis of the acyl group yields the active hydrazine. Thereafter, the action of the enzyme is thought to produce the active, irreversibly inhibiting agent, as described in the discussion of phenylzine. For isocarboxazid, the agent would be the corresponding benzyl radical.

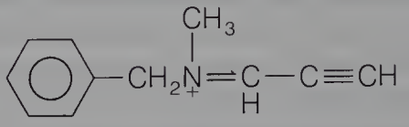
Tranylcypromine Sulfate, USP

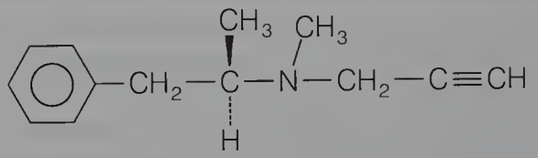
(±)-*trans*-2-Phenylcyclopropylamine sulfate (Parnate). Tranylcypromine was synthesized to be an amphetamine analogue (visualize the α-methyl of amphetamine condensed onto the β-carbon atom).[27] It does have some amphetamine-like properties, which probably relate to the fact that the agent has more immediate CNS stimulant effects than do agents that act by MAO inhibition alone. For MAO inhibition, there may be two components to the action. One component is thought to arise because the agent has structural features (the basic nitrogen and the quasi-π character of the α- and β-cyclopropane carbon atoms) that approximate the transition state in a route of metabolism of β-arylamines.[28,29] As α- and β-hydrogen atoms are removed from the substrate, the quasi-π character develops over the α–β-carbon system. Duplication of the transition state permits extremely strong, but reversible, attachment to the enzyme. Additionally, tranylcypromine is a mechanism-based inactivator. It is metabolized by MAO, with one electron of the nitrogen pair lost to flavin. This, in turn, produces a homolytic fission of a carbon–carbon bond of cyclopropane, one electron from the fission pairing with the remaining lone nitrogen electron to generate an imine (protonated), the other residing on a methylene carbon. Thus, a free radical ($C_6H_6\dot{C}HCH_2CNH^+_2$) is formed, reacts covalently either with the enzyme or with reduced flavin to inactivate the enzyme.[30]

Pargyline Hydrochloride, USP

N-Methyl-*N*-2-propynylbenzylamine hydrochloride (Eutonyl). Pargyline is said to be specific for an MAO that prefers phenylethylamine substrates over 5-HT substrates. Although it has CNS-stimulating properties, it usually is used for its hypotensive properties. Hypotensive properties for the MAOI are said, as stated earlier, to arise from a bretylium-like effect and need not be a consequence of MAO inhibition. For example, *N*-demethylpargyline is an active MAOI but reportedly is not hypotensive.

Pargyline, as are other propargylamines, is a mechanism-based inactivator of MAO. It is converted by the enzyme to the Michael acceptor below which can react with the Michael donor, the number 5 nitrogen of flavin.[24]

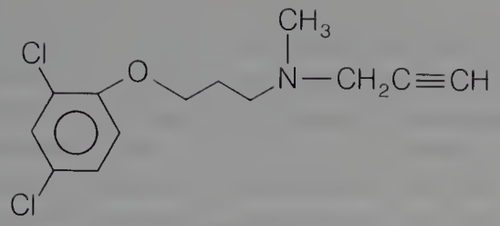




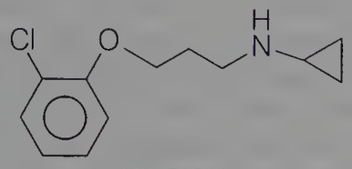
L-Deprenyl (selegiline) has received attention in recent years as an agent that might spare liver MAO and, thereby, avoid the "cheese effect," or hypertensive response, to tyramine and other dietary pressor amines. There appears to be avoidance of the effect, but it has been suggested that an effect on tyramine other than the sparing of liver MAO may be involved. The agent has been employed in Parkinson's disease to decrease the severity of the "on–off" phenomenon, one of a variety of abrupt changes in motor status believed to be caused by fluctuations in DA levels. It has been introduced into therapy as selegeline (Eldepryl). It appears to be the first drug that slows progression of the disease. The compound's neuronal inhibition of DA oxidation, which inhibits formation of free radicals including superoxide, is considered responsible.[31] It also has undergone trials for antidepressant therapy.

Clorgyline and Lilly 51641

In both of these compounds, a halogenated phenyl in conjugation with the amino nitrogen produces selectivity for 5-HT-metabolizing enzymes. The four-atom chain between the aromatic ring and nitrogen could fold to a two-atom chain when the nitrogen is protonated, and the proton can H-bond with the ether oxygen. Finally, the nitrogen is substituted with a group that produces a mechanism-based inactivating effect, in one case a propargyl group and in the other a cyclopropyl group. The agents have undergone trials as potential antidepressant agents.



Clorgyline



Lilly 51641

TRICYCLIC (AND MECHANISTICALLY RELATED) ANTIDEPRESSANT COMPOUNDS

This heading attempts to bring under one umbrella what was originally a small group of closely related agents, the tricyclic antidepressants (TCAs), which now are known to be chemically diverse. Almost all of the original agents block neuronal uptake of NE, 5-HT, and DA. Some agents appear not to block uptake of these transmitters but still share with the uptake (reuptake) blockers the property of increasing synaptic availability of NE, 5-HT, and DA. As to which amine is mainly responsible for antidepressant activity, various opinions exist. Many studies have implied that 5-HT is primarily responsible.[32] Others have cited NE as the likely prime agent.[33] A number of SSRIs are proving to be effective antidepressants. Some feel a combination of the two is desirable. One new 5-HT and NE reuptake inhibitor (SNRI), Effexor, has been introduced. The involvement of DA in antidepressant effects is less certain. Some investigators would exclude it altogether. One basis for exclusion is that drugs that act largely by blocking uptake of DA, such as methylphenidate and cocaine, tend to produce euphoria or dysphoria and psychotic effects and do not have highly beneficial antidepressant properties.[33]

The SARs for the TCAs are compiled in detail in the eighth edition of this text.[34] The interested reader is referred to this compilation. Overall, these SARs appear to be summarizable as a large, bulky group encompassing two aromatic rings, preferably held in a skewed arrangement by a third central ring and a three- or sometimes two-atom chain to an aliphatic amino group that is monomethyl- or dimethyl-substituted. The features can be visualized by consulting the structures of imipramine and desipramine as examples. The overall arrangement has features that approximate a fully extended *trans* conformation of the β-aryl amines. To relate these features to the mechanism of action, block of reuptake, it can be visualized that the same basic arrangement is present as is found in the β-arylamines, plus extra bulky groups that block the uptake process. The overall concept of a β-arylamine-like system with added structural bulk appears to be applicable to many newer compounds that do not have a tricyclic grouping.

Still another way of rationalizing the SARs may be useful. Many of the antidepressant drugs are close structural relatives of postsynaptic DA (antipsychotic) and NE (sedative) blockers. Conceivably, as a consequence of small structural changes, an agent can begin to gain the ability to block a presynaptic event (uptake) and then lose the ability to effect a postsynaptic block. In fact, in many tricyclics there is some retention of postsynaptic effects. Many antidepressants retain appreciable antipsychotic and sedative properties, which may be due largely to postsynaptic DA and NE blocks, respectively.

The TCAs and related agents are usually preferred for treatment of major depressions. Some of the newer agents differ in side effects from the TCAs, and these side effects will be discussed under the headings for the individual agents. The TCAs are structurally related to each other and, consequently, possess related biologic properties that can be summarized as characteristic of the group.

The dimethylamino compounds tend to be sedative, whereas the monomethyl relatives tend to be stimulatory. The dimethyl compounds tend toward higher ratios of 5-HT/NE uptake block; in the monomethyl, the proportion of NE uptake block tends to be higher. The compounds have anticholinergic properties, and these are usually higher in the dimethylamino compounds. When treatment is begun with a dimethyl compound, with time there is a significant accumulation of the monomethyl compound as *N*-demethylation proceeds.

As with the MAOIs, there is a time lag before antidepressant effects are seen. It was once thought that the antidepressant effect depended on the buildup of the nor-metabolite. This is now known to be not true. Current views on the time lag focus on down-regulation or decrease in sensitivity of receptors (α_2, β-, 5-HT_2) as a sequela of increased levels of NE and 5-HT being responsible for actual antidepressant action.[35–39]

The TCAs are extremely lipophilic and, accordingly, very highly tissue-bound outside the CNS. They do have anticholinergic and noradrenergic effects, both central and peripheral, that are often unpleasant and sometimes dangerous. In overdose, the combination of anticholinergic and antiadrenergic effects, as well as a quinidine-like cardiac depressant effect, can be lethal. Overdose is complicated because the agents are so highly protein-bound that dialysis is ineffective.

PRODUCTS

Imipramine Hydrochloride, USP

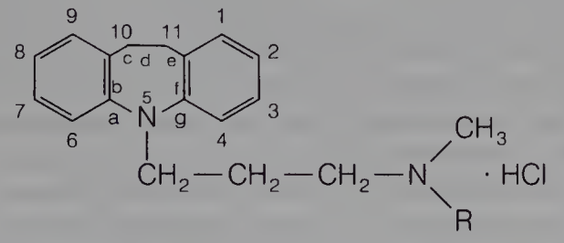
5-[3-(Dimethylamino)propyl]-10, 11-dihydro-5*H*-dibenz[*b*,*f*]azepine monohydrochloride (Tofranil). Imipramine

may be considered the parent compound of the TCAs. It is also a close relative of the antipsychotic phenothiazines (replace the 10–11 bridge with sulfur and the compound is the discontinued antipsychotic agent promazine). Relative to promazine, it has weak D-2 postsynaptic blocking activity, and mainly presynaptic effects on amines (5-HT, NE, and DA) are seen. As is typical of dimethylamino compounds, anticholinergic and sedative effects tend to be marked. The compound has a tendency toward a high 5-HT/NE uptake block ratio. Metabolic inactivation proceeds mainly by oxidative hydroxylation in the 2-position, followed by conjugation with glucuronic acid of the conjugate. Urinary excretion predominates (about 75%), but some biliary excretion (up to 25%) can occur, probably because of the large nonpolar grouping. Oxidative hydroxylation is not as rapid or complete as that of the more nucleophilic ring phenothiazine antipsychotics; consequently, appreciable *N*-demethylation with a buildup of norimipramine (or desimipramine) occurs.

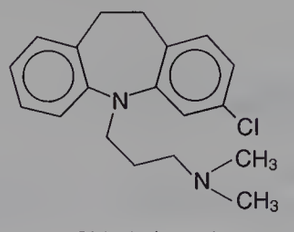
The demethylated metabolite is less anticholinergic, less sedative, and more stimulatory and has a higher NE than 5-HT uptake-blocking capability. Consequently, a patient treated with imipramine has at least two major bioactive metabolites that contribute to activity. The activity of des- or norimipramine is terminated by 2-hydroxylation, followed by conjugation and excretion. A second *N*-demethylation also can occur, which in turn is followed by 2-hydroxylation, conjugation, and excretion.

Desipramine Hydrochloride, USP

10,11-Dihydro-*N*-methyl-5*H*-dibenz[*b,f*]azepine-5-propanamine monohydrochloride; 5-(3-methylaminopropyl)-10,11-dihydro-5*H*-dibenz[*b,f*]azepine hydrochloride (Norpramin, Pertofrane). The structure of this agent as well as its salient properties are discussed under "Imipramine," above. In choosing an antidepressant drug for a patient, desipramine would be considered when few anticholinergic effects or a low level of sedation are important.



Imipramine: R = CH_3
Desipramine: R = H



Chlorimipramine

Imipramine is the parent TCA. Chlorimipramine (Anafranil), a much later introduction, is, however, an easier compound with which to visualize how changes in structure lead from antipsychotic to antidepressant effects. Chlorimipramine is related to chlorpromazine. As with chlorpromazine, protonation of the dimethylamino group and H-bonding with an unshared pair of electrons of the chloro substituent can generate a protonated β-arylamine-like grouping. Replacement of the sulfur atom with a $-CH_2-CH_2-$ bridge loses a receptor-binding atom (S). Also, the change destroys a crease present along the S–N axis. This crease allows one ring and S (and possibly N) to bind to the receptor, while the second aromatic ring is held at an angle slightly up and away from the receptor, not interfering with the binding of the first ring in the β-arylamine-like portion. With the crease gone, the second aromatic ring can be partly in the plane of the first ring, thus interfering with receptor binding. With postsynaptic binding decreased, presynaptic events can predominate. Serotonin and NE are, like DA, β-arylamines, and reuptake can be blocked in those systems also. Because of the bulky group (the bulge) on the side of the β-arylamine-like group, reuptake processes can be "plugged" and concentrations of NE and 5-HT built up in their respective synapses.

Chlorimipramine is up to 50 times as potent as imipramine. Increased distribution to the CNS could account for some of the potency increase, but other factors, such as –Cl H-bonding to help stabilize β-arylamine-like conformations, might be operative. Chlorimipramine is an antidepressant. It is used often in obsessive–compulsive disorder (OCD), which may have a component of depression.

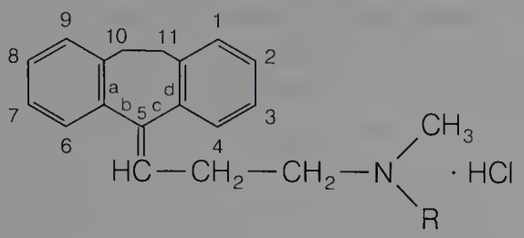
Amitriptyline Hydrochloride, USP

3-(10,11-Dihydro-5*H*-dibenzo [*a,d*]cyclohepten-5-ylidene)-*N, N*-dimethyl-1-propanamine hydrochloride; 5-(3-dimethylaminopropylidene)-10, 11-dihydro-5*H*-dibenzo[*a,d*]cycloheptene hydrochloride (Elavil). Amitriptyline is one of the most anticholinergic and sedative of the TCAs. Because it lacks the ring electron-enriching nitrogen atom of imipramine, metabolic inactivation mainly proceeds not at the analogous 2-position but at the benzylic 10-position (i.e., toluene-like metabolism predominates). Because of the 5-exocyclic double bond, *E*- and *Z*-hydroxy isomers are produced by oxidation metabolism. Conjugation produces excretable metabolites. As is typical of the dimethyl compounds, *N*-demethylation occurs, and nortriptyline is produced, which has a less anticholinergic, less sedative, and more stimulant action than amitriptyline.

Nortriptyline Hydrochloride, USP

3-(10,11-Dihydro-5*H*-dibenzo[*a, d*]cyclohepten-5-ylidene)-*N*-methyl-1-propanamine hydrochloride; 5-(3-methyl-aminopropylidene)-10,11-hydro-5*H*-dibenzo [*a,d*]cycloheptene

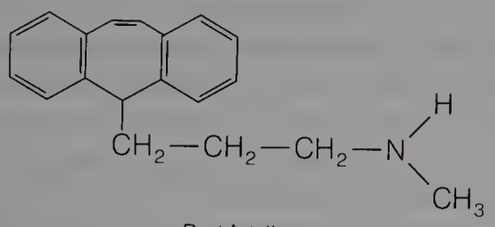
hydrochloride (Aventyl, Pamelor). Pertinent biologic and chemical properties for this agent are given in the foregoing discussion for amitriptyline. Metabolic inactivation and elimination are analogous with those of amitriptyline.



Amitriptyline: R = CH_3
Nortriptyline: R = H

Protriptyline Hydrochloride, USP

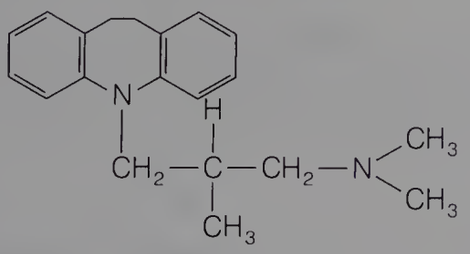
N-Methyl-5*H*-dibenzo[*a,d*]cycloheptene-5-propylamine hydrochloride;5-(3-methylaminopropyl)-5*H*-dibenzo-[*a, d*]cycloheptene hydrochloride (Vivactil). As with the other compounds under consideration, protriptyline is an effective antidepressant. The basis for its chemical naming can be seen by consulting the naming and the structure for imipramine. It is a structural isomer of nortriptyline. Inactivation can be expected to involve the 10–11 double bond. Because it is a monomethyl compound, the sedative potential is low.



Protriptyline

Trimipramine Maleate (Surmontil)

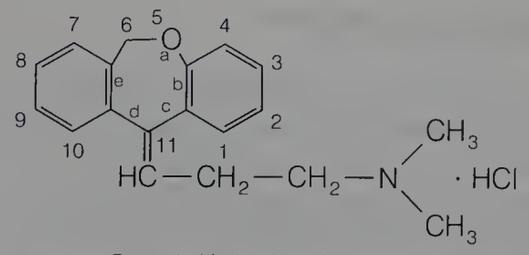
For details on chemical nomenclature, consult the description of imipramine. Replacement of hydrogen with an α-methyl substituent produces a chiral carbon, and the compound is employed as the racemic mixture. Biologic properties are said to resemble those of imipramine.



Trimipramine

Doxepin Hydrochloride, USP

3-Dibenz[*b,e*]-oxepin-11 (6*H*)ylidine-*N,N*-dimethyl-1-propanamine hydrochloride; *N,N*-dimethyl-3-(dibenz[*b,e*]oxepin-11(6*H*)-ylidene)propylamine (Sinequan, Adapin). Doxepin is an oxa congener of amitriptyline, as can be seen from its structure.

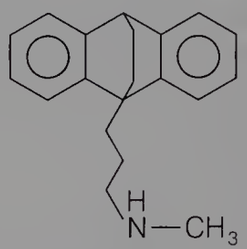


Doxepin Hydrochloride

The oxygen is interestingly placed and should influence oxidative metabolism as well as post- and presynaptic binding affinities. The (*Z*)-isomer is the more active; however, the drug is marketed as the mixture of isomers. The drug is an NE- and 5-HT-uptake blocker and has significant anticholinergic and sedative properties. It can be anticipated that the nor- or des-metabolite will contribute to the overall activity pattern.

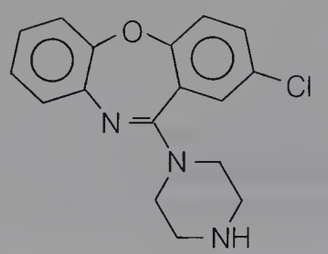
Maprotiline Hydrochloride, USP

N-Methyl-9,10-ethanoanthracene-9(10*H*)- propanamine hydrochloride (Ludiomil). Maprotiline sometimes is described as a tetracyclic, rather than a tricyclic, antidepressant. The description is chemically accurate, but the compound, nonetheless, conforms to the overall TCA pharmacophore. It is a dibenzobicyclooctadiene and can be viewed as a TCA with an ethylene-bridged central ring. The compound is not strongly anticholinergic and has been noted to have stimulant properties. The drug can have effects on the cardiovascular system.



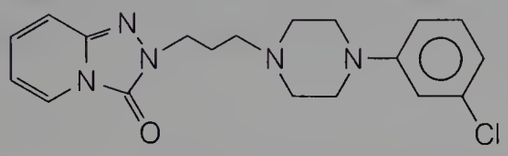
Amoxapine

2-Chloro-11-(1-piperazinyl)dibenz-[*b,f*] [1,4]oxazepine (Asendin). Consideration of the structure of amoxapine reinforces the observation that many TCAs bear a very close resemblance to antipsychotics; indeed, some have significant effects at D-2 receptors. The *N*-methyl-substituted relative is the antipsychotic loxapine (Loxitane). It is reported that the 8-hydroxy metabolite of amoxapine is active as an antidepressant and as a D-2 receptor blocker, as is, for the latter, amoxapine.

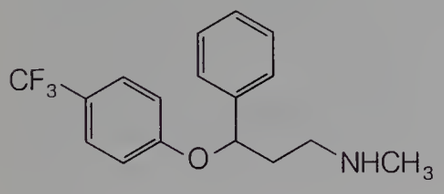


Trazodone Hydrochloride

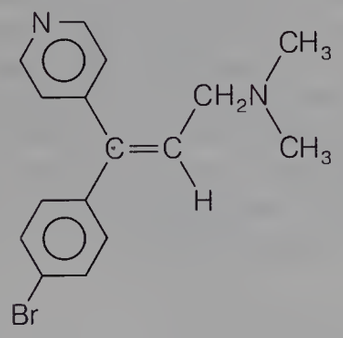
2-[3-[4-(3-Chlorophenyl)-1-piperazinyl] propyl]-1,2,4-triazolo [4,3-α]-pyridin-3(2*H*)-one (Desyrel). Consideration of the structure of trazodone reveals certain similarities with the fluorobutyrophenone antipsychotics, just as many TCAs relate to the phenothiazine and other tricyclic antipsychotics. Whereas the fluorobutyrophenone antipsychotics block DA postsynaptically, trazodone as well as its major metabolite *m*-chloro-4-phenylpiperazine block presynaptic uptake of 5-HT. The agent is an effective antidepressant. As can be expected from the structure, unlike the TCA, anticholinergic effects are not usually a problem. The usual side effect is sedation.



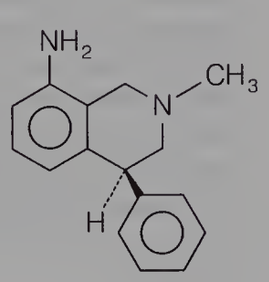
Finally, interesting examples of amine-uptake blockers that deviate from the fundamental TCA structure are **zimelidine** and **nomifensin.** They conform to the overall pharmacophore of a β-arylamine-like moiety with a lump or bulge on the side. Zimelidine was employed in Europe but reportedly produced Guillain-Barré syndrome and, accordingly, was withdrawn from use. Nomifensin was introduced in the United States but withdrawn because of reports of production of hemolytic anemia. The serotonin reuptake inhibitor fluoxetine (Prozac) has received much favorable attention as an antidepressant. It looks suspiciously like the protonated amino group, H-bonding to the ether oxygen to generate the β-aryl amino-like group with the bulge on the side.



Fluoxetine

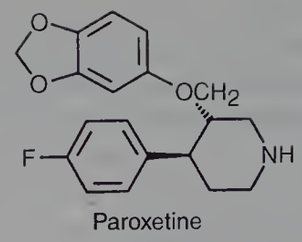


Zimelidine

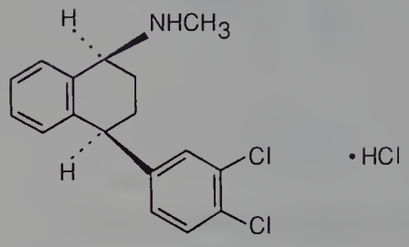


Nomifensin

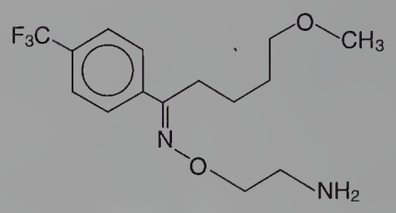
The SSRI paroxetine (Paxil) seems to conform to the general structural features of the group, as do the SSRIs sertraline (Zoloft) and fluvoxamine (Luvox).



Paroxetine



Sertraline

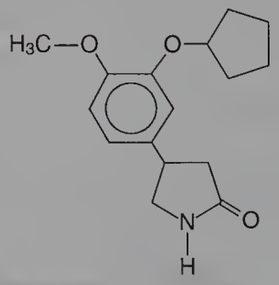


Fluvoxamine

Luvox is used especially in OCD.

Additionally, it has been reported that α_2-adrenoreceptor antagonists (e.g., agents that antagonize clonidine) are potential antidepressants.[40] Also, it appears that several β_2-NE stimulants (e.g., salbutamol and atenolol) are effective antidepressants.[41,42] As cited in Chap. 14, buspar and its relatives (partial agonists and agonists of 5-HT on 5-HT_{1A} receptors) are proving to be antidepressant as well as anxiolytic.

The phosphodiester type IV (PDE-IV) inhibitor rolipram was tried in depression but discontinued because it lacked adequate efficacy.[43] A major problem was the potency. It has been found that molecular changes in rolipram-type compounds can raise potency severalfold.[44] It is likely that trials of the more potent PDE-IV inhibitors (to elevate cell levels of cAMP) in treating depression will be made.



Volipram

PSYCHEDELICS

The term "*psychedelic*" refers to agents subjectively described as producing an increased awareness and enhanced

perception of sensory stimuli. Additionally, the stimuli may be perceived in unusual or novel ways (e.g., sound may be perceived as color). Also, the user may both experience the sensation and feel as though participating as an observer. These are the so-called mind-expanding drugs.

Additionally, the drugs can produce anxiety, fear, panic, hallucinations, and an overall symptomatology resembling a psychosis. Hence, they can be classed as hallucinogens and psychotomimetics.

Also included in this discussion are drugs that are mainly hallucinogenic (phencyclidine; PCP) and euphoriant (cocaine) and mainly depressant or intoxicating (Δ^9- or Δ^1-tetrahydrocannabinol; THC).

Psychedelics are broadly groupable into those possessing an indolethylamine moiety and those with a phenylethylamine moiety. In the first group, there is a structural resemblance to the central neurotransmitter 5-HT and in the second to NE and DA. This resemblance is intriguing, and indeed, there may be some effects on the respective transmitter systems. However, with structures of the complexity found in many of these agents, there is also a likelihood that a given structure may affect not just the closest structurally related neurotransmitter but other systems as well. Thus, a phenethylamine system can affect not only NE and DA systems but also 5-HT systems, and an indolethylamine system could affect not only 5-HT but also NE and DA systems.

INDOLETHYLAMINES

Dimethyltryptamine

This compound is a rather weak hallucinogen, active only by inhalation or injection, with a short duration of action. It possesses pronounced sympathomimetic side effects.

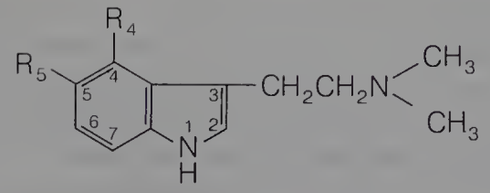
Bufotenine

This compound is 5-hydroxydimethyltryptamine and is probably not a hallucinogen, as it was once thought to be. Rather, it acts as a cardiovascular stimulant by release of 5-HT.[45]

Psilocybin and Psilocyn

Psilocybin is the phosphoric acid ester of psilocyn and appears to be converted to psilocyn as the active species in vivo. It occurs in a mushroom, *Psilocybe mexicana*. Both drugs are orally active and of short duration of action.

Synthetic α-methyl-substituted relatives have a much longer duration of action and enhanced oral potency.[46]

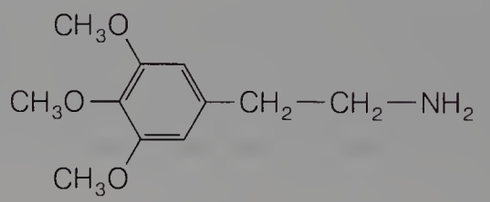


Dimethyltryptamine: $R_4 = R_5 = H$
Bufotenine: $R_4 = H$; $R_5 = OH$
Psilocybin: $R_4 = OPO(OH)_2$; $R_5 = H$
Psilocyn: $R_4 = OH$; $R_5 = H$

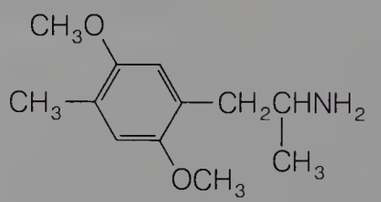
2-PHENYLETHYLAMINES

Mescaline

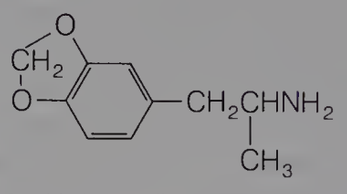
3,4,5-Trimethoxyphenethylamine is a much-studied hallucinogen with many complex effects on the CNS. It occurs in the peyote cactus. The oral dose required for its hallucinogenic activities is very high, as much as 500 mg of the sulfate salt. The low oral potency is probably because of facile metabolism by MAO. α-Methylation increases activity. Synthetic α-methyl-substituted relatives, such as the two illicit drugs DOM and MDA, are more potent than mescaline.[46,47]



Mescaline



1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane
(DOM, STP)



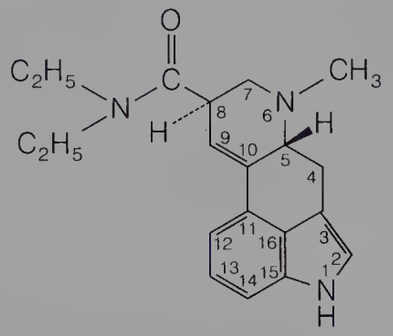
3,4-Methylenedioxyamphetamine
(MDA)

AGENT POSSESSING BOTH AN INDOLETHYLAMINE AND A PHENYLETHYLAMINE MOIETY

(+)-Lysergic Acid Diethylamide (LSD)

In the structure of the extraordinarily potent hallucinogen LSD can be seen both an indolethylamine group and a phenyl-

ethylamine group. The stereochemistry is exceedingly important. Chirality, as shown, must be maintained or activity is lost; likewise, the location of the double bond, as shown, is required.[48] Experimentally, LSD has marked effects on serotoninergic neurons. However, the bases for all of its complex CNS actions are not completely understood.

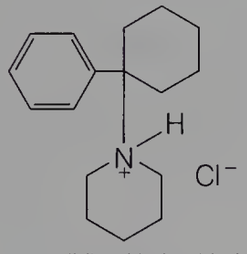


Lysergic Acid Diethylamide

DISSOCIATIVE AGENTS

Phencyclidine (PCP)

PCP was introduced as a dissociative anesthetic for animals. Its close structural relative ketamine is still so employed. The drug produces a sense of intoxication; there are hallucinogenic experiences, not unlike those produced by the anticholinergic hallucinogens, and often amnesia.



Phencyclidine Hydrochloride

The drug affects many systems, including those of NE, DA, and 5-HT. It has been proposed that PCP (and certain other psychotomimetics) produce a unique pattern of activation of ventral tegumental area dopaminergic neurons.[49] It reportedly blocks glutaminergic *N*-methyl-D-aspartate receptors.[50] This probably is the basis for many of its CNS effects. PCP itself appears to be the main active agent, producing its many CNS effects.

EUPHORIANT-STIMULANT

Cocaine

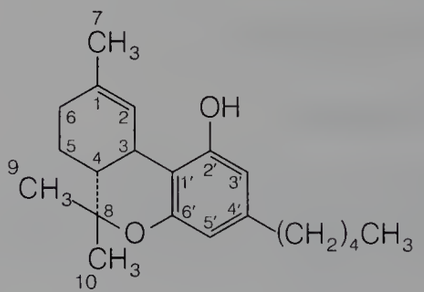
Cocaine as a euphoriant-stimulant, psychotomimetic, and drug of abuse could as well be discussed with amphetamine and methamphetamine, with which it shares many biologic properties. At low doses, it produces feelings of well-being, decreased fatigue, and increased alertness. The drug tends to produce compulsive drug-seeking behavior, and a full-blown toxic psychosis may emerge. Many of these effects appear to be related to the effects of increased availability of DA for interaction with postsynaptic receptors. Cocaine is a potent DA-uptake blocker. (The amphetamines largely increase availability of DA by release.) As with many uptake blockers, the structural requirements do not appear to be highly stringent. A phenethylamine moiety with a bulky group may suffice. If an interaction between a hydrogen atom on the nitrogen of the protonated form of cocaine and an oxygen of the ester group or, alternatively, between the unshared electron pair of the free-base nitrogen and the carbonyl of the ester group occurs, this grouping could be approximated.

For detailed discussions of the toxicology of cocaine including peripheral effects, which appear to be mediated by noradrenergic mechanisms, a review may be consulted.[51]

DEPRESSANT-INTOXICANT

Δ^1-Tetrahydrocannabinol (THC) or Δ^9-THC

There are two conventions for numbering THC: that arising from terpinoid chemistry produces Δ^1-THC, and that based on the dibenzopyran system results in a Δ^9-THC designation. The terpinoid convention is employed in the structure.



(−)-Δ^1-*trans*-Tetrahydrocannabinol

The drug is a depressant with stimulant sensations arising from depression of higher centers. Many effects, reputedly subjectively construed as pleasant, are evident at low doses. The interested reader may consult a pharmacology text for a detailed account of these effects. At higher doses, psychotomimetic actions, including dysphoria, hallucinations, and paranoia, can be marked. Structural features associated with activity have been reviewed.[52] Notably, the phenolic OH is required for activity. Some SARs (especially separation of potency between enantiomers) for cannabinoids suggest action at receptors.[53] The bulk of early evidence suggests that actions are mediated through effects on cell membranes.[54] A receptor for THC has been discovered.[55] The most prominent candidate for the natural ligand is an amide derivative of a product of the arachidonic acid cascade, anandamide.[56]

Medically, in some areas of the United States, THC is available as an effective antiemetic in patients undergoing cancer chemotherapy. Also, the drug has served as a model for other potentially useful agents, such as anticonvulsants.

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CHAPTER 16

Adrenergic Agents

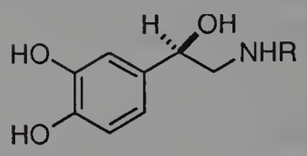
Rodney L. Johnson

Adrenergic drugs are chemical agents that exert their principal pharmacological and therapeutic effects by either enhancing or reducing the activity of the various components of the sympathetic division of the autonomic nervous system. In general, substances that produce effects similar to stimulation of sympathetic nervous activity are known as *sympathomimetics* or *adrenergic stimulants*. Those that decrease sympathetic activity are referred to as *sympatholytics, antiadrenergics*, or *adrenergic-blocking agents*. Because of the important role that the sympathetic nervous system plays in the normal functioning of the body, adrenergic drugs find wide use in the treatment of a number of diseases. In addition to their effects on sympathetic nerve activity, a number of adrenergic agents produce important effects on the central nervous system (CNS). In this chapter, those agents that affect adrenergic neurotransmission and those that act directly on the various types of adrenergic receptors are discussed.

ADRENERGIC NEUROTRANSMITTERS

STRUCTURE AND PHYSICOCHEMICAL PROPERTIES

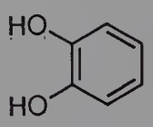
Norepinephrine (NE) is the neurotransmitter of the postganglionic sympathetic neurons. As a result of sympathetic nerve stimulation, it is released from sympathetic nerve endings into the synaptic cleft, where it interacts with specific presynaptic and postsynaptic adrenergic receptors. Another endogenous adrenergic receptor agonist is epinephrine. This compound is not released from peripheral sympathetic nerve endings, as is NE. Rather, it is synthesized and stored in the adrenal medulla, from which it is released into the circulation. Thus, epinephrine is often referred to as a neurohormone. Epinephrine is also biosynthesized in certain neurons of the CNS, where both it and NE serve as neurotransmitters.



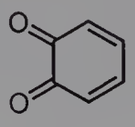
Norepinephrine: R = H

Epinephrine: R = CH_3

Epinephrine and NE belong to the chemical class of substances known as the *catecholamines*. This name was given to these compounds because they contain an amino group attached to an aromatic ring that contains two hydroxyl groups situated *ortho* to each other, the same arrangement of hydroxyl groups as found in catechol. Aromatic compounds that contain such an arrangement of hydroxyl substituents are highly susceptible to oxidation. Catecholamines, such as epinephrine and NE, undergo oxidation in the presence of oxygen (air) or other oxidizing agents to produce *ortho*-quinone-like compounds, which undergo further reactions to give mixtures of colored products. Hence, solutions of catecholamine drugs often are stabilized by the addition of an antioxidant (reducing agent), such as ascorbic acid or sodium bisulfite.



Catechol



ortho-Quinone

Epinephrine and NE each possess a chiral carbon atom; thus, each can exist as an enantiomeric pair of isomers. The enantiomer with the *(R)*-configuration is biosynthesized by the body and possesses the biological activity.

Catecholamines are polar substances that contain both acidic (the aromatic hydroxyls) and basic (the aliphatic amine) functional groups. For example, the pK_a values for the epinephrine cation are 8.7 and 9.9 and are attributed to the phenolic hydroxyl group and the protonated amino group, respectively. Ganellin[1] has calculated the relative

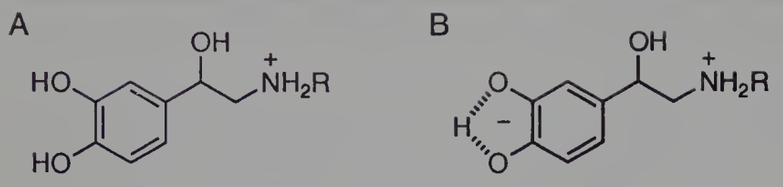


FIG. 16-1. Cationic **A** *and zwitterionic* **B** *forms of norepinephrine (R = H) and epinephrine (R = CH_3).*

populations of the various ionized and non-ionized species of NE and epinephrine at *p*H 7.4 and has found that the cation form (Fig. 16-1A) is present to an extent slightly greater than 95% for both catecholamines. The zwitterionic form (Fig. 16-1B), in which the aliphatic amine is protonated and one of the phenolic hydroxyl groups is ionized, is present to an extent of about 3%. Thus, at physiological *p*H, less than 2% of either epinephrine or NE exists in the non-ionized form. This largely accounts for the high degree of water solubility of these compounds, as well as other catecholamines, such as isoproterenol and dopamine.

BIOSYNTHESIS

The biosynthesis of the catecholamines dopamine, NE, and epinephrine involves a sequence of enzymatic reactions,[2] as illustrated in Fig. 16-2. Catecholamine biosynthesis takes place in adrenergic and dopaminergic neurons in the CNS, in sympathetic neurons of the autonomic nervous system, and in the adrenal medulla. The amino acid L-tyrosine serves as the precursor for the catecholamines. It is transported actively into the axoplasm, where it is acted upon by tyrosine-3-monooxygenase (tyrosine hydroxylase) to form L-dihydroxyphenylalanine (L-dopa). Tyrosine hydroxylase is an Fe^{2+}-containing enzyme that requires molecular oxygen and uses tetrahydrobiopterin as a cofactor. The enzyme plays a key role in the regulation of catecholamine biosynthesis as it is the rate-limiting step. For example, adrenergic nerve stimulation leads to activation of a protein kinase which phosphorylates tyrosine hydroxylase, thereby increasing its activity.[3] In addition, through end-product inhibition, NE markedly reduces tyrosine hydroxylase activity. The basis of this feedback inhibition is believed to be a competition between the catecholamine product and the pterin cofactor.

The second enzymatic step in the catecholamine biosynthesis is the decarboxylation of L-dopa to give dopamine. The enzyme which carries out this transformation is L-aromatic amino acid decarboxylase (dopa decarboxylase). It is a cytoplasmic enzyme that uses pyridoxal phosphate as a cofactor. In addition to being found in catecholaminergic neurons, L-aromatic amino acid decarboxylase is found in high concentrations in many other tissues, including the liver and kidneys. It exhibits broad substrate specificity in that aromatic amino acids, such as L-tyrosine, L-phenylalanine, L-histidine, and L-tryptophan, in addition to L-dopa and L-5-hydroxytryptophan, serve as substrates.

The dopamine formed in the cytoplasm of the neuron is actively transported into storage vesicles, where it is hydroxylated stereospecifically by the Cu^{2+}-containing enzyme dopamine β-monooxygenase (dopamine β-hydroxylase) to give NE. Dopamine β-hydroxylase requires molecular oxygen and uses ascorbic acid as a cofactor. It exhibits a rather

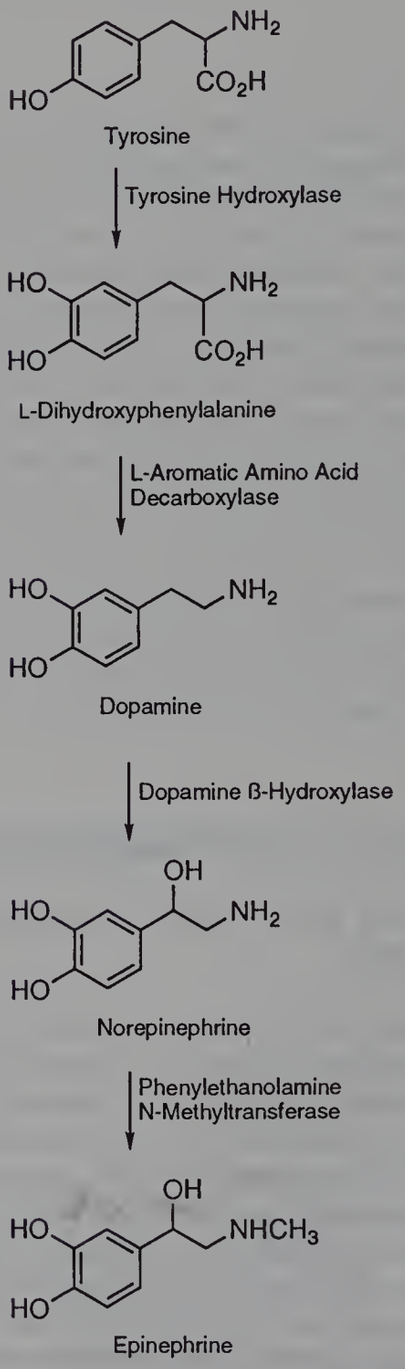


FIG. 16-2. Biosynthesis of the catecholamines dopamine, norepinephrine, and epinephrine.

wide degree of substrate specificity. The NE formed is stored in the vesicles until depolarization of the neuron initiates the process of vesicle fusion with the plasma membrane and extrusion of NE into the synaptic cleft. Adenosine triphosphate (ATP) and the protein chromogranin A are released along with NE.

In the adrenal medulla, NE is converted to epinephrine. This reaction, which involves the transfer of a methyl group from *S*-adenosyl methionine to NE, is catalyzed by phenylethanolamine-*N*-methyltransferase (PNMT) and occurs in the cell cytoplasm. Although PNMT is highly localized in the adrenal medulla, it is also present in small amounts in heart and brain tissues. The epinephrine formed is held in the storage granules of the chromaffin cells.

UPTAKE AND METABOLISM

The action of NE at adrenergic receptors is terminated by a combination of processes, including uptake into the neuron and into extraneuronal tissues, diffusion away from the synapse, and metabolism. Usually the primary mechanism for termination of the action of NE is reuptake of the catecholamine into the nerve terminal. This process is termed "uptake-1" and involves a membrane energy-requiring pump system that has a high affinity for NE.[4] This uptake system will also transport certain amines other than NE into the nerve terminal, and it is the site of action of cocaine and some of the tricyclic antidepressants. Some of the NE that reenters the sympathetic neuron is transported into storage granules, where it is held in a stable complex with ATP and protein until sympathetic nerve activity or some other stimulus causes it to be released into the synaptic cleft.

In addition to the neuronal uptake of NE discussed above, there exists an extraneuronal uptake process, uptake-2. This uptake process is present in a wide variety of cells, including glial, hepatic, and myocardial cells. It has relatively low affinity for NE. Although its physiological significance is unknown, it may play a role in the disposition of circulating catecholamines since catecholamines that are taken up into extraneuronal tissues are metabolized rapidly.

The two principal enzymes involved in catecholamine metabolism are monoamine oxidase (MAO) and catechol *O*-methyltransferase (COMT).[5] Both of these enzymes are distributed throughout the body, with high concentrations found in the liver and kidneys. MAO is associated primarily with the outer membrane of the mitochondria, while COMT is found primarily in the cytoplasm. The wide tissue distribution of MAO and COMT indicates that both act upon catecholamines that enter the circulation and the extraneuronal tissues after being released from nerves or the adrenal gland or after being administered exogenously. In addition, the fact that COMT is not present in sympathetic neurons, whereas the neuronal mitochondria do contain MAO, indicates that MAO also has a role in the metabolism of intraneuronal catecholamines.

Neither COMT nor MAO exhibits a high degree of substrate specificity. MAO oxidatively deaminates a variety of compounds that contain an amino group attached to a terminal carbon. There are two types of MAO, and these exhibit different substrate selectivity. For example, MAO-A shows substrate preference for NE and serotonin, while MAO-B shows substrate selectivity for β-phenylethylamine and benzylamine. Similarly, COMT catalyzes the methylation of a variety of catechol-containing molecules. The lack of substrate specificity of COMT and MAO is manifested in the metabolic disposition of NE and epinephrine, shown in Fig. 16-3. Not only do both MAO and COMT utilize NE and epinephrine as substrates, but each also acts upon the metabolites produced by the other.

The results of extensive research on catecholamine metabolism indicate that in the adrenergic neurons of human brain and peripheral tissues NE is deaminated oxidatively by MAO to give 3,4-dihydroxyphenylglycolaldehyde, which then is reduced by aldehyde reductase to 3,4-dihydroxyphenylethylene glycol. It is primarily this glycol metabolite that is released into the circulation, where it undergoes methylation by the COMT that it encounters in non-neuronal tissues. The product of methylation, 3-methoxy-4-hydroxyphenylethylene glycol, is oxidized by alcohol dehydrogenase and aldehyde dehydrogenase to give 3-methoxy-4-hydroxymandelic acid. This metabolite commonly is referred to as vanillylmandelic acid (VMA), and although it can be the end product of several pathways of NE metabolism, 3-methoxy-4-hydroxyphenylethylene glycol is its principal precursor. In the oxidative deamination of NE and epinephrine at extraneuronal sites such as the liver, the aldehyde which is formed is oxidized usually by aldehyde dehydrogenase to give 3,4-dihydroxymandelic acid.

Methylation by COMT occurs almost exclusively on the *meta*-hydroxyl group of the catechol, regardless of whether the catechol is NE, epinephrine, or one of the metabolic products. For example, the action of COMT upon NE and epinephrine gives normetanephrine and metanephrine, respectively. A converging pattern of NE metabolism of NE and epinephrine in which 3-methoxy-4-hydroxymandelic acid and 3-methoxy-4-hydroxyphenylethylene glycol are common end products thus occurs, regardless of whether the first metabolic step is oxidation by MAO or methylation by COMT.

Under normal circumstances, 3-methoxy-4-hydroxymandelic acid is the principal urinary metabolite of NE, though substantial amounts of 3-methoxy-4-hydroxyphenylethylene glycol are excreted along with varying quantities of other metabolites, both in the free form and as sulfate or glucuronide conjugates. Endogenous epinephrine is excreted primarily as metanephrine and 3-methoxy-4-hydroxymandelic acid.

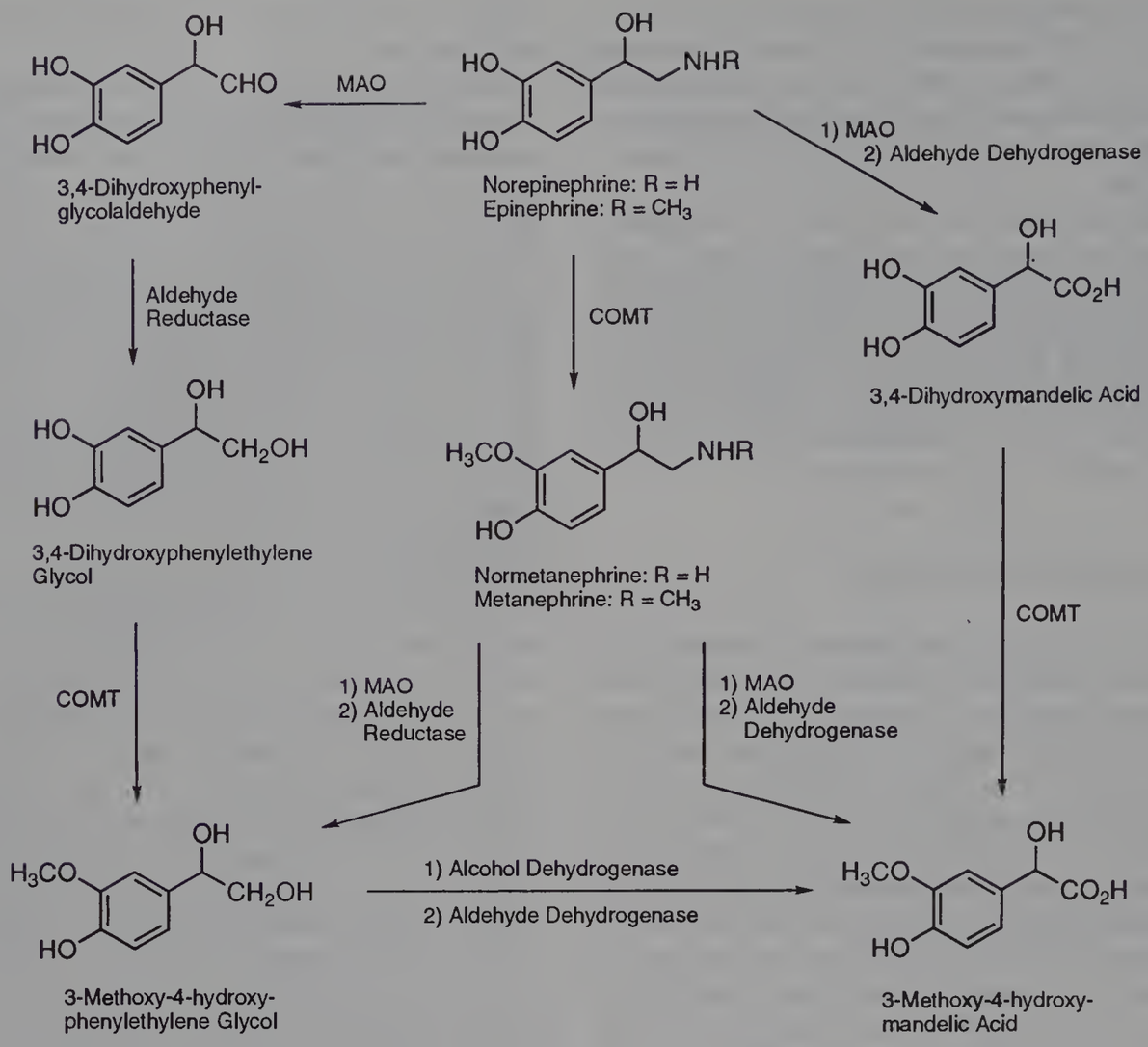


FIG. 16-3. *Metabolism of norepinephrine and epinephrine by MAO and COMT.*

ADRENERGIC RECEPTORS

α-ADRENERGIC RECEPTORS

Ahlquist was the first to propose the existence of two general types of adrenergic receptors (adrenoceptors) in mammalian tissues.[6] He designated these adrenergic receptors α and β. His hypothesis was based on the differing relative potencies of a series of adrenergic receptor agonists on various smooth muscle preparations. In the early 1970s, the discovery that certain adrenergic agonists and antagonists exhibited various degrees of selectivity for presynaptic and postsynaptic adrenergic α-receptors led to the proposal that postsynaptic α-receptors be designated α_1 and that presynaptic α-receptors be referred to as α_2.[7] Later, a functional classification of the α-receptors was proposed wherein α_1-receptors were designated as those that were excitatory in nature, while α_2-receptors purportedly mediated inhibitory responses.[8] Further developments revealed, however, that both α_1- and α_2-receptors could be either presynaptic or postsynaptic and either excitatory or inhibitory in their responses. Thus, it became clear that neither an anatomical nor a functional classification system was as generally useful in classifying adrenergic receptors as a pharmacological classification based upon the relative potency of a series of receptor agonists and antagonists.[9] Pharmacological and molecular biological methods have shown that it is possible to subdivide the α_1- and α_2-receptors into additional subtypes. Although the subtyping of adrenergic receptors continues to evolve, at present, the α_1- and α_2-receptors each have been divided into at least three subtypes, which have been designated as α_{1A}, α_{1B}, and α_{1D} and α_{2A}, α_{2B}, α_{2C}, respectively.[10–12]

The molecular basis by which activation of α-adrenergic receptors produces the appropriate tissue responses has been studied extensively. Both receptor subtypes belong to a superfamily of membrane receptors whose general structure consists of seven transmembrane α-helical segments and whose signal-transduction mechanisms involve coupling to guanine nucleotide-regulatory proteins (G-proteins).[13] They differ from each other, however, in the second-messenger system that is affected.[14,15] The α_1-adrenergic receptor is

coupled to the enzyme phospholipase C via a G-protein, G_q. When stimulated by activation of the α_1-adrenergic receptor, phospholipase C hydrolyzes phosphatidylinositol-4,5-bisphosphate to give the second messengers inositol-1,4,5-triphosphate [Ins(1,4,5)P_3] and 1,2-diacylglycerol (DAG). Ins(1,4,5)P_3 stimulates the release of Ca^{2+} from the sarcoplasmic reticulum, while DAG activates protein kinase C, an enzyme that phosphorylates proteins. α_1-Receptor activation also can increase the influx of extracellular Ca^{2+} via voltage-dependent as well as non-voltage-dependent Ca^{2+} channels. Activation of α_2-adrenergic receptors leads to a reduction in the catalytic activity of adenylyl cyclase, which in turn results in a lowering of intracellular levels of cyclic-3′,5′-adenosine monophosphate (cAMP). The α_2-adrenergic receptor-mediated inhibition of adenylyl cyclase is regulated by the G-protein G_i.

α-Adrenergic receptors of the CNS and in peripheral tissues affect a number of important physiological functions.[14] In particular, α-receptors are involved in control of the cardiovascular system. For example, constriction of vascular smooth muscle is mediated by both postjunctional α_1- and α_2-adrenergic receptors, though the predominant receptor mediating this effect is α_1.[16] In the heart, activation of α_1-receptors results in a selective inotropic response with little or no change in heart rate.[17] This is in contrast to the β_1-receptor, which is the predominant postjunctional receptor in the heart, mediating both inotropic and chronotropic effects. In the brain, activation of postjunctional α_2-receptors reduces sympathetic outflow from the CNS, which in turn causes a lowering of blood pressure.[18] The prototypical α_2-receptor is the presynaptic α-receptor found on the terminus of the sympathetic neuron.[7,8,19] Interaction of this receptor with agonists such as NE and epinephrine results in inhibition of NE release from the neuron. Thus, α_2-receptors play a role in the regulation of NE release. This receptor also mediates inhibition of acetylcholine release from parasympathetic nerves. Both α_1- and α_2-adrenergic receptors also play an important role in the regulation of a number of metabolic processes, such as insulin secretion and glycogenolysis.[20]

β-ADRENERGIC RECEPTORS

In 1967, almost 20 years after Ahlquist's landmark paper proposing the existence of α- and β-adrenergic receptors, Lands and co-workers[21] suggested that β-receptors also could be subdivided into β_1 and β_2 types. Seventeen years later, Arch et al.[22] identified a third subtype of β-receptor in brown adipose tissue. They initially referred to this as an atypical β-receptor, but it later became designated the β_3 subtype.[12] These β-adrenergic receptor subtypes differ in terms of the rank order of potency of the adrenergic receptor agonists NE, epinephrine, and isoproterenol. β_1-Receptors exhibit the agonist potency order isoproterenol > epinephrine = NE, while β_2-receptors exhibit the agonist potency order isoproterenol > epinephrine $\gg$ NE. For the β_3-receptor, the agonist potency order is isoproterenol = NE > epinephrine.

β_1-Receptors are located mainly in the heart, where they mediate the positive inotropic and chronotropic effects of the catecholamines. They are also found on the juxtaglomerular cells of the kidney, where they are involved in increasing renin secretion. β_2-Receptors are located on smooth muscle throughout the body, where they are involved in relaxation of the smooth muscle, producing such effects as bronchodilation and vasodilation. They are also found in the liver, where they promote glycogenolysis. The β_3-receptor is located on brown adipose tissue and is involved in the stimulation of lipolysis.

Like the α-adrenergic receptors, the β-adrenergic receptors belong to the superfamily of membrane receptors whose general structure consists of seven transmembrane α-helical segments and whose signal-transduction mechanisms involve coupling to G-proteins.[13] All three β-receptors are coupled to adenylyl cyclase, which catalyzes the conversion of ATP to cAMP. This coupling is via the guanine nucleotide protein G_s.[23,24] In the absence of agonist, guanosine diphosphate (GDP) is bound reversibly to the G_s protein. Interaction of the agonist with the receptor is believed to bring about a conformational change in the protein receptor, which causes a reduction in the affinity of the G_s protein for GDP and a concomitant increase in affinity for guanosine triphosphate (GTP). The α_s-subunit of the G_s protein, with GTP bound to it, dissociates from the receptor–G-protein ternary complex, binds to adenylyl cyclase, and activates the enzyme. The bound GTP then undergoes hydrolysis to GDP, and the receptor–G_s protein complex returns to the basal state.

The intracellular function of the second messenger cAMP appears to be activation of protein kinases, which phosphorylate specific proteins, thereby altering their function. Thus, the phosphorylated proteins mediate the actions of cAMP, which functions as the mediator of the action of the drug or neurotransmitter that originally interacted with the β-receptor.[25] The action of cAMP is terminated by a class of enzymes known as phosphodiesterases, which catalyze the hydrolysis of cAMP to AMP.

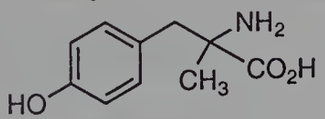
Cloning of the gene and cDNA for the mammalian β-adrenergic receptor has made it possible to explore through single point mutations and the construction of chimeric receptors the structure–function relationships of the receptor.[26] Through such studies, it has been proposed that the adrenergic agonist-binding site is within the transmembrane-spanning regions, while the cytoplasmic regions of the receptor interact with the G_s protein. Specifically, aspartic acid residue 113 in transmembrane region III acts as the counterion to the cationic amino group of the adrenergic agonist, while two serine residues, at positions 204 and 207 in transmembrane region V, form hydrogen bonds with the catechol hydroxyls of the adrenergic agonists. The β-hydroxyl group of adrenergic agonists interacts with the phenylalanine resi-

due at position 290 in transmembrane region VI. Information such as this will no doubt aid in the future design and synthesis of new and improved adrenergic receptor agonists and antagonists.

DRUGS AFFECTING ADRENERGIC NEUROTRANSMISSION

DRUGS AFFECTING CATECHOLAMINE BIOSYNTHESIS

Many agents that affect catecholamine biosynthesis are known, but only a few are used as therapeutic agents. One example of a catecholamine-biosynthesis inhibitor in clinical use is **metyrosine** (α-methyl-*p*-tyrosine, Demser).[27] Metyrosine differs structurally from tyrosine only in the presence of an α-methyl group. It is a competitive inhibitor of tyrosine hydroxylase, the first and rate-limiting step in catecholamine biosynthesis. As such, metyrosine is a much more effective inhibitor of epinephrine and NE production than agents that inhibit any of the other enzymes involved in catecholamine biosynthesis. Although metyrosine is used as a racemic mixture, it is the (−)-isomer that possesses the inhibitory activity. Metyrosine, which is given orally in dosages ranging from 0.6 to 4 g/day, is used for the preoperative management of pheochromocytoma. This condition involves chromaffin cell tumors that produce large amounts of NE and epinephrine. Although these tumors, which occur in the adrenal medulla, are often benign, patients frequently suffer hypertensive episodes. Metyrosine reduces the frequency and severity of these episodes by lowering catecholamine production. The drug is excreted mainly unchanged in the urine. Its most serious side effect is crystalluria.

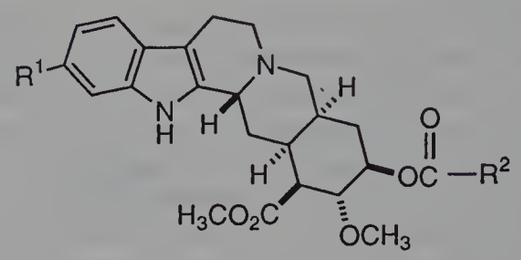


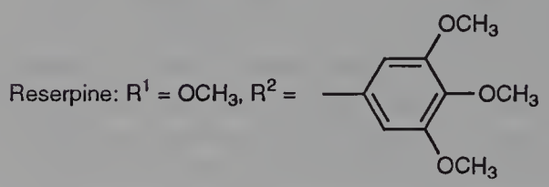
Metyrosine

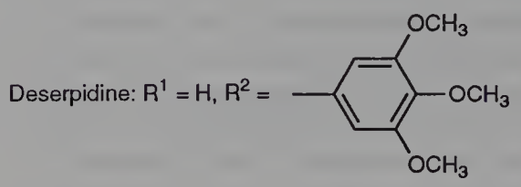
DRUGS AFFECTING CATECHOLAMINE STORAGE AND RELEASE

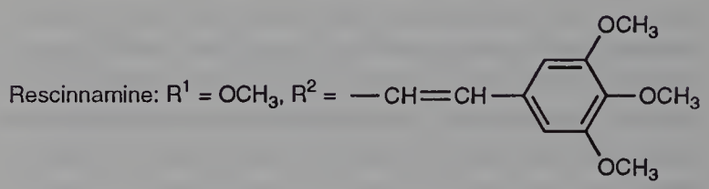
Reserpine (Serpasil) is the prototypical drug affecting the vesicle storage of NE in sympathetic neurons and neurons of the CNS and of epinephrine in the adrenal medulla. Its actions are not limited to NE and epinephrine, however, as it also affects the storage of serotonin and dopamine in their respective neurons in the brain. Reserpine is an indole alkaloid obtained from the root of *Rauwolfia serpentina*, a climbing shrub found in India. Reserpine binds extremely tightly with the ATP-driven monoamine transporter that transports NE and other biogenic amines from the cytoplasm into the storage vesicles.[28] This binding leads to a blockade of the transporter. Thus, in sympathetic neurons NE, which normally is transported into the storage vesicles, is instead metabolized by mitochondrial MAO in the cytoplasm. In addition, there is a gradual loss of vesicle-stored NE as it is used up by release resulting from sympathetic nerve activity. It is thought that the storage vesicles eventually are destroyed. The end result is a depletion of NE in the sympathetic neuron. Analogous effects are seen in the adrenal medulla with epinephrine and in serotonergic neurons.

Reserpine exhibits a slow onset of action and a sustained effect up to several weeks after the last dose has been given. It is indicated for the treatment of hypertension and psychotic disorders such as schizophrenia in patients who cannot tolerate the phenothiazines. Other available preparations include the powdered whole root of *R. serpentina* (Raudixin, Rauverid), **deserpidine** (Harmonyl), and **rescinnamine** (Moderil).





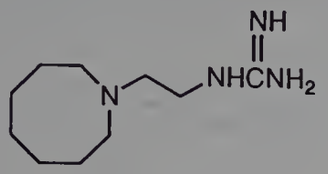




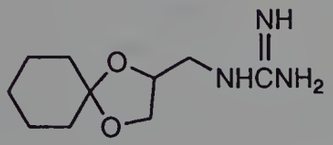
Neuronal blocking agents are drugs that produce their pharmacological effects primarily by preventing the release of NE from sympathetic nerve terminals. Drugs of this type enter the adrenergic neuron by way of the uptake-1 process and accumulate within the neuronal storage vesicles. There, they stabilize the neuronal storage vesicle membranes, making them less responsive to nerve impulses. The ability of the vesicles to fuse with the neuronal membrane is diminished, resulting in inhibition of NE release into the synaptic cleft. These agents also cause the release of stored NE and with time can produce a depletion of NE stores in sympathetic neurons.

Structurally, the neuronal blocking drugs typically possess a guanidino moiety [—NHC(=NH)NH_2], which is attached to either an alicyclic or an aromatic lipophilic group. These structural features are seen in **guanethidine** (Ismelin) and

guanadrel (Hylorel), which are used clinically in the treatment of hypertension. The presence of the very basic guanidino group ($pK_a > 12$) in these drugs means that at physiological *p*H they are essentially completely protonated. Thus, these agents do not get into the CNS.



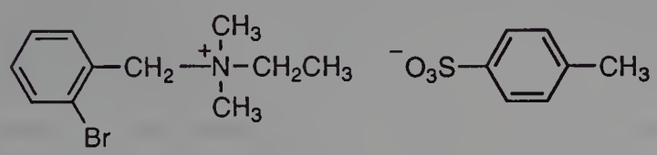
Guanethidine



Guanadrel

Although guanethidine and guanadrel have virtually the same mechanism of action on sympathetic neurons, they differ in their pharmacokinetic properties. For example, while guanethidine is absorbed incompletely after oral administration (3% to 50%), guanadrel is well absorbed, with a bioavailability of 85%.[29] These two agents also differ in terms of half-life: guanethidine has a half-life of around 5 days, while the half-life of guanadrel is 12 hr.

Another neuronal blocking agent is the aromatic quaternary ammonium compound **bretylium tosylate** (Bretylol). This agent is used as an antiarrhythmic drug. Its antiarrhythmic actions are not believed to be due to its neuronal blocking effects, however. This agent is discussed in more detail in Chapter 19.



Bretylium Tosylate

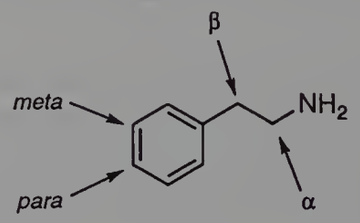
SYMPATHOMIMETIC AGENTS

Sympathomimetic agents produce effects resembling those produced by stimulation of the sympathetic nervous system. They may be classified as agents that produce effects by a direct, indirect, or mixed mechanism of action. Direct-acting agents elicit a sympathomimetic response by interacting directly with adrenergic receptors. Indirect-acting agents produce effects primarily by causing the release of NE from adrenergic nerve terminals; the NE that is released by the indirect-acting agent activates the receptors to produce the response. Compounds with a mixed mechanism of action interact directly with adrenergic receptors and cause the release of NE. As described below, the mechanism by which an agent produces its sympathomimetic effect is related intimately to its chemical structure.

DIRECT-ACTING SYMPATHOMIMETICS

Structure–Activity Relationships

Structure–activity relationships for α- and β-adrenergic receptor agonists have been reviewed.[30–32] The parent structure for many of the sympathomimetic drugs is β-phenylethylamine. The manner in which β-phenylethylamine is substituted on the *meta*- and *para*-positions of the aromatic ring and on the amino, α, and β positions of the ethylamine side chain influences not only the mechanism of sympathomimetic action but also the receptor selectivity of the drug. For the direct-acting sympathomimetic amines, maximal activity is seen in β-phenylethylamine derivatives containing hydroxyl groups in the *meta*- and *para*-positions of the aromatic ring (a catechol) and a β-hydroxyl group of the correct stereochemical configuration on the ethylamine portion of the molecule. Such structural features are seen in the prototypical direct-acting compounds NE, epinephrine, and isoproterenol.



β-Phenylethylamine

A critical factor in the interaction of adrenergic agonists with their receptors is stereoselectivity. Direct-acting sympathomimetics that exhibit chirality by virtue of the presence of a β-hydroxyl group (phenylethanolamines) invariably exhibit a high degree of stereoselectivity in producing their agonistic effects; that is, one enantiomeric form of the drug has greater affinity for the receptor than the other form. This is true for both α- and β-receptor agonists. For epinephrine, NE, and related compounds, the more potent enantiomer has the (*R*)-configuration. This enantiomer is typically several hundredfold more potent than the enantiomer with the (*S*)-configuration. It appears that for all direct-acting, β-phenylethylamine–derived agonists that are structurally similar to NE, the more potent enantiomer is capable of assuming a conformation that results in the arrangement in space of the catechol group, the amino group, and the β-hydroxyl group in a fashion resembling that of (−)-(*R*)-NE. This explanation of stereoselectivity is based on the presumed interaction of these three critical pharmacophoric groups with three complementary binding areas on the receptor and is known as the Easson-Stedman hypothesis.[15,33] This three-point interaction is supported by recent site-directed mutagenesis studies[26] on the adrenergic receptor and is illustrated in Fig. 16-4.

The presence of the amino group in phenylethylamines is important for direct agonist activity. The amino group should be separated from the aromatic ring by two carbon atoms

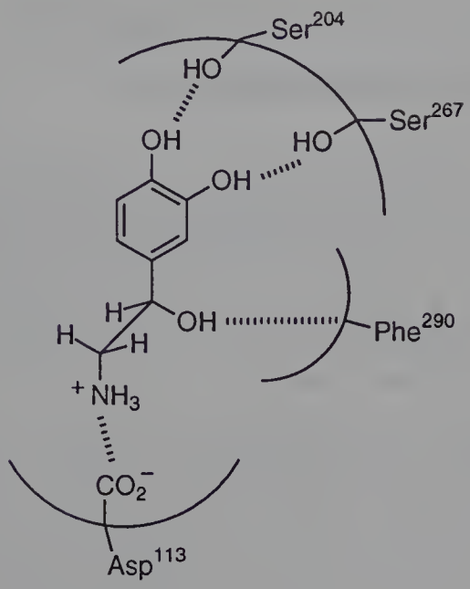
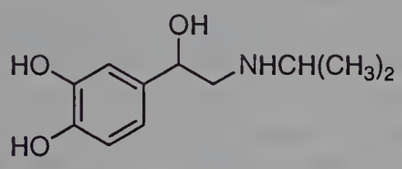
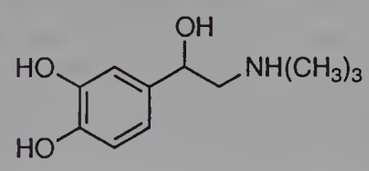


FIG. 16-4. *Illustration of the Easson-Stedman hypothesis representing the interaction of three critical pharmacophoric groups of norepinephrine with the complementary binding areas on the adrenergic receptor as suggested by site-directed mutagenesis studies.*

for optimal activity. Both primary and secondary amines are found among the potent direct-acting agonists, but tertiary or quarternary amines tend to be poor direct agonists. The nature of the amino substituent dramatically affects the receptor selectivity of the compound. In general, as the bulk of the nitrogen substituent increases, α-receptor agonist activity decreases and β-receptor activity increases. Thus, NE, which is an effective β_1-receptor agonist, is also a potent α-agonist, while epinephrine is a potent agonist at α-, β_1-, and β_2-receptors. Isoproterenol, however, is a potent β_1- and β_2-receptor agonist but has little affinity for α-receptors. The nature of the substituent can also affect β_1/β_2-receptor selectivity. In several instances, it has been shown that an *N-tert*-butyl group enhances β_2-selectivity. For example, *N-tert*-butylnorepinephrine (Colterol) is nine to ten times as potent an agonist at tracheal β_2-receptors than at cardiac β_1-receptors. Large substituents on the amino group also protect the amino group from undergoing oxidative deamination by MAO.

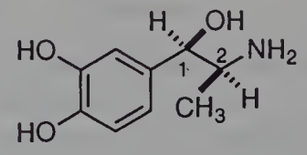


Isoproterenol



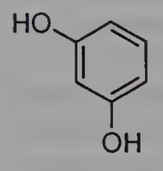
N-*tert*-Butylnorepinephrine (Colterol)

Methyl or ethyl substitution on the α-carbon of the ethylamine side chain reduces direct receptor agonist activity at both α- and β-receptors. Importantly, however, an α-alkyl group increases the duration of action of the phenylethylamine agonist by making the compound resistant to metabolic deamination by MAO. Such compounds often exhibit enhanced oral effectiveness and greater CNS activity than their counterparts that do not contain an α-alkyl group. α-Substitution also significantly affects receptor selectivity. In the case of β-receptors, for example, α-methyl or ethyl substitution results in compounds with selectivity toward the β_2-receptor, while in the case of α-receptors, α-methyl substitution gives compounds with selectivity toward the α_2-receptor. Another effect of α-substitution is the introduction of a chiral center, which has pronounced effects on the stereochemical requirements for activity. For example, with α-methylnorepinephrine, it is the *erythro* (1*R*,2*S*)-isomer that possesses significant activity at α-receptors.

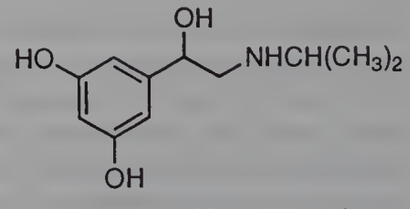


(1*R*,2*S*)-α-Methylnorepinephrine

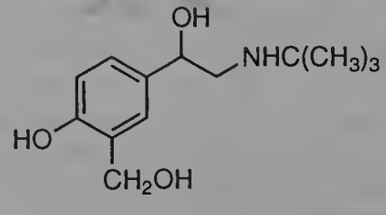
Although the catechol moiety is an important structural feature in terms of yielding compounds with maximal agonist activity at adrenergic receptors, it can be replaced with other substituted phenyl moieties to provide selective adrenergic agonists. In particular, this approach has been used in the design of selective β_2-receptor agonists. For example, replacement of the catechol function of isoproterenol with the resorcinol structure gives the drug metaproterenol, which is a selective β_2-receptor agonist. Furthermore, since the resorcinol ring is not a substrate for COMT, β-agonists that contain this ring structure tend to have better absorption characteristics and a longer duration of action than their catechol-containing counterparts. In another approach, replacement of the *meta*-hydroxyl of the catechol structure with a hydroxymethyl group gives agents, such as albuterol, which show selectivity to the β_2-receptor. As in the case of the resorcinol modification, this type of substitution gives agents that are not metabolized by COMT and thus show improved oral bioavailability.



Resorcinol

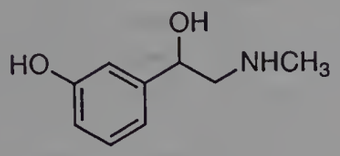


Metaproterenol



Albuterol

Modification of the catechol ring can also bring about selectivity at α-receptors as it appears that the catechol moiety is more important for agonist activity at α_2-receptors than at α_1-receptors. For example, removal of the *para*-hydroxyl group from epinephrine gives phenylephrine, which, in contrast to epinephrine, is selective for the α_1-adrenergic receptor.



Phenylephrine

In addition to the β-phenylethylamine class of adrenergic receptor agonists, there is a second chemical class of compounds, the imidazolines, that give rise to α-adrenergic receptor agonists. Structurally, imidazolines for the most part have the heterocyclic imidazoline nucleus linked to a substituted aromatic moiety via some type of bridging unit (Fig. 16-5).[31] Although modification of the imidazoline ring generally results in compounds with significantly reduced agonist activity, there are examples of so-called open-ring imidazolines, which are highly active. Examples of these are described below. The optimum bridging unit (X) is usually a single amino or methylene group. The nature of the aromatic moiety, as well as how it is substituted, is quite flexible. However, agonist activity is enhanced when the aromatic ring is substituted with halogen substituents like Cl or small alkyl groups like methyl, particularly when they are placed in the two *ortho*-positions. Since the structure–activity relationships of the imidazolines are quite different from those of the β-phenylethylamines, it has been postulated that the imidazolines interact with α-adrenergic receptors differently from the way the β-phenylethylamines do, particularly with regard to the aromatic moiety.

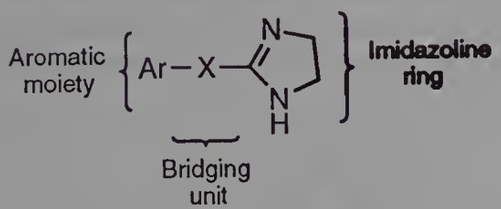
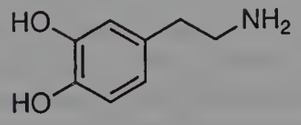


FIG. 16-5. *General structural features of the imidazoline* α-adrenergic receptor agonists.

Endogenous Catecholamines

The three naturally occurring catecholamines dopamine, NE, and epinephrine are used as therapeutic agents. **Dopamine** (Intropin) is used in the treatment of shock. It is ineffective orally in large part because it is substrate for both MAO and COMT. Thus, it is used intravenously. In contrast with the catecholamines NE and epinephrine, dopamine increases blood flow to the kidney in doses that have no chronotropic effect on the heart or that cause no increase in blood pressure. The increased blood flow to the kidneys enhances glomerular filtration rate, Na^+ excretion, and, in turn, urinary output. The dilation of renal blood vessels produced by dopamine is the result of its agonist action on the D_1-dopamine receptor.



Dopamine

In doses slightly higher than those required to increase renal blood flow, dopamine stimulates the β_1-receptors of the heart to increase cardiac output. Some of the effects of dopamine on the heart are also due to NE release. Infusion at a rate greater than 10 μg/kg/minute results in stimulation of α_1-receptors, leading to vasoconstriction and an increase in arterial blood pressure.

Norepinephrine (Levophed) is used to maintain blood pressure in acute hypotensive states resulting from surgical or nonsurgical trauma, central vasomotor depression, and hemorrhage. Like the other endogenous catecholamines, it is a substrate for both MAO and COMT and, thus, is not effective by the oral route of administration. It is given by intravenous injection. Sodium bisulfite is often used in preparations of NE to protect it against oxidation.

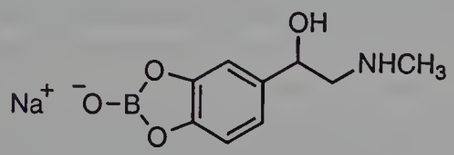
Epinephrine (Adrenalin) finds use in a number of situations because of its potent stimulatory effects on both α- and β-adrenergic receptors. Like the other catecholamines, epinephrine is light-sensitive and easily oxidized on exposure to air because of the catechol ring system. The development of a pink to brown color is indicative of oxidative breakdown. To minimize oxidation, solutions of the drug are stabilized by the addition of reducing agents such as sodium bisulfite. As the free amine, it is used in aqueous solution for inhalation. Like other amines, it forms salts with acids; for example, those now used include the hydrochloride, nitrate, and bitartrate. The bitartrate has the advantage of being less acidic and, therefore, is used in the eye because its solutions have a *p*H close to that of lacrimal fluid. Epinephrine is destroyed readily in alkaline solutions, and by metals (e.g., Cu, Fe, Zn), weak oxidizing agents, and oxygen of the air. It is not effective by the oral route due to poor absorption and rapid metabolism by MAO and COMT.

Although intravenous infusion of epinephrine has pronounced effects on the cardiovascular system, its use in the treatment of heart block or circulatory collapse is limited because of its tendency to induce cardiac arrhythmias. It increases systolic pressure by increasing cardiac output, and it lowers diastolic pressure by causing an overall decrease in peripheral resistance; the net result is little change in mean blood pressure.

Epinephrine is of value as a constrictor in hemorrhage or nasal congestion. Also, it is used to enhance the activity of local anesthetics. Its use in these two situations takes advantage of the drug's potent stimulatory effects on α-re-

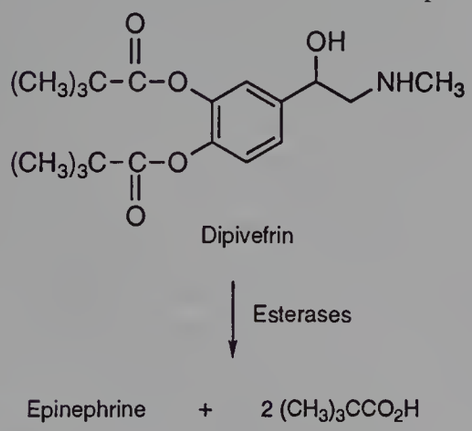
ceptors. The ability of epinephrine to stimulate β_2-receptors has led to its use by injection and by inhalation to relax bronchial smooth muscle in asthma and in anaphylactic reactions. Several over-the-counter preparations (e.g., Primatene, Bronkaid) used for treating bronchial asthma employ epinephrine.

Epinephrine is used in the treatment of open-angle glaucoma, where it apparently reduces intraocular pressure by increasing the rate of outflow of aqueous humor from the anterior chamber of the eye. The irritation often experienced upon instillation of epinephrine into the eye has led to the development of other preparations of the drug that potentially are not as irritating. One such example is the soluble complex between boric acid and epinephrine, **epinephryl borate** (Epinal). This complex forms at neutral or slightly alkaline *p*H. A buffered solution has a *p*H of about 7.4, which it is claimed causes less stinging upon application to the eye. In the lacrimal fluid, epinephryl borate immediately dissociates to yield free epinephrine.



Epinephryl Borate

Dipivefrin (Propine) is the pivalic acid ester prodrug of epinephrine. It is formed by the esterification of the catechol hydroxyl groups of epinephrine with pivalic acid. Dipivefrin is much more lipophilic than epinephrine, and it achieves a much better penetration of the eye when administered topically as an aqueous solution for the treatment of primary open-angle glaucoma. It is converted to epinephrine by esterases in the cornea and anterior chamber. Dipivefrin offers the advantage of being less irritating to the eye than epinephrine, and because of its more efficient transport into the eye, it can be used in lower concentrations than epinephrine.

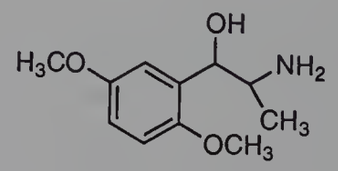


Dipivefrin

α-Adrenergic Receptor Agonists

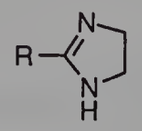
Phenylephrine (Neo-Synephrine, structure shown under "Structure–Activity Relationships," above) is the prototypical selective direct-acting α_1-receptor agonist. It is a potent vasoconstrictor and is active when given orally. Its duration of action is about twice that of epinephrine. It is metabolized by MAO, but since it lacks the catechol moiety, it is not metabolized by COMT. It is relatively nontoxic and produces little CNS stimulation. When applied to mucous membranes, it reduces congestion and swelling by constricting the blood vessels of the membranes. Thus, one of its main uses is in the relief of nasal congestion. In the eye, it is used to dilate the pupil and to treat open-angle glaucoma. It also is used in spinal anesthesia, to prolong the anesthesia and to prevent a drop in blood pressure during the procedure. Another use is in the treatment of severe hypotension due either to shock or to drug administration.

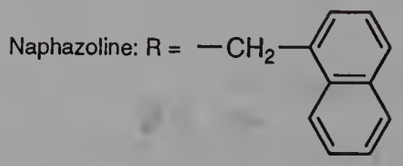
Another selective direct-acting α_1-receptor agonist used therapeutically is **methoxamine** (Vasoxyl). This drug is a potent vasoconstrictor that has no stimulant action on the heart. In fact, it tends to slow the ventricular rate because of an activation of the carotid sinus reflex. It is used primarily during surgery to maintain adequate arterial blood pressure, especially in conjunction with spinal anesthesia. It does not stimulate the CNS.

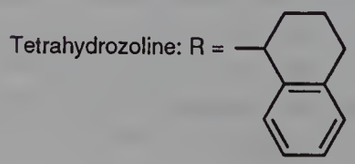


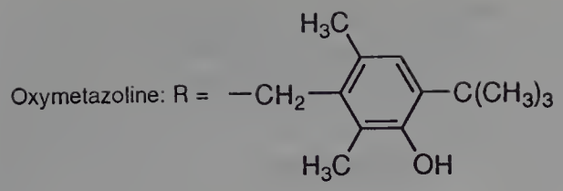
Methoxamine

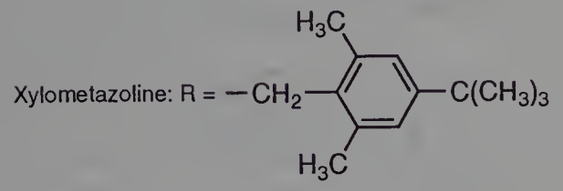
The 2-aralkylimidazolines **naphazoline** (Privine), **tetrahydrozoline** (Tyzine, Visine), **xylometazoline** (Ortrivin), and **oxymetazoline** (Afrin) are partial agonists at both α_1- and α_2-adrenergic receptors. These agents are used for their vasoconstrictive effects as nasal and ophthalmic decongestants. They have limited access to the CNS since they essentially exist in an ionized form at physiological *p*H because of the very basic nature of the imidazoline ring (pK_a = 9 to 10).



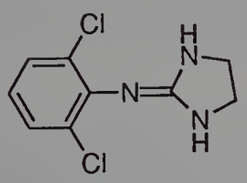








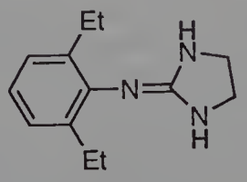
Clonidine (Catapres) is an example of a (phenylimino)imidazolidine derivative that possesses selectivity for the α_2-adrenergic receptor. Under certain conditions, such as intravenous infusion, clonidine can briefly exhibit vasoconstrictive activity as a result of stimulation of peripheral α-adrenergic receptors. However, this hypertensive effect, if it occurs, is followed by a much longer-lasting hypotensive effect as a result of the ability of clonidine to enter into the CNS and stimulate α_2-receptors located in regions of the brain, such as the nucleus tractus solitarius. Stimulation of these α_2-receptors brings about a decrease in sympathetic outflow from the CNS, which in turn leads to decreases in peripheral vascular resistance and blood pressure.[18,34] Bradycardia is also produced by clonidine as a result of a centrally induced facilitation of the vagus nerve and stimulation of cardiac prejunctional α_2-adrenergic receptors.[35] These pharmacological actions have made clonidine quite useful in the treatment of hypertension.



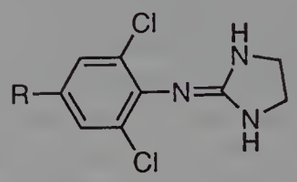
Clonidine

The ability of clonidine and its analogues to exert an antihypertensive effect depends on the ability of these compounds to not only interact with the α_2-receptor but also to gain entry into the CNS. For example, in the case of clonidine, the basicity of the guanidine group (typically pK_a = 13.6) is decreased to 8.0 (the pK_a of clonidine) because of its direct attachment to the dichlorophenyl ring. Thus, at physiological *p*H, clonidine will exist to a significant extent in the non-ionized form required for passage into the CNS.

Substitutions on the aromatic ring also affect the ability of clonidine and its analogues to gain entry into the CNS to produce an antihypertensive effect. For example, although various halogen and alkyl substitutions can be placed at the two *ortho*-positions of the (phenylimino)imidazolidine nucleus without affecting the affinity of the derivatives toward α_2-receptors, such substitutions have a marked effect on the lipophilicity of the compound. Halogen substituents such as chlorine seem to provide the optimal characteristics in this regard. For example, clonidine with its two *ortho* chloro-substituents has a partition coefficient of 3.0 and, thus, distributes into the CNS quite well. The diethyl analogue ST-91, however, possesses a partition coefficient of 0.06. Even though ST-91 has very good affinity toward the α_2-receptor, it is not effective as an antihypertensive agent since it is not able to get into the CNS to any great extent.[36] This distributive phenomenon is also seen with one of the metabolites of clonidine, 4-hydroxyclonidine. This compound has good affinity for α_2-receptors, but it is not an effective antihypertensive agent since it is too polar to get into the CNS.



ST-91

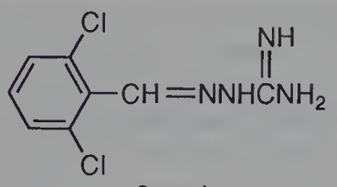


4-Hydroxyclonidine: R = OH

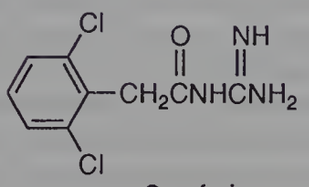
Apraclonidine: R = NH_2

A derivative of clonidine, **apraclonidine** (Iopidine), in which there is an amino group at the 4-position, is also a selective α_2-receptor agonist. It has a very specific use, to control elevations in intraocular pressure that can occur after laser surgery on the eye.

Two analogues of clonidine, **guanabenz** (Wytensin) and **guanfacine** (Tenex), are also employed as antihypertensive drugs. Their mechanism of action is the same as that of clonidine. Structurally, these two compounds can be considered "open-ring imidazolidines." In these compounds, the 2,6-dichlorophenyl moiety found in clonidine is connected to a guanidino group by a two-atom bridge. In the case of guanabenz, this bridge is a —CH=N— group, while for guanfacine it is a $—CH_2CO—$ moiety. For both compounds, conjugation of the guanidino moiety with the bridging moiety helps to decrease the pK_a of this normally very basic group so that at physiological *p*H a significant portion of each drug exists in its non-ionized form. A major difference between clonidine and its two analogues is their plasma half-life values: the plasma half-life of clonidine ranges from 12 to 16 hr, while that for guanfacine is around 17 hr. Guanabenz has the shortest duration of action of these three agents, with a half-life of around 6 hr.



Guanabenz



Guanfacine

A phenylethylamine derivative that shows selectivity toward the α_2-receptor is α-methylnorepinephrine (Fig. 16-6). As discussed under "Structure–Activity Relationships" above, the presence of an α-methyl group in the correct configuration on the phenylethylamine nucleus yields compounds with increased potency at α_2-receptors and decreased potency at α_1-receptors. Although α-methylnorepinephrine is not given as a drug, it is the metabolic product of the drug **methyldopa** (L-α-methyl-3,4-dihydroxyphenylalanine, Aldomet). Since methyldopa is a close structural analogue of L-dopa, it is treated as an alternate substrate by the enzyme L-aromatic amino acid decarboxylase. The product of this initial enzymatic reaction is α-methyldopamine. This intermediate in turn is acted upon by dopamine β-hydroxylase to give the diastereoisomer of α-methylnorepinephrine, which possesses the *(R)*-configuration at the carbon with the β-hydroxyl group and the *(S)*-configuration at the carbon with the α-methyl substituent (Fig. 16-6). It is postulated that α-methylnorepinephrine acts on α_2-receptors in the CNS in the same manner as clonidine, to decrease sympathetic outflow and lower blood pressure.[34] Since methyldopa serves as an alternate substrate to L-aromatic amino acid decarboxylase, it ultimately decreases the concentration of dopamine, NE, epinephrine, and serotonin in the CNS and periphery.

Methyldopa is used only by oral administration since its zwitterionic character limits its solubility. Absorption can range from 8% to 62% and appears to involve an amino acid transporter. Entry into the CNS also appears to involve an active-transport process. The ester hydrochloride salt of methyldopa, **methyldopate** (Aldomet Ester), was developed as a highly water-soluble derivative that could be used to make parenteral preparations. Methyldopate is converted to methyldopa in the body through the action of esterases (Fig. 16-6).

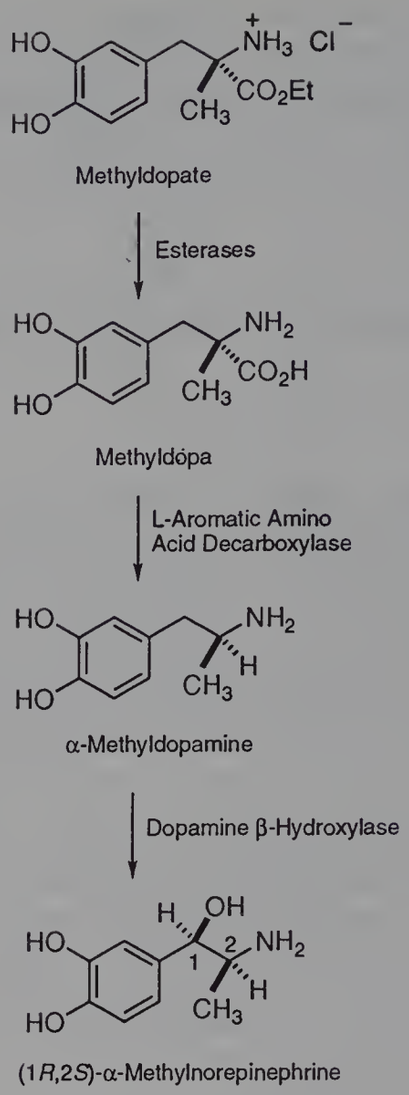


FIG. 16-6. *Metabolic conversion of methyldopate and methyldopa to* α-methylnorepinephrine.

β-Adrenergic Receptor Agonists

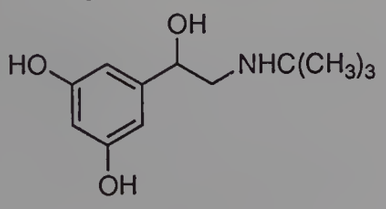
Isoproterenol (Isuprel, structure shown under "Structure–Activity Relationships," above) is the prototypical β-adrenergic receptor agonist. Because of an isopropyl substitution on the nitrogen atom, it has virtually no effect on α-receptors. However, it does act on both β_1- and β_2-receptors. It thus can produce an increase in cardiac output by stimulating cardiac β_1-receptors and can bring about bronchodilation through stimulation of β_2-receptors in the respiratory tract. It also produces the metabolic effects expected of a potent β-agonist. Isoproterenol is available for use by inhalation, injection, and sublingual tablets. Its principal clinical use is for the relief of bronchospasms associated with bronchial asthma. In fact, it is one of the most potent bronchodilators available. Cardiac stimulation is an undesirable, and occasionally dangerous, side effect in its use. However, advantage sometimes is taken of the effect of isoproterenol on the heart by using it in the treatment of heart block.

After oral administration, the absorption of isoproterenol is rather erratic and undependable. The drug has a duration of action of only a few minutes, regardless of the route of

administration. The principal reason for its poor absorption characteristics and short duration of action is its facile metabolic transformation by sulfate and glucoronide conjugation of the ring hydroxyls and methylation by COMT. Unlike epinephrine and NE, isoproterenol does not appear to undergo oxidative deamination by MAO. Since it is a catechol, it is sensitive to light and air. Aqueous solutions become pink on standing.

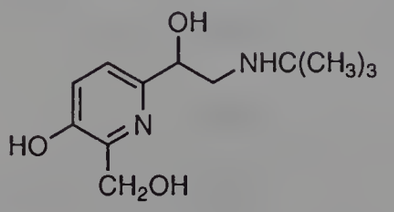
The problems of lack of β-receptor selectivity and rapid metabolic inactivation associated with isoproterenol have been overcome at least partially by the design and development of a number of selective β_2-adrenergic receptor agonists. These agents relax smooth muscle of the bronchi, uterus, and skeletal muscle vascular supply. They find their primary use as bronchodilators in the treatment of acute and chronic bronchial asthma and other obstructive pulmonary diseases.

As pointed out in the discussion of structure–activity relationships, modification of the catechol portion of a β-agonist has resulted in the development of selective β_2-receptor agonists. For example, **metaproterenol** (Alupent, Metaprel, structure shown under "Structure–Activity Relationships," above) and **terbutaline** (Bricanyl, Brethine) are resorcinol derivatives which are β_2-selective. Although these agents have a lower affinity for β_2-receptors than isoproterenol, they are much more effective when given orally and have a longer duration of action. This is because they are not metabolized by either COMT or MAO. Instead, their metabolism primarily involves glucuronide conjugation. Although both metaproterenol and terbutaline exhibit significant β_2-receptor selectivity, the common cardiovascular effects associated with other adrenergic agents can also be seen with these drugs when high doses are used.

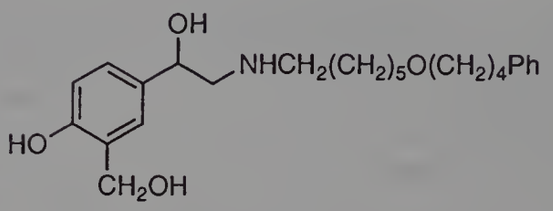


Terbutaline

Albuterol (Proventil, Ventolin, structure shown under "Structure–Activity Relationships," above), **pirbuterol** (Maxair), and **salmeterol** (Serevent) are examples of selective β_2-receptor agonists whose selectivity results from replacement of the *meta*-hydroxyl group of the catechol ring with a hydroxymethyl moiety. Pirbuterol is closely related structurally to albuterol. The only difference between the two is that pirbuterol contains a pyridine ring instead of a benzene ring. As in the case of metaproterenol and terbutaline, these drugs are not metabolized by either COMT or MAO. They thus are active orally and exhibit a much longer duration of action than isoproterenol. Salmeterol, in fact, is very long-acting (12 hr), an effect attributed to the lipophilic phenylalkyl substituent on the nitrogen atom.

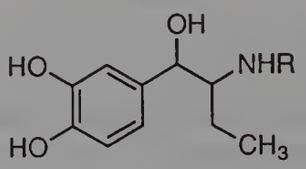


Pirbuterol



Salmeterol

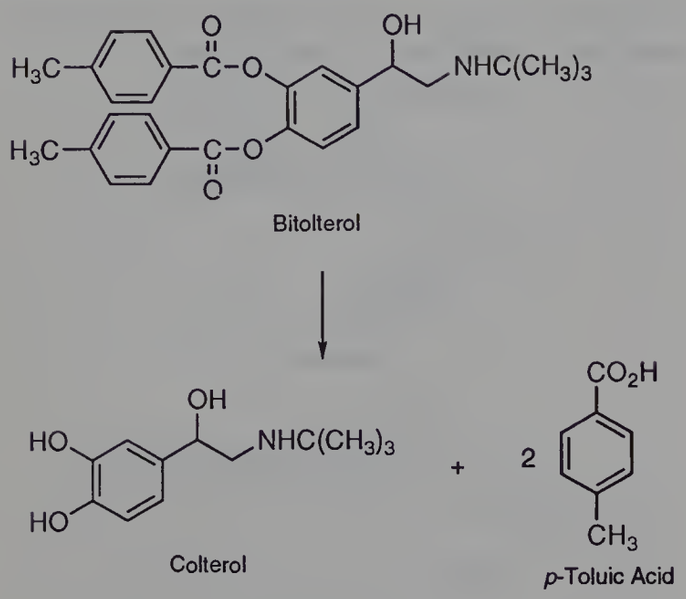
Two additional sympathomimetic drugs that find use as bronchodilators are the α-ethyl catecholamines **isoetharine** (Bronkosol) and **ethylnorepinephrine** (Bronkephrine). Both of these are weaker than isoproterenol at stimulating β_2-receptors. In addition, their β_2-selectivity is not as great as that seen with drugs such as terbutaline or albuterol. Because of the presence of the α-ethyl group, isoetharine and ethylnorepinephrine are not metabolized by MAO. However, since both contain the catechol ring system, they are metabolized quite effectively by COMT, and thus, their duration of action is similar to that seen with isoproterenol.



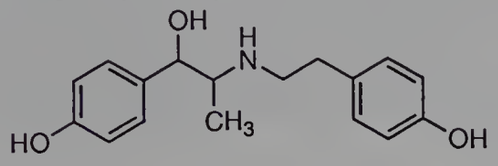
Ethylnorepinephrine: R = H

Isoetharine: R = $CH(CH_3)_2$

Bitolterol (Tornalate) is a prodrug of the β_2-selective adrenergic agonist colterol, the *N-tert*-butyl analogue of NE. The presence of the two *p*-toluic acid esters in bitolterol makes it considerably more lipophilic than colterol. Bitolterol is administered by inhalation for bronchial asthma and reversible bronchospasm. It is hydrolyzed by esterases in the lung and other tissues to produce the active agent, colterol. Bitolterol has a longer duration of action than isoproterenol and is metabolized, after hydrolysis of the esters, by COMT and conjugation.

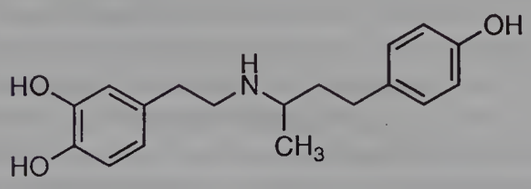


Ritodrine (Yutopar) is a selective β_2-receptor agonist used to control premature labor and to reverse fetal distress caused by excessive uterine activity. Its uterine inhibitory effects are more sustained than its effects on the cardiovascular system, which are minimal compared with those caused by nonselective β-agonists. The cardiovascular effects usually associated with its administration are mild tachycardia and slight diastolic pressure decrease. It usually is administered initially by intravenous infusion to stop premature labor. Subsequently, it may be given orally.



Ritodrine

Dobutamine (Dobutrex) is a compound that structurally can be viewed as an analogue of dopamine, in which a 1-(methyl)-3-(4-hydroxyphenyl)propyl substituent has been placed on the amino group. This substitution gives a compound that possesses an asymmetric carbon atom. Thus, dobutamine exists as a pair of enantiomers, with each enantiomer possessing a distinct pharmacology.[37] The (+)-enantiomer is a potent full agonist at both β_1- and β_2-receptors. In contrast, the (−)-enantiomer is some 10 times less potent at β_1- and β_2-receptors. The (−)-enantiomer is, however, a potent agonist at α_1-receptors. Dobutamine does not act as an agonist at the dopaminergic receptors that mediate renal vasodilation.

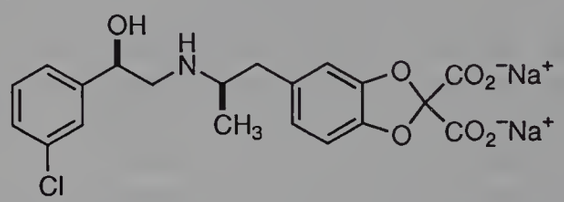


Dobutamine

In vivo, racemic dobutamine increases the inotropic activity of the heart to a much greater extent than it increases chronotropic activity. This pharmacological profile has led to its use in treating congestive heart failure. Since β_1-receptors are involved positively in both inotropic and chronotropic effects of the heart, the selective inotropic effect seen with dobutamine cannot be due simply to its activity at β_1-receptors. Rather, this effect is the result of a combination of the inotropic effect of (+)-dobutamine on β_1-receptors and that of (−)-dobutamine mediated through α_1-receptors.[38] Thus, this is a case where a racemic mixture provides a more desirable pharmacological and therapeutic effect than would either enantiomer alone.

Dobutamine is given by intravenous infusion since it is not effective orally. Solutions of the drug can exhibit a slight pink color as a result of oxidation of the catechol function. It has a plasma half-life of about 2 minutes. It is metabolized by COMT and conjugation but not by MAO.

Several direct-acting agonists for the β_3-adrenergic receptor have been developed. These agents are phenylethanolamine derivatives; however, they lack hydroxyl groups on the aromatic ring. Instead, the aromatic ring usually is substituted with a halogen atom. One example of these agents is CL 316,243.[39] Because stimulation of the β_3-receptor promotes lipolysis, these agents are being developed as potential antiobesity drugs and as drugs for the treatment of non–insulin-dependent diabetes.

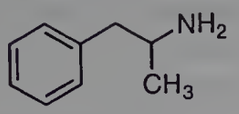


CL 316,243

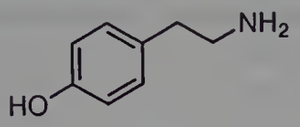
INDIRECT-ACTING SYMPATHOMIMETICS

Indirect-acting sympathomimetics act by releasing endogenous NE. They enter the nerve ending by way of the active-uptake process and displace NE from its storage granules. Certain structural characteristics tend to impart indirect sympathomimetic activity to phenylethylamines. As with the direct-acting agents, the presence of the catechol hydroxyls enhances the potency of indirect-acting phenylethylamines. However, the indirect-acting drugs that are used therapeutically are not catechol derivatives and, in most cases, do not even contain a hydroxyl moiety. In contrast with the direct-acting agents, the presence of a β-hydroxyl group decreases, and an α-methyl group increases, the effectiveness of indirect-acting agents. The presence of nitrogen substituents decreases indirect activity, with substituents larger than methyl rendering the compound virtually inactive. Phenylethylamines that contain a tertiary amino group are also ineffective as NE-releasing agents. Given the foregoing structure–activity considerations, it is easy to understand why amphetamine

and *p*-tyramine are often cited as prototypical indirect-acting sympathomimetics. Since amphetamine-type drugs exert their primary effects on the CNS, they are discussed in more detail in Chapter 15. In this chapter, those agents that exert their effects primarily on the periphery are discussed.

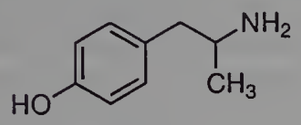


Amphetamine



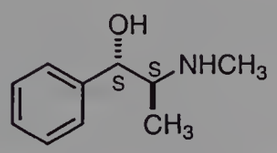
p-Tyramine

Although *p*-tyramine is not a clinically useful agent, its α-methylated derivative, **hydroxyamphetamine** (Paredrine), is an effective, indirect-acting sympathomimetic drug. Hydroxyamphetamine has little or no ephedrine-like, CNS-stimulating action. It is used to dilate the pupil for diagnostic eye examinations and for surgical procedures on the eye. It is used sometimes with cholinergic blocking drugs like atropine to produce a mydriatic effect, which is more pronounced than that produced by either drug alone.



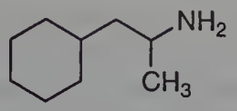
Hydroxyamphetamine

L-(+)-**Pseudoephedrine** (Sudafed, Afrinol, Drixoral) is the *(S,S)*-diastereoisomer of ephedrine. Whereas ephedrine has a mixed mechanism of action, pseudoephedrine acts by an indirect mechanism. The structural basis for this difference in mechanism is the stereochemistry of the carbon atom possessing the β-hydroxyl group. In pseudoephedrine, this carbon atom possesses the *(S)*-configuration, which is the wrong stereochemistry at this center for a direct-acting effect at adrenergic receptors. This agent is found in many over-the-counter preparations used as nasal decongestants. Although it is less prone to increase blood pressure than ephedrine, it should be used with caution in hypertensive individuals.



L-(+)-Pseudoephedrine

Propylhexedrine (Benzedrex) is an analogue of amphetamine in which the aromatic ring has been replaced with a cyclohexane ring. This drug produces vasoconstriction and a decongestant effect on the nasal membranes, but it has only about one-half the pressor effect of amphetamine and produces decidedly fewer effects on the CNS. Its major use is for a local vasoconstrictive effect on nasal mucosa in the symptomatic relief of nasal congestion caused by the common cold, allergic rhinitis, or sinusitis.

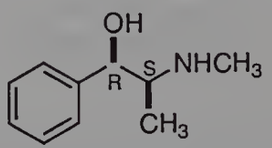


Propylhexedrine

SYMPATHOMIMETICS WITH A MIXED MECHANISM OF ACTION

Those phenylethylamines considered to have a mixed mechanism of action usually have no hydroxyls on the aromatic ring but do have a β-hydroxyl group. D-(−)-**Ephedrine** is the classic example of a sympathomimetic with a mixed mechanism of action. This drug is an alkaloid that can be obtained from the stems of various species of *Ephedra*. The plant mahuang, containing ephedrine, was known to the Chinese in 2000 B.C., but the active principle, ephedrine, was not isolated until 1885.

Ephedrine has two asymmetric carbon atoms; thus, there are four optically active forms. The *erythro*-racemate is called "ephedrine," and the *threo*-racemate is known as "*pseudo*ephedrine" (ψ-ephedrine). Natural ephedrine is the D(−)-isomer, and it is the most active of the four isomers as a pressor amine (Table 16-1). This is due largely to the fact that this isomer has the correct *(R)*-configuration at the carbon atom bearing the hydroxyl group and the desired *(S)*-configuration at the carbon bearing the methyl group for optimal direct action at adrenergic receptors.



D-(-)-Ephedrine

Ephedrine decomposes gradually and darkens when exposed to light. The free alkaloid is a strong base, and an aqueous solution of the free alkaloid has a *p*H above 10. The salt form has a pK_a of 9.6.

The pharmacological activity of ephedrine resembles that of epinephrine. The drug acts on both α- and β-adrenergic

TABLE 16-1

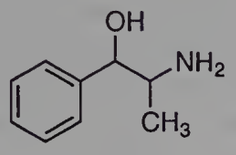
RELATIVE PRESSOR ACTIVITY OF THE ISOMERS OF EPHEDRINE

Isomer	*Relative Activity*
D-(−)-Ephedrine	36
DL-(±)-Ephedrine	26
L-(+)-Ephedrine	11
L-(+)-Pseudoephedrine	7
DL-(±)-Pseudoephedrine	4
D-(−)-Pseudoephedrine	1

receptors. Although it is less potent than epinephrine, its pressor and local vasoconstrictive actions are of greater duration. It also causes more pronounced stimulation of the CNS than epinephrine, and it is effective when given orally. The drug is not metabolized by either MAO or COMT. Rather, it is *para*-hydroxylated and *N*-demethylated by cytochrome P-450 mixed function oxidases.

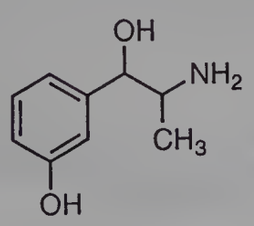
Ephedrine and its salts are used orally, intravenously, intramuscularly, and topically for a variety of conditions, such as allergic disorders, colds, hypotensive conditions, and narcolepsy. It is employed locally to constrict the nasal mucosa and cause decongestion and to dilate the pupil or the bronchi. Systemically, it is effective for asthma, hay fever, and urticaria.

Phenylpropanolamine (Propadrine) is similar in structure to ephedrine except that it is a primary instead of a secondary amine. This modification gives an agent that has slightly higher vasopressive action and lower toxicity and central stimulatory action than ephedrine. It can be used in place of ephedrine for most purposes and is used widely as a nasal decongestant. For the latter purpose, it is applied locally to shrink swollen mucous membranes; its action is more prolonged than that of ephedrine. It also is stable when given orally. Phenylpropanolamine is commonly the active component in over-the-counter appetite suppressants.

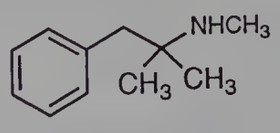


Phenylpropanolamine

Metaraminol (Aramine) and **mephentermine** (Wyamine) possess a mixed mechanism of action and are used parenterally as vasopressors in the treatment and prevention of the acute hypotensive state occurring with spinal anesthesia. Metaraminol is structurally similar to phenylephrine except that it is a primary instead of a secondary amine. Its direct-acting effects are mainly on α-adrenergic receptors. Mephentermine exhibits a prolonged duration of action.



Metaraminol



Mephentermine

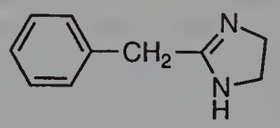
ADRENERGIC RECEPTOR ANTAGONISTS

α-ADRENERGIC RECEPTOR ANTAGONISTS

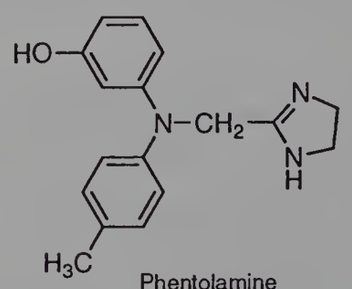
Unlike the β-adrenergic receptor antagonists, which bear clear structural similarities to the adrenergic agonists NE, epinephrine, and isoproterenol, the α-adrenergic receptor antagonists consist of a number of compounds of diverse chemical structure that bear little obvious resemblance to the α-adrenergic receptor agonists.[31]

Imidazolines

The imidazoline α-antagonists are competitive (reversible) blocking agents that are structurally similar to the imidazoline α-agonists, such as naphazoline, tetrahydrozoline, and xylometazoline. The type of group attached to the imidazoline ring dictates whether an imidazoline is an agonist or an antagonist. The two representatives of the imidazoline α-antagonists that are used therapeutically are **tolazoline** (Priscoline) and **phentolamine** (Regitine). Phentolamine is the more effective α-antagonist, but neither drug is useful as an antihypertensive agent. Theoretically, the vasodilatory effects of an α-antagonist should be beneficial in the management of hypertension. However, tolazoline and phentolamine have both α_1- and α_2-antagonistic activity and produce tachycardia. Presumably, the antagonistic actions of these agents at presynpatic α_2-receptors contribute to their cardiac stimulant effects by enhancing the release of NE. Both agents have a direct vasodilatory action on vascular smooth muscle that may be more prominent than their α-receptor antagonistic effects.



Tolazoline



Phentolamine

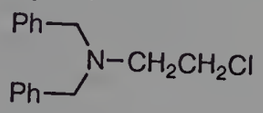
The antagonistic action of tolazoline is relatively weak, but its histamine-like and acetylcholine-like agonistic actions probably contribute to its vasodilatory activity. Its histamine-like effects include stimulation of gastric acid secretion, rendering it inappropriate for administration to patients who have gastric or peptic ulcers. It has been used to treat Raynaud's syndrome and other conditions involving peripheral vasospasm. Tolazoline is available in an injectable form

and is indicated for use in persistent pulmonary hypertension of the newborn when supportive measures are not successful.

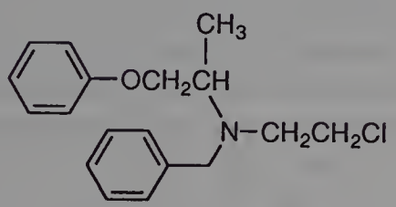
Phentolamine is used to prevent or control hypertensive episodes that occur in patients with pheochromocytoma. It can be used as an aid in the diagnosis of pheochromocytoma, but measurement of catecholamine levels is a safer and more reliable method of diagnosis.

β-Haloalkylamines

Agents in this class, when given in adequate doses, produce a slowly developing, prolonged adrenergic blockade that is not overcome by epinephrine. In essence, they are irreversible blockers of the α-adrenergic receptor. Although dibenamine is the prototypical agent in this class, it is **phenoxybenzamine** (Dibenzyline) that is used therapeutically today.



Dibenamine



Phenoxybenzamine

The mechanism whereby β-haloalkylamines produce a long-lasting, irreversible α-adrenergic receptor blockade is depicted in Fig. 16-7. The initial step involves the formation of an intermediate aziridinium ion (ethylene iminium ion), which then forms an initial reversible complex with the receptor. The positively charged aziridinium ion electrophile then reacts with a nucleophilic group on the receptor, resulting in the formation of a covalent bond between the drug and the receptor. Although the aziridinium ion intermediate has long been believed to be the active receptor-alkylating species, only in recent years has it been demonstrated unequivocally that the aziridinium ions derived from dibenamine and phenoxybenamine are capable of α-receptor alkylation.[40]

The action of phenoxybenzamine has been described as representing a "chemical sympathectomy" because of its selective blockade of the excitatory responses of smooth muscle and of the heart muscle. Although phenoxybenzamine is capable of blocking acetylcholine, histamine, and serotonin receptors, its primary pharmacological effects, especially vasodilation, may be attributed to its α-adrenergic-blocking capability. As would be expected of a drug that produces such a profound α-blockade, administration is frequently associated with reflex tachycardia, increased cardiac output, and postural hypotension. There is also evidence in-

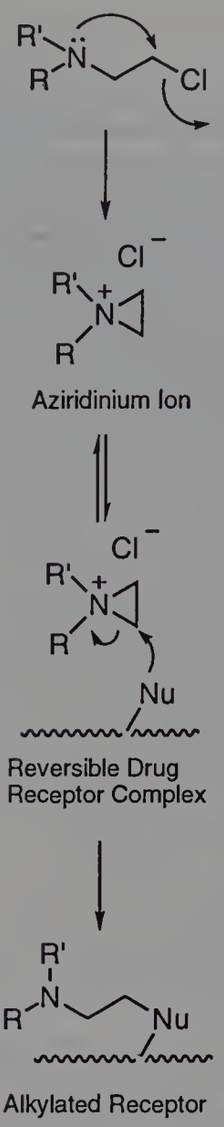


FIG. 16-7. *Mechanism of inactivation of* α-adrenergic receptors by β-haloalkylamines.

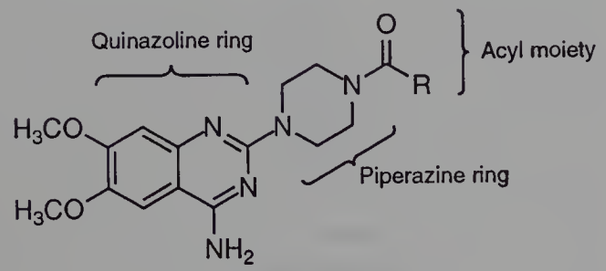
dicating that blockade of presynaptic α_2-receptors contributes to the increased heart rate produced by phenoxybenzamine.

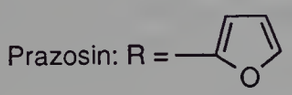
The onset of action of phenoxybenzamine is slow, but the effects of a single dose of drug may last 3 to 4 days since new receptors essentially need to be made to replace those that have been inhibited irreversibly. The principal effects following its administration are an increase in peripheral blood flow, an increase in skin temperature, and a lowering of blood pressure. It has no effect on the parasympathetic system and little effect on the gastrointestinal tract. The most common side effects are miosis, tachycardia, nasal stuffiness, and postural hypotension, all of which are related to the production of adrenergic blockade.

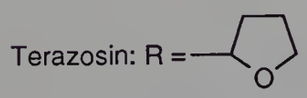
Oral phenoxybenzamine is used for the preoperative management of patients with pheochromocytoma and in the chronic management of patients whose tumors are not amenable to surgery. Only about 20% to 30% of an oral dose is absorbed.

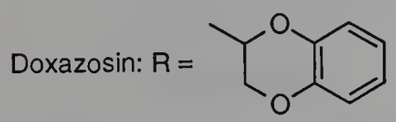
Quinazolines

Agents in this class are highly selective competitive antagonists of the α_1-adrenergic receptor. Examples include **prazosin** (Minipress), **terazosin** (Hytrin), and **doxazosin** (Cardura). Structurally, these three agents consist of three components: the quinazoline ring, the piperazine ring, and the acyl moiety. The 4-amino group on the quinazoline ring is very important for α_1-receptor affinity. Although prazosin, terazosin, and doxazosin possess a piperazine moiety attached to the quinazoline ring, this group can be replaced with other heterocyclic moieties (e.g., piperidine moiety) without loss of affinity. The nature of the acyl group has a significant effect on the pharmacokinetic properties.[41]









These drugs are used in the treatment of hypertension. They dilate both arterioles and veins. Agents in this class offer distinct advantages over the other α-blockers because they produce peripheral vasodilation without an increase in heart rate or cardiac output. This advantage, at least in part, is attributed to the fact that prazosin blocks postjunctional α_1-receptors selectively without blocking presynaptic α_2-receptors. These agents also find use in the treatment of benign prostatic hyperplasia, where they help improve urine flow rates.

Although the side effects of these drugs are usually minimal, the most frequent one, known as the *first-dose phenomenon*, is sometimes severe. This is a dose-dependent effect characterized by marked excessive postural hypotension and syncope. This phenomenon can be minimized by initially giving a low dose at bedtime.

The main difference between prazosin, terazosin, and doxazosin lies in their pharmacokinetic properties. As mentioned above, these differences are dictated by the nature of the acyl moiety attached to the piperazine ring. A comparison of these three agents with respect to their bioavailability, half-life, and duration of action is shown in Table 16-2. These drugs are metabolized extensively and excreted mainly in the bile.

TABLE 16-2

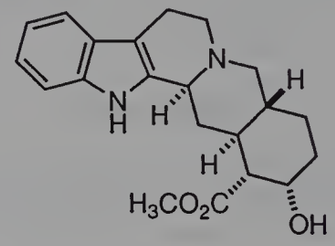
PHARMACOKINETIC PROPERTIES OF PRAZOSIN, TERAZOSIN, AND DOXAZOSIN

Agent	*Bioavailability (%)*	*Half-Life (hours)*	*Duration of Action (hours)*
Prazosin	55–60	3	4–6
Terazosin	90	12	18
Doxazosin	65	20	36

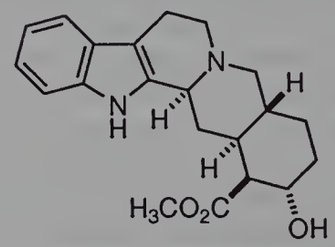
Yohimbanes

This class is comprised of a number of isomeric indole alkaloids that exhibit different degrees of selectivity toward the α_1- and α_2-adrenergic receptors depending on their stereochemistry. For example, **yohimbine** (Yocon) is a selective antagonist of the α_2-receptor, while corynanthine is a selective antagonist of the α_1-receptor. The only difference between these two compounds is the relative stereochemistry of the carbon containing the carbomethoxy substituent. In yohimbine, this group lies in the plane of the alkaloid ring system, while in corynanthine it lies in an axial position and thus is out of the plane of the rings.[42]

Yohimbine increases heart rate and blood pressure as a result of its blockade of α_2-receptors in the CNS. It is being used experimentally to treat male erectile impotence.



Yohimbine



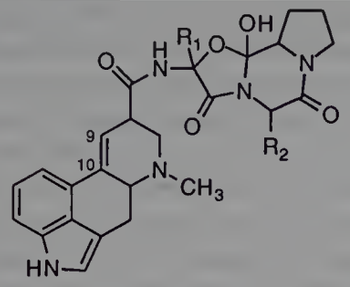
Corynanthine

Ergot Alkaloids

Although the ergot alkaloids were among the first α-adrenergic receptor antagonists identified, their therapeutic use today, as discussed below, takes advantage of some of their many other pharmacological actions. The alkaloids are the product of the grain (e.g., rye) fungus *Claviceps purpurea*. The active alkaloids are amides of *d*-lysergic acid. Pharmacologically, the naturally occurring ergot alkaloids may be placed in two classes: (1) the water-insoluble, polypeptide-

TABLE 16-3

NATURALLY OCCURRING POLYPEPTIDE-LIKE ERGOT ALKALOIDS

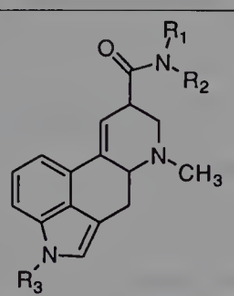


Compound	R_1	R_2
Ergotamine group		
Ergotamine	CH_3	CH_2Ph
Ergosine	CH_3	$CH_2CH(CH_3)_2$
Ergotoxine group		
Ergocristine	$CH(CH_3)_2$	CH_2Ph
Ergocryptine	$CH(CH_3)_2$	$CH_2CH(CH_3)_2$
Ergocornine	$CH(CH_3)_2$	$CH(CH_3)_2$

like group comprising ergocryptine, ergocornine, ergocristine (ergotoxine group), ergosine, and ergotamine (Table 16-3) and (2) the water-soluble alkaloid ergonovine (Table 16-4). The members of the water-insoluble group are typical adrenergic-blocking agents in that they inhibit responses to the stimulation of adrenergic nerves and block the effects of circulating epinephrine. In addition, they cause a rise in blood pressure by constriction of the peripheral blood vessels owing to a direct action on the smooth muscle of the vessels. The most important action of these alkaloids, however, is their strong stimulating action on the smooth muscle of the uterus, especially the gravid or puerperal uterus. This activity develops more slowly and lasts longer when the water-insoluble alkaloids are used than when ergonovine is administered. Toxic doses and the too-frequent use of these alkaloids in small doses are responsible for the symptoms of ergotism.

TABLE 16-4

LYSERGIC ACID AMIDES



Agent	R_1	R_2	R_3
Ergonovine	H	$CH(CH_3)CH_2OH$	H
Methylergonovine	H	$CH(CH_2CH_3)CH_2OH$	H
LSD	CH_2CH_3	CH_2CH_3	H
Methysergide	H	$CH(CH_2CH_3)CH_2OH$	CH_3

These alkaloids are rendered water-soluble by preparing salts of them with organic acids, such as tartaric, maleic, or methylsufonic acid.

Semisynthetic derivatives of *d*-lysergic acid have been prepared. Examples include the amides methylergonovine, methysergide, and the potent hallucinogen lysergic acid diethylamide (LSD) (Table 16-4). Hydrogenation of the C-9 to C-10 double bond in the lysergic acid portion of either ergotamine or the ergotoxine group of alkaloids gives derivatives with enhanced adrenergic-blocking activity.

Ergonovine (Ergotrate) and **methylergonovine** (Methergine) have little or no activity as adrenergic-blocking agents. However, they possess a strong oxytocic action, which is used to bring about a prompt and sustained contraction of the uterus to prevent and treat postpartum and postabortion hemorrhage.

Ergotamine (Ergostat) has both direct vasoconstrictive properties and an α-receptor antagonistic action. It is a potent emetic and oxytocic agent, used in the treatment of migraine headaches. It is of no value in other types of headache and sometimes fails to abort migraine headaches. It has no prophylactic value. The drug is usually administered sublingually or by inhalation because it is not well absorbed after oral administration. It is available in combination with caffeine (Cafergot, Wigraine), in both tablets and suppositories. Caffeine appears to enhance the absorption of ergotamine.

Dihydroergotamine (DHE 45) is produced by hydrogenation of the easily reducible C9 to C10 double bond in the lysergic acid portion of the ergotamine molecule. Dihydroergotamine, although very closely related to ergotamine, has less vasoconstrictive activity and less uterine stimulant activity than ergotamine. However, the adrenergic-blocking action is stronger. One of its principal uses has been in the relief of migraine headache in a manner similar to ergotamine. Because the drug is not particularly effective orally, it is administered intravenously or intramuscularly.

Methysergide (Sansert) is a semisynthetic derivative that is closely related in structure to methylergonovine; however, it does not possess the potent oxytocic action of the latter. In addition, it is a weak α-receptor antagonist and emetic agent. It is a potent blocker of the effects of serotonin in certain tissues, while in other tissues and areas of the brain it acts as an agonist. The principal use of the drug is in the prevention of migraine headache. It is not useful in the treatment of acute attacks. Although the exact mechanism of prevention has not been elucidated, the fact that serotonin has been implicated in the pathophysiology of migraine headache suggests that the mechanism of action of methysergide may be related to its effects on serotonergic receptors.

Methylsergide produces a variety of untoward side effects, though most of them are mild and will disappear with continued use. However, when administered on a long-term, uninterrupted basis, it appears to induce retroperitoneal fibrosis,

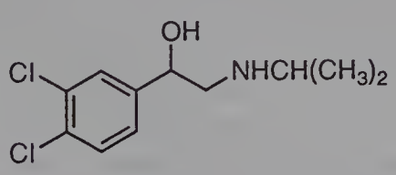
pleuropulmonary fibrosis, and fibrotic thickening of cardiac valves. As a consequence of these potential fibrotic manifestations, the drug has been reserved for prophylaxis in patients whose vascular headaches are frequent and/or severe and uncontrollable. Because of its side effects, it should not be administered continuously for longer than a 6-month period without a drug-free interval of 3 to 4 weeks between each 6-month course of treatment. Furthermore, the dosage should be reduced gradually during the last 2 to 3 weeks of the 6-month treatment period to avoid "headache rebound." The drug is not recommended for children.

Ergoloid Mesylates (Hydergine, Deapril-ST). This preparation contains equal parts of the dihydrogenated alkaloids dihydroergocornine, dihydroergocristine, and dihydroergocryptine. These alkaloids are not vasoconstrictors. The preparation is used in the treatment of senile dementias. The rationale for this use is based on the assumptions that the symptoms of senile dementia may be due to cerebral arteriosclerosis and that the condition may be alleviated by the cerebral vasodilatory effect of the dihydrogenated ergot alkaloids. The benefits of the drug are modest, and the mechanism of action has not been proved to involve cerebrovascular dilatation. Thus, the use of this drug for the treatment of senile dementias is controversial.

β-ADRENERGIC RECEPTOR ANTAGONISTS

Structure–Activity Relationships

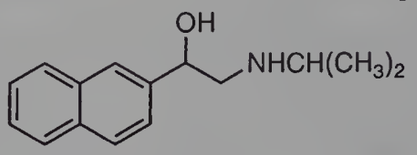
Although the α-adrenergic receptor antagonistic action of agents such as the ergot alkaloids was discovered many years ago, the first β-blocker was not reported until 1958, when Powell and Slater described the activity of dichloroisoproterenol (DCI).[43] The structure of DCI is identical to that of isoproterenol, with the exception that the catechol hydroxyl groups have been replaced by two chloro groups. This simple structural modification, involving the replacement of the aromatic hydroxyl groups, has provided the basis for nearly all of the approaches employed in subsequent efforts to design and synthesize therapeutically useful β-receptor antagonists.[32] Unfortunately, DCI is not a pure antagonist but a partial agonist. The substantial direct sympathomimetic action of DCI precluded its development as a clinically useful drug.



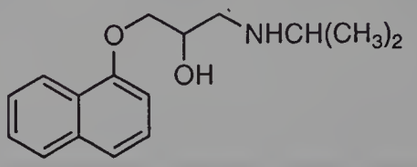
Dichloroisoproterenol

Pronethalol was the next important β-antagonist to be described. Although it had much less intrinsic sympathomimetic activity than DCI, it was withdrawn from clinical testing because of reports that it caused thymic tumors in mice. However, within 2 years of this report, Black and Stephenson[44] described the β-blocking actions of propranolol, a close structural relative of pronethalol. Propranolol has become one of the most thoroughly studied and widely used drugs in the therapeutic armamentarium. It is the standard against which all other β-antagonists are compared.

Propranolol belongs to the group of β-blocking agents known as "*aryloxypropanolamines.*" This term reflects the fact that an —OCH_2— group has been incorporated into the molecule between the aromatic ring and the ethylamino side chain. Because this structural feature is frequently found



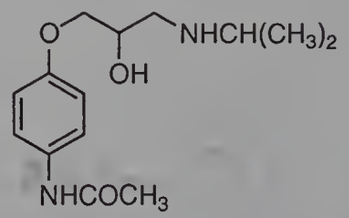
Pronethalol



Propranolol

in β-antagonists, the assumption is made that the —OCH_2— group is responsible for the antagonistic properties of the molecules. However, this is not true; in fact, the —OCH_2— group is present in several compounds that are potent β-agonists.[45] This latter fact again leads to the conclusion that it is the nature of the aromatic ring and its substituents that is the primary determinant of β-antagonistic activity. The aryl group also affects the absorption, excretion, and metabolism of the β-blockers.[46]

The nature of the aromatic ring is also a determinant in the β_1-selectivity of the antagonists. One common structural feature of many cardioselective antagonists is the presence of a *para*-substituent of sufficient size on the aromatic ring along with the absence of *meta*-substituents. Practolol is the prototypical example of a β_1-antagonist of this structural type. Although it was not released for use in the United States, it was the first cardioselective β_1-antagonist to be used extensively in humans. However, because it produced several toxic effects, it is no longer in general use in most countries.



Practolol

As in the sympathomimetics, bulky aliphatic groups, such as the *tert*-butyl and isopropyl groups, are normally found on

the amino function of the aryloxypropanolamine β-receptor antagonists. It must be a secondary amine for optimal activity.

The β-blocking agents exhibit a high degree of stereoselectivity in the production of their β-blocking effects. As with the sympathomimetic agents, the configuration of the hydroxyl-bearing carbon of the aryloxypropanolamine side chain plays a critical role in the interaction of β-antagonist drugs with β-receptors. This carbon must possess the *(S)*-configuration for optimal affinity to the β-receptor. The enantiomer with the *(R)*-configuration is typically 100 times less potent. The available data indicate that the pharmacologically more active enantiomer interacts with the receptor recognition site in a manner analogous to that of the agonists. However, the structural features of the aromatic portion of the antagonist appear to perturb the receptor or to interact with it in a manner that inhibits activation. In spite of the fact that nearly all of the β-antagonistic activity resides in one enantiomer, propranolol and most other β-blockers are used clinically as racemic mixtures. The only exceptions are levobunolol, timolol, and penbutolol, where the *(S)*-enantiomer is used.

Nonselective β-Blockers

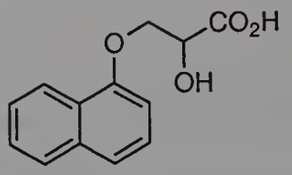
Propranolol (Inderal) is the prototypical β-adrenergic receptor antagonist. It is nonselective in that it blocks the β_1- and β_2-receptors equally well. Propranolol, similar to the other β-receptor antagonists that are discussed, is a competitive antagonist, the receptor-blocking actions of which can be reversed with sufficient concentrations of β-agonists. Currently, propranolol is approved for use in the United States for treating hypertension, cardiac arrythmias, angina pectoris caused by coronary atherosclerosis, hypertrophic subaortic stenosis, myocardial infarction, pheochromocytoma, and essential tremor, as well as for prophylaxis of migraine headache. In addition, propranolol is under investigation for the treatment of a variety of other conditions, including anxiety, schizophrenia, alcohol withdrawal syndrome, and aggressive behavior.

Some of the most prominent effects of propranolol are on the cardiovascular system. By blocking the β-receptors of the heart, propranolol slows the heart, reduces the force of contraction, and reduces cardiac output. Because of reflex sympathetic activity and blockade of vascular β_2-receptors, administration may result in increased peripheral resistance. The antihypertensive action, at least in part, may be attributed to its ability to reduce cardiac output, as well as to its suppression of renin release from the kidney. Because it exhibits no selectivity for β_1-receptors, it is contraindicated in the presence of conditions such as asthma and bronchitis.

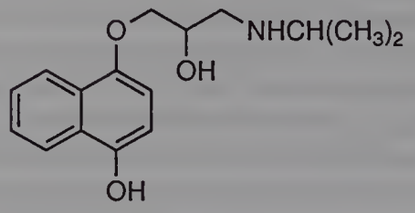
A facet of the pharmacological action of proranolol that has received a good deal of attention is its so-called *membrane-stabilizing activity*. This is a nonspecific effect (i.e., not mediated by a specific receptor), which is also referred to as a *local anesthetic* effect or a *quinidine-like* effect. Both enantiomers possess membrane-stabilizing activity. Since the concentrations required to produce this effect far exceed those obtained with normal therapeutic doses of propranolol and related β-blocking drugs, it is most unlikely that the nonspecific membrane-stabilizing activity plays any role in the clinical efficacy of β-blocking agents.

The metabolism of propranolol is a topic that has received intense study. Propranolol is well absorbed after oral administration, but it undergoes extensive first-pass metabolism before it reaches the systemic circulation. The term "*first-pass metabolism*" refers to the fact that the compound is extracted efficiently from the portal vein by the liver, where it undergoes biotransformation. Lower doses are extracted more efficiently than higher doses, indicating that the extraction process may become saturated at higher doses. In addition, the active enantiomer is cleared more slowly than the inactive enantiomer.[47]

Numerous metabolites of propranolol have been identified, but the major metabolite in people, after a single oral dose, is naphthoxylactic acid, which is formed by a series of metabolic reactions involving *N*-dealkylation, deamination, and oxidation of the resultant aldehyde. One metabolite of particular interest is 4-hydroxypropranolol. This compound is a potent β-antagonist that has some intrinsic sympathomimetic activity. It is not known what contribution, if any, 4-hydroxypropranolol makes to the pharmacological effects seen after administration of propranolol. The half-life after a single oral dose is 3 to 4 hr, which increases to 4 to 6 hr after long-term therapy.

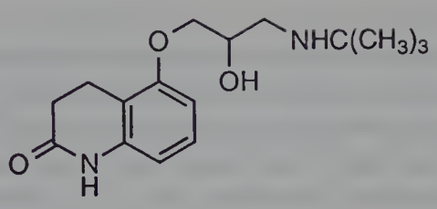


Naphthoxylactic Acid

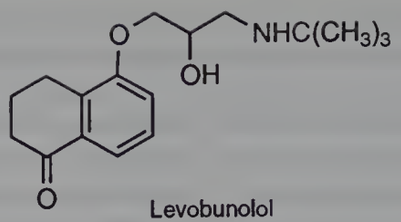


4-Hydroxypropranolol

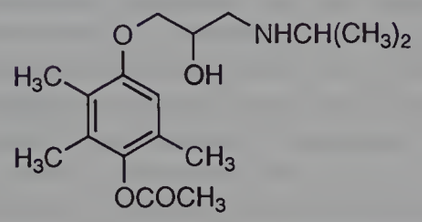
Several other nonselective β-blockers are used clinically. These include **nadolol** (Corgard), **pindolol** (Visken), **penbutolol** (Levatol), **carteolol** (Cartrol, Ocupress), **timolol** (Blocadren, Timoptic), **levobunolol** (Betagan), and **metipranolol** (OptiPranolol). Structures of these compounds are shown in Fig. 16-8. The first five of these agents are used to treat hypertension. Nadolol is also used in the long-term



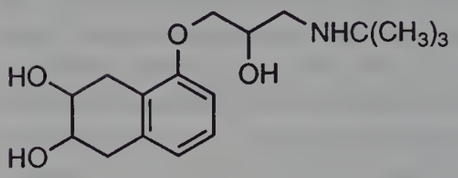
Carteolol



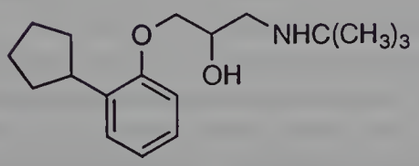
Levobunolol



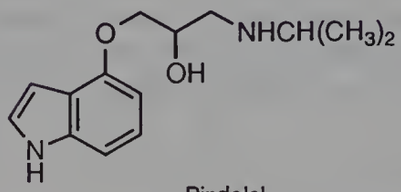
Metipranolol



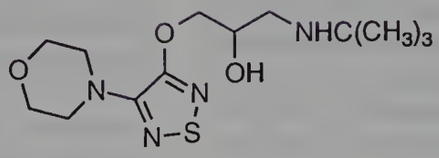
Nadolol



Penbutolol



Pindolol



Timolol

FIG. 16-8. *Nonselective β-blockers.*

management of angina pectoris, while timolol finds use in the prophylaxis of migraine headaches and in the therapy following myocardial infarction.

Carteolol, timolol, levobunolol, and metipranolol are used topically to treat open-angle glaucoma. These agents lower intraocular pressure with virtually no effect on pupil size or accommodation. They thus offer an advantage over many of the other drugs used in the treatment of glaucoma. Although the precise mechanism whereby β-blockers lower intraocular pressure is not known with certainty, it is believed that they may reduce the production of aqueous humor. Even though these agents are administered into the eye, systemic absorption can occur, producing such side effects as bradycardia and acute bronchospasm in patients with bronchospastic disease.

Pindolol possesses modest membrane-stabilizing activity and significant intrinsic β-agonistic activity. Penbutolol and carteolol also have partial agonistic activity but not to the degree that pindolol does. β-Antagonists with partial agonistic activity cause less slowing of the resting heart rate than do agents without this capability. The partial agonistic activity may be beneficial in patients who are likely to exhibit severe bradycardia or who have little cardiac reserve.

Timolol, pindolol, penbutolol, and carteolol have half-life values in the same range as propranolol. The half-life of nadolol, however, is around 20 hr, making it one of the longest-acting β-blockers. Timolol undergoes first-pass metabolism but not to the same extent that propranolol does. Timolol and penbutolol are metabolized extensively, with little or no unchanged drug excreted in the urine. Pindolol is metabolized by the liver to the extent of 60%, with the remaining 40% being excreted in the urine unchanged. In contrast, nadolol undergoes very little hepatic metabolism. Most of the drug is excreted unchanged in the urine.

β_1-Selective Blockers

The discovery that β-blocking agents are useful in the treatment of cardiovascular disease, such as hypertension, stimu-

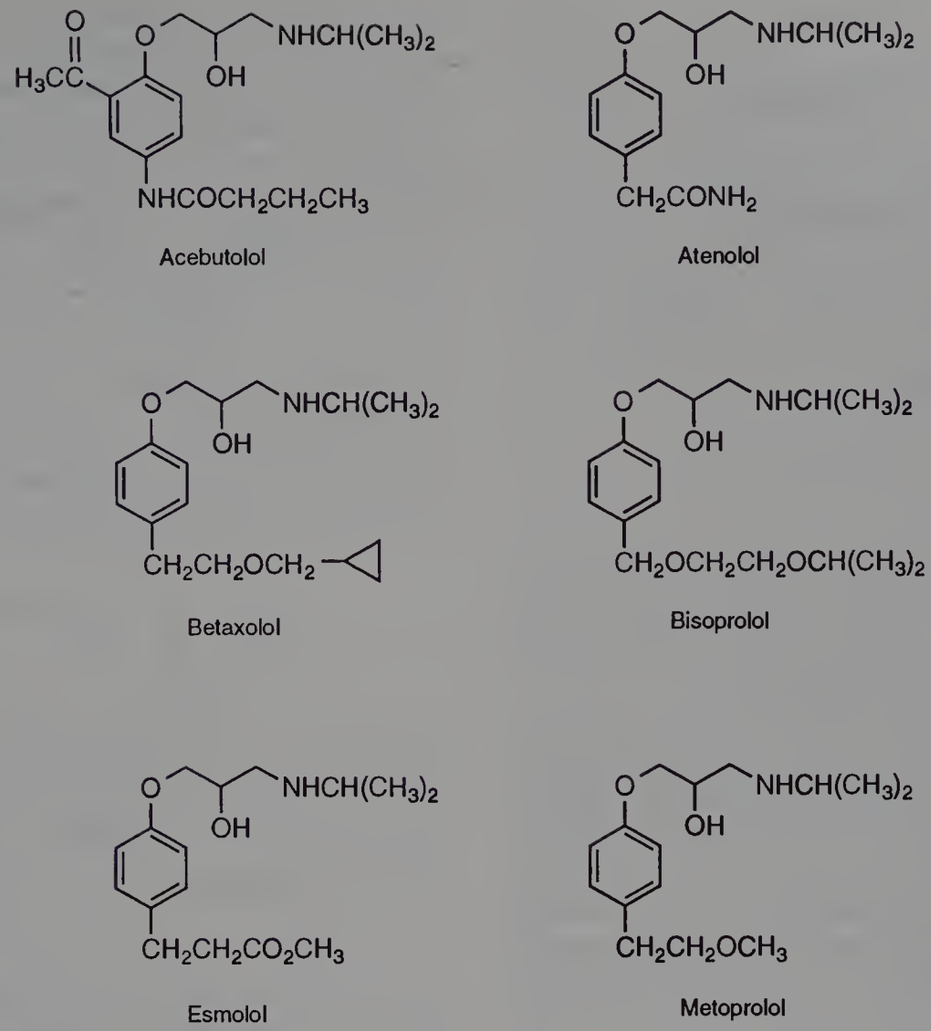


FIG. 16-9. β_1-Selective blockers.

lated a search for cardioselective β-blockers. Cardioselective β-antagonists are drugs that have a greater affinity for the β_1-receptors of the heart than for β_2-receptors in other tissues. Such cardioselective agents should provide two important therapeutic advantages. The first advantage would be the lack of an antagonistic effect on the β_2-receptors in the bronchi. Theoretically, this would make β_1-blockers safe for use in patients who have bronchitis or bronchial asthma. The second advantage would be the absence of blockade of the vascular β_2-receptors, which mediate vasodilation. This would be expected to reduce or eliminate the increase in peripheral resistance that sometimes occurs after the administration of nonselective β-antagonists. Unfortunately, cardioselectivity is usually observed with β_1-antagonists at only relatively low doses. At normal therapeutic doses, much of the selectivity is lost.

At present, the following β_1-selective agents are used therapeutically: **acebutolol** (Sectral), **atenolol** (Tenormin), **betaxolol** (Kerlone, Betoptic), **bisoprolol** (Zebeta), **esmolol** (Brevibloc), and **metoprolol** (Lopresor). Structures of these agents are depicted in Fig. 16-9. All of these agents but esmolol are indicated for the treatment of hypertension. Atenolol and metoprolol are also approved for use in treating angina pectoris and in therapy following myocardial infarction. Betaxolol is the only β_1-selective blocker indicated for the treatment of glaucoma.

Acebutolol and esmolol are indicated for treating certain cardiac arrhythmias. Esmolol was designed specifically to possess a very short duration of action; it has an elimination half-life of 9 minutes. This agent is administered by continuous intravenous infusion for control of ventricular rate in patients with atrial flutter, atrial fibrillation, or sinus tachycardia. Its rapid onset and short duration of action render it useful during surgery, postoperatively, or during emergency situations for short-term control of heart rates. Its effects disappear within 20 to 30 minutes after the infusion is discontinued. Esmolol must be diluted with an injection solution before administration; it is incompatible with sodium bicarbonate.

The short duration of action of esmolol is the result of rapid hydrolysis of its ester functionality by esterases present in erythrocytes (Fig. 16-10). The resultant carboxylic acid is an extremely weak β-antagonist that does not appear to exhibit clinically significant effects. The acid metabolite has an elimination half-life of 3 to 4 hr and is excreted primarily by the kidneys.

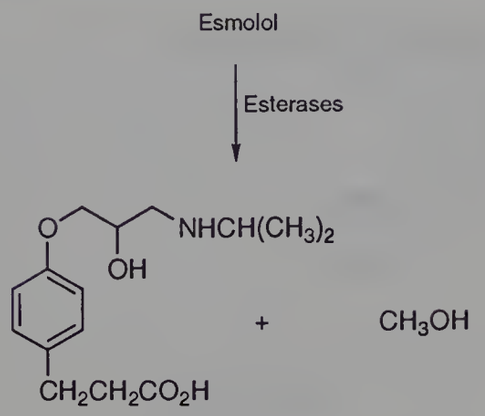


FIG. 16-10. *Metabolism of esmolol.*

In the class of β_1-selective blockers, only acebutolol possesses intrinsic sympathomimetic activity. This activity is very weak, however. Acebutolol and betaxolol possess membrane-stabilizing activity, but the activity is much weaker than that seen with propranolol.

The half-life values of acebutolol and metoprolol are comparable to that seen with propranolol, and those of atenolol and bisoprolol are about twice that of propranolol. Betaxolol, with a half-life ranging between 14 and 22 hr, has the longest duration of action of the β_1-selective blockers. Like propranolol, the bioavailability of metoprolol is low because of significant first-pass metabolism. Although the bioavailability of betaxolol is very high, it is metabolized extensively by the liver, with very little of the unchanged drug excreted in the urine. Atenolol, like nadolol, has a low lipid solubility and does not readily cross the blood–brain barrier. It is absorbed incompletely from the gastrointestinal tract, the oral bioavailability being approximately 50%. Little of the absorbed portion of the dose is metabolized; most of it is excreted unchanged in the urine. In the case of bisoprolol, about 50% of a dose undergoes hepatic metabolism, while the remaining 50% is excreted in the urine unchanged.

Acebutolol is one of the very few β-blockers whose metabolite plays a significant role in the pharmacological actions of the drug. This drug is absorbed well from the gastrointestinal tract, but it undergoes extensive first-pass metabolic conversion to diacetolol. Diacetolol is formed by hydrolytic conversion of the amide group to the amine, followed by acetylation of the amine (Fig. 16-11). After oral administration, plasma levels of diacetolol are higher than those of acebutolol. Diacetolol is also a selective β_1-receptor antagonist with partial agonistic activity; it has little membrane-stabilizing activity. It has a longer half-life (8 to 12 hr) than the parent drug and is excreted by the kidneys.

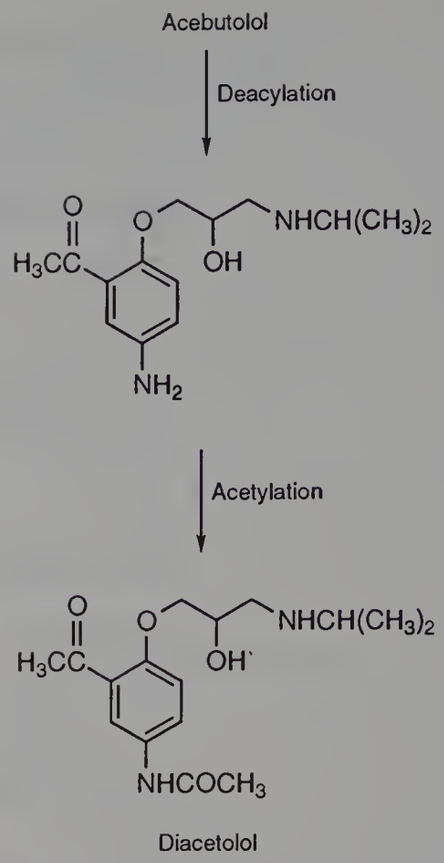
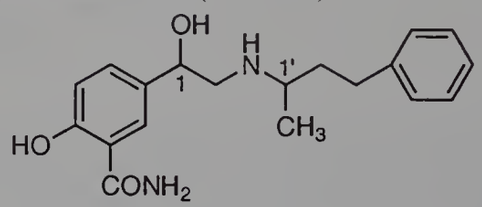
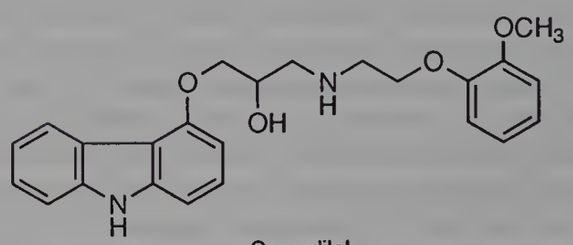


FIG. 16-11. *Metabolism of acebutolol.*

β-Blockers with α_1-Receptor Antagonistic Activity

Several drugs have been developed that possess both β- and α-receptor–blocking activities within the same molecule. Two examples of such molecules are **labetalol** (Normodyne, Trandate) and **carvedilol** (Dilatrend).





Labetalol is a phenylethanolamine derivative that is a competitive inhibitor at both β_1- and β_2-adrenergic receptors and at the α_1-adrenergic receptor. It is a more potent β-antagonist than α-antagonist. Since it has two asymmetric carbon atoms (1 and 1′), it exists as a mixture of four isomers. It is this mixture that is used clinically. The different isomers, however, possess different α- and β-antagonistic activities. The β-blocking activity resides solely in the (1*R*, 1′*R*)-isomer, while the α_1-antagonistic activity is seen in the (1*S*,

1′*R*)- and (1*S*, 1′*S*)-isomers, with the (1*S*, 1′*R*)-isomer possessing the greater activity.[48] Labetalol is a clinically useful antihypertensive agent. The rationale for its use in the management of hypertension is that its α-receptor–blocking effects produce vasodilation and its β-receptor–blocking effects prevent the reflex tachycardia usually associated with vasodilation. Although labetalol is very well absorbed, it undergoes extensive first-pass metabolism.

Carvedilol, like labetalol, is a β-blocker that possesses α_1-adrenergic receptor-blocking activity. Only the (*S*)-enantiomer possesses the β-blocking activity, while both enantiomers are antagonists of the α_1-adrenergic receptor.[49] This drug is also unique in that it possesses antioxidant activity and an antiproliferative effect on vascular smooth muscle cells. It thus has a neuroprotective effect and the ability to provide major cardiovascular organ protection.[50]

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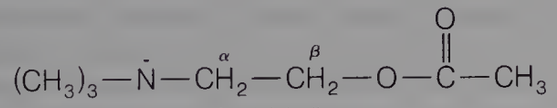
CHAPTER 17

Cholinergic Drugs and Related Agents

George H. Cocolas

Few systems, if any, have been studied as extensively as those innervated by neurons that release acetylcholine (ACh) at their endings. Since the classic studies of Dale,[1] who described the actions of the esters and ethers of choline on isolated organs and their relationship to muscarine, pharmacologists, physiologists, chemists, and biochemists have applied their knowledge to understand the actions of the cholinergic nerve and its neurotransmitter. Recent applications of biotechnology and chemistry have developed probes that have uncovered the complexity of the action of ACh on cholinergic neurons and receptors, unknown when ACh was first demonstrated in the frog heart in 1921 by Loewi as the substance released by vagus nerve stimulation.[2]

This chapter includes the drugs and chemicals that act on cholinergic nerves or the tissues they innervate to either mimic or block the action of ACh. Drugs that mimic the action of ACh do so either by acting directly on the cholinergic receptors in the tissue or by inhibiting acetylcholinesterase (AChE), the enzyme that inactivates ACh at the nerve terminal. Cholinergic neurotransmission may be blocked by chemicals that bind or compete with ACh for binding to the receptor



Acetylcholine

Cholinergic nerves are found in the peripheral nervous system and central nervous system (CNS) of humans. Synaptic terminals in the cerebral cortex, corpus striatum, hippocampus, and several other regions in the CNS are rich in ACh and in the enzymes that synthesize and hydrolyze this neurotransmitter. Many experiments show that agonists and antagonists of cholinergic receptors can modify the output of neurotransmitters, including ACh, from brain preparations. Although the function of ACh in the brain and brain stem is not clear, it has been implicated in memory and behavioral activity in humans.[3] The *peripheral nervous system* consists of those nerves outside the cerebrospinal axis and includes the somatic nerves and the autonomic nervous system. The *somatic nerves* are made up of a sensory (afferent) nerve and a motor (efferent) nerve. The *motor nerves* arise from the spinal cord and project uninterrupted throughout the body to all skeletal muscle. ACh mediates transmission of impulses from the motor nerve to skeletal muscle (i.e., neuromuscular junction).

The *autonomic nervous system* is composed of two divisions: *sympathetic* and *parasympathetic*. ACh serves as a neurotransmitter at both sympathetic and parasympathetic preganglionic nerve endings, postganglionic nerve fibers in the parasympathetic division, and some postganglionic fibers (e.g., salivary and sweat glands) in the sympathetic division of the autonomic nervous system. The autonomic nervous system regulates the activities of smooth muscle and glandular secretions. These, as a rule, function below the level of consciousness (e.g., respiration, circulation, digestion, body temperature, metabolism). The two divisions have contrasting effects on the internal environment of the body. The sympathetic division frequently discharges as a unit, especially during conditions of rage or fright, and expends energy. The parasympathetic division is organized for discrete and localized discharge and stores and conserves energy.

Drugs and chemicals that cause the parasympathetic division to react are termed *parasympathomimetic*, whereas those blocking the actions are called *parasympatholytic*. Agents that mimic the sympathetic division are *sympathomimetic*, and those that block the actions are *sympatholytic*. Another classification used to describe drugs and chemicals acting on the nervous system or the structures that the fibers innervate is based on the neurotransmitter released at the nerve ending. Drugs acting on the autonomic nervous system are divided into *adrenergic*, for those postganglionic sympathetic fibers that release norepinephrine and epinephrine, and *cholinergic*, for the remaining fibers in the autonomic nervous system and the motor fibers of the somatic nerves that release ACh.

CHOLINERGIC RECEPTORS

There are two distinct receptor types for ACh that differ in composition, location, and pharmacologic function and have specific agonists and antagonists. Cholinergic receptors have been characterized as *nicotinic* and *muscarinic* on the basis of their ability to be bound by the naturally occurring alkaloids nicotine and muscarine, respectively. Receptor subtypes that differ in location and specificity to agonists and antagonists have been identified for both the nicotinic and muscarinic receptors.

TABLE 17-1

RADII OF ALKALI AND ALKALI EARTH CATIONS

Ion	*Ionic Radius (Å)*	*Effective Hydrated Radius (Å)*
Li^+	0.60	4.5
Na^+	0.95	3.4
K^+	1.33	2.2
Rb^+	1.48	1.9
Cs^+	1.69	1.9
Mg^{2+}	0.65	5.9
Ca^{2+}	0.99	4.5
Sr^{2+}	1.13	3.7
Ba^{2+}	1.35	3.7

From Triggle, D. J.: Neurotransmitter–Receptor Interactions. San Diego, Academic Press, 1971.

NICOTINIC RECEPTORS

Nicotinic receptors are coupled directly to ion channels and mediate very rapid responses when activated by ACh. Ion channels are responsible for the electrical excitability of nerve and muscle cells and for sensitivity of sensory cells. The channels are pores that open or close in an all-or-nothing fashion on time scales ranging from 0.1 to 10 milliseconds to provide aqueous pathways through the plasma membrane that ions can transverse. Factors affecting selectivity of ion pores include both the charge and size of the ion. Ions in aqueous solution are hydrated. The water around the ion is characterized by the presence of two distinct water structures: a tightly bound, highly ordered layer immediately surrounding the ion and a second, less structured layer[4] (Fig. 17-1). Ion transport through a channel requires some denuding of the surrounding water shell. The degree of organization of the water structure determines the energy required to remove the hydration shell and is a factor in the selectivity of that ion channel.[5] Table 17-1 lists the effective radii of alkali and alkaline earth cations.

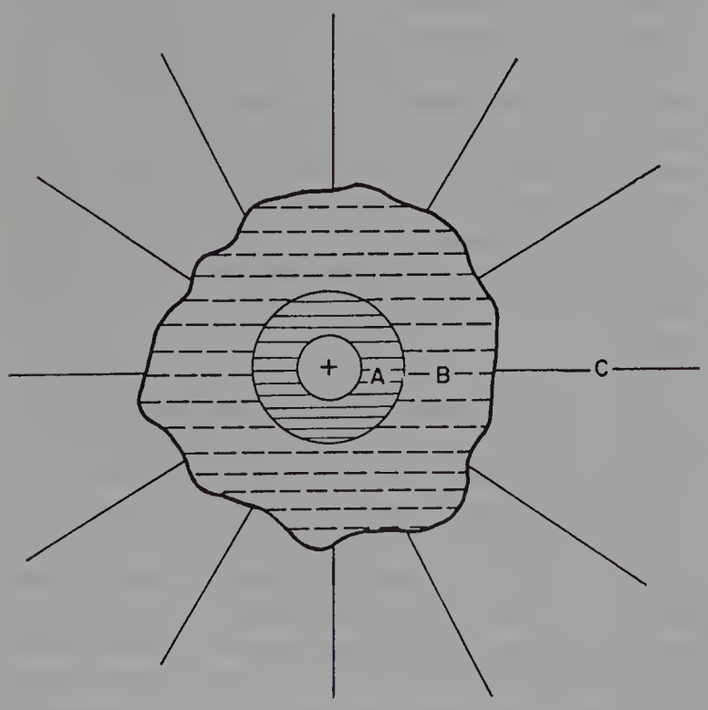


FIG. 17-1. Hydrated cation showing a highly structured shell of water around **(A)** *the cation,* **(B)** *a less structured layer surrounding the inner water shell, and* **(C)** *water in a "normal" state. With permission from the author and the Royal Society of Chemistry.*

The nicotinic ACh receptor was the first neurotransmitter isolated and purified in an active form.[6] It is a glycoprotein embedded into the polysynaptic membrane that can be obtained from the electric organs of the marine ray *Torpedo californa* and the electric eel *Electrophorus electricus*. The receptor is pictured as a cyclindric protein of about 250,000 Da and consists of five subunit polypeptide chains, of which two appear to be identical.[7,8] The subunit stoichiometry of the polypeptide units from the *Torpedo* receptor is α_2, β, γ, δ.[9] The peptide chains of the receptor are arranged to form an opening in the center, which is the ion channel. Each α-chain contains a negatively charged binding site for the quaternary ammonium group of ACh. The receptor appears to exist as a dimer of the two five-subunit polypeptide chain monomers linked through a disulfide bond between δ-chains. A structural protein of molecular weight 43,000 binds the nicotinic receptor to the membrane (Fig. 17-2).

When the neurotransmitter ACh binds to the nicotinic receptor, it causes a change in the permeability of the membrane to allow passage of small cations, Ca^{2+}, Na^+, and K^+. The physiologic effect is to temporarily depolarize the end plate. This depolarization results in muscular contraction at a neuromuscular junction or, as occurs in autonomic ganglia, continuation of the nerve impulse. Neuromuscular nicotinic ACh receptors are of interest as targets for autoimmune antibodies in myasthenia gravis and muscle relaxants used during the course of surgical procedures. Nicotinic receptors in autonomic ganglia, when blocked by drugs, can play a role in the control of hypertension.

Nicotinic Receptor Subtypes

Nicotinic receptors located in the neuromuscular junction differ from those on neurons, such as those in the CNS and autonomic ganglia, in that they have different ligand speci-

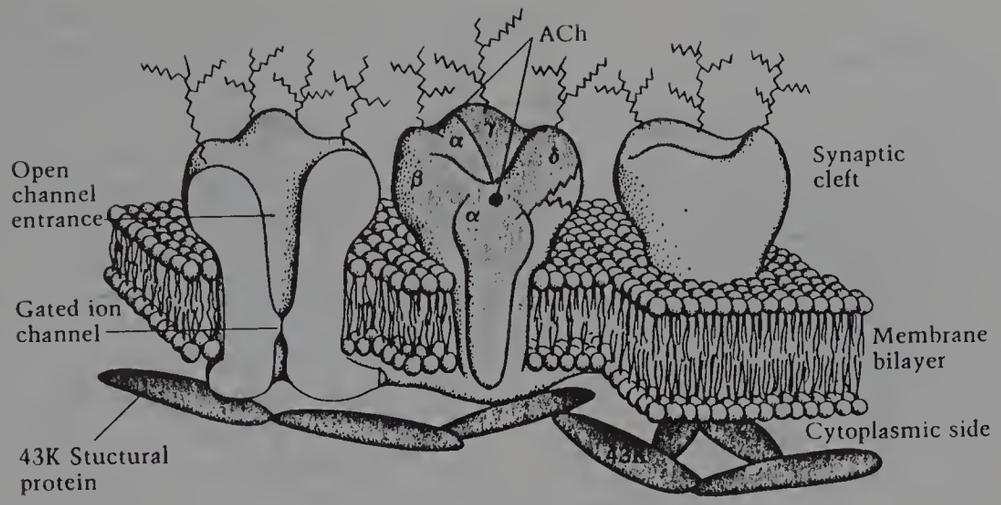
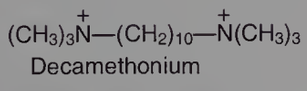


FIG. 17-2. *A model of the nicotinic receptor consisting of five protein subunits embedded in a cell membrane based on electron microscopy and neutron scattering data. Jagged lines represent oligosaccharide chains on the upper part of the receptor. A 43K protein is bound to the receptor on the cytosolic side of the cell membrane. The ACh-binding sites are shown on the two-subunit proteins. (Reprinted with permission from Lindstrom, J. M. et al.: Cold Spring Harbor Symposium on Quantitative Biology, 48:93, 1983.)*

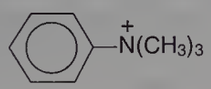
ficities. Nicotinic receptors at the neuromuscular junction, termed N_1, are blocked by succinylcholine, *d*-tubocurarine, and decamethonium and stimulated by phenyltrimethylammonium. N_2 nicotinic receptors are found in autonomic ganglia. They are blocked by hexamethonium and trimethaphan but stimulated by tetramethylammonium and dimethyl-4-phenylpiperazinium (DMPP). Nicotinic receptor subtypes have also been identified in many regions of the CNS; however, their pharmacologic function is not yet understood clearly.[10]

the respiratory tract. It inhibits contraction of the heart and relaxes smooth muscle of blood vessels.

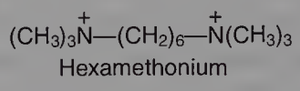
As early as 1980, it became apparent that the actions of ACh could not be mediated by a single muscarinic receptor type. Research on cholinergic receptors has increased since the 1980s, as these receptors represent potential targets for useful drugs for disease states that are becoming more prevalent because of our increasing population of aged. The outcome of these studies has been the discovery of several muscarinic receptor subtypes.



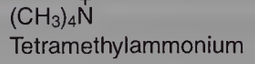
Decamethonium



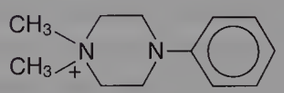
Phenyltrimethylammonium



Hexamethonium



Tetramethylammonium



DMPP

MUSCARINIC RECEPTORS

Muscarinic receptors play an essential role in regulating the functions of organs innervated by the autonomic nervous system to maintain homeostasis of the organism. The action of ACh on muscarinic receptors can result in stimulation or inhibition of the organ system affected. ACh stimulates secretions from salivary and sweat glands, secretions and contraction of the gut, and constriction of the airways of

Muscarinic receptors mediate their effects by activating guanosine triphosphate (GTP)-binding proteins (G-proteins). These receptors have seven protein helixes that transcend the plasma membrane, creating four extracellular domains and four intracellular domains (Fig. 17-3). The extracellular domain of the receptor contains the binding site for ACh. The intracellular domain couples with G-proteins to initiate biochemical changes that result in pharmacologic action from receptor activation.

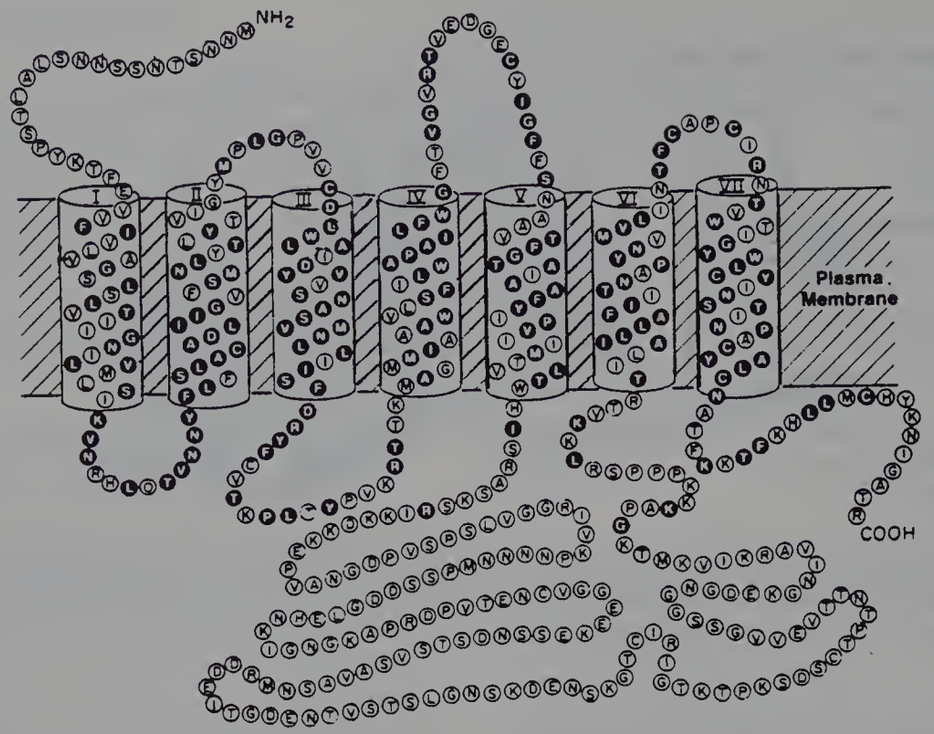
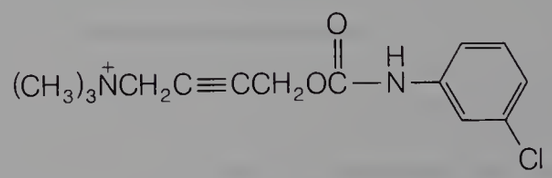


FIG. 17-3. Hypothetical model of a muscarinic receptor showing the location of the transmembrane helical protein domains and the extracellular and intracellular domains connecting the seven alpha helical proteins in the membrane. (Reprinted from Goyal, R. K.: N. Engl. J. Med. 321:1024, 1989, with permission from the author and the Massachusetts Medical Society.)

Muscarinic Receptor Subtypes

Evidence from both pharmacologic and biochemical studies shows that subtypes of muscarinic receptors are located in the CNS and peripheral nervous system.[11,12] Molecular cloning studies have revealed the existence of five different molecular mammalian muscarinic receptor proteins. The cloned receptors have been identified as m_1–m_5. In another method of identification, muscarinic receptor subtypes have been defined on the basis of their affinity for selective agonists and antagonists and the pharmacologic effects they cause. These receptors are designated using capital letters and subscript numbers, M_1–M_5. The nomenclature convention adopted for these receptors is that the pharmacologically defined subtypes M_1, M_2, and M_3 correspond to the genetically defined subtypes m_1, m_2, and m_3. The m_4 gene-derived protein is referred to as the M_4 subtype and has many pharmacologic properties similar to the M_2 subtype. The m_5 receptor gene product does not have an equivalent pharmacologic profile.

M_1 Receptors. Even though molecules do not have exclusive selectivity on muscarinic receptor subtypes, M_1 receptors have been defined as those with high affinity for pirenzapine and low affinity for compounds such as AF-DX 116. They have been termed "neural" because of their distribution within particular brain structures. In addition to the CNS, M_1 receptors are located in exocrine glands and autonomic ganglia. In humans, these receptors seem to affect arousal attention, rapid eye movement (REM) sleep, emotional responses, affective disorders including depression, and modulation of stress. They are believed to participate in higher brain functions, such as memory and learning. Alzheimer's disease research has implicated cholinergic neurons and receptors, but evidence does not show conclusively that these are the primary causes of the disease. M_1 receptors have been identified in submucosal glands and some smooth muscle. They are located in parietal cells in the gastrointestinal (GI) tract and in peripheral autonomic ganglia, such as the intramural ganglia of the stomach wall. When stimulated, M_1 receptors cause gastric secretion.[13] While McN-A-343 is a selective agonist, pirenzapine HCl acts as an antagonist and has been used outside the United States for the treatment of peptic ulcer disease.



McN-A-343

M_2 Receptors. M_2 receptors are identified by their high affinity for methoctramine, a polyamine, and low affinity for pirenzapine. M_2 receptors are also called "cardiac" muscarinic receptors because they are located in the atria and conducting tissue of the heart. Their stimulation causes a decrease in the strength and rate of cardiac muscle contraction. The manner in which these effects are produced may be by affecting intracellular K^+ and Ca^{2+} levels in heart tissue. M_2 receptors activate K^+ channels to cause hyperpolarization of cardiac cells, resulting in bradycardia. These receptors may also act through an inhibitory G-protein (G_i)

to reduce adenylate cyclase activity and lower cyclic 3′,5′-adenosine monophosphate (cAMP) levels in cardiac cells. Lower cAMP levels decrease the amount of free Ca^{2+} in cardiac cells and slow down the heart rate.[14] M_2 receptors can also serve as autoreceptors on presynaptic terminals of postganglionic cholinergic nerves to inhibit ACh release. The size of the airway of the smooth muscle in the bronchioles is determined by the balance of the effects of multiple muscarinic receptor subtypes. Contraction is primarily the result of the action of ACh on M_3 receptors (see below) following stimulation of the vagus. At the same time, ACh stimulates inhibitor M_2 autoreceptors located on nerve endings to limit release of ACh. In asthmatics, neuronal M_2 receptors in the lungs do not function normally.[15]

M_3 Receptors. M_3 receptors, referred to as "glandular" muscarinic receptors, are located in exocrine glands and smooth muscle. Their effect on these organ systems is mostly stimulatory. Glandular secretions from lacrimal, salivary, bronchial, pancreatic, and mucosal cells in the GI tract are characteristic of M_3 receptor activation. Contraction of visceral smooth muscle is also a result of M_3 receptor stimulation. These stimulant effects are mediated through G-protein activation of phospholipase C (PLC) to form the second messengers inositol triphosphate (IP_3) and diacylglycerol (DG). Discoveries in the past decade have revealed that the endothelium can control the tone of vascular smooth muscle by the synthesis of a potent relaxant, endothelium-derived relaxing factor (EDRF), now identified as nitric oxide (NO), and a vasoconstrictor substance, endothelium-derived contracting factor (EDCF). The synthesis and release of these substances contribute to the tone of the vascular epithelium. M_3 receptors, when activated in endothelial cells, cause the release of EDRF and contribute to vasodilation.[16]

M_4 Receptors. M_4 receptors, like M_2 receptors, act through G_i protein to inhibit adenylate cyclase. They also function by a direct regulatory action on K^+ and Ca^{2+} ion channels. M_4 receptors in tracheal smooth muscle, when stimulated, inhibit the release of ACh[17] in the same manner that M_2 receptors do.

Biochemical Effects of Muscarinic Receptor Stimulation

Transmission at the synapse involving second messengers is much slower, about 100 milliseconds, compared with the few milliseconds at synapses where ion channels are activated directly. The delayed reaction to receptor stimulation is due to a cascade of biochemical events, which must occur to cause the pharmacologic response (Fig. 17-4). The sequence of events in these second-messenger systems begins with activation of the receptors by an agonist and involves the activation of G-proteins that are bound to a portion of the intracellular domain of the muscarinic receptor.[18] G-proteins are so called because of their interaction with the guanine nucleotides GTP and guanosine diphosphate (GDP). They translate drug–receptor interactions at the surface of the cell

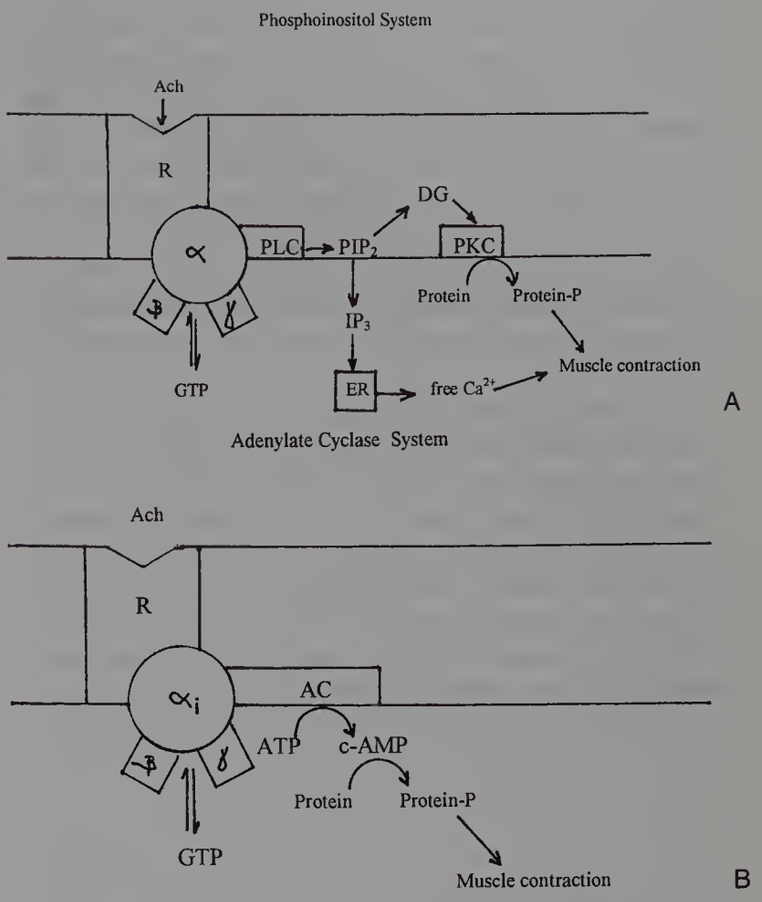


FIG. 17-4. *Proposed biochemical mechanisms of cholinergic receptor action.* **A:** *ACh activates a G-protein (α, β, γ) in the phospholipase system to activate the membrane enzyme phospholipase C (PLC), enhancing muscle contraction.* **B:** *Inhibition of adenylate cyclase system through an inhibitory G-protein (α_1) to cause muscle relaxation.*

to components inside the cell to create the biologic response. G-proteins consist of three subunits, α, β, and γ. When the receptor is occupied, the α-subunit, which has enzymatic activity, catalyzes the conversion of GTP to GDP. The α-subunit bound with GTP is the active form of the G-protein that can associate with various enzymes (i.e., PLC and adenylate cyclase) and ion channels (K^+ and Ca^{2+}). G-proteins are varied, and the α-subunit may cause activation (G_s) or inactivation (G_i) of the enzymes or channels. Recent studies suggest that β- and γ-subunits also contribute to pharmacologic effects.[19]

A single drug–receptor complex can activate several G-protein molecules, and each in turn can remain associated with a target molecule—for example, an enzyme—and cause the production of many molecules, amplifying the result of the initial drug–receptor combination. M_1, M_3, and M_5 receptors activate PLC, causing the release of IP_3 and DG, which in turn release intracellular Ca^{2+} and activate protein kinases, respectively. M_2 and M_4 receptors produce inhibition of adenylate cyclase.

Phosphoinositol System. The phosphoinositol system requires the breakdown of membrane-bound phosphatidylinositol 4,5-diphosphate (PIP_2) by PLC to IP_3 and DG,

which serve as second messengers in the cell. IP_3 mobilizes Ca^{2+} from intracellular stores in the endoplasmic recticulum to elevate cytosolic free Ca^{2+}. The Ca^{2+} activates Ca^{2+}-dependent kinases (e.g., troponin C in muscle) directly or binds to the Ca^{2+}-binding protein calmodulin, which activates calmodulin-dependent kinases. These kinases phosphorylate cell-specific enzymes to cause muscle contraction. DG is lipid-like and acts in the plane of the membrane through activation of protein kinase C to cause the phosphorylation of cellular proteins, also leading to muscle contraction (Fig. 17-4).[20,21]

Adenylate Cyclase. Adenylate cyclase, a membrane enzyme, is another target of muscarinic receptor activation. The second messenger cAMP is synthesized within the cell from adenosine triphosphate (ATP) by the action of adenylate cyclase. The regulatory effects of cAMP are many as it can activate a variety of protein kinases. Protein kinases catalyze the phosphorylation of enzymes and ion channels, altering the amount of calcium entering the cell and, thus, affecting muscle contraction. Muscarinic receptor activation causes lower levels of cAMP, reducing cAMP protein-dependent kinase activity, and a relaxation of muscle contraction. It has been suggested by some that a GTP-inhibitory protein (G_i) reduces the activity of adenylate cyclase, causing smooth muscle relaxation (Fig. 17-4).[18,22]

Ion Channels. In addition to the action of protein kinases that phosphorylates ion channels and modifies ion conductance, G-proteins are coupled directly to ion channels to regulate their action.[22] The Ca^{2+} channel on the cell membrane is activated by G-proteins without the need of a second messenger to allow Ca^{2+} to enter the cell. The α-subunit of the G-protein in heart tissue acts directly to open the K^+ channel, producing hyperpolarization of the membrane and slowing down the heart rate.

CHOLINERGIC NEUROCHEMISTRY

Cholinergic neurons synthesize, store, and release ACh (Fig. 17-5). The neurons also form choline acetyltransfer-

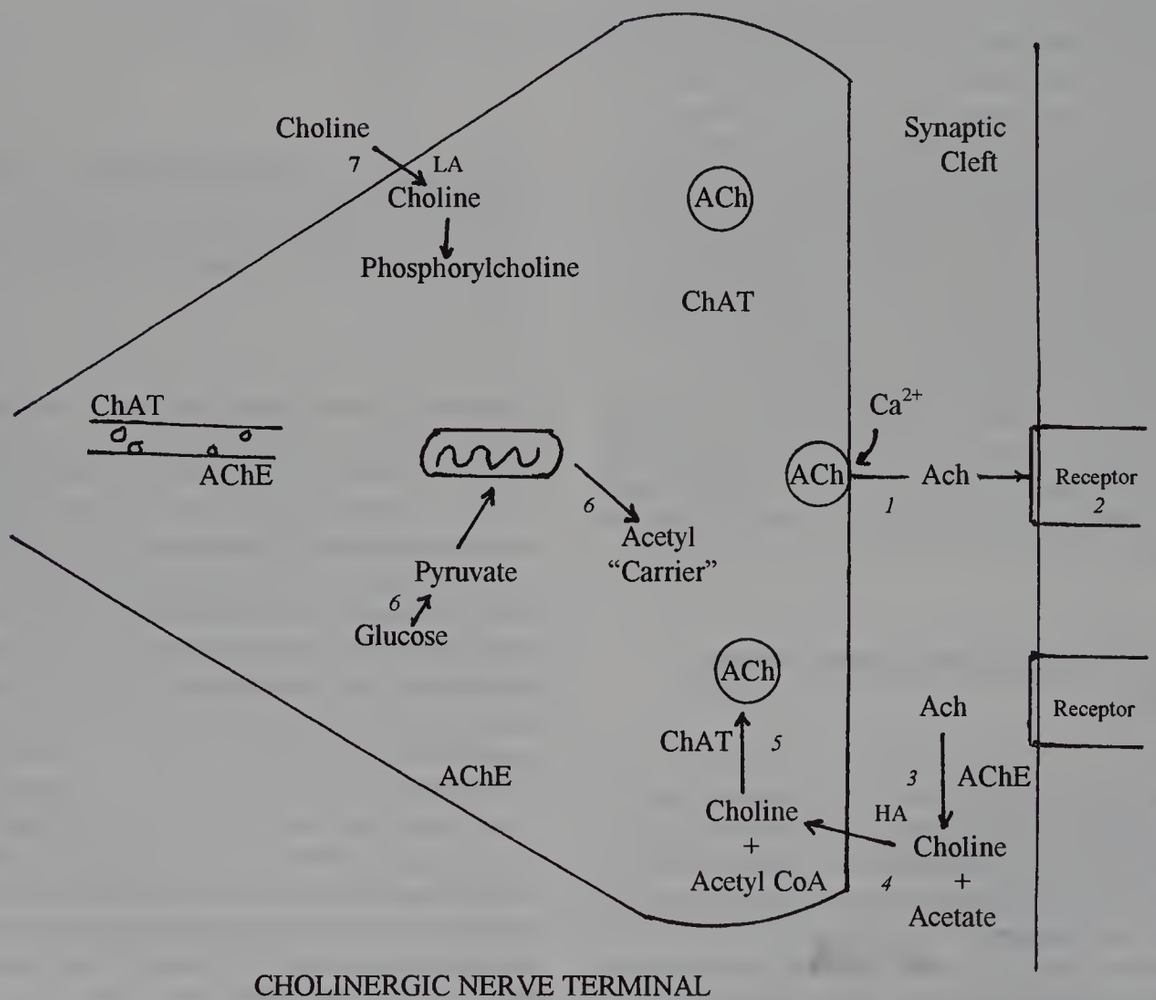
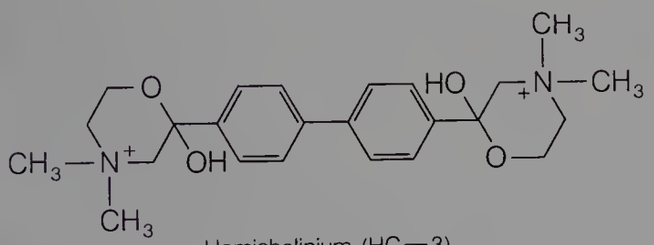


FIG. 17-5. Hypothetical model of synthesis, storage, and release of ACh. (1) *ACh is released from storage granules under the influence of the nerve action potential and* Ca^{2+}. (2) *ACh acts on postsynaptic cholinergic cholinergic receptors.* (3) *Hydrolysis of ACh by AChE occurs in the synaptic cleft.* (4) *A high-affinity uptake system returns choline into the cytosol.* (5) *ChAT synthesizes ACh in the cytosol, and the ACh is stored in granules.* (6) *Glucose is converted to pyruvate, which is converted to acetyl-CoA in the mitochondria. Release of acetyl-CoA from the mitochondria is by an acetyl carrier.* (7) *Choline is also taken up into the neuron by a low-affinity uptake system and converted partly to phosphorylcholine.*

ase (ChAT) and AChE. These enzymes are synthesized in the soma of the neuron and distributed throughout the neuron by axoplasmic flow. AChE is also located outside the neuron and associated with the neuroglial cells in the synaptic cleft. ACh is prepared in the nerve ending by the transfer of an acetyl group from acetyl-coenzyme A (CoA) to choline. The reaction is catalyzed by ChAT. Cell fractionation studies show that much of the ACh is contained in synaptic vesicles in the nerve ending but that some is also free in the cytosol. Choline is the limiting substrate for the synthesis of ACh. The majority of choline for ACh synthesis comes from the hydrolysis of ACh in the synapse. Choline is recaptured by the presyaptic terminal as part of a high-affinity uptake system under the influence of sodium ions[23] to synthesize ACh.

Several quaternary ammonium bases act as competitive inhibitors of choline uptake. Hemicholinium (HC-3), a bis-quaternary cyclic hemiacetal, and the triethyl analogue of choline, 2-hydroxyethyltriethylammonium, act at the presynaptic membrane to inhibit the high-affinity uptake of choline into the neuron. These compounds cause a delayed paralysis at repetitively activated cholinergic synapses and can produce respiratory paralysis in test animals. The delayed block is due to the depletion of stored ACh, which may be reversed by choline. The acetyl group used for the synthesis of ACh is obtained by conversion of glucose to pyruvate in the cytosol of the neuron and eventual formation of acetyl-CoA. Owing to the impermeability of the mitochondrial membrane to acetyl-CoA, this substrate is brought into the cytosol by the aid of an acetyl "carrier."



Hemicholinium (HC—3)

$HO—CH_2CH_2—\overset{+}{N}(C_2H_5)_3$

2-Hydroxyethyltriethylammonium

The synthesis of ACh from choline and acetyl-CoA is catalyzed by ChAT. Transfer of the acetyl group from acetyl-CoA to choline may be by a random or by an ordered reaction of the Theorell-Chance type. In the ordered sequence, acetyl-CoA first binds to the enzyme, forming a complex (EA) that then binds to choline. The acetyl group is transferred, and the ACh formed dissociates from the enzyme active site. The CoA is then released from the enzyme complex, EQ, to regenerate the free enzyme. The scheme is diagrammed in Fig. 17-6. ChAT is inhibited in vitro by *trans-N*-methyl-4-(1-naphthylvinyl)pyridinium iodide;[24] however, its inhibitory activity in whole animals is unreliable.[25]

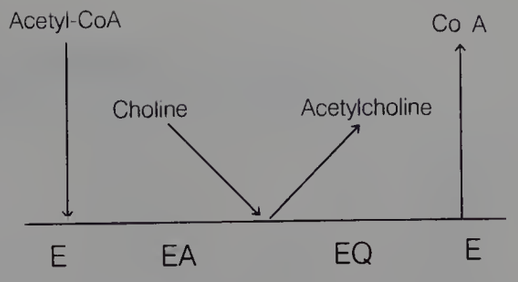
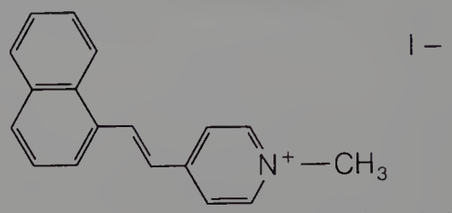


FIG. 17-6. *Ordered synthesis of acetylcholine by cholineacetyl transferase.*



trans-N-Methyl-4(1-naphthylvinyl)pyridinium iodide

Newly formed ACh is released from the presynaptic membrane when a nerve action potential invades a presynaptic nerve terminal.[26] The release of ACh results from a depolarization of the nerve terminal by the action potential, which alters membrane permeability to Ca^{2+}. Calcium enters the nerve terminal and causes release of the contents of several synaptic vesicles containing ACh into the synaptic cleft. This burst, or quantal release, of ACh causes depolarization of the postsynaptic membrane. The number of quanta of ACh released may be as high as several hundred at a neuromuscular junction, with each quantum containing between 12,000 and 60,000 molecules. ACh is also released spontaneously in small amounts from presynaptic membranes. This small amount of neurotransmitter maintains muscle tone by acting on the cholinergic receptors on the postsynaptic membrane.

After ACh has been released into the synaptic cleft, its concentration decreases rapidly. It is generally accepted that there is enough AChE at nerve endings to hydrolyze into choline and acetate any ACh that has been liberated. For example, there is sufficient AChE in the nerve junction of rat intercostal muscle to hydrolyze about 2.7×10^8 ACh molecules in 1 millisecond; this far exceeds the 3×10^6 molecules released by one nerve impulse.[27]

CHOLINERGIC AGONISTS

CHOLINERGIC STEREOCHEMISTRY

Three techniques have been used to study the conformational properties of ACh and other cholinergic chemicals: x-ray crystallography, nuclear magnetic resonance (NMR), and molecular modeling by computation. Each of these methods may report the spatial distribution of atoms in a molecule in terms of torsion angles. A *torsion angle* is defined as the angle formed between two planes, for example, by the O1-C5-C4-N atoms in ACh. The angle between the oxygen and

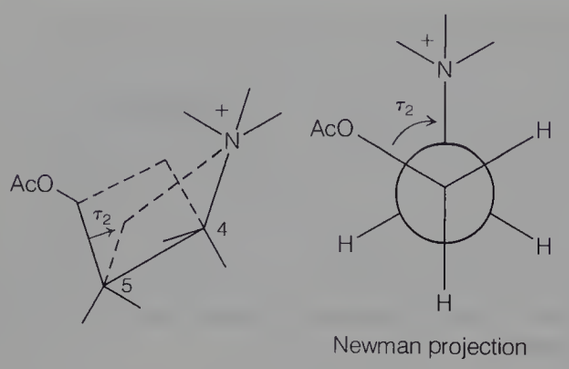


FIG. 17-7. *Spatial orientation of O1-C5-C4-N atoms in ACh.*

nitrogen atoms is best depicted by means of Newman projections (Fig. 17-7). A torsion angle has a positive sign when the bond of the front atom is rotated to the right to eclipse the bond of the rear atom. The spatial orientation of ACh is described by four torsion angles (Fig. 17-8).

The conformation of the choline moiety of ACh has drawn the most attention in studies relating structure and pharmacologic activity. The torsion angle (τ_2) determines the spatial orientation of the cationic head of ACh to the ester group. X-ray diffraction studies have shown that the torsion angle (τ_2) on ACh has a value of +77°. Many compounds that are muscarinic receptor agonists containing a choline component [e.g., O-C-C-$N^+(CH_3)_3$] have a preferred synclinal (gauche) conformation, with τ_2 values ranging from 68° to 89° (Table 17-2). Intermolecular-packing forces in the crystal as well as electrostatic interactions between the charged nitrogen group and the ether oxygen of the ester group are probably the two dominant factors that lead to a preference for the synclinal conformation in the crystal state. Some choline esters display an antiperiplanar (*trans*) conformation between the onium and ester groups. For example, carbamoylcholine chloride (τ_2 + 178°) is stabilized in this *trans* conformation by several hydrogen bonds. Acetylthiocholine iodide (τ_2 + 171°) is in this conformation because of the presence of the more bulky and less electronegative sulfur atom, and (+) *trans*-(1*S*,2*S*)-acetoxycyclopropyl trimethylammonium iodide (τ_2 + 137°) is fixed in this conformation by the rigidity of the cyclopropyl ring.

NMR spectroscopy of cholinergic molecules in solution is more limited than crystallography in delineating the conformation of compounds and is restricted to determining the

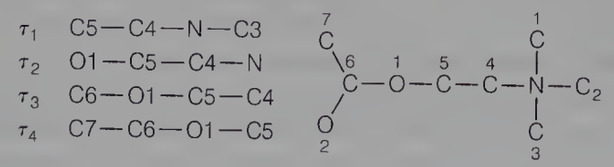


FIG. 17-8. *ACh torsion angles.*

TABLE 17-2

CONFORMATIONAL PROPERTIES OF SOME CHOLINERGIC AGENTS

Compound	*O1-C5-C4-N Torsion Angle*
Acetylcholine bromide	+77
Acetylcholine chloride	+85
(+)-2*S*, 3*R*, 5*S*-Muscarine iodide	+73
Methylfurmethide iodide	+83
(+) Acetyl(*S*)β-methylcholine iodide	+85
(−) Acetyl(*R*)α-methylcholine iodide	
Crystal form A	+89
Crystal form B	−150
(+) *cis*(2*S*)-methyl-(4*R*)-trimethylammonium-1,3-dioxolane iodide	+68
(+) *trans*(1*S*, 2*S*)-acetoxycyclopropyltrimethylammonium iodide	+137
Carbamoylcholine bromide	+178
Acetylthiocholine bromide	+171
Acetyl(*Rα*, *Sβ*)-dimethylcholine iodide (*erythro*)	+76

From Shefter, E.: In Triggle, D. J., Moran, J. F., Barnard, E. A. (eds.). Cholinergic Ligand Interactions. New York, Academic Press, 1971.

torsion angle O1-C5-C4-N. Most NMR data are in agreement with the results of x-ray diffraction studies. NMR studies indicate that ACh and methacholine apparently are not in their most stable *trans* conformation but exist in one of two gauche conformers[28] (Fig. 17-9). This may be the result of strong intramolecular interactions that stabilize the conformation of these molecules in solution.[29]

Molecular orbital calculations based on the principles of quantum mechanics may be used to determine energy minima of rotating bonds and to predict preferred conformations for the molecule. By means of molecular mechanics, theoretical conformational analysis has found that ACh has an energy minimum for the τ_2 torsion angle at about 84° and that the preferred conformation of ACh corresponds closely in aqueous solution to that found in the crystal state.

The study of interactions between bimolecules and small molecules is of great interest and importance toward the understanding of drug action. These studies are challenging because of the large size of at least one molecule. For the first time, the conformation of a neurotransmitter has been

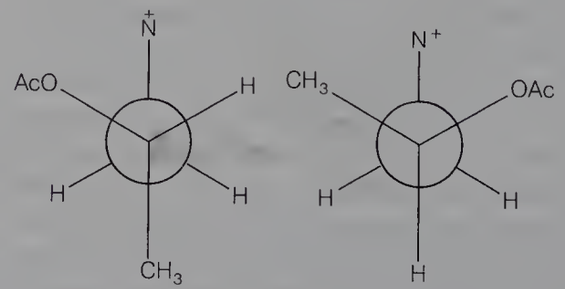


FIG. 17-9. *Gauche conformers of methacholine.*

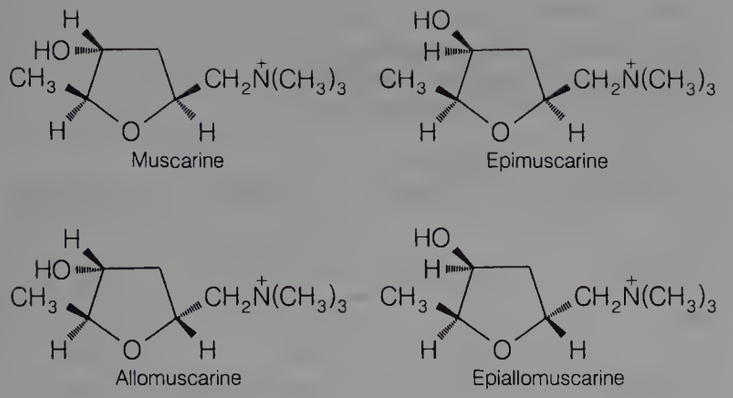


FIG. 17-10. *Geometric isomers of muscarine.*

determined for a molecular in the bound state. ACh is transformed from the gauche conformation in the free state to a nearly *trans* conformation when bound to the nicotinic receptor.[30] The active conformation of muscarinic agonists on their receptor has a dihedral angle of τ_2 between 110° and 117°.[31]

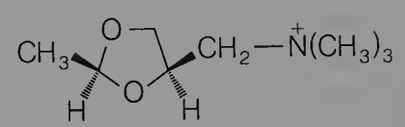
The parasympathomimetic effects of muscarine were first reported in 1869,[32] but its structure was not elucidated until 1957.[33] Muscarine has four geometric isomers: muscarine, epimuscarine, allomuscarine, and epiallomuscarine (Fig. 17-10). None has a center or plane of symmetry. Each geometric isomer can exist as an enantiomeric pair. The activity of muscarine, a nonselective muscarinic receptor agonist, resides primarily in the naturally occurring (+)-muscarine enantiomer. It is essentially free of nicotinic activity and apparently has the optimal stereochemistry to act on the muscarinic receptor subtypes. Synthetic molecules having a substituent on the carbon atom that corresponds to the β-carbon of ACh also show great differences in muscarinic activity between their isomers. Acetyl (+) (*S*) β-methylcholine, (+)*cis*-(2*S*)-methyl-(4*R*)-tri-methylammonium-1,3-dioxolane, (+)*trans*-(1*S*, 2*S*)-acetoxycyclopropyltrimethyl-ammonium, and naturally occurring (+)(2*S*, 3*R*, 5*S*)muscarine are more potent than their enantiomers and have very high ratios of activity between the (*S*)- and (*R*)-isomers (Table 17-3). A similar observation may be made of (+) acetyl (*S*)-β-methylcholine, (+)*cis*-(2*S*)-methyl-(4*R*)-trimethylammonium-1,3-dioxolane, and (+)*trans*-(1*S*, 2*S*)-acetoxycyclopropyltrimethyl-ammonium, all of which have an (*S*)-configuration at the carbon atom that corresponds to the β-carbon of ACh. Each of these active muscarinic molecules may be deployed on the receptor in the same manner as ACh and (+)-muscarine. Their (*S*)/(*R*) ratios (Table 17-3) show the greatest degree of stereoselectivity of the muscarinic receptor in guinea pig ileum for the configuration at the carbon adjacent to the ester group. In contrast, the nicotinic receptors are not considered as highly stereoselective as their muscarinic counterparts.

TABLE 17-3

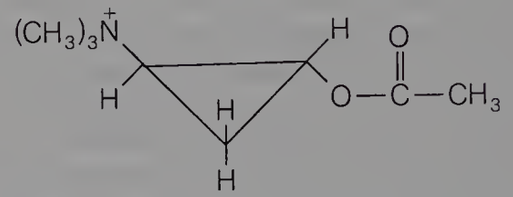
EQUIPOTENT MOLAR RATIOS OF ISOMERS ON GUINEA PIG ILEUM: RATIOS RELATIVE TO ACETYLCHOLINE

Compound	*Guinea Pig Ileum*	*(S)/(R) Ratio*
(+) Acetyl(*S*)β-methylcholine chloride	1.0[a]	24
(−) Acetyl(*R*)β-methylcholine chloride	24.0[a]	
(+) (2*S*, 3*R*, 5*S*)-Muscarine iodide	0.33[b]	394
(−) (2*R*, 3*S*, 5*R*)-Muscarine iodide	130[b]	
(+) *cis*(2*S*)-Methyl-(4*R*)-trimethylammonium-1,3-dioxolane iodide	6.00[c]	100
(−) *cis*(2*R*)-Methyl-(4*S*)-trimethylammonium-1,3-dioxolane iodide	0.06[c]	
(+) *trans*(1*S*, 2*S*)-Acetoxycyclopropyltrimethylammonium iodide	0.88[d]	517
(−) *trans*(1*R*, 2*R*)-Acetoxycyclopropyltrimethylammonium iodide	455[d]	

[a] Beckett, A. H., et al. Nature 189:671, 1961. [b] Waser, P. I.: Pharmacol. Rev. 13:465, 1961. [c] Belleau, B., Puranen, J.: J. Med. Chem. 6:235–328, 1963. [d] Armstrong, P. D., Cannon, J. G., Long, J. P.: Nature 220:65–66, 1968.



cis-2-Methyl-4-trimethylammonium-1,3-dioxolane



trans-2-Acetoxycyclopropyltrimethylammonium

STRUCTURE–ACTIVITY RELATIONSHIPS

Although muscarinic receptors have been cloned and the amino acid sequences are known, their three-dimensional structures remain unresolved. Thus, it is not possible to use this information alone to design specific drug molecules. Scientists still employ pharmacologic and biochemical tests to determine optimal structural requirements for activity. ACh is a relatively simple molecule. The chemistry and ease of testing for ACh biologic activity have allowed numerous chemical derivatives to be made and studied. Alterations on the molecule may be divided into three categories: the onium group, the ester function, and the choline moiety.

The onium group is essential for intrinsic activity and contributes to the affinity of the molecule for the receptors, partially through the binding energy and partially because of its action as a detecting and directing group. Molecular modeling data show the binding site to be a negatively

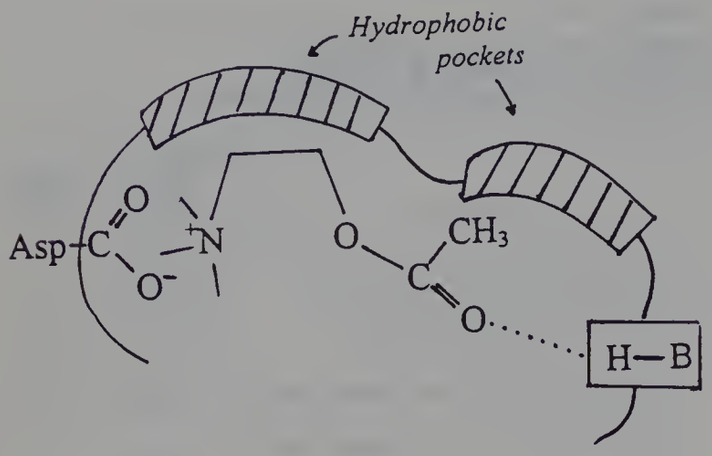


FIG. 17-11. Hypothetical structure of the muscarinic receptor.

charged aspartic acid residue in the third of the seven transmembrane helixes of the muscarinic receptor.[34] Hydrophobic pockets are located in helices 4, 5, 6, and 7 of the muscarinic receptor (Fig. 17-11).[35] The trimethylammonium group is the optimal functional moiety for activity, though some significant exceptions are known (e.g., pilocarpine, arecoline, nicotine, and oxotremorine). Phosphonium, sulfonium, arsenonium isosteres, or substituents larger than methyl on the nitrogen increase the size of the onium moiety, produce diffusion of the positive charge, and sterically interfere with proper drug–receptor interaction, resulting in a decrease in activity (Table 17-4).

The ester group in ACh contributes to the binding of the compound to the muscarinic receptor because of hydrogen bond formation with threonine and asparagine residues at the receptor site. A comparison of the cholinergic activity of a series of alkyl trimethylammonium compounds [$R-N^+(CH_3)$, R = C_1–C_9] shows *n*-amyltrimethylammonium,[36] which may be considered to have a size and mass similar to ACh and to be one magnitude weaker as a muscarinic agonist. The presence of the acetyl group in ACh is not as critical as the size of the molecule. In studying a series of *n*-alkyltrimethylammonium salts, it was noted[37] that for maximal muscarinic activity the quaternary ammonium group should be followed by a chain of five atoms; this has been referred to as the *five-atom rule.*

Shortening or lengthening the chain of atoms that separates the ester group from the onium moiety reduces muscarinic activity. α-Substitution on the choline moiety decreases both nicotinic and muscarinic activity, but muscarinic activity is decreased to a greater extent. Nicotinic activity is decreased to a greater degree by substitution on the β-carbon. Therefore, acetyl α-methylcholine, although less potent than ACh, has more nicotinic than muscarinic activity, while acetyl β-methylcholine (methacholine) exhibits more muscarinic than nicotinic activity. Hydrolysis by AChE is affected more by substitutions on the β- than the α-carbon. The hydrolysis rate of racemic acetyl β-methylacetylcholine is about 50% of that of ACh; racemic acetyl α-ACh is hydrolyzed about 90% as fast.

TABLE 17-4

ACTIVITY OF ACETOXYETHYL ONIUM SALTS: EQUIPOTENT MOLAR RATIOS RELATIVE TO ACETYLCHOLINE

$CH_3COOCH_2CH_2$	Cat Blood Pressure	Intestine	Frog Heart
$\overset{+}{N}Me_3$	1	1 (Rabbit)	1
$\overset{+}{N}Me_2H$	50	40	50
$\overset{+}{N}MeH_2$	500	1000	500
$\overset{+}{N}H_3$	2000	20,000	40,000
$\overset{+}{N}Me_2Et$	3	2.5 (Guinea pig)	2
$\overset{+}{N}MeEt_2$	400	700	1500
$\overset{+}{N}Et_3$	2000	1700	10000[a]
$\overset{+}{P}Me_3$	13	12 (Rabbit)	12
$As\overset{+}{}Me_3$	66	90	83
$\overset{+}{S}Me_2$	50	30 (Guinea pig)	96
	Size of quaternary atom:[b]		
N	d = 1.47 Å		d′ = 2.4 Å
P	1.87		3.05
S	1.82		—
As	1.98		3.23

[a] Reduces effect of acetylcholine.

[b] From Barlow, R. B.: Introduction to Chemical Pharmacology, London, Methuen and Co., 1964.

Welsh, A. D., Roepke, M. H.: J. Pharmacol. Exp. Ther. 55:118, 1935; Stehle, K. L., Melville, K. J., Oldham, F. K.: J. Pharmacol. Exp. Ther. 56:473, 1936; Holton, P., Ing, H. R.: Br. J. Pharmacol. 4:190, 1949; Ing, H. R., Kordik, P., Tudor Williams, D. P. H.: Br. J. Pharmacol. 7:103, 1952.

Oxotremorine

Oxotremorine [1-(-pyrrolidono)-4-pyrrolidino-2-butyne] has been regarded as a CNS muscarinic stimulant. Its action on the brain produces tremors in experimental animals. It increases ACh brain levels in rats up to 40% and has been studied as a drug in the treatment of Alzheimer's disease. While earlier studies suggested that this approach of elevating levels of ACh to treat Alzheimer's disease is useful, more recent data dispute this. Nevertheless, oxotremorine, as a cholinergic agonist, has been shown to facilitate memory storage.[38] Although it possesses groups that do not occur in other highly active muscarinic agents, its *trans*-conformation shows that distances between possible active centers correspond with (+)-muscarine (Fig. 17-12).[39]

Arecoline

Arecoline is an alkaloid obtained from the seeds of the betel nut (*Areca catechu*). The betel nut has been consumed by natives of the East Indies as a euphoria-creating substance.

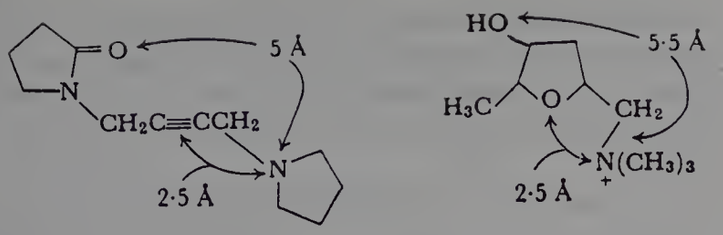
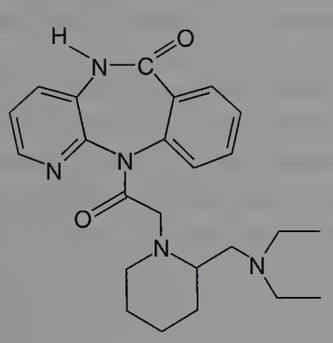


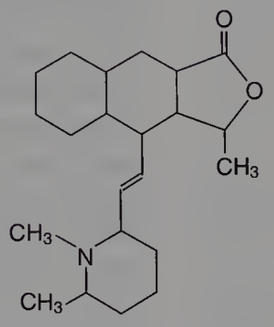
FIG. 17-12. Comparison of the geometries of oxotremorine and muscarine.

CHOLINERGIC RECEPTOR ANTAGONISTS

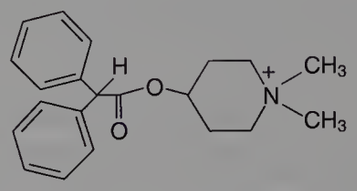
Characterization of muscarinic receptors can now be extended beyond the pharmacologic observations on organ systems (e.g., smooth muscle, heart) to determine structure–activity relationships. Dissociation constants of antagonists from radioligand-binding experiments on the various muscarinic receptors have played a major role in identifying these receptors and the selectivity of antagonists to the five muscarinic receptor subtypes. However, antagonists with high affinity for one receptor coupled with low affinity for the other four receptor types are very limited, and many antagonists bind to several subtypes with equal affinity. M_1 receptors have been identified as those with high affinity for pirenzapine and low affinity for a compound such as AF-DX 116. Pirenzapine can distinguish between M_1 and M_2, M_3, or M_5 but has significant affinity for M_4 receptors. Himbacine can distinguish between M_1 and M_4 receptors. Methoctramine, a polymethylenetetramine, not only discriminates between M_1 and M_2 receptors but also has good selectivity for M_2 muscarinic receptors. M_2 receptors bind to AF-DX 116 and gallamine, a neuromuscular blocking agent. M_3 receptors have a high affinity for 4-diphenylacetoxy-*N*-methyl-piperidine (4-DAMP) and hexahydrosiladifenidol (HHSiD) but also exhibit affinity for M_1 and M_2 receptors.[19] Tropicamide has been reported to be a putative M_4 receptor antagonist. Figure 17-13 includes structures of some receptor subtype antagonists.



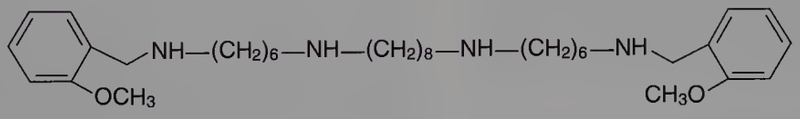
AF—DX 116



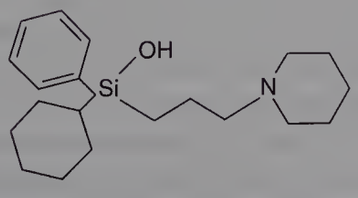
Himbacine



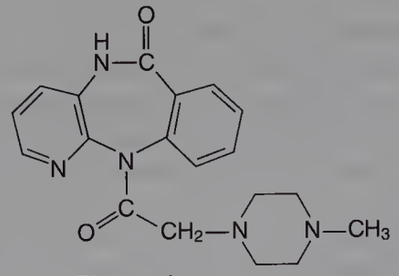
4-DAMP



Methoctramine



Hexahydrosiladiphenidol



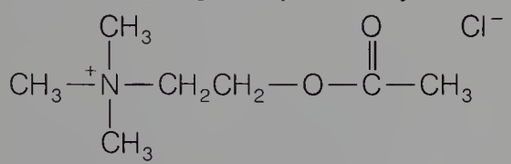
Pirenzepine

FIG. 17-13. Chemical structures of partially selective muscarinic antagonists.

PRODUCTS

Acetylcholine Chloride

ACh chloride exerts a powerful stimulant effect on the parasympathetic nervous system. Attempts have been made to use it as a cholinergic agent, but its duration of action is too short for sustained effects, owing to rapid hydrolysis by esterases and lack of specificity when administered for systemic effects. It is a cardiac depressant and an effective vasodilator. Stimulation of the vagus and the parasympathetic nervous system produces a tonic action on smooth muscle and induces a flow from the salivary and lacrimal glands. Its cardiac-depressant effect results from (1) a negative chronotropic effect that causes a decrease in heart rate and (2) a negative inotropic action on heart muscle that produces a decrease in the force of myocardial contractions. The vasodilatory action of ACh is primarily on the arteries and the arterioles, with distinct effect on the peripheral vascular system. Bronchial constriction is a characteristic side effect when the drug is given systemically.



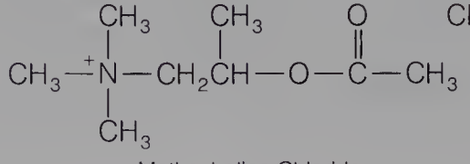
Acetylcholine Chloride

One of the most effective antagonists to the action of ACh is atropine, a nonselective muscarinic antagonist. Atropine blocks the depressant effect of ACh on cardiac muscle and its production of peripheral vasodilation (i.e., muscarinic effects) but does not affect the skeletal muscle contraction (i.e., nicotinic effect) produced.

ACh chloride is a hygroscopic powder that is available in an admixture with mannitol to be dissolved in sterile water for injection shortly before use. It is a short-acting miotic when introduced into the anterior chamber of the eye and is especially useful after cataract surgery during the placement of sutures. When applied topically to the eye, it has little therapeutic value because of poor corneal penetration and rapid hydrolysis by AChE.

Methacholine Chloride, USP

Acetyl β-methylcholine chloride; (2-hydroxypropyl)trimethylammonium chloride acetate. Methacholine is the acetyl ester of β-methylcholine. Unlike ACh, methacholine has sufficient stability in the body to give sustained parasympathetic stimulation. This action is accompanied by little (1/1000 that of ACh) or no nicotinic effect.



Methacholine Chloride

Methacholine can exist as (*S*)- and (*R*)-enantiomers. Although the chemical is used as the racemic mixture, its muscarinic activity resides principally in the (*S*)-isomer. The (*S*)/(*R*) ratio of muscarinic potency for these enantiomers is 240:1.

(+)-Acetyl-(*S*)-β-methylcholine is hydrolyzed by AChE, whereas the (*R*)(−)-isomer is not. (−)-Acetyl-(*R*)-β-methylcholine is a weak competitive inhibitor (K_i 4 × 10^{-4} *M*) of AChE obtained from the electric organ of the eel (*Electrophorus electricus*). The hydrolysis rate of the (*S*)(+)-isomer is about 54% that of ACh. This rate probably compensates for any decreased association (affinity) owing to the β-methyl group with the muscarinic receptor site and may account for the fact that ACh and (+)-acetyl-β-methylcholine have equimolar muscarinic potencies in vivo. (−)-Acetyl-(*R*)-β-methylcholine weakly inhibits AChE and slightly reinforces the muscarinic activity of the (*S*)(+)-isomer in the racemic mixture of acetyl-β-methylcholine.

In the hydrolysis of the acetyl α- and β-methylcholines, the greatest stereochemical inhibitory effects occur when the choline is substituted in the β-position. This also appears to be true of organophosphorus inhibitors. The (*R*)(−)- and (*S*)(+)-isomers of acetyl-α-methylcholine are hydrolyzed at 78% and 97% of the rate of ACh, respectively.

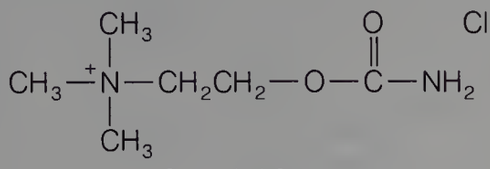
Methacholine chloride occurs as colorless or white crystals or as a white crystalline powder. It is odorless or has a slight odor and is very deliquescent. It is freely soluble in water, alcohol, or chloroform, and its aqueous solution is neutral to litmus and bitter. It is hydrolyzed rapidly in alkaline solutions. Solutions are relatively stable to heat and will keep for at least 2 or 3 weeks when refrigerated to delay growth of molds.

Carbachol

Choline chloride carbamate is nonspecific in its action on muscarinic receptor subtypes. The pharmacologic activity of carbachol is similar to that of ACh. It is an ester of choline and, thus, possesses both muscarinic and nicotinic properties by cholinergic receptor stimulation. It can also act indirectly by promoting release of ACh and by its weak anticholinesterase activity. Carbachol forms a carbamyl ester in the active site of AChE, which is hydrolyzed more slowly than an acetyl ester. This slower hydrolysis rate reduces the amount of free enzyme and prolongs the duration of ACh in the synapse. Carbachol also stimulates the autonomic ganglia and causes contraction of skeletal muscle but differs from a true muscarinic agent in that it does not have cardiovascular activity despite the fact that it seems to affect M_2 receptors.[40]

Carbachol is a miotic and has been used to reduce the intraocular tension of glaucoma when a response cannot be obtained with pilocarpine or neostigmine. Penetration of the cornea is poor but can be enhanced by the use of a wetting agent in the ophthalmic solution. In addition to its topical

use for glaucoma, carbachol is used during ocular surgery, when a more prolonged miosis is required than that which can be obtained with ACh chloride.



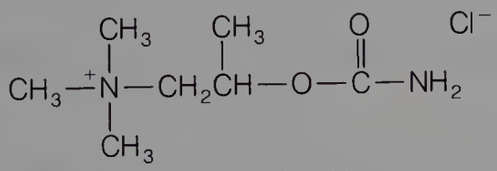
Carbachol Chloride

Carbachol differs chemically from ACh in its stability to hydrolysis. The carbamyl group of carbachol decreases the electrophilicity of the carbonyl and, thus, can form resonance structures more easily than can ACh. The result is that carbachol is less susceptible to hydrolysis and, therefore, more stable in aqueous solutions.

Bethanechol Chloride, U.S.P.

β-Methylcholine carbamate chloride; (2-hydroxypropyl)trimethylammonium chloride carbamate; carbamylmethylcholine chloride (Urecholine). Bethanechol is nonspecific in its action on muscarinic receptor subtypes but appears to be more effective at eliciting pharmacologic action of M_3 receptors.[41] It has pharmacologic properties similar to methacholine. Both are esters of β-methylcholine and have feeble nicotinic activity. Bethanechol is inactivated more slowly by AChE in vivo than is methacholine. It is a carbamyl ester and is expected to have similar stability in aqueous solutions as carbachol.

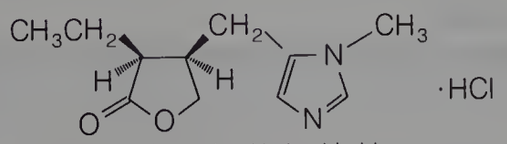
The main use of bethanechol chloride is in the relief of urinary retention and abdominal distention after surgery. The drug is used orally and by subcutaneous injection. It must never be administered by intramuscular or intravenous injection because of the danger from cholinergic overstimulation and loss of selective action. Proper administration of the drug is associated with low toxicity and no serious side effects. Bethanechol chloride should be used with caution in asthmatic patients; when used for glaucoma, it produces frontal headaches from the constriction of the sphincter muscle in the eye and from ciliary muscle spasms. Its duration of action is 1 hr.



Bethanechol Chloride

Pilocarpine Hydrochloride, USP

Pilocarpine monohydrochloride is the hydrochloride of an alkaloid obtained from the dried leaflets of *Pilocarpus jaborandi* or *P. microphyllus*, in which it occurs to the extent of about 0.5% together with other alkaloids.



Pilocarpine Hydrochloride

It occurs as colorless, translucent, odorless, faintly bitter crystals that are soluble in water (1:0.3), alcohol (1:3), and chloroform (1:360). It is hygroscopic and affected by light; its solutions are acid to litmus and may be sterilized by autoclaving. Alkalies saponify the lactone group to give the pharmacologically inactive hydroxy acid (pilocarpic acid). Base-catalyzed epimerization at the ethyl group position occurs to an appreciable extent and is another major pathway of degradation.[42] Both routes result in loss of pharmacologic activity.

Pilocarpine is a nonselective agonist on the muscarinic receptors. Despite this, it has been reported to act on M_3 receptors in smooth muscle to cause contractions in the gut, trachea, and eye.[43,44] In the eye, it produces pupillary constriction (miosis) and a spasm of accommodation. These effects are valuable in the treatment of glaucoma. The pupil constriction and spasm of the ciliary muscle reduce intraocular tension by establishing a better drainage of ocular fluid through the canal of Schlemm, located near the corner of the iris and cornea. Pilocarpine is used as a 0.5% to 0.6% solution (i.e., of the salts) in treating glaucoma. Systemic effects include copious sweating, salivation, and gastric secretion.

Pilocarpine Nitrate, USP

Pilocarpine mononitrate occurs as shining white crystals that are not hygroscopic but are light-sensitive. It is soluble in water (1:4) and alcohol (1:75) but insoluble in chloroform and ether. Aqueous solutions are slightly acid to litmus and may be sterilized in the autoclave. The alkaloid is incompatible with alkalies, iodides, silver nitrate, and reagents that precipitate alkaloids.

CHOLINESTERASE INHIBITORS

There are two types of cholinesterases in humans, AChE and butyrylcholinesterase (BuChE). The cholinesterases differ in their location in the body and their substrate specificity. AChE is associated with the outside surface of glial cells in the synapse and catalyzes the hydrolysis of ACh to choline and acetic acid. Inhibition of AChE prolongs the duration of the neurotransmitter in the junction and produces pharmacologic effects similar to those observed when ACh is administered. These inhibitors are indirect-acting cholinergic agonists. AChE inhibitors have been used in the treatment of myasthenia gravis, atony in the gastrointestinal tract, and glaucoma. They have also been employed as agricultural insecticides and nerve gases.

TABLE 17-5

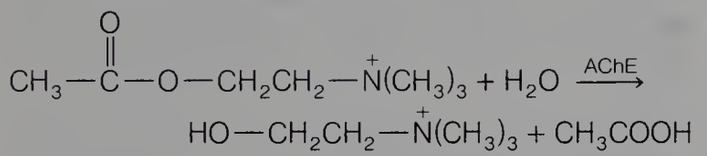
HYDROLYSIS OF VARIOUS SUBSTRATES BY AChE AND BuChE

	AChE		BuChE	
Enzyme Substrate	*Source*	*Relative Rate**	*Source*	*Relative Rate**
Acetylcholine	Human or bovine RBC	100	Human or horse plasma	100
Acetylthiocholine	Bovine RBC	149	Horse plasma	407
Acetyl β-methylcholine	Bovine RBC	18	Horse plasma	0
Propionylcholine	Human RBC	80	Horse plasma	170
Butyrylcholine	Human RBC	2.5	Horse plasma	250
Butyrylthiocholine	Bovine RBC	0	Horse plasma	590
Benzoylcholine	Bovine RBC	0	Horse plasma	67
Ethyl acetate	Human RBC	2	Human plasma	1
3,3-Dimethylbutyl acetate	Human RBC	60	Human plasma	35
2-Chloroethyl acetate	Human RBC	37	Human plasma	10
Isoamyl acetate	Human RBC	24	Horse plasma	7
Isoamyl propionate	Human RBC	10	Horse plasma	13
Isoamyl butyrate	Human RBC	1	Horse plasma	14

* Relative rates at approximately optimal substrate concentration; rate with acetylcholine = 100.

Adapted from Heath, D. F.: Organophosphorus Poisons—Anticholinesterases and Related Compounds, 1st ed. New York, Pergamon Press, 1961.

BuChE (psuedocholinesterase) is located in human plasma. Although its biologic function is not clear, it has catalytic properties similar to AChE. The substrate specificity is broader (Table 17-5), and it may hydrolyze dietary esters and drug molecules in the blood.

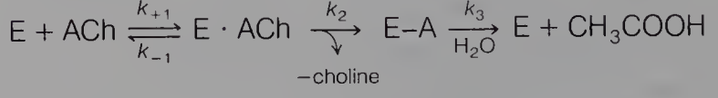


Three different chemical groupings—acetyl, carbamyl, and phosphoryl—may react with the esteratic site of AChE. Although the chemical reactions are similar, the kinetic parameters for each type of substrate differ and result in differences between toxicity and usefulness.

The initial step in the hydrolysis of ACh by AChE is a reversible enzyme–substrate complex formation. The association rate (k_{+1}) and dissociation rate (k_{-1}) are relatively large. The enzyme–substrate complex, E·ACh, may also form an acetyl–enzyme intermediate at a rate (k_2) that is slower than either the association or dissociation rates. Choline is released from this complex with the formation of the acetyl–enzyme intermediate, EA. This intermediate is then hydrolyzed to regenerate the free enzyme and acetic acid. The acetylation rate, k_2, is the slowest step in this sequence and is rate-limiting (see later discussion).

Kinetic studies with different substrates and inhibitors suggest that the active center of AChE consists of several major domains: an anionic site, to which the trimethylammonium group binds; an esteratic site, which causes hydrolysis of the ester portion of ACh; and hydrophobic sites, which bind aryl substrates, other uncharged ligands, and the alkyl portion of the acyl moiety of ACh. There is also a peripheral anionic site, removed by at least 20 Å from this active center, which allosterically regulates activity at the esteratic site.[45] The anionic site was believed to have been formed by the γ-carboxylate group of a glutamic acid residue,[46] but more recent studies suggest that the aromatic moieties of tryptophan and phenylalanine residues bind the quaternary ammonium group of ACh in the anionic site through cation-π interactions.[47] The location and spatial organization in the esteratic site by serine, histidine, and glutamic acid residues constitute the esteratic site. The triad of these amino acid residues contributes to the high catalytic efficiency of AChE (Fig. 17-14).[48]

AChE attacks the ester substrate through a serine hydroxyl, forming a covalent acyl–enzyme complex. The serine is activated as a nucleophile by the glutamic acid and histidine residues that serve as the proton sink to attack the carbonyl carbon of ACh. Choline is released, leaving the acetylated serine residue on the enzyme. The acetyl–enzyme intermediate is cleaved by a general base catalysis mechanism to regenerate the free enzyme. The rate of the deacetylation step is indicated by k_3.



Carbamates such as carbachol are also able to serve as substrates for AChE, forming a carbamylated enzyme intermediate (E–C). The rate of carbamylation (k_2) is slower than the rate of acetylation. Hydrolysis (k_3, decarbamylation) of the carbamyl–enzyme intermediate is 10^7 times slower than that of its acetyl counterpart. The slower hydrolysis rate limits the optimal functional capacity of AChE, allowing carbamate substrates to be semireversible inhibitors of AChE.

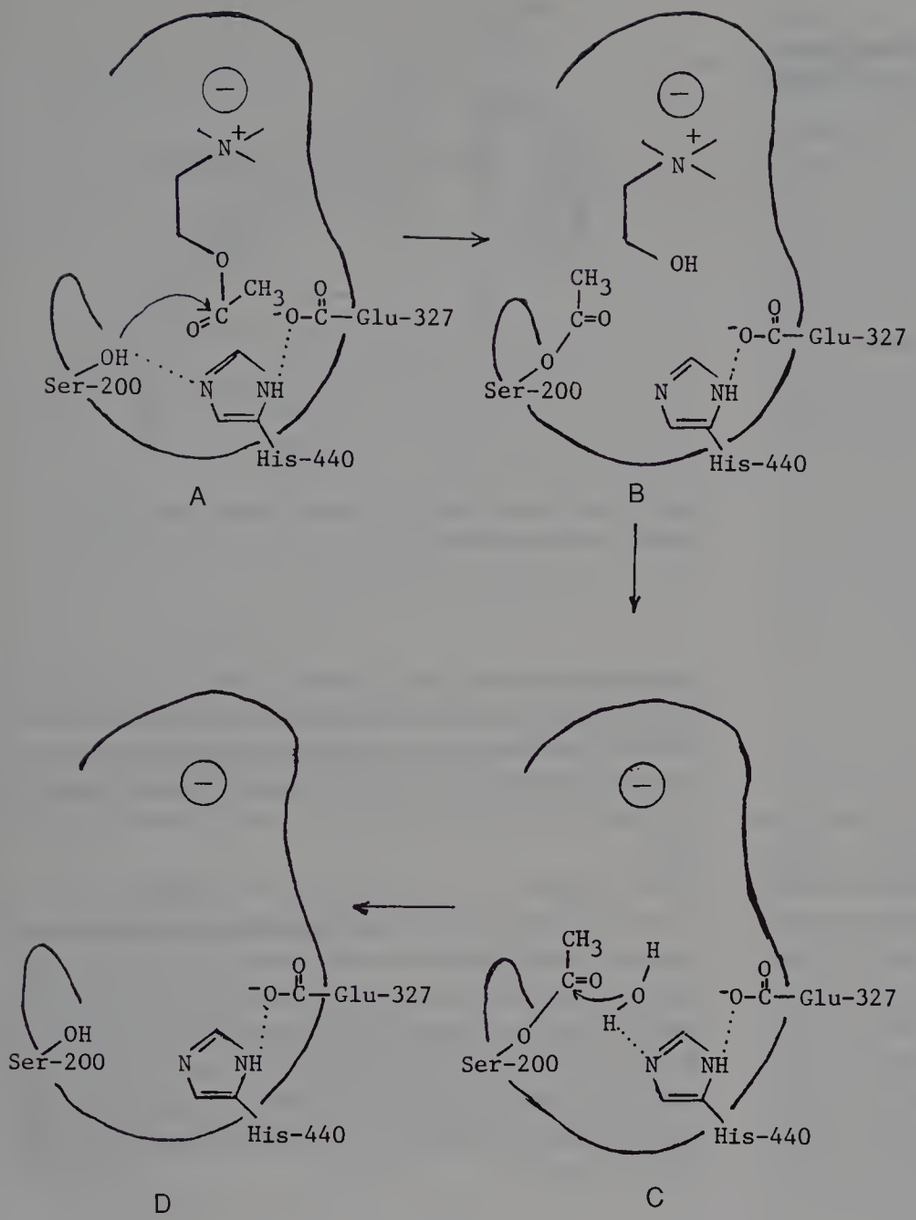
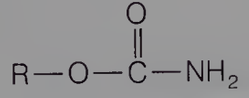


FIG. 17-14. *Mechanism of hydrolysis of ACh by AchE.* **(A)** *ACh–AChE-reversible complex.* **(B)** *Acetylation of esteratic site.* **(C)** *General base-catalyzed hydrolysis of acetylated enzyme.* **(D)** *Free enzyme.*

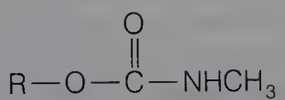
$$E + CX \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} E \cdot CX \overset{k_2}{\underset{X}{\rightleftharpoons}} E\text{–}C \xrightarrow{k_3} E + C$$

where CX = carbamylating substrate

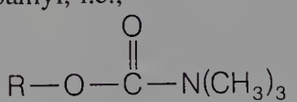
In the mechanism above, k_3 is the rate-limiting step. The rate of k_2 depends not only on the nature of the alcohol moiety of the ester but also on the type of carbamyl ester. Esters of carbamic acid, i.e.,



are better carbamylating agents of AChE than the methylcarbamyl, i.e.,



and dimethylcarbamyl, i.e.,



analogues.[49]

Organophosphate esters of selected compounds are also able to esterify the serine residue in the active site of AChE. The hydrolysis rate (k_3) of the phosphorylated serine is extremely slow, and hydrolysis to the free enzyme and phosphoric acid derivative is so limited that the inhibition is considered irreversible. These organophosphorus compounds are used in the treatment of glaucoma, as agricultural insecticides, and, at times, as nerve gases in warfare. Table 17-6 shows the relative potencies of several AChE inhibitors.

$$E + PX \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} E \cdot PX \overset{k_2}{\underset{X}{\rightleftharpoons}} E\text{–}P \xrightarrow{k_3} E + P$$

where PX = phosphorylating substrate

TABLE 17-6

INHIBITION CONSTANTS FOR ANTICHOLINESTERASE POTENCY OF ACETYLCHOLINESTERASE INHIBITORS

Reversible and Semireversible Inhibitors	K_1 *(M)*
Ambenonium	4.0×10^{-8}
Carbachol	1.0×10^{-4}
Demecarium	1.0×10^{-10}
Edrophonium	3.0×10^{-7}
Neostigmine	1.0×10^{-7}
Physostigmine	1.0×10^{-8}
Pyridostigmine	4.0×10^{-7}
Irreversible Inhibitors	K_2 *(mol/min)*
Isoflurophate	1.9×10^{4}
Echothiophate	1.2×10^{5}
Paraoxon	1.1×10^{6}
Sarin	6.3×10^{7}
Tetraethylpyrophosphate	2.1×10^{8}

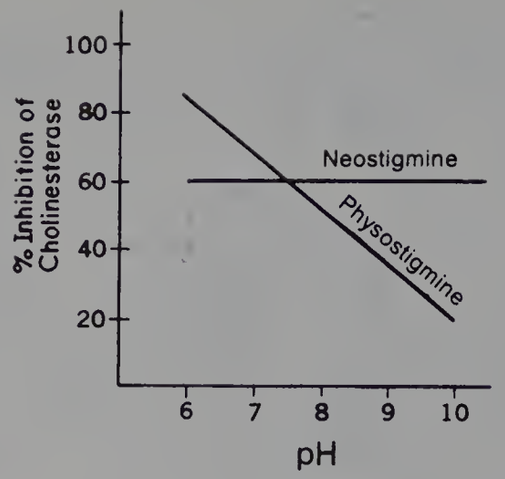


FIG. 17-15. *Effect of pH* on inhibition of cholinesterase by physostigmine and neostigmine.

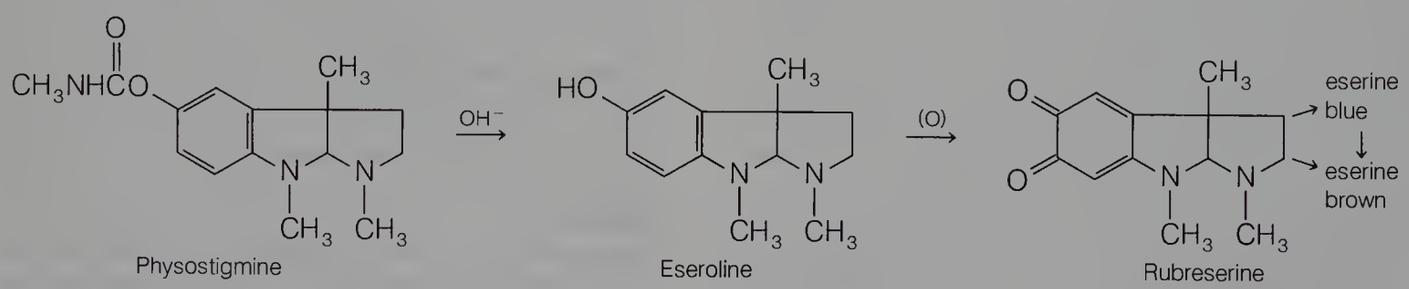
REVERSIBLE INHIBITORS

Physostigmine, USP

Physostigmine is an alkaloid obtained from the dried ripe seed of *Physostigma venenosum*. It occurs as a white, odorless, microcrystalline powder that is slightly soluble in water and freely soluble in alcohol, chloroform, and the fixed oils. The alkaloid, as the free base, is quite sensitive to heat, light, moisture, and bases, undergoing rapid decomposition. In solution it is hydrolyzed to methyl carbamic acid and eseroline, neither of which inhibits AChE. Eseroline is oxidized to a red compound, rubreserine,[50] and then further decomposed to eserine blue and eserine brown. Addition of sulfite or ascorbic acid prevents oxidation of the phenol, eseroline, to rubreserine. Hydrolysis does take place, however, and the physostigmine is inactivated. Solutions are most stable at *p*H 6 and should never be sterilized by heat.

Physostigmine is a relatively poor carbamylating agent of AChE and is often considered a reversible inhibitor of the enzyme. Its cholinesterase-inhibiting properties vary with the *p*H of the medium (Fig. 17-15). The conjugate acid of physostigmine has a pK_a of about 8, and as the *p*H of the solution is lowered, more is present in the protonated form. Inhibition of cholinesterase is greater in acid media, suggesting that the protonated form makes a contribution to the inhibitory activity well as its carbamylation of the enzyme.

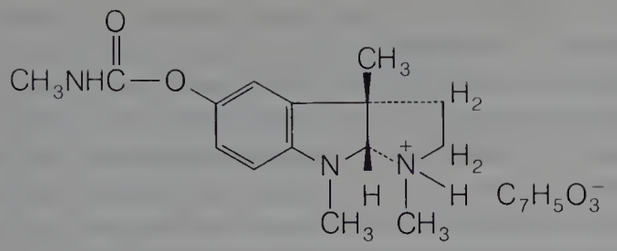
Physostigmine was used first as a topical application in the treatment of glaucoma. Its lipid solubility properties permit adequate absorption from ointment bases. It is used systemically as an antidote for atropine poisoning and other anticholinergic drugs by increasing the duration of action of ACh at cholinergic sites through inhibition of AChE. Physostigmine, along with other cholinomimetic drugs acting in the CNS, has been studied for use in the treatment of Alzheimer's disease.[51]



Physostigmine — Eseroline — Rubreserine

Physostigmine Salicylate, USP (Eserine Salicylate)

The salicylate of physostigmine may be prepared by neutralizing an ethereal solution of the alkaloid with an ethereal solution of salicylic acid. Excess salicylic acid is removed from the precipitated product by washing it with ether. The salicylate is less deliquescent than the sulfate.



Physostigmine Salicylate

Physostigmine salicylate occurs as a white, shining, odorless crystal or white powder that is soluble in water (1:75), alcohol (1:16), or chloroform (1:6) but much less soluble in ether (1:250). Upon prolonged exposure to air and light, the crystals turn red. The red may be removed by washing the crystals with alcohol, although this causes loss of the compound as well. Aqueous solutions are neutral or slightly acidic and take on a red coloration after a period. The coloration may be taken as an index of the loss of activity of physostigmine solutions.

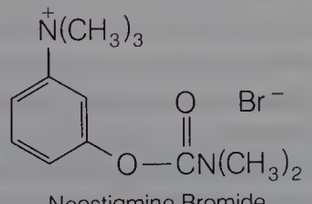
Solutions of physostigmine salicylate are incompatible with the usual reagents that precipitate alkaloids (alkalies) and with iron salts. Incompatibility also occurs with benzalkonium chloride and related wetting agents because of the salicylate ion.

Physostigmine Sulfate, USP

Physostigmine sulfate occurs as a white, odorless, microcrystalline powder that is deliquescent in moist air. It is soluble in water (1:4), alcohol (1:0.4), and ether (1:1200). It has the advantage over the salicylate salt in that it is compatible in solution with benzalkonium chloride and related compounds.

Neostigmine Bromide

(*m*-Hydroxyphenyl)trimethylammonium bromide dimethylcarbamate; dimethylcarbamic ester of 3-hydroxyphenyltrimethylammonium bromide (Prostigmin bromide). Neostigmine is used as an antidote to nondepolarizing neuromuscular blocking drugs and in the treatment of myasthenia gravis. It occurs as a bitter, odorless, white, crystalline powder. It is soluble in water and alcohol. The crystals are much less hygroscopic than those of neostigmine methylsulfate and, thus, may be used in tablets. Solutions are stable and may be sterilized by boiling. Aqueous solutions are neutral to litmus.



Neostigmine Bromide

Use of physostigmine as a prototype of an indirect-acting parasympathomimetic drug led to the development of stigmine, in which a trimethylamine group was placed *para* to a dimethyl carbamate group in benzene. Better inhibition of cholinesterase was observed when these groups were placed *meta* to each other as in neostigmine, a more active and useful agent. Although physostigmine contains a methyl carbamate functional group, greater chemical stability toward hydrolysis was obtained with the dimethylcarbamyl group in neostigmine.[52]

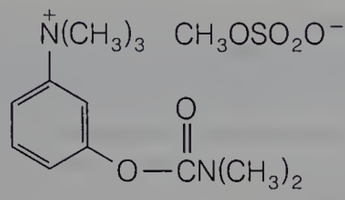
Neostigmine has a half-life of about 50 minutes after oral or intravenous administration. About 80% of a single intramuscular dose is excreted in the urine within 24 hours, approximately 40% unchanged and the remainder as metabolites. Of the neostigmine that reaches the liver, 98% is metabolized in 10 minutes to 3-hydroxyphenyltrimethyl ammonium, which has activity similar to, but weaker than, neostigmine. Its transfer from plasma to liver cells and then to bile is probably passive. Because cellular membranes permit the passage of plasma proteins synthesized in the liver into the bloodstream through capillary walls or lymphatic vessels, they may not present a barrier to the diffusion of quaternary amines such as neostigmine. The rapid hepatic metabolism of neostigmine may provide a downhill gradient for the continual diffusion of this compound.[53] A certain amount is hydrolyzed slowly by plasma cholinesterase.

Neostigmine has a mechanism of action quite similar to that of physostigmine. It effectively inhibits cholinesterase at about 10^{-6} *M* concentration. Its activity does not vary with *p*H, and at all ranges it exhibits similar cationic properties (Fig. 17-15). Skeletal muscle is also stimulated by neostigmine, a property that physostigmine does not have.

The uses of neostigmine are similar to those of physostigmine but differ in that there is greater miotic activity, fewer and less unpleasant local and systemic manifestations, and greater chemical stability. The most frequent application of neostigmine is to prevent atony of the intestinal, skeletal, and bladder musculature. An important use is in the treatment of myasthenia gravis, a condition caused by an autoimmune mechanism that requires an increase in ACh in the neuromuscular junction to sustain normal muscular activity.

Neostigmine Methylsulfate

(*m*-Hydroxyphenyl)trimethylammonium methylsulfate dimethylcarbamate; dimethylcarbamic ester of 3-hydroxyphenyltrimethylammonium methylsulfate (Prostigmin methylsulfate). This compound is a bitter, odorless, white, crystalline powder. It is very soluble in water and soluble in alcohol. Solutions are stable and can be sterilized by boiling. The compound is too hygroscopic for use in a solid form and, thus, always is used as an injection. Aqueous solutions are neutral to litmus.

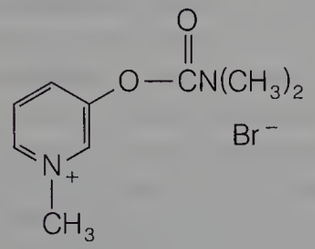


Neostigmine Methylsulfate

The methylsulfate salt is used postoperatively as a urinary stimulant and in the diagnosis and treatment of myasthenia gravis.

Pyridostigmine Bromide, USP

3-Hydroxy-1-methylpyridinium bromide dimethylcarbamate, pyridostigminium bromide (Mestinon). This compound occurs as a white, hygroscopic, crystalline powder having an agreeable, characteristic odor. It is freely soluble in water, alcohol, and chloroform.



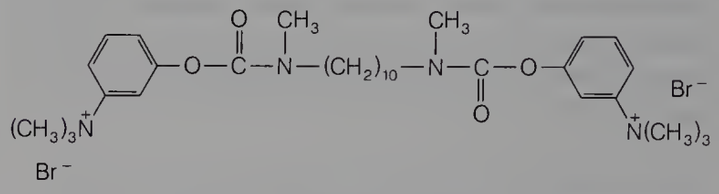
Pyridostigmine Bromide

Pyridostigmine bromide is about one-fifth as toxic as neostigmine. It appears to function in a manner similar to that of neostigmine and is the most widely used anticholinesterase agent employed to treat myasthenia gravis. The drug is metabolized by the liver enzymes and plasma cholinesterase. The principal metabolite is 3-hydroxy-*N*-methylpyridinium. Pyridostigmine has a half-life of 90 minutes and a duration of action between 3 and 6 hours when administered orally.

This drug acts by suppressing the activity of AChE. It possesses a relatively prolonged duration of action and causes fewer side effects in the gastrointestinal tract than the other anticholinesterase agents. The dosage requirements vary considerably, and the dosage must be individualized according to the response and tolerance of the patient. Because of its quaternary ammonium structure, ambenonium chloride is absorbed poorly from the gastrointestinal tract. In moderate doses, the drug does not cross the blood–brain barrier. Ambenonium chloride is not hydrolyzed by cholinesterases.

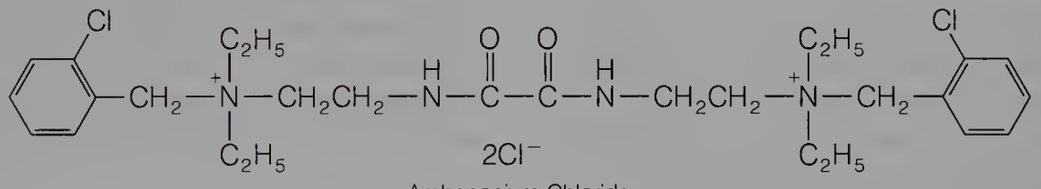
Demecarium Bromide, USP

(*m*-Hydroxyphenyl)trimethylammonium bromide decamethylenebis[methylcarbamate] (Humorsol) is the diester of (*m*-hydroxyphenyl)trimethylammonium bromide with decamethylene bis(methylcarbamic acid) and, thus, is comparable to a bis-prostigmine molecule.



Demecarium Bromide

It occurs as a slightly hygroscopic powder that is freely soluble in water or alcohol. Ophthalmic solutions of the drug have a *p*H of 5 to 7.5. Aqueous solutions are stable and may be sterilized by heat. Its efficacy and toxicity are comparable with those of other potent anticholinesterase inhibitor drugs. It is a long-acting miotic used to treat wide-angle glaucoma and accommodative esotropia. Maximal effect occurs hours after administration, and the effect may persist for days.



Ambenonium Chloride

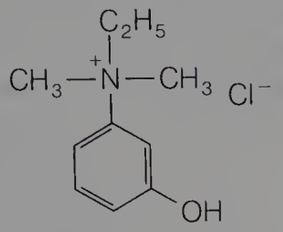
Ambenonium Chloride

[Oxalylbis(iminoethylene)]bis[(*o*-chlorobenzyl)diethyl ammonium] dichloride (Mytelase chloride). This compound is a white, odorless powder, soluble in water and alcohol, slightly soluble in chloroform, and practically insoluble in ether and acetone. Ambenonium chloride is used for the treatment of myasthenia gravis in patients who do not respond satisfactorily to neostigmine or pyridostigmine.

Edrophonium Chloride, USP

Ethyl(*m*-hydroxyphenyl)dimethylammonium chloride (Tensilon). Edrophonium chloride is a reversible anticholinesterase agent. It is bitter and very soluble in water and alcohol. Edrophonium chloride injection has a *p*H of 5.2 to 5.5. On parenteral administration, edrophonium has a more rapid onset and shorter duration of action than neostigmine, pyridostigmine, or ambenonium. It is a specific anticurare agent

and acts within 1 minute to alleviate overdose of *d*-tubocurarine, dimethyl *d*-tubocurarine, or gallamine triethiodide. The drug is also used to terminate the action of any one of these drugs when the physician so desires. However, it is of no value in terminating the action of the depolarizing (i.e., noncompetitive) blocking agents, such as decamethonium and succinylcholine. In addition to inhibiting AChE, edrophonium chloride has a direct cholinomimetic effect on skeletal muscle, which is greater than that of most other anticholinesterase drugs.

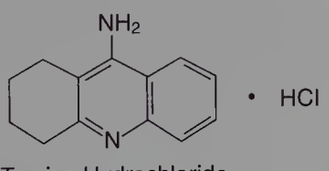


Edrophonium Chloride

Edrophonium chloride is structurally related to neostigmine methylsulfate and has been used as a potential diagnostic agent for myasthenia gravis. This is the only degenerative neuromuscular disease that can be temporarily improved by administration of an anticholinesterase agent. Edrophonium chloride brings about a rapid increase in muscle strength without significant side effects.

Tacrine Hydrochloride

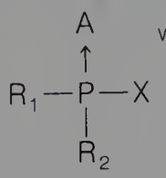
1,2,3,4-Tetrahydro-9-aminoacridine hydrochloride (THA) is a reversible cholinesterase inhibitor that has been used in the treatment of Alzheimer's disease. The drug has been used to increase the levels of ACh in patients with the disease based on observations from autopsies that concentrations of ChAT and AChE are markedly reduced in the brain, while the number of muscarinic receptors is almost normal. The use of the drug is not without controversy, as conflicting results on efficacy have been reported.[54,55] The drug has been used in mild to moderate Alzheimer's dementia.



Tacrine Hydrochloride

IRREVERSIBLE INHIBITORS

Both AChE and BuChE are inhibited irreversibly by a group of phosphate esters that are highly toxic (LD_{50} for humans is 0.1 to 0.001 mg/kg). These chemicals are nerve poisons and have been used in warfare and as agricultural insecticides. They permit ACh to accumulate at nerve endings and produce an exacerbation of ACh-like actions. The compounds belong to a class of organophosphorous esters. A general formula for such compounds is as follows:



where R_1 = alkoxyl
R_2 = alkoxyl, alkyl, or tertiary amine
X = a good leaving group (e.g. F, CN, thiomalate, *p*-nitrophenoxy)

A is usually oxygen or sulfur but may also be selenium. When *A* is other than oxygen, biologic activation is required before the compound becomes effective as an inhibitor of cholinesterases. Phosphorothionates [$R_1R_2P(S)X$] have much poorer electrophilic character than their oxygen analogues and are much weaker hydrogen bond-forming molecules because of the sulfur atom.[56] Their anticholinesterase activity is 10^5-fold weaker than their oxygen analogues. *X* is the leaving group when the molecule reacts with the enzyme. Typical leaving groups include fluoride, nitrile, and *p*-nitrophenoxy. The *R* groups may be alkyl, alkoxy, aryl, aryloxy, or amino. The *R* moiety imparts lipophilicity to the molecule and contributes to its absorption through the skin. Inhibition of AChE by organophosphorous compounds takes place in two steps, association of enzyme and inhibitor and the phosphorylation step, completely analogous to acylation by the substrate (Fig. 17-16). Stereospecificity is mainly due to interactions of enzyme and inhibitor at the esteratic site.

The serine residue at the esteratic site forms a stable phosphoryl ester with the organophosphorous inhibitors. This stability permits labeling studies[57] to be carried out on this and other enzymes (e.g., trypsin, chymotrypsin) that have the serine hydroxyl as part of their active site.

Although insecticides and nerve gases are irreversible inhibitors of cholinesterases by forming a phosphorylated serine at the esteratic site of the enzyme, it is possible to reactivate the enzyme if action is taken soon after exposure to these poisons has occurred. Several compounds can provide a nucleophilic attack on the phosphorylated enzyme and cause regeneration of the free enzyme. Substances such as choline, hydroxylamine, and hydroxamic acid have led to the development of more effective cholinesterase reactivators, such as nicotinic hydroxamic acid and pyridine-2-aldoxime methiodide (2-PAM). A proposed mode of action for the reactivation of cholinesterase that has been inactivated by isoflurophate by 2-PAM is shown in Figure 17-16.

Cholinesterases that have been exposed to phosphorylating agents (e.g., sarin) become refractory to reactivation by cholinesterase reactivators. The process is called aging and occurs both in vivo and in vitro with AChE and BuChE. Aging occurs by partial hydrolysis of the phosphorylated moiety that is attached to the serine residue at the esteratic site of the enzyme (Fig. 17-17).

Phosphate esters used as insecticidal agents are toxic and must be handled with extreme caution. Symptoms of toxicity are nausea, vomiting, excessive sweating, salivation, miosis, bradycardia, low blood pressure, and respiratory difficulty, which is the usual cause of death.

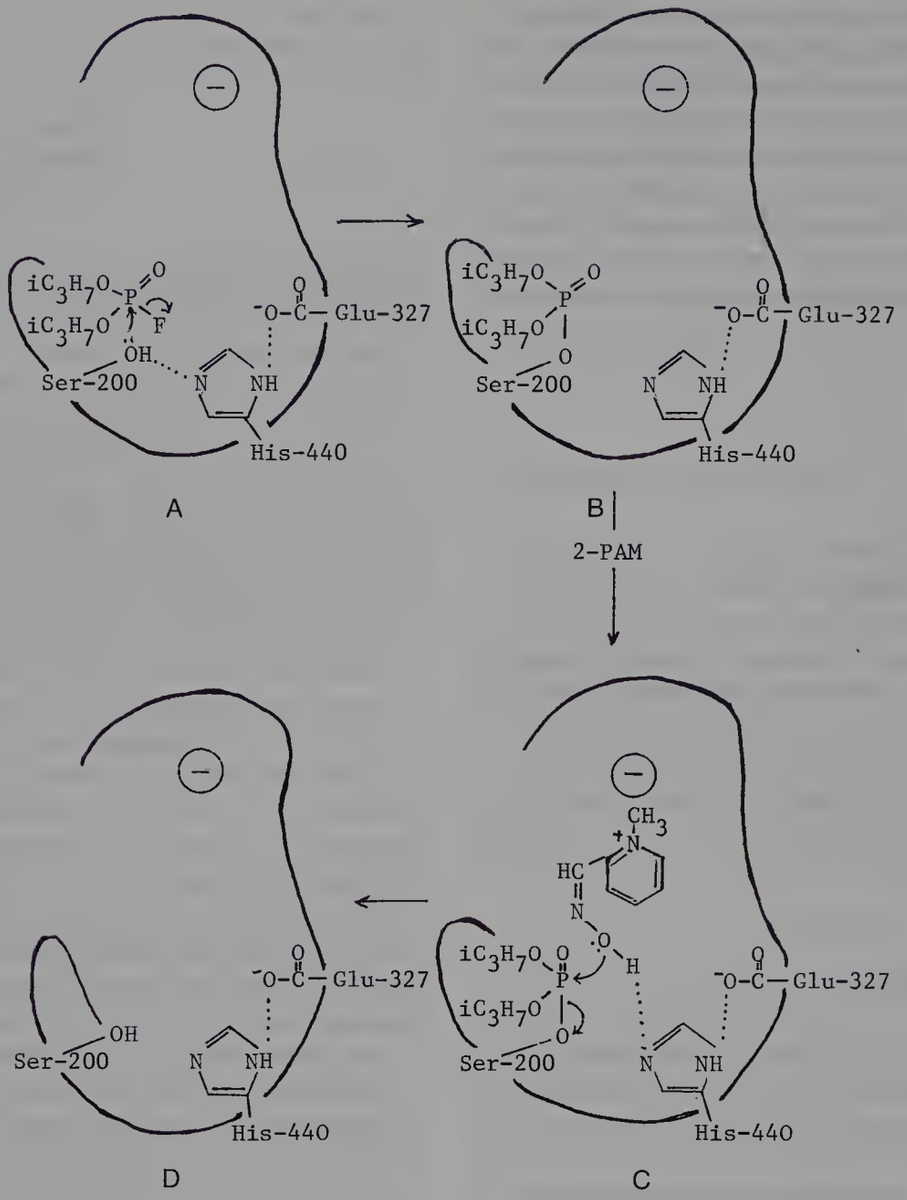


FIG. 17-16. *Phosphorylation and reactivation of cholinesterase.* **(A)** *Phosphorylation of serine by isofluorphate.* **(B)** *Phosphorylated serine at the esteratic site.* **(C)** *Nucleophilic attack on phosphorylated residue by 2-PAM.* **(D)** *Free enzyme.*

The organophosphate insecticides of low toxicity, such as malathion, generally cause poisoning only by ingestion of relatively large doses. However, parathion or methylparathion cause poisoning by inhalation or dermal absorption. Because these compounds are so long-acting, cumulative and serious toxic manifestations may result after several small exposures.

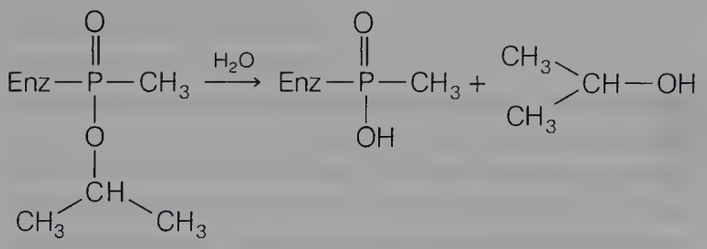
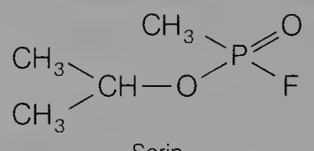


FIG. 17-17. *Aging of phosphorylated enzyme.*



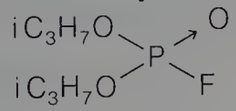
Sarin

PRODUCTS

Isofluorphate, USP

Diisopropyl phosphorofluoridate (Floropryl). Isoflurophate is a colorless liquid, soluble in water to the extent of 1.54% at 25°C, which decomposes to give a *p*H of 2.5. It is soluble in alcohol and to some extent in peanut oil. It is stable in peanut oil for a period of 1 year but decomposes in water in a few days. Solutions in peanut oil can be sterilized by

autoclaving. The compound should be stored in hard glass containers. Continued contact with soft glass is said to hasten decomposition, as evidenced by a discoloration.



Isofluorphate

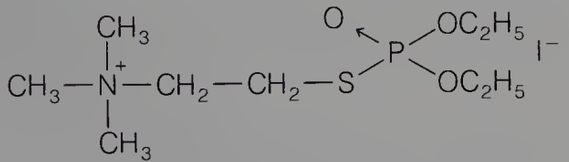
Isofluorphate must be handled with extreme caution. Contact with eyes, nose, mouth, and even the skin should be avoided because it can be absorbed readily through intact epidermis and more so through mucous tissues.

Since isofluorphate irreversibly[58] inhibits cholinesterase, its activity lasts for days or even weeks. During this period, new cholinesterase may be synthesized in plasma, erythrocytes, and other cells.

A combination of atropine sulfate and magnesium sulfate gives protection in rabbits against the toxic effects of isofluorphate. Atropine sulfate counteracts the muscarinic effect and magnesium sulfate the nicotinic effect of the drug.[59] Isofluorphate has been used in the treatment of glaucoma.

Echothiophate Iodide, USP

(2-Mercaptoethyl)trimethylammonium iodide, *S*-ester with *O,O*-diethyl phosphorothioate (Phospholine iodide) occurs as a white, crystalline, hygroscopic solid that has a slight mercaptan-like odor. It is soluble in water (1: 1) and dehydrated alcohol (1: 25); aqueous solutions have a *p*H of about 4 and are stable at room temperature for about 1 month.

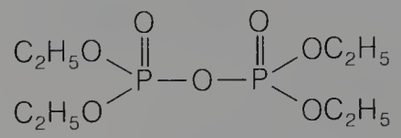


Echothiophate Iodide

Echothiophate iodide is a long-lasting cholinesterase inhibitor of the irreversible type, such as isofluorphate. However, unlike the latter, it is a quaternary salt, and when applied locally, its distribution in tissues is limited, which can be very desirable. It is used as a long-acting anticholinesterase agent in the treatment of glaucoma.

Hexaethyltetraphosphate (HETP) and Tetraethylpyrophosphate (TEPP)

These two substances are compounds that also show anticholinesterase activity. HETP was developed by the Germans during World War II and is used as an insecticide against aphids. When used as insecticides, these compounds have the advantage of being hydrolyzed rapidly to the relatively nontoxic, water-soluble compounds phosphoric acid and ethyl alcohol. Fruit trees or vegetables sprayed with this type of compound retain no harmful residue after a period of a few days or weeks, depending on the weather conditions. Workers spraying with these agents should use extreme caution so that the vapors are not breathed and that none of the vapor or liquid comes in contact with the eyes or skin.



Tetraethylpyrophosphate

Malathion

2-[(Dimethoxyphosphinothioyl)thio]-butanedioic acid diethyl ester is a water-insoluble phosphodithioate ester that has been used as an agricultural insecticide. Malathion is a poor inhibitor of cholinesterases. Its effectiveness as a safe insecticide is due to the different rates at which humans and insects metabolize the chemical. Microsomal oxidation, which causes desulfuration, occurs slowly to form the phosphothioate (malaoxon), which is 10,000 times more active than the phosphodithioate (malathion) as a cholinesterase inhibitor. Insects detoxify the phosphothioate by a phosphatase-forming dimethylphosphorothioate, which is inactive as an inhibitor. Humans, however, are able to rapidly hydrolyze malathion through a carboxyesterase enzyme yielding malathion acid, a still poorer inhibitor of AChE. Phosphatases and carboxyesterases further metabolize malathion acid to dimethylphosphothioate. The metabolic reactions are shown in Fig. 17-18.

Parathion

O,O-diethyl *O*-*p*-nitrophenyl phosphorothioate (Thiophos). This compound is a yellow liquid that is freely soluble in aromatic hydrocarbons, ethers, ketones, esters, and alcohols but practically insoluble in water, petroleum ether, kerosene, and the usual spray oils. It is decomposed at a *p*H above 7.5. Parathion is used as an agricultural insecticide. It is a relatively weak inhibitor of cholinesterase; however, enzymes present in liver microsomes and insect tissues convert parathion ($pI_{50} < 4$) to paraoxon, a more potent inhibitor of cholinesterase ($pI_{50} > 8$).[60] Parathion is also metabolized by liver microsomes to yield *p*-nitrophenol and diethylphosphate, the latter of which is inactive as an irreversible cholinesterase inhibitor.[61]

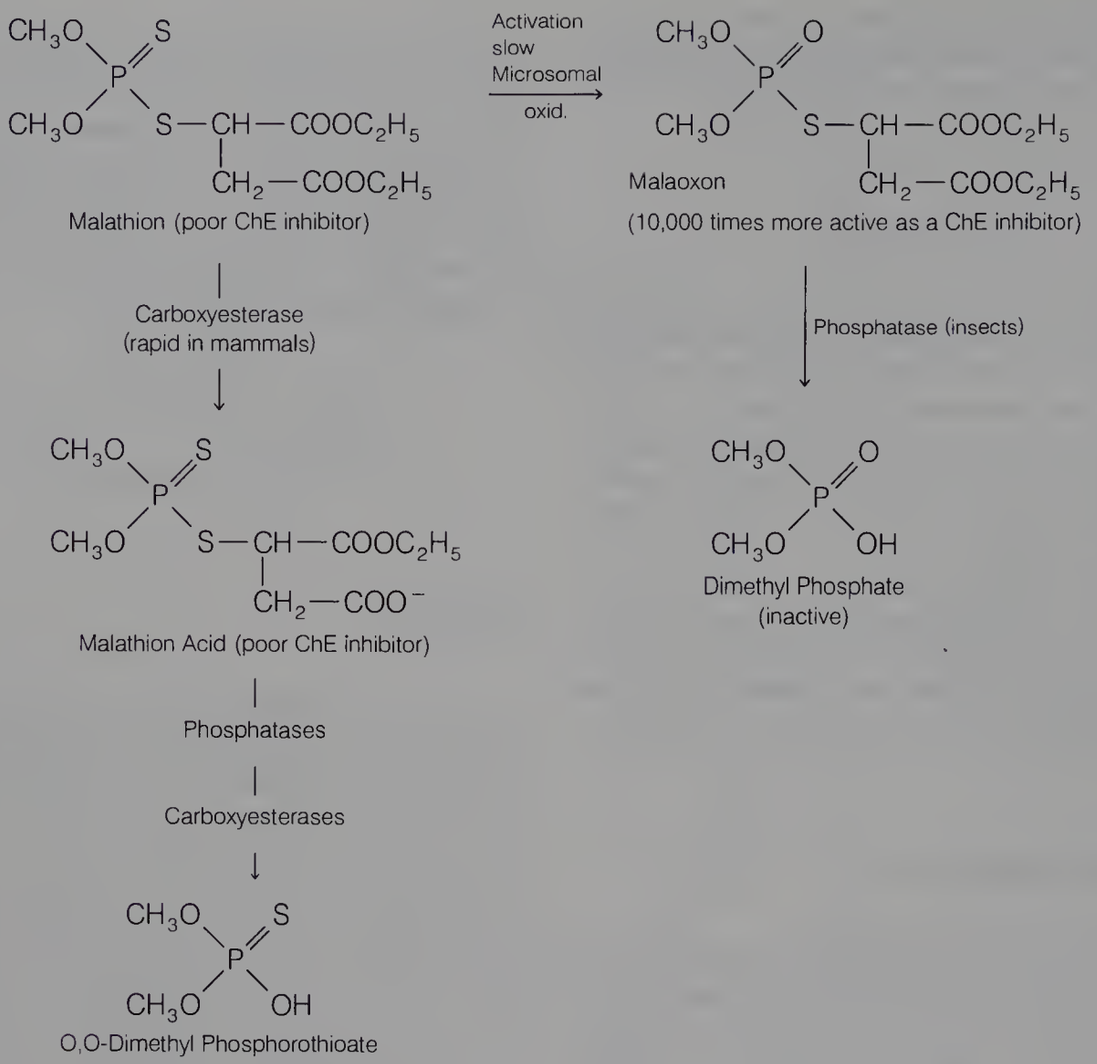
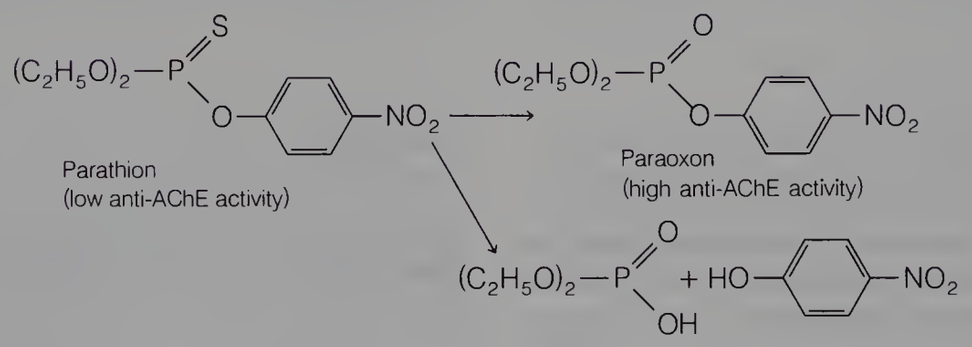


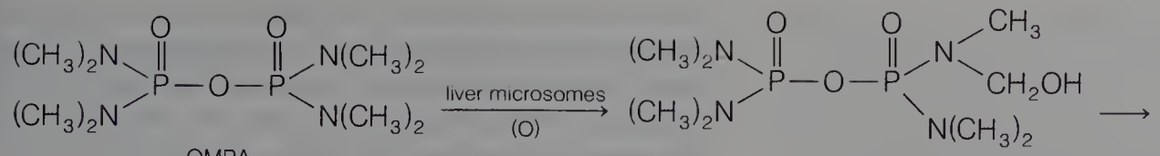
FIG. 17-18. *Comparative metabolism of malathion between mammals and insects.*



Schradan

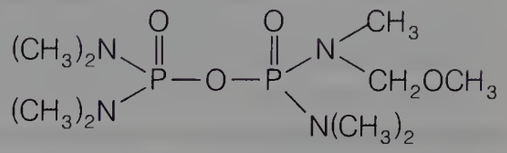
Octamethylpyrophosphoramide; OMPA; *bis*[bisdimethylaminophosphonous] anhydride (Pestox III). This compound is a viscous liquid that is miscible with water and soluble in most organic solvents. It is not hydrolyzed by alkalies or water but is hydrolyzed by acids. Schradan is used as a systemic insecticide for plants, being absorbed by the plants without appreciable injury. Insects feeding on the plant are incapacitated.

Schradan is a weak inhibitor of cholinesterases in vitro. In vivo it is metabolized to the very strong inhibitor hydroxymethyl OMPA. Hydroxymethyl OMPA is not stable and is metabolized further to the *N*-methoxide, which is a weak inhibitor of cholinesterase.[62]



OMPA
(weak cholinesterase inhibitor)

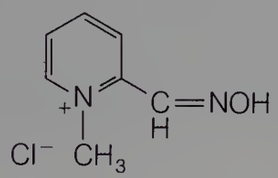
hydroxymethyl OMPA
(strong cholinesterase inhibitor)



OMPA-*N*-methoxide
(weak cholinesterase inhibitor)

Pralidoxime Chloride, USP

2-Formyl-1-methylpyridinium chloride oxime; 2-PAM chloride; 2-pyridine aldoxime methyl chloride (Protopam chloride). Pralidoxime chloride occurs as a white, nonhygroscopic, crystalline powder that is soluble in water, 1 g in less than 1 mL.



Pralidoxime Chloride

Pralidoxime chloride is used as an antidote for poisoning by parathion and related pesticides. It may be effective against some phosphates that have a quaternary nitrogen. It is also an effective antagonist for some carbamates, such as neostigmine methylsulfate and pyridostigmine bromide.

The mode of action of pralidoxime chloride is described in Fig. 17-16.

The biologic half-life of 2-PAM chloride in humans is about 2 hr, and its effectiveness is a function of its concentration in plasma, which reaches a maximum in 2 to 3 hr after oral administration.

Pralidoxime chloride, a quaternary ammonium compound, is most effective by intramuscular, subcutaneous, or intravenous administration. Treatment of poisoning by an anticholinesterase will be most effective if given within a few hours. Little will be accomplished if the drug is used more than 36 hr after parathion poisoning has occurred.

CHOLINERGIC BLOCKING AGENTS

A wide variety of tissues respond to ACh released by the neuron or exogenously administered chemicals to mimic this neurotransmitter's action. Peripheral cholinergic receptors are located at parasympathetic postganglionic nerve endings in smooth muscle, sympathetic and parasympathetic ganglia, and neuromuscular junctions in skeletal muscle. Although these receptors are activated by ACh, there are antagonists that are selective for each. Atropine is an effective blocking agent at parasympathetic postganglionic terminals. Like most classic blocking agents, it acts on all muscarinic receptor subtypes. *d*-Tubocurarine blocks the effect of ACh on skeletal muscle, which is activated by N_1 nicotinic receptors. Hexamethonium blocks transition at N_2 nicotinic receptors located in autonomic ganglia.

Anticholinergic action by drugs and chemicals is apparently dependent upon their ability to reduce the number of free receptors that can interact with ACh. The theories of Stephenson[63] and Ariens[64] have explained the relationship between drug–receptor interactions and the observed biologic response (see Chap. 2). These theories indicate that the quantity of a drug–receptor complex formed at a given time depends upon the affinity of the drug for the receptor and that a drug that acts as an agonist must also possess another property, called "*efficacy*" or "*intrinsic activity.*" Another explanation of drug–receptor interactions, the Paton rate theory,[65] defines a biologic stimulus as being proportional to the rate of drug–receptor interactions (see Chap. 2).

Both of these theories are compatible with the concept that a blocking agent that has a high affinity for the receptor may decrease the number of available free receptors and the efficiency of the endogenous neurotransmitter.

STRUCTURE–ACTIVITY RELATIONSHIPS

A wide variety of compounds possess anticholinergic activity. The development of such compounds has been largely empiric and based principally on atropine as the prototype. Nevertheless, structural permutations have resulted in compounds that do not have obvious relationships to the parent molecule. The following classification delineates the major chemical types encountered:

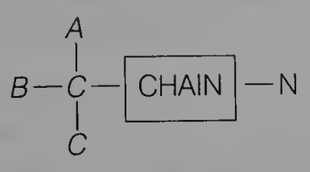
- Solanaceous alkaloids and synthetic analogues
- Synthetic aminoalcohol esters
- Aminoalcohol ethers

- Aminoalcohols
- Aminoamides
- Miscellaneous
- Papaveraceous

The chemical classification of anticholinergics acting on parasympathetic postganglionic nerve endings is complicated somewhat because some agents, especially the quaternary ammonium derivatives, act on the ganglia that have a muscarinic component to their stimulation pattern and, at high doses, at the neuromuscular junction in skeletal muscle.

There are several ways in which the structure–activity relationship could be considered, but in this discussion we follow, in general, the considerations of Long et al.,[66] who based their postulations on the l-hyoscyamine molecule as being one of the most active anticholinergics and, therefore, having an optimal arrangement of groups.

Anticholinergic compounds may be considered as chemicals that have some similarity to ACh but contain additional substituents that enhance their binding to the cholinergic receptor.



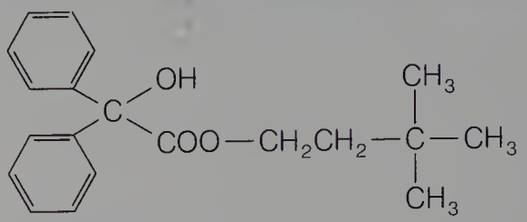
A, *B* = bulky groups, e.g. cycloalkyl, aromatic, etc.

C = H, OH, carboxamide

As depicted above, an anticholinergic may contain a quaternary ammonium function or a tertiary amine that is protonated in the biophase to form a cationic species. The nitrogen is separated from a pivotal carbon atom by a chain that may include an ester, ether, or hydrocarbon moiety. The substituent groups *A* and *B* contain at least one aromatic moiety capable of van der Waals' interactions to the receptor surface and one cycloaliphatic or other hydrocarbon moiety for hydrophobic bonding interactions. *C* may be hydroxyl or carboxamide to undergo hydrogen bonding with the receptor.

The Cationic Head

It is generally considered that the anticholinergic molecules have a primary point of attachment to cholinergic sites through the *cationic head* (i.e., the positively charged nitrogen). For quaternary ammonium compounds, there is no question of what is implied, but for tertiary amines, one assumes, with good reason, that the cationic head is achieved by protonation of the amine at physiologic *p*H. The nature of the substituents on this cationic head is critical insofar as a parasympathomimetic response is concerned. Steric factors that cause a diffusion of the onium charge or produce a less than optimal drug–receptor interaction result in a decrease of parasympathomimetic properties and allow the drug to act as an antagonist because of other bonding interactions. Ariens[64] has shown that carbocholines (e.g., benzilylcarbocholine) engage in a typical competitive action with ACh, though they are less effective than the corresponding compounds possessing a cationic head, suggesting that hydrophobic bonding may play an important role in these drug–receptor interactions.



Benzilylcarbocholine

The Hydroxyl Group

Although not a requisite for activity, a suitably placed alcoholic hydroxyl group enhances antimuscarinic activity over a similar compound without the hydroxyl group. The position of the hydroxyl group relative to the nitrogen appears to be fairly critical, with the diameter of the receptive area being estimated at about 2 to 3 Å. It is assumed that the hydroxyl group contributes to the strength of binding, probably by hydrogen-bonding to an electron-rich portion of the receptor surface.

The Esteratic Group

Many of the highly potent antimuscarinic compounds possess an ester grouping, and this may be a contributing feature for effective binding. This is reasonable because the agonist (i.e., ACh) possesses a similar function for binding to the same site. That an esteratic function is not necessary for activity is illustrated amply by the several types of compounds not possessing such a group (e.g., ethers, aminoalcohols).

Cyclic Substitution

It will be apparent from an examination of the active compounds discussed in the following sections that at least one cyclic substituent (phenyl, thienyl, or other) is a common feature in almost all anticholinergic molecules. Aromatic substitution often is used in connection with the acidic moiety of the ester function. However, virtually all acids employed are of the aryl-substituted acetic acid variety. Use of aromatic acids leads to low activity of these compounds as anticholinergics but potential activity as local anesthetics.

In connection with the apparent need for a cyclic group, it is instructive to consider the postulations of Ariens.[64] He points out that the "mimetic" molecules, richly endowed

with polar groups, undoubtedly require a complementary polar receptor area for effective binding. As a consequence, it is implied that a relatively nonpolar area surrounds such sites. Thus, by increasing the binding of the molecule in this peripheral area by means of introducing flat, nonpolar groups (e.g., aromatic rings), it should be possible to achieve compounds with excellent affinity but without intrinsic activity. This postulate is consistent with most antimuscarinic drugs, whether they possess an ester group or not.

PARASYMPATHETIC POSTGANGLIONIC BLOCKING AGENTS

These blocking agents are also known as antimuscarinic, anticholinergic, parasympatholytic, or cholinolytic drugs. Antimuscarinic drugs act by competitive antagonism of ACh binding to muscarinic receptors. Endogenous neurotransmitters, including ACh, are relatively small molecules. It was noted by Ariens[64] that competitive reversible antagonists generally are larger molecules capable of additional binding to the receptor surface. The most potent anticholinergic drugs are derived from muscarinic agonists that contain one or sometimes two large or bulky groups. Ariens[64] suggested that molecules that act as competitive reversible antagonists generally are capable of binding to the active site of the receptor but that they have an additional binding interaction that increases receptor affinity but does not contribute to the intrinsic activity (efficacy) of the drug. Several three-dimensional models of G-protein-coupled receptors, including the muscarinic receptor, have been reported. Despite knowledge of their amino acid sequences, it is not yet possible to provide an unambiguous description of the docking of molecules to these receptors. The concepts of Ariens[64] and others, however, appear consistent with the binding site models proposed. Bebbington and Brimblecombe[39] proposed in 1965 that there is a relatively large area lying outside the agonist–receptor binding site, where van der Waals' interactions can take place between the agonist and the receptor area. This too is not inconsistent with contemporary theories on cholinergic receptor interaction with small molecules.

THERAPEUTIC ACTIONS

Organs controlled by the autonomic nervous system usually are innervated by both the sympathetic and the parasympathetic systems. There is a continual state of dynamic balance between the two systems. Theoretically, one should achieve the same end result by either stimulation of one of the systems or blockade of the other. Unfortunately, there is usually a limitation to this type of generalization. However, there are three predictable and clinically useful results from blocking the muscarinic effects of ACh.

1. *Mydriatic effect:* dilatation of the pupil of the eye; and *cycloplegia,* a paralysis of the ciliary structure of the eye, resulting in a paralysis of accommodation for near vision
2. *Antispasmodic effect:* lowered tone and motility of the GI tract and the genitourinary tract
3. *Antisecretory effect:* reduced salivation (*antisialagogue*), reduced perspiration (*anhidrotic*), and reduced acid and gastric secretion

These three general effects of parasympatholytics can be expected in some degree from any of the known drugs, though occasionally it is necessary to administer rather heroic doses to demonstrate the effect. The mydriatic and cycloplegic effects, when produced by topical application, are not subject to any great undesirable side effects because of limited systemic absorption. This is not true for the systemic antispasmodic effects obtained by oral or parenteral administration. It is generally understood that drugs having effective blocking action on the GI tract are seldom free of undesirable side effects on the other organs. The same is probably true of drugs used for their antisecretory effects. Perhaps the most common side effects experienced from the oral use of these drugs, under ordinary conditions, are dryness of the mouth, mydriasis, and urinary retention.

Mydriatic and cycloplegic drugs are generally prescribed or used in the office by ophthalmologists. The principal purpose is for refraction studies in the process of fitting lenses. This permits the physician to examine the eye retina for possible abnormalities and diseases, as well as to provide controlled conditions for the proper fitting of glasses. Because of the inability of the iris to contract under the influence of these drugs, there is a definite danger to the patient's eyes during the period of drug activity unless they are protected from strong light by the use of dark glasses. These drugs also are used to treat inflammation of the cornea (keratitis), inflammation of the iris and the ciliary organs (iritis and iridocyclitis), and inflammation of the choroid (choroiditis). A dark-colored iris appears to be more difficult to dilate than a light-colored one and may require more concentrated solutions. Caution in the use of mydriatics is advisable because of their demonstrated effect in raising the intraocular pressure. Pupil dilation tends to cause the iris to restrict drainage of fluid through the canal of Schlemm by crowding the angular space, thereby leading to increased intraocular pressure. This is particularly true for patients having glaucomatous conditions.

Atropine is used widely as an antispasmodic because of its marked depressant effect on parasympathetically innervated smooth muscle. It appears to block all muscarinic receptor subtypes. However, atropine is the standard by which other similar drugs are measured. Also, atropine has a blocking action on the transmission of the nerve impulse, rather than a depressant effect directly on the musculature. This action is termed *neurotropic,* in contrast with the action of an anti-

spasmodic such as papaverine, which appears to act by depression of the muscle cells and is termed *musculotropic.*

Papaverine is the standard for comparison of musculotropic antispasmodics and, although not strictly a parasympatholytic, is treated together with its synthetic analogues later in this chapter. The synthetic antispasmodics appear to combine neurotropic and musculotropic effects in greater or lesser measure, together with a certain amount of ganglion-blocking activity for the quaternary derivatives.

Anticholinergic drugs have a minor role in the management of peptic ulcer disease.[67] For the present, the most rational therapy used by clinicians employing anticholinergic drugs seems to be a combination of a nonirritating diet to reduce acid secretion, antacid therapy, and reduction of emotional stress. Most of the anticholinergic drugs are offered either as the chemical alone or in combination with a CNS depressant, such as phenobarbital, or with a neuroleptic drug to reduce the CNS contribution to parasympathetic hyperactivity. In addition to the antisecretory effects of anticholinergics on hydrochloric acid and gastric acid secretion, there have been some efforts to employ them as antisialagogues and anhidrotics.

Paralysis agitans or parkinsonism (Parkinson's disease), first described by the English physician James Parkinson in 1817, is another condition that is treated often with anticholinergic drugs. It is characterized by tremor, "pill rolling," cog-wheel rigidity, festinating gait, sialorrhea, and mask-like facies. Fundamentally, it represents a malfunction of the extrapyramidal system. Parkinsonism is characterized by a progressive and selective degeneration of dopaminergic neurons, which originate in the substantia nigra of the midbrain and terminate in the basal ganglia, i.e., caudate nucleus, putamen, and pallidum. Skeletal muscle movement is controlled to a great degree by patterns of excitation and inhibition, resulting from the feedback of information to the cortex, and mediated through the pyramidal and extrapyramidal pathways. The basal ganglia structures, such as the pallidum, corpus striatum, and substantia nigra, serve as data processors for the pyramidal pathways and the structures through which the extrapyramidal pathways pass on their way from the spinal cord to the cortex. Lesions of the pyramidal pathways lead to a persistent increase in muscle tone, resulting in an excess of spontaneous involuntary movements, along with changes in the reflexes. It is apparent, therefore, that the basal ganglia are functional in maintaining normal motor control. In parkinsonism there is a degeneration of the substantia nigra and corpus striatum, which are involved with controlled integration of muscle movement. The neurons in the substantia nigra and basal ganglia utilize the neurotransmitter dopamine and interact with short cholinergic interneurons. When dopamine neurons degenerate, the balance between them is altered. The inhibitory influence of dopamine is reduced and the activity of cholinergic neurons increased. The principal goal of anticholinergic drugs in the treatment of parkinsonism is to decrease the activity of cholinergic neurons in the basal ganglia.

The usefulness of the belladonna group of alkaloids for the treatment of parkinsonism was an empiric discovery. Since then chemists have prepared many synthetic analogues of atropine in an effort to retain the useful antitremor and antirigidity effects of the belladonna alkaloid while at the same time reducing the undesirable side effects. In this process, it was discovered that antihistamine drugs (e.g., diphenhydramine) reduced tremor and rigidity. The antiparkinson-like activity of antihistamines has been attributed to their anticholinergic properties. The activity of these drugs is confined to those that can pass through the blood–brain barrier.

SOLANACEOUS ALKALOIDS AND ANALOGUES

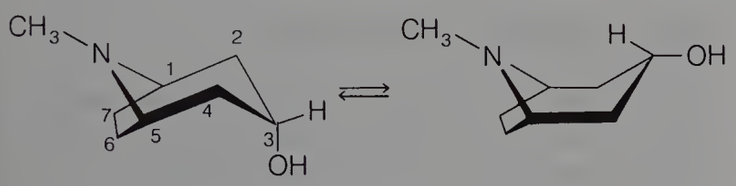
The solanaceous alkaloids, represented by (−)-hyoscyamine, atropine [(±)-hyoscyamine], and scopolamine (hyoscine), are the forerunners of the class of antimuscarinic drugs. These alkaloids are found principally in henbane (*Hyoscyamus niger*), deadly nightshade (*Atropa belladonna*), and jimson weed (*Datura stramonium*). There are other alkaloids that are members of the solanaceous group (e.g., apoatropine, noratropine, belladonnine, tigloidine, meteloidine) but are not of sufficient therapeutic value to be considered in this text.

Crude drugs containing these alkaloids have been used since early times for their marked medicinal properties, which depend largely on inhibition of the parasympathetic nervous system and stimulation of the higher nervous centers. Belladonna, probably as a consequence of the weak local anesthetic activity of atropine, has been used topically for its analgesic effect on hemorrhoids, certain skin infections, and various itching dermatoses. Application of sufficient amounts of belladonna or of its alkaloids results in mydriasis. Internally, the drug causes diminution of secretions, increases the heart rate (by depression of the vagus nerve), depresses the motility of the gastrointestinal tract, and acts as an antispasmodic on various smooth muscles (ureter, bladder, and biliary tract). In addition, it directly stimulates the respiratory center. The multiplicity of actions exerted by the drug causes it to be looked upon with some disfavor because the physician seeking one type of response unavoidably also obtains the others. The action of scopolamine-containing drugs differs from those containing hyoscyamine and atropine in that there is no CNS stimulation, but rather a narcotic or sedative effect predominates. The use of this group of drugs is accompanied by a fairly high incidence of reactions because of individual idiosyncrasies; death from overdosage usually results from respiratory failure. A complete treatment of the pharmacology and the uses of these drugs is not within the scope of this text. The introductory pages of this chapter have reviewed briefly some of the more pertinent points in connection with the major activities of these drug types.

STRUCTURAL CONSIDERATIONS

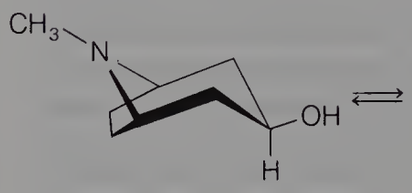
All of the solanaceous alkaloids are esters of the bicyclic aminoalcohol 3-hydroxytropane or of related aminoalcohols.

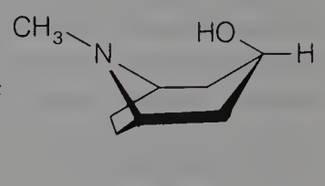
The structural formulas that follow show the piperidine ring system in the commonly accepted chair conformation because this form has the lowest energy requirement. However, the alternate boat form can exist under certain conditions because the energy barrier is not great. Inspection of the 3-hydroxytropane formula also indicates that, even though there is no optical activity because of the plane of symmetry, two stereoisomeric forms (tropine and pseudotropine) can exist because of the rigidity imparted to the molecule through the ethylene chain across the 1,5-positions. In tropine, the axially oriented hydroxyl group, *trans* to the nitrogen bridge, is designated as α, and the alternate *cis* equatorially oriented hydroxyl group is β. The aminoalcohol derived from scopolamine, namely scopine, has the axial orientation of the 3-hydroxyl group but, in addition, a β-oriented epoxy group bridged across the 6,7-positions, as shown. Of the several different solanaceous alkaloids known, it has been indicated that (−)-hyoscyamine, atropine, and scopolamine are the most important. Their structures are indicated, but it can be pointed out that antimuscarinic activity is associated with all of the solanaceous alkaloids that possess the tropine-like axial orientation of the esterified hydroxyl group. It will be noted in studying the formulas that tropic acid is, in each case, the esterifying acid. Tropic acid contains an easily racemized asymmetric carbon atom, the moiety accounting for optical activity in these compounds in the absence of racemization. The proper enantiomorph is necessary for high antimuscarinic activity, as illustrated by the potent (−)-hyoscyamine in comparison with the weakly active (+)-hyoscyamine. The racemate, atropine, has an intermediate activity. The marked difference in antimuscarinic potency of the optical enantiomorphs apparently does not extend to the action on the CNS, inasmuch as both seem to have the same degree of activity.[68]



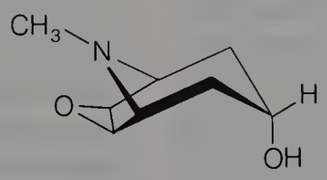
(chair) ⇌ (boat)

TROPINE
(3α-Hydroxytropane or
3α-tropanol)

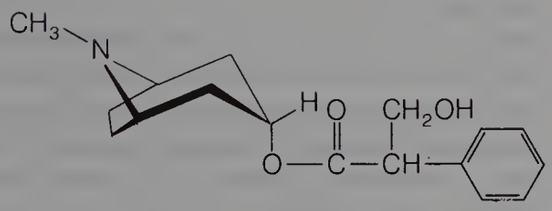


(chair) ⇌  (boat)

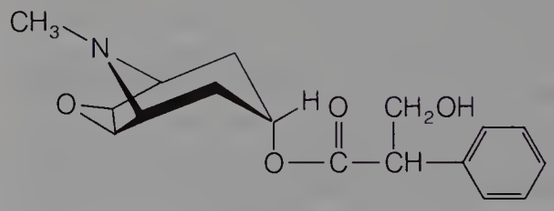
PSEUDOTROPINE
(3β-Hydroxytropane or
3β-tropanol)



SCOPINE
(6 : 7 β-Epoxy-3α-hydroxytropane
or 6 : 7 β-Epoxy-3α-tropanol)



ATROPINE
(or Hyoscyamine)



SCOPOLAMINE
(or Hyoscine)

The solanaceous alkaloids have been modified by preparing other esters of 3α-tropanol or making a quaternary of the nitrogen in tropanol or scopine with a methyl halide. These compounds represent some of the initial attempts to separate the varied actions of atropine and scopolamine. It should be pointed out that few aminoalcohols have been found that impart the same degree of neurotropic activity as that exhibited by the ester formed by combination of tropine with tropic acid. Similarly, the tropic acid portion is highly specific for the anticholinergic action, and substitution by

other acids results in decreased neurotropic potency, though the musculotropic action may increase. The earliest attempts to modify the atropine molecule retained the tropine portion and substituted various acids for tropic acid.

Besides changing the acid residue, other changes have been directed toward the quaternization of the nitrogen. Examples of this type of compound are methscopolamine bromide, homatropine methylbromide, and anisotropine methylbromide. Quaternization of the tertiary amine produces variable effects in terms of increasing potency. Decreases in activity are apparent in comparing atropine with methylatropine (no longer used) and scopolamine with methscopolamine. Ariens et al.[69] ascribed decreased activity, especially when the groups attached to nitrogen are larger than methyl, to a possible decrease in affinity for the anionic site on the cholinergic receptor. They attributed this decreased affinity to a combination of greater electron repulsion by such groups and greater steric interference to the approach of the cationic head to the anionic site. In general, however, the effect of quaternization is much greater in reduction of parasympathomimetic than parasympatholytic action. This may be partially due to the additional blocking at the parasympathetic ganglion induced by quaternization, which could offset the decreased affinity at the postganglionic site. However, it should also be noted that quaternization increases the curariform activity of these alkaloids and aminoesters, a usual consequence of quaternizing alkaloids. Another disadvantage in converting an alkaloidal base to the quaternary form is that the quaternized base is absorbed more poorly through the intestinal wall, with the consequence that the activity becomes erratic and, in some instances, unpredictable. Bases (such as alkaloids) are absorbed through the lipoidal gut wall only in the dissociated form, which can be expected to exist for a tertiary base, in the small intestine. However, quaternary nitrogen bases cannot revert to an undissociated form, even in basic media and, presumably, may have difficulty passing through the gut wall. That quaternary compounds can be absorbed indicates that other less efficient mechanisms for absorption probably prevail. Quaternary ammonium compounds combine reversibly with endogenous substances in the gut, such as mucin, to form neutral ion-pair complexes. These complexes penetrate the lipid membrane by passive diffusion.

PRODUCTS

Atropine, USP

Atropine is the tropine ester of racemic tropic acid and is optically inactive. It possibly occurs naturally in various Solanaceae, though some claim, with justification, that whatever atropine is isolated from natural sources results from racemization of (−)-hyoscyamine during the isolation process. Conventional methods of alkaloid isolation are used to obtain a crude mixture of atropine and hyoscyamine from the plant material. This crude mixture is racemized to atropine by refluxing in chloroform or by treatment with cold dilute alkali. Because atropine is made by the racemization process, an official limit is set on the hyoscyamine content by restricting atropine to a maximum levorotation under specified conditions.

Atropine occurs in the form of optically inactive, white, odorless crystals possessing a bitter taste. It is not very soluble in water (1:460, 1:90 at 80°C) but is more soluble in alcohol (1:2, 1:1.2 at 60°C). It is soluble in glycerin (1:27), in chloroform (1:1), and in ether (1:25).* Saturated aqueous solutions are alkaline in reaction (approximate *p*H 9.5). The free base is useful when nonaqueous solutions are to be made, such as in oily vehicles and ointment bases. Atropine has a plasma half-life of about 2 to 3 hr. It is metabolized in the liver to several products, including tropic acid and tropine.

Atropine Sulfate, USP (Atropisol)

Atropine sulfate is prepared by neutralizing atropine in acetone or ether solution with an alcoholic solution of sulfuric acid, care being exercised to prevent hydrolysis.

The salt occurs as colorless crystals or as a white, crystalline powder. It is efflorescent in dry air and should be protected from light to prevent decomposition.

Atropine sulfate is freely soluble in water (1:0.5), in alcohol (1:5, 1:2.5 at boiling point), and in glycerin (1:2.5). Aqueous solutions are not very stable, though solutions may be sterilized at 120°C (15 lb pressure) in an autoclave if the *p*H is kept below 6. Sterilization probably is best effected by the use of aseptic techniques and a bacteriologic filter. It has been suggested that no more than a 30-day supply of an aqueous solution should be made and that, for small quantities, the best procedure is to use hypodermic tablets and sterile distilled water.[69] Kondritzer and Zvirblis[70] have studied the kinetics of alkaline and proton-catalyzed hydrolyses of atropine in aqueous solution. The region of maximal stability lies between *p*H 3 and approximately 5. They have also proposed an equation to predict the half-life of atropine undergoing hydrolysis at constant *p*H and temperature.

The action of atropine or its salts is the same. It produces a mydriatic effect by paralyzing the iris and the ciliary muscles and, for this reason, is used by the oculist in iritis and corneal inflammations and lesions. Its use is rational in these conditions because one of the first rules in the treatment of inflammation is rest, which, of course, is accomplished by the paralysis of muscular motion. Its use in the eye (0.5% to 1% solutions or gelatin disks) for fitting glasses is widespread. Atropine is administered in small doses before general anesthesia to lessen oral and air passage secretions and,

* In this chapter, a solubility expressed as 1:460 indicates that 1 g is soluble in 460 mL of the solvent at 25°C. Solubilities at other temperatures will be so indicated.

when morphine is administered with it, to lessen the respiratory depression induced by morphine.

Atropine causes restlessness, prolonged pupillary dilation, and loss of visual accommodation and, furthermore, gives rise to arrhythmias such as atrioventricular dissociation, ventricular extrasystoles, and even ventricular fibrillation. Even though there has been a gradual replacement of ether with other anesthetics, thereby eliminating problems with respiratory secretions caused by ether and thus requiring atropine, surgeons and anesthesiologists today continue to use it as an anesthetic premedicant to reduce excessive salivary and airway secretions and to prevent vagal reflexes.

Its ability to dry secretions has also been utilized in the so-called rhinitis tablets for symptomatic relief in colds. In cathartic preparations, atropine or belladonna has been used as an antispasmodic to lessen the smooth-muscle spasm (griping) often associated with catharsis.

Atropine may be used to treat some types of arrhythmias. It increases the heart rate by blocking the effects of ACh on the vagus. In this context, it is used to treat certain reversible bradyarrhythmias that may accompany acute myocardial infarction. It is also used as an adjunct to anesthesia to protect against bradycardia, hypotension, and even cardiac arrest induced by the skeletal muscle relaxant succinylcholine chloride.

Still another use for atropine sulfate has emerged following the development of the organophosphates, which are potent inhibitors of AChE. Atropine is a specific antidote to prevent the muscarinic effects of ACh accumulation, such as vomiting, abdominal cramps, diarrhea, salivation, sweating, bronchoconstriction, and excessive bronchial secretions. It is used intravenously but does not protect against respiratory failure caused by depression of the respiratory center and the muscles of respiration.

Hyoscyamine, USP

Hyoscyamine is a levorotatory alkaloid obtained from various solanaceous species. One of the commercial sources is Egyptian henbane (*Hyoscyamus muticus*), in which it occurs to the extent of about 0.5%. Usually, it is prepared from the crude drug in a manner similar to that used for atropine and is purified as the oxalate. The free base is obtained easily from this salt.

It occurs as white needles that are sparingly soluble in water (1:281), more soluble in ether (1:69) or benzene (1:150), very soluble in chloroform (1:1) and freely soluble in alcohol. It is used as the sulfate and hydrobromide. The principal reason for the popularity of the hydrobromide has been its nondeliquescent nature. The salts have the advantage over the free base in being quite water-soluble.

Hyoscyamine is the *levo*-form of the racemic mixture known as atropine. The *dextro*-form does not exist naturally but has been synthesized. Comparison of the activities of (−)-hyoscyamine, (+)-hyoscyamine, and the racemate (atropine) was carried out by Cushny[71] in 1904, who found a greater peripheral potency for the (−)-isomer and twice the potency of the racemate. All later studies have essentially borne out these observations, namely, that the (+)-isomer is only weakly active and that the (−)-isomer is, in effect, the active portion of atropine. Inspection of the relative doses of atropine sulfate and hyoscyamine sulfate illustrates the differences very nicely. The principal criticism offered against the use of hyoscyamine sulfate exclusively is that it tends to racemize to atropine sulfate rather easily in solution so that atropine sulfate then becomes the more stable of the two. All of the isomers behave very much the same in the CNS.

Hyoscyamine is used to treat disorders of the urinary tract more so than any other antispasmodic, though there is no evidence that it has any advantages over the other belladonna preparations and the synthetic anticholinergics. It is used to treat spasms of the bladder and, in this manner, serves as a urinary stimulant. It is used together with a narcotic to counteract the spasm produced by the narcotic when the latter is used to relieve the pain of urethral colic. Hyoscyamine preparations are also used as antispasmodics in the therapy of peptic ulcers.

Hyoscyamine Sulfate, USP (Levsin Sulfate)

This salt is a white, odorless, crystalline compound of a deliquescent nature that also is affected by light. It is soluble in water (1:0.5) and alcohol (1:5) but almost insoluble in ether. Solutions of hyoscyamine sulfate are acidic to litmus.

This drug is used as an anticholinergic in the same manner and for the same indications as atropine and hyoscyamine, but it possesses the disadvantage of being deliquescent.

Scopolamine (Hyoscine)

This alkaloid is found in various members of the Solanaceae (e.g., *H. niger, Duboisia myoporoides, Scopolia* sp., and *Datura metel*). Scopolamine usually is isolated from the mother liquor remaining from the isolation of hyoscyamine.

"*Hyoscine*" is the older name for this alkaloid, though "*scopolamine*" is the accepted name in the United States. Scopolamine is the *levo*-component of the racemic mixture, which is known as "*atroscine.*" The alkaloid is racemized readily in the presence of dilute alkali.

The alkaloid occurs in the form of a levorotatory, viscous liquid that is only slightly soluble in water but very soluble in alcohol, chloroform, or ether. It forms crystalline salts with most acids, the hydrobromide being the most stable and the most popularly accepted. An aqueous solution of the hydrobromide, containing 10% mannitol, is said to be less prone to decomposition than unprotected solutions. The

commercially available transdermal system of scopolamine comprises an outer layer of polymer film and a drug reservoir containing scopolamine, polyisobutylene, and mineral oil, which is interfaced with a microporous membrane to control diffusion of the drug. In this dosage form, scopolamine is effective in preventing motion sickness. The action is believed to be on the cortex or the vestibular apparatus. Whereas atropine stimulates the CNS, causing restlessness and talkativeness, scopolamine usually acts as a CNS depressant.

Scopolamine Hydrobromide, USP (Hyoscine Hydrobromide)

This salt occurs as white or colorless crystals or as a white, granular powder. It is odorless and tends to effloresce in dry air. It is freely soluble in water (1:1.5), soluble in alcohol (1:20), only slightly soluble in chloroform, and insoluble in ether.

Scopolamine is a competitive blocking agent of the parasympathetic nervous system as is atropine, but it differs markedly from atropine in its action on the higher nerve centers. Both drugs readily cross the blood–brain barrier and, even in therapeutic doses, cause confusion, particularly in the elderly.

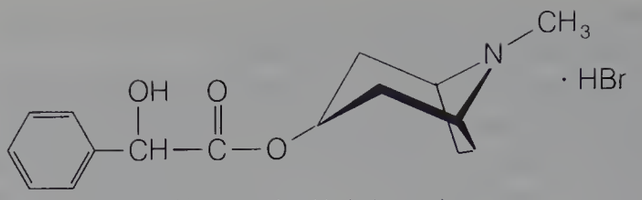
A sufficiently large dose of scopolamine will cause an individual to sink into a restful, dreamless sleep for about 8 hr, followed by a period of approximately the same length in which the patient is in a semiconscious state. During this time, the patient does not remember events that take place. When scopolamine is administered with morphine, this temporary amnesia is termed "*twilight sleep*."

Homatropine Hydrobromide, USP

1αH, 5αH-Tropan-3α-ol mandelate (ester) hydrobromide (Homatrocel). Homatropine hydrobromide occurs as white crystals or as a white, crystalline powder that is affected by light. It is soluble in water (1:6) and alcohol (1:40), less soluble in chloroform (1:420), and insoluble in ether.

Solutions are incompatible with alkaline substances, which precipitate the free base, and with the common reagents that precipitate alkaloids. As in atropine, solutions are sterilized best by filtration through a bacteriologic filter.

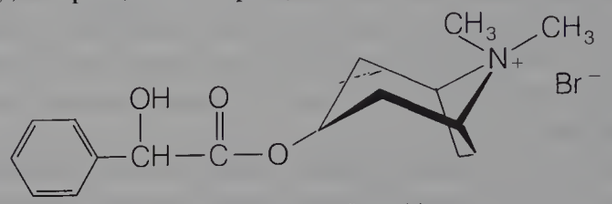
Homatropine hydrobromide is used topically to paralyze the ciliary structure of the eye (cycloplegia) and to effect mydriasis. It behaves very much like atropine but is weaker and less toxic. In the eye, it acts more rapidly but less persistently than atropine. Dilation of the pupil takes place in about 15 to 20 minutes, and the action subsides in about 24 hr. By utilizing a miotic, such as physostigmine, it is possible to restore the pupil to normality in a few hours.



Homatropine Hydrobromide

Homatropine Methylbromide, USP

3α-Hydroxy-8-methyl-1αH,5αH-tropanium bromide mandelate (Novatropine, Mesopin). Homotropine methylbromide occurs as a bitter, white, odorless powder and is affected by light. The compound is readily soluble in water and alcohol but insoluble in ether. The *p*H of a 1% solution is 5.9 and that of a 10% solution is 4.5. Although a solution of the compound yields a precipitate with alkaloidal reagents, such as mercuric-potassium-iodide test solution, addition of alkali hydroxides or carbonates does not cause the precipitate that occurs with nonquaternary nitrogen salts (e.g., atropine, homatropine).

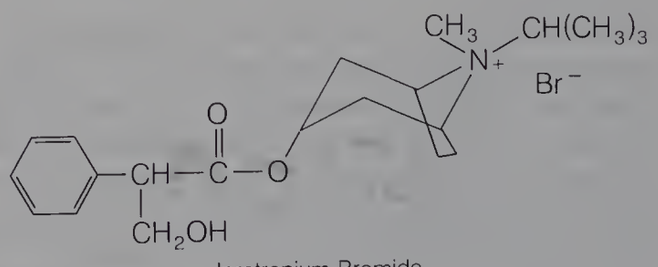


Homatropine Methylbromide

Homatropine methylbromide is transported poorly across the blood–brain barrier because of its quaternary ammonium group and, therefore, has far fewer stimulant properties than atropine. It does have all the characteristic peripheral parasympathetic depressant properties of atropine and is used to reduce oversecretion and to relieve GI spasms.

Ipratropium Bromide

3-(3-Hydroxy-1-oxo-2-phenylpropoxy)-8-methyl-8-(1-methylethyl)-8-aza-bicyclo[3.2.1]octane bromide (Atrovent). Ipratropium bromide is a quaternary ammonium derivative of atropine. It is freely soluble in water and ethanol but insoluble in chloroform and ether. The salt is stable in neutral and acidic solutions but rapidly hydrolyzed in alkaline solutions.



Ipratropium Bromide

Ipratropium bromide is used for inhalation therapy to produce dilation of bronchial smooth muscle for acute asthmatic

attacks. The drug produces bronchodilation by competitive inhibition of cholinergic receptors bound to smooth muscle of the bronchioles. Ipratropium may also act on the surface of mast cells to inhibit ACh-enhanced release of chemical mediators. The drug has a slow onset of action, within 5 to 15 minutes, after being administered by inhalation and should not be used alone for acute asthmatic attacks. The peak therapeutic effect from one dose is observed between 1 and 2 hr. The effects of the drug last for about 6 hr. It has a half-life of 3.5 hr.

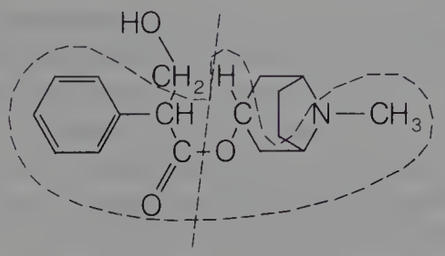
SYNTHETIC CHOLINERGIC BLOCKING AGENTS

AMINOALCOHOL ESTERS

It is generally agreed that the solanaceous alkaloids are potent parasympatholytics but that they have the undesirable property of producing a wide range of effects through their nonspecific blockade of autonomic functions. Efforts to use the antispasmodic effect of the alkaloids most often result in side effects such as dryness of the mouth and fluctuations in pulse rate. Therefore, synthesis of compounds possessing specific cholinolytic actions has been a very desirable field of study. Few prototypical drugs were as avidly dissected in the minds of researchers as atropine in attempts to modify its structure to separate the numerous useful activities (i.e., antispasmodic, antisecretory, mydriatic, and cycloplegic). Most early research was carried out in the pre- and post-World War II era before muscarinic receptor subtypes were known.

Efforts at synthesis started with rather minor deviations from the atropine molecule, but a review of the commonly used drugs today indicates a marked departure from the rigid tropane aminoalcohols and tropic acid residues. An examination of the structures of antispasmodics shows that the acid portion has been designed to provide a large hydrophobic moiety rather than the stereospecific requirement of (*S*)-tropic acid in (−)-hyoscyamine that was once considered important. One of the major developments in the field of aminoalcohol esters was the successful introduction of the quaternary ammonium derivatives as contrasted with the tertiary amine-type esters synthesized originally. Although there are some effective tertiary amine esters in use today, the quaternaries, as a group, represent the most popular type and appear to be slightly more potent than their tertiary amine counterparts.

The accompanying formula shows the portion of the atropine molecule (enclosed in the curved dotted line) believed to be responsible for its major activity. This is sometimes called the "spasmophoric" group and compares with the "anesthesiophoric" group obtained by similar dissection of the cocaine molecule. The validity of this conclusion has been amply borne out by the many active compounds having only a simple diethylaminoethyl residue replacing the tropine portion.



Tropic Acid Tropine

The aminoalcohol portion of eucatropine may be considered a simplification of the atropine molecule. In eucatropine, the bicyclic tropine has been replaced by a monocyclic aminoalcohol and mandelic acid replaces tropic acid (see under "Products").

Although simplification of the aminoalcohol portion of the atropine prototype has been a guiding principle in most research, many of the anticholinergics now used still include a cyclic aminoalcohol moiety. The aminoalcohol-ester anticholinergics are used primarily as antispasmodics or mydriatics, and cholinolytic compounds classed as aminoalcohol or aminoalcohol ether analogues of atropine are, with few exceptions, employed as antiparkinsonian drugs.

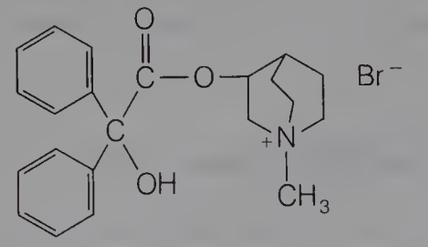
Another important feature in many of the synthetic anticholinergics used as antispasmodics is that they contain a quaternary nitrogen, presumably to enhance activity. The initial synthetic quaternary compound methantheline bromide has served as a forerunner for many others. These compounds combine anticholinergic activity of the antimuscarinic type with some ganglionic blockade to reinforce the parasympathetic blockade. However, formation of a quaternary ammonium moiety introduces the possibility of blockade of voluntary synapses (curariform activity); this can become evident with sufficiently high doses.

PRODUCTS

The antimuscarinic compounds now in use are described in the following monographs:

Clidinium Bromide, USP

3-Hydroxy-1-methylquinuclidinium bromide benzilate (Quarzan). Clidinium bromide is a white or nearly white, almost odorless, crystalline powder that is optically inactive. It is soluble in water and alcohol but only very slightly soluble in ether and benzene.

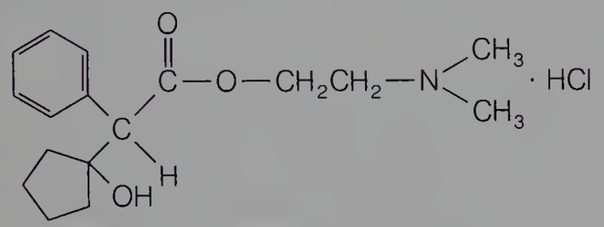


Clidinium Bromide

This anticholinergic agent is marketed alone and in combination with the minor tranquilizer chlordiazepoxide (Librium), the resultant product being known as Librax. The rationale of the combination for the treatment of GI complaints is the use of an anxiety-reducing agent together with an anticholinergic based on the recognized contribution of anxiety to the development of the diseased condition. It is suggested for peptic ulcer, hyperchlorhydria, ulcerative or spastic colon, anxiety states with GI manifestations, nervous stomach, irritable or spastic colon, and others. Clidinium bromide is contraindicated in glaucoma and other conditions that may be aggravated by the parasympatholytic action, such as prostatic hypertrophy in the elderly man, which could lead to urinary retention.

Cyclopentolate Hydrochloride, USP

2-(Dimethylamino)ethyl 1-hydroxy-α-phenylcyclopentaneacetate hydrochloride (Cyclogyl). Cyclopentolate hydrochloride is a crystalline, white, odorless solid that is very soluble in water, easily soluble in alcohol, and only slightly soluble in ether. A 1% solution has a *p*H of 5.0 to 5.4.



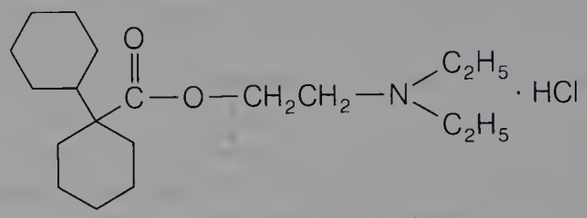
Cyclopentolate Hydrochloride

It is used only for its effects on the eye, where it acts as a parasympatholytic. It quickly produces cycloplegia and mydriasis when placed in the eye. Its primary field of usefulness is in refraction studies. However, cyclopentolate hydrochloride can be used as a mydriatic in the management of iritis, iridocyclitis, keratitis, and choroiditis. Although it does not seem to affect intraocular tension significantly, it is desirable to be very cautious with patients with high intraocular pressure and with elderly patients with possible unrecognized glaucomatous changes.

Cyclopentolate hydrochloride has one-half the antispasmodic activity of atropine and has been shown to be nonirritating when instilled repeatedly into the eye. If not neutralized after the refraction studies, its effect dissipates within 24 hr. Neutralization with a few drops of pilocarpine nitrate solution, 1% to 2%, often results in complete recovery in 6 hr. It is supplied as a ready-made ophthalmic solution in concentrations of either 0.5% or 2%.

Dicyclomine Hydrochloride, USP

2-(Diethylamino)ethyl bicyclohexyl-1-carboxylate hydrochloride (Bentyl). Dicyclomine hydrochloride has some muscarinic receptor subtype selectivity. It binds more firmly to M_1 and M_3 than, to M_2 and M_4 receptors.[72]

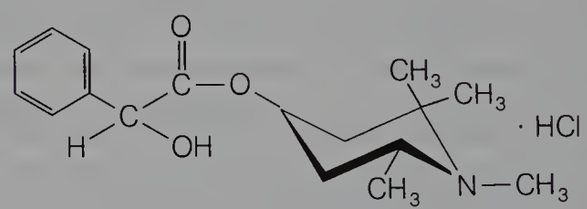


Dicyclomine Hydrochloride

Dicyclomine hydrochloride has one-eighth the neurotropic activity of atropine and approximately twice the musculotropic activity of papaverine. This preparation, first introduced in 1950, has minimized the undesirable side effects associated with the atropine-type compounds. It is used for its spasmolytic effect on various smooth-muscle spasms, particularly those associated with the gastrointestinal tract. It is also useful in dysmenorrhea, pylorospasm, and biliary dysfunction.

Eucatropine Hydrochloride, USP

Euphthalmine hydrochloride; 1,2,2,6-tetramethyl-4-piperidyl mandelate hydrochloride. This compound possesses the aminoalcohol moiety characteristic of one of the early local anesthetics (e.g., β-eucaine) but differs in the acidic portion of the ester by being a mandelate instead of a benzoate. The salt is an odorless white granular powder, providing solutions that are neutral to litmus. It is very soluble in water, freely soluble in alcohol and chloroform, but almost insoluble in ether.

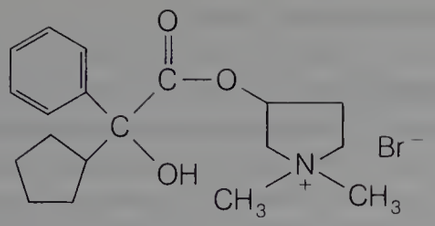


Eucatropine Hydrochloride

The action of eucatropine hydrochloride closely parallels that of atropine, though it is much less potent than the latter. It is used topically in a 0.1 mL dose as a mydriatic in 2% solution or in the form of small tablets. However, the use of concentrations of from 5% to 10% is not uncommon. Dilation, with little impairment of accommodation, takes place in about 30 minutes, and the eye returns to normal in 2 to 3 hr.

Glycopyrrolate, USP

3-Hydroxy-1,1-dimethylpyrrolidinium bromide α-cyclopentylmandelate (Robinul). This drug occurs as a white, crystalline powder that is soluble in water or alcohol but practically insoluble in chloroform or ether.



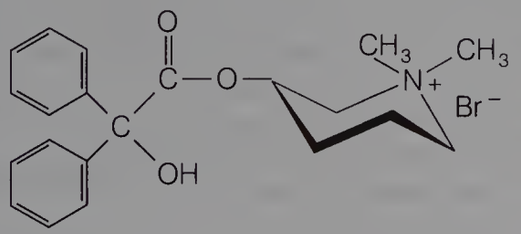
Glycopyrrolate

Glycopyrrolate is a typical anticholinergic and possesses, at adequate dosage levels, the atropine-like effects characteristic of this class of drugs. It has a spasmolytic effect on the musculature of the GI tract as well as the genitourinary tract. It diminishes gastric and pancreatic secretions and the quantity of perspiration and saliva. Its side effects are typically atropine-like also (i.e., dryness of the mouth, urinary retention, blurred vision, constipation). Glycopyrrolate is more potent an antagonist on M_1 than on M_2 and M_3 receptors. The low affinity of M_2 receptors may, in part, explain the low incidence of tachycardia occurring in the use of this drug as an antispasmodic.[73] Because of its quaternary ammonium character, glycopyrrolate rarely causes CNS disturbances, though in sufficiently high dosage it can bring about ganglionic and myoneural junction block.

The drug is used as an adjunct in the management of peptic ulcer and other GI ailments associated with hyperacidity, hypermotility, and spasm. In common with other anticholinergics, its use does not preclude dietary restrictions or use of antacids and sedatives if these are indicated.

Mepenzolate Bromide

3-Hydroxy-1,1-dimethylpiperidinium bromide benzilate (Cantil). Mepenzolate bromide has an activity about one-half that of atropine in reducing ACh-induced spasms of the guinea pig ileum. The selective action on colonic hypermotility is said to relieve pain, cramps, and bloating and to help curb diarrhea.

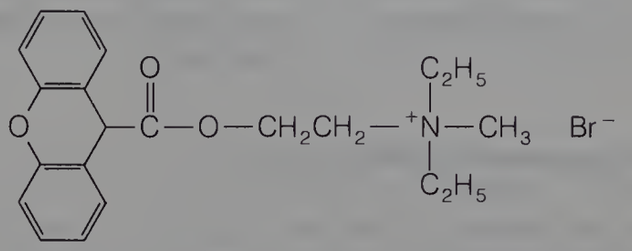


Mepenzolate Bromide

Methantheline Bromide, USP

Diethyl(2-hydroxyethyl)methylammonium bromide xanthene-9-carboxylate (Banthine). Methantheline bromide is a white, slightly hygroscopic, crystalline salt that is soluble in water to produce solutions with a *p*H of about 5. Aqueous solutions are not stable and hydrolyze in a few days. The bromide form is preferable to the very hygroscopic chloride.

This drug, introduced in 1950, is a potent anticholinergic agent and acts at the nicotinic cholinergic receptors of the



Methantheline Bromide

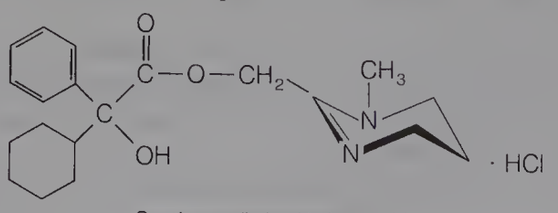
sympathetic and parasympathetic systems, as well as at the myoneural junction of the postganglionic cholinergic fibers. Similar to other quaternary ammonium drugs, methantheline bromide is absorbed incompletely from the GI tract.

Among the conditions for which methantheline bromide is indicated are gastritis, intestinal hypermotility, bladder irritability, cholinergic spasm, pancreatitis, hyperhidrosis, and peptic ulcer, all of which are manifestations of parasympathotonia.

Side reactions are atropine-like (mydriasis, cycloplegia, dryness of mouth). The drug is contraindicated in glaucoma. Toxic doses may bring about a curare-like action, a not too surprising fact when it is considered that ACh is the mediating factor for neural transmission at the somatic myoneural junction. This side effect can be counteracted with neostigmine methylsulfate.

Oxyphencyclimine Hydrochloride

(1,4,5,6-Tetrahydro-1-methyl-2-pyrimidinyl)methyl α-phenylcyclohexaneglycolate monohydrochloride (Daricon, Vistrax). Oxyphencyclimine hydrochloride, introduced in 1958, was promoted as a peripheral anticholinergic–antisecretory agent, with little or no curare-like activity and little or no ganglionic blocking activity. That these activities are absent is probably because of the tertiary character of the molecule. This activity is in contrast with compounds that couple antimuscarinic action with ganglionic blocking action. The tertiary character of the nitrogen promotes intestinal absorption of the molecule. Perhaps the most significant activity of this compound is its marked ability to reduce both the volume and the acid content of the gastric juices, a desirable action in view of the more recent hypotheses pertaining to peptic ulcer therapy. Another important feature of this compound is its low toxicity in comparison with many of the other available anticholinergics. Oxyphencyclimine hydrochloride is hydrolyzed in the presence of excessive moisture and heat. It is absorbed from the GI tract and has a duration of action of up to 12 hr.

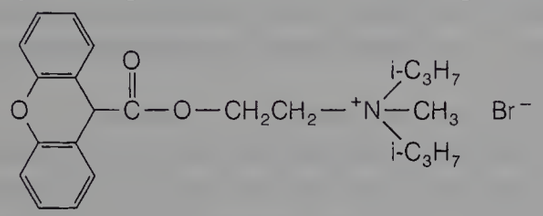


Oxyphencyclimine Hydrochloride

Oxyphencyclimine hydrochloride is suggested for use in peptic ulcer, pylorospasm, and functional bowel syndrome. It is contraindicated, as are other anticholinergics, in patients with prostatic hypertrophy and glaucoma.

Propantheline Bromide, USP

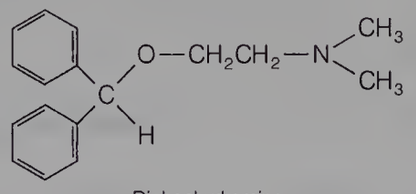
(2-Hydroxy-ethyl)diisopropylmethylammonium bromide xanthene-9-carboxylate (Pro-Banthine). The method of preparation of this compound is exactly analogous to that used for methantheline bromide. It is a white, water-soluble, crystalline substance, with properties quite similar to those of methantheline bromide. Its chief difference from methantheline bromide is in its potency, which has been estimated variously as being from two to five times as great.



Propantheline Bromide

AMINOALCOHOL ETHERS

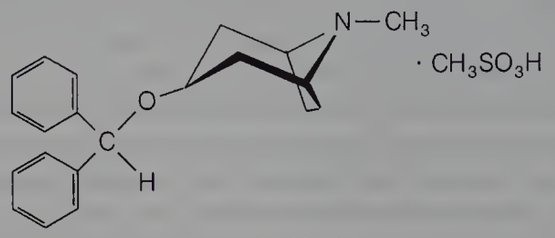
The aminoalcohol ethers thus far introduced have been used as antiparkinsonian drugs rather than as conventional anticholinergics (i.e., as spasmolytics or mydriatics). In general, they may be considered closely related to the antihistaminics and, indeed, do possess antihistaminic properties of a substantial order. In turn, the antihistamines possess anticholinergic activity and have been used as antiparkinsonian agents. Comparison of chlorphenoxamine and orphenadrine with the antihistaminic diphenhydramine illustrates the close similarity of structure. The use of diphenhydramine in parkinsonism has been cited earlier. Benztropine may also be considered a structural relative of diphenhydramine, though the aminoalcohol portion is tropine and, therefore, more distantly related than chlorphenoxamine and orphenadrine. In the structure of benztropine, a three-carbon chain intervenes between the nitrogen and oxygen functions, whereas in the others, a two-carbon chain is evident. However, the rigid ring structure possibly orients the nitrogen and oxygen functions into more nearly the two-carbon chain interprosthetic distance than is apparent at first glance. This, combined with the flexibility of the alicyclic chain, would help to minimize the distance discrepancy.



Diphenhydramine

Benztropine Mesylate, USP

3α-(Diphenylmethoxy)-1αH,5αH-tropane methanesulfonate (Cogentin). Benztropine mesylate has anticholinergic, antihistaminic, and local anesthetic properties. Its anticholinergic effect makes it applicable in its use as an antiparkinsonian agent. It is about as potent as atropine as an anticholinergic and shares some of the side effects of this drug, such as mydriasis and dryness of mouth. Importantly, however, it does not produce central stimulation but instead exerts the characteristic sedative effect of the antihistamines.



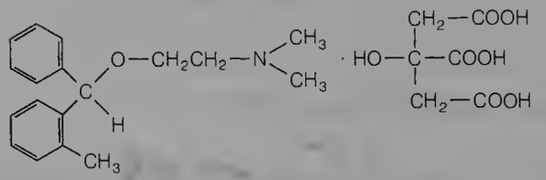
Benztropine Mesylate

The tremor and rigidity characteristic of parkinsonism are relieved by benztropine mesylate, and it is of particular value for those patients who cannot tolerate central excitation (e.g., aged patients). It also may have a useful effect in minimizing drooling, sialorrhea, mask-like facies, oculogyric crises, and muscular cramps.

The usual caution exercised with any anticholinergic in glaucoma and prostatic hypertrophy is observed with this drug.

Orphenadrine Citrate

N,N-Dimethyl-2-(*o*-methyl-α-phenylbenzyloxy)ethylamine citrate (1 : 1) (Norflex). Although this compound, introduced in 1957, is closely related to diphenhydramine structurally, it has a much lower antihistaminic activity and a much higher anticholinergic action. Likewise, it lacks the sedative effects characteristic of diphenhydramine. Pharmacologic testing indicates that it is not primarily a peripherally acting anticholinergic because it has only weak effects on smooth muscle, on the eye, and on secretory glands. However, it does reduce voluntary muscle spasm by a central inhibitory action on cerebral motor areas, a central effect similar to that of atropine.



Orphenadrine Citrate

The drug is used for the symptomatic treatment of Parkinson's disease. It relieves rigidity better than it does tremor, and in certain cases it may accentuate the latter. The drug combats mental sluggishness, akinesia, adynamia, and lack of mobility, but this effect seems to diminish rather rapidly

on prolonged use. It is best used as an adjunct to the other agents, such as benztropine, procyclidine, cycrimine, and trihexyphenidyl, in the treatment of paralysis agitans. Orphenadrine citrate is also used as an adjunct to rest, physiotherapy, and other measures to relieve pain of local muscle spasm (e.g., nocturnal leg cramps).

The drug has a low incidence of the usual side effects for this group, namely, dryness of mouth, nausea, and mild excitation.

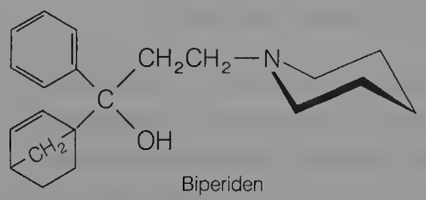
AMINOALCOHOLS

The development of aminoalcohols as parasympatholytics took place in the 1940s. It was soon established, however, that these antispasmodics were equally efficacious in parkinsonism.

Several of the drugs in this class of antimuscarinic agents have the structural characteristic of possessing bulky groups in the vicinity of hydroxyl and cyclic amino functional groups. These compounds are similar to the classic aminoester anticholinergic compounds derived from atropine. The presence of the alcohol group seems to substitute adequately as a prosthetic group for the carboxyl function in creating an effective parasympathetic blocking agent. The aminoester group, per se, is not a necessary adjunct to cholinolytic activity, provided that other polar groupings, such as the hydroxyl, can substitute as a prosthetic group for the carboxyl function. Another structural feature common to all aminoalcohol anticholinergics is the γ-aminopropanol arrangement, with three carbons intervening between the hydroxyl and amino functions. All of the aminoalcohols used for paralysis agitans are tertiary amines. Because the desired locus of action is central, formation of a quaternary ammonium moiety destroys the antiparkinsonian properties. However, quaternization of these aminoalcohols has been utilized to enhance the anticholinergic activity to produce an antispasmodic and antisecretory compound, such as tridihexethyl chloride.

Biperiden, USP

α-5-Norbornen-2-yl-α-phenyl-1-piperidinepropanol (Akineton). Biperiden, introduced in 1959, has a relatively weak visceral anticholinergic, but a strong nicotinolytic, action in terms of its ability to block nicotine-induced convulsions. Therefore, its neurotropic action is rather low on intestinal musculature and blood vessels. It has a relatively strong musculotropic action, which is about equal to that of papaverine, in comparison with most synthetic anticholinergic drugs. Its action on the eye, although mydriatic, is much less than that of atropine. These weak anticholinergic effects add to its usefulness in Parkinson's syndrome by minimizing side effects.



Biperiden

The drug is used in all types of Parkinson's disease (postencephalitic, idiopathic, arteriosclerotic) and helps to eliminate akinesia, rigidity, and tremor. It is also used in drug-induced extrapyramidal disorders to eliminate symptoms and permit continued use of tranquilizers. Biperiden is also of value in spastic disorders not related to parkinsonism, such as multiple sclerosis, spinal cord injury, and cerebral palsy. It is contraindicated in all forms of epilepsy.

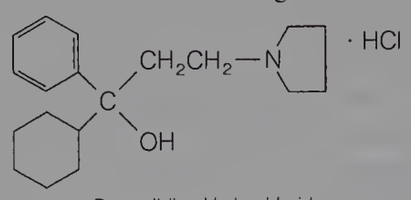
Biperiden Hydrochloride, USP

α-5-Norbornen-2-yl-α-phenyl-1-piperidinepropanol hydrochloride (Akineton hydrochloride). Biperiden hydrochloride is a white, optically inactive, crystalline, odorless powder that is slightly soluble in water, ether, alcohol, and chloroform and sparingly soluble in methanol.

Biperiden hydrochloride has all of the actions described for biperiden. The hydrochloride is used for tablets because it is better suited to this dosage form than is the lactate salt. As with the free base and the lactate salt, xerostomia (dryness of the mouth) and blurred vision may occur.

Procyclidine Hydrochloride, USP

α-Cyclohexyl-α-phenyl-1-pyrrolidinepropanol hydrochloride (Kemadrin). Although procyclidine hydrochloride, introduced in 1956, is an effective peripheral anticholinergic and, indeed, has been used for peripheral effects similarly to its methochloride (i.e., tricyclamol chloride), its clinical usefulness lies in its ability to relieve spasticity of voluntary muscle by its central action. Therefore, it has been employed with success in the treatment of Parkinson's syndrome. It is said to be as effective as trihexyphenidyl and is used for reduction of muscle rigidity in postencephalitic, arteriosclerotic, and idiopathic types of the disease. Its effect on tremor is not predictable and probably should be supplemented by combination with other similar drugs.

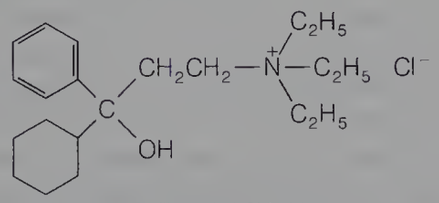


Procyclidine Hydrochloride

The toxicity of the drug is low, but side effects are noticeable when the dosage is high. At therapeutic dosage levels, dry mouth is the most common side effect. The same care should be exercised with this drug as with all other anticholinergics when administered to patients with glaucoma, tachycardia, or prostatic hypertrophy.

Tridihexethyl Chloride, USP

(3-Cyclohexyl-3-hydroxy-3-phenylpropyl)triethylammoniumchloride (Pathilon). Tridihexethyl chloride is a white, bitter, crystalline powder possessing a characteristic odor. The compound is freely soluble in water and alcohol, aqueous solutions being nearly neutral in reaction.



Tridihexethyl Chloride

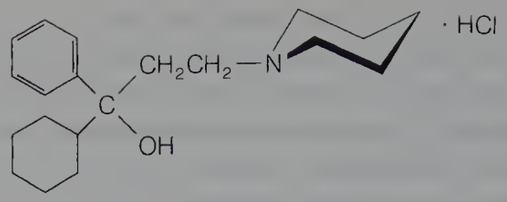
Although this drug, introduced in 1958, has ganglion-blocking activity, its peripheral atropine-like activity predominates; therefore, its therapeutic application has been based on the latter activity. It possesses the antispasmodic and the antisecretory activities characteristic of this group, but because of its quarternary character, it is valueless in relieving Parkinson's syndrome.

The drug is useful for adjunctive therapy in a wide variety of GI diseases, such as peptic ulcer, gastric hyperacidity, and hypermotility, and spastic conditions, such as spastic colon, functional diarrhea, pylorospasm, and other related conditions. Because its action is predominantly antisecretory, it is most effective in gastric hypersecretion rather than in hypermotility and spasm. It is best administered intravenously for the latter conditions.

The side effects usually found with effective anticholinergic therapy occur with the use of this drug. These are dryness of mouth, mydriasis, and such. As with other anticholinergics, care should be exercised when administering the drug in glaucomatous conditions, cardiac decompensation, and coronary insufficiency. It is contraindicated in patients with obstruction at the bladder neck, prostatic hypertrophy, stenosing gastric and duodenal ulcers, or pyloric or duodenal obstruction.

Trihexyphenidyl Hydrochloride, USP

α-Cyclohexyl-α-phenyl-1-piperidinepropanol hydrochloride (Artane, Tremin, Pipanol). Introduced in 1949, trihexyphenidyl hydrochloride is approximately one-half as active as atropine as an antispasmodic but is claimed to have milder side effects, such as mydriasis, drying of secretions, and cardioacceleration. It has a good margin of safety, though it is about as toxic as atropine. It has found a place in the treatment of parkinsonism and is claimed to provide some measure of relief from the mental depression often associated with this condition. However, it does exhibit some of the side effects typical of the parasympatholytic-type preparation, though these may often be eliminated by adjusting the dose carefully.



Trihexyphenidyl Hydrochloride

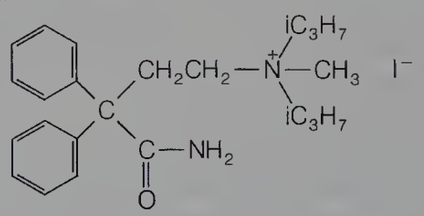
AMINOAMIDES

From a structural standpoint, the aminoamide type of anticholinergic represents the same type of molecule as the aminoalcohol group, with the important exception that the polar amide group replaces the corresponding polar hydroxyl group. Aminoamides retain the same bulky structural features as are found at one end of the molecule or the other in all of the active anticholinergics. Isopropamide iodide is the only drug of this class currently in use.

Another amide-type structure is that of tropicamide, formerly known as bistropamide, a compound having some of the atropine features.

Isopropamide Iodide, USP

(3-Carbamoyl-3,3-diphenylpropyl)diisopropylmethylammonium iodide (Darbid). Isopropamide iodide occurs as a bitter, white to pale yellow, crystalline powder that is only sparingly soluble in water but freely soluble in chloroform and alcohol.



Isopropamide Iodide

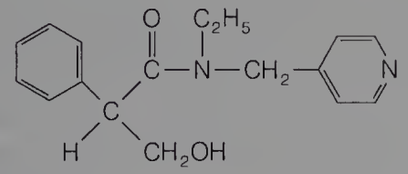
This drug, introduced in 1957, is a potent anticholinergic, producing atropine-like effects peripherally. Even with its quaternary nature, it does not cause sympathetic blockade at the ganglionic level except in high-level dosage. Its principal distinguishing feature is its long duration of action. A single dose can provide antispasmodic and antisecretory effects for as long as 12 hours.

It is used as adjunctive therapy in the treatment of peptic ulcer and other conditions of the gastrointestinal tract associated with hypermotility and hyperacidity. It has the usual side effects of anticholinergics (dryness of mouth, mydriasis, difficult urination) and is contraindicated in glaucoma, prostatic hypertrophy, etc.

Tropicamide, USP

N-Ethyl-2-phenyl-*N*-(4-pyridylmethyl)hydracrylamide (Mydriacyl). This drug is an effective anticholinergic for oph-

thalmic use where mydriasis is produced by relaxation of the sphincter muscle of the iris, allowing the adrenergic innervation of the radial muscle to dilate the pupil. Its maximum effect is achieved in about 20 to 25 minutes and lasts for about 20 minutes, with complete recovery being noted in about 6 hr. Its action is more rapid in onset and wears off more rapidly than that of most other mydriatics. To achieve mydriasis, either 0.5% or 1.0% concentration may be used, though cycloplegia is achieved only with the stronger solution. Its uses are much the same as those described earlier for mydriatics in general, but opinions differ on whether or not the drug is as effective as homatropine, for example, in achieving cycloplegia. For mydriatic use, however, in examination of the fundus and treatment of acute iritis, iridocyclitis, and keratitis, it is quite adequate; and because of its shorter duration of action, it is less prone to initiate a rise in intraocular pressure than the more potent, longer-lasting drugs. However, as with other mydriatics, pupil dilation can lead to increased intraocular pressure. In common with other mydriatics, it is contraindicated in cases of glaucoma, either known or suspected, and should not be used in the presence of a shallow anterior chamber. Thus, far, allergic reactions or ocular damage have not been observed with this drug. The ability to clone the various muscarinic receptor subtypes has allowed for the observation that tropicamide has modest selectivity for the M_4 receptor.[74]



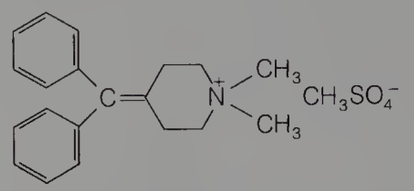
Tropicamide

MISCELLANEOUS

Further structural modification of classic antimuscarinic agents can be found in the drugs described below. Each of them has the typical bulky group that is characteristic of the musual anticholinergic molecule. One modification is represented by the diphenylmethylene moiety (e.g., diphemanil); a second, by a phenothiazine (e.g., ethopropazine); and a third, by a thioxanthene structure (e.g., methixene).

Diphemanil Methylsulfate, USP

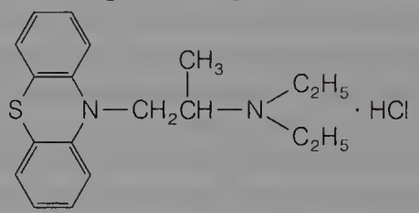
4-(Diphenyl-methylene)-1, 1-dimethylpiperidinium methylsulfate (Prantal). Diphemanil methylsulfate is a potent cholinergic blocking agent. In the usual dosage range, it acts as an effective parasympatholytic by blocking nerve impulses at the parasympathetic ganglia, but it does not invoke a sympathetic ganglionic blockade. It is claimed to be highly specific in its action upon those innervations that activate gastric secretion and GI motility. Although this drug is capable of producing atropine-like side effects, they rarely occur at recommended doses. The highly specific nature of its action on gastric functions makes the drug useful in the treatment of peptic ulcer, and its lack of atropine-like effects makes this use much less distressing than other antispasmodic drugs. In addition to its action in decreasing gastric hypermotility, diphemanil methylsulfate is valuable in hyperhidrosis in low doses (50 mg twice daily) or topically. The drug is not well absorbed from the GI tract, particularly in the presence of food, and should be administered between meals. The methylsulfate salt was chosen as the best because the chloride is hygroscopic and the bromide and iodide ions have exhibited toxic manifestations in clinical use.



Diphemanil Methylsulfate

Ethopropazine Hydrochloride, USP

10-[2-(Diethylamino)propyl]phenothiazine monohydrochloride (Parsidol). Ethopropazine hydrochloride was introduced to therapy in 1954. It has antimuscarinic activity and is especially useful in the symptomatic treatment of parkinsonism. In this capacity, it has value in controlling rigidity, and it also has a favorable effect on tremor, sialorrhea, and oculogyric crises. It is used often in conjunction with other antiparkinsonian drugs for complementary activity.



Ethopropazine Hydrochloride

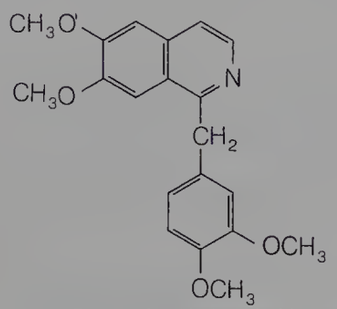
Side effects are common with this drug but are not usually severe. Drowsiness and dizziness are the most common side effects at ordinary dosage levels, and as the dose increases, xerostomia, mydriasis, and others become evident. It is contraindicated in conditions such as glaucoma because of its mydriatic effect.

Papaverine Hydrochloride, USP

6,7-Dimethoxy-1-veratrylisoquinoline hydrochloride. This alkaloid was isolated by Merck in 1848 from opium, in which it occurs to the extent of about 1%. Although its natural origin is closely related to morphine, the pharmacologic actions of papaverine hydrochloride are unlike those of morphine. Its main effect is as a spasmolytic on smooth muscle, acting as a direct, nonspecific relaxant on vascular, cardiac, and other smooth muscle. Because of its broad antispas-

modic action on ACh muscarinic receptors, it is often called a nonspecific antagonist. Papaverine hydrochloride has been used in the treatment of peripheral vascular disorders but its use is limited due to lack of potency.

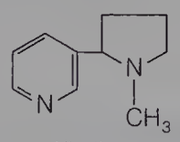
Papaverine hydrochloride interferes with the mechanism of muscle contraction by inhibiting the cyclic nucleotide phosphodiesterases in smooth muscle cells responsible for converting cAMP and cyclic guanosine monophosphate (cGMP) to 5′-AMP and 5′-GMP, respectively. The increased levels of cAMP and cGMP are associated with muscle relaxation through their phosphorylation of myosin light chain kinase.



Papaverine Hydrochloride

GANGLIONIC BLOCKING AGENTS

Autonomic ganglia have been a subject of interest for many years in the study of interactions occurring between drugs and nervous tissues. The first important account[75] was given by Langley and described the stimulating and blocking actions of nicotine on sympathetic ganglia. It was found that small amounts of nicotine stimulated ganglia and then produced a blockade of ganglionic transmission because of persistent depolarization. From these experiments, Langley was able to outline the general pattern of innervation of organs by the autonomic nervous system. *Parasympathetic* ganglia usually are located near the organ they innervate and have preganglionic fibers that stem from the cervical and thoracic regions of the spinal cord. *Sympathetic* ganglia consist of 22 pairs that lie on either side of the vertebral column to form lateral chains. These ganglia are connected both to each other by nerve trunks and to the lumbar or sacral regions of the spinal cord.



Nicotine

Using the sympathetic cervical ganglion as a model, it has been found that transmission in the autonomic ganglion is more complex than formerly believed. Traditionally, stimulation of autonomic ganglia by ACh has been considered the nicotinic action of the neurotransmitter. It is now understood that stimulation by ACh produces a triphasic response in sympathetic ganglia. Impulse transmission through the ganglion occurs when ACh is released from preganglionic fibers and activates the N_2 nicotinic receptors of the neuronal membrane. This triggers an increase in sodium and potassium conductances of a subsynaptic membrane, resulting in an initial excitatory postsynaptic potential (EPSP) with a latency of 1 millisecond, followed by an inhibitory postsynaptic potential (IPSP) with a latency of 35 milliseconds, and, finally, a slowly generating EPSP with a latency of several hundred milliseconds. The ACh released by preganglionic fibers also activates M_1 muscarinic receptors of the ganglion and probably of the small-intensity fluorescent (SIF) cell. This results in the appearance of a slow IPSP and a slow EPSP in the neurons of the ganglion.[76] The initial EPSP is blocked by conventional competitive nondepolarizing ganglionic blocking agents, such as hexamethonium, and is considered the primary pathway for ganglionic transmission.[77] The slowly generating or late EPSP is blocked by atropine but not by the traditional ganglionic blocking agents. This receptor has muscarinic properties because methacholine causes generation of the late EPSP without causing the initial spike characteristic of ACh. Atropine also blocks the late EPSP produced by methacholine. There may be more than one type of muscarinic receptor in sympathetic ganglia. Atropine blocks both high-affinity (M_1) and low-affinity (M_2) muscarinic receptors in the ganglion.[78] In addition to the cholinergic pathways, the cervical sympathetic ganglion was found to have a neuron that contains a catecholamine.[79] These neuronal cells, identified initially by fluorescence histochemical studies and shown to be smaller than the postganglionic neurons, are now referred to as SIF cells. Dopamine has been identified as the fluorescent catecholamine in the SIF cells that are common to many other sympathetic ganglia. Dopamine apparently mediates an increase in cAMP, which causes hyperpolarization of postganglionic neurons (Fig. 17-19). The IPSP phase of the transmission of sympathetic ganglia following ACh administration can be blocked by both atropine and α-adrenergic blocking agents.[76]

If a similar nontraditional type of ganglionic transmission occurs in the parasympathetic ganglia, it has not yet become evident.

With the anatomic and physiologic differences between sympathetic and parasympathetic ganglia, it should be no surprise that ganglionic agents may show some selectivity between the two types of ganglia. Although we do not have drug classifications such as "parasympathetic ganglionic blockers" and "sympathetic ganglionic blockers," we do find that certain ganglia have a predominant effect over certain organs and tissues and that a nondiscriminant blockade of autonomic ganglia results in a change in the effect of the autonomic nervous system on that organ (Table 17-7). None of the commonly known ganglionic blockers has yet been identified as a selective blocker of parasympathetic ganglia.

Van Rossum[80,81] has reviewed the mechanisms of ganglionic synaptic transmission, the mode of action of ganglionic stimulants, and the mode of action of ganglionic blocking agents. They have been classified as blocking agents in the following manner.

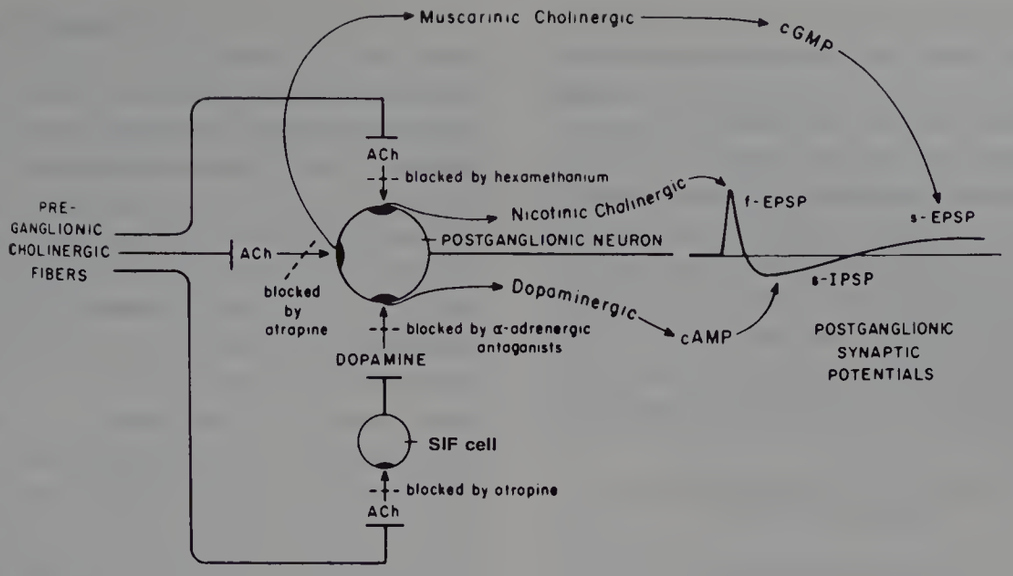


FIG. 17-19. *Neurotransmission at the sympathetic cervical ganglion.*

DEPOLARIZING GANGLIONIC BLOCKING AGENTS

These blocking agents are actually ganglionic stimulants. Thus, for nicotine, small doses give an action similar to that of the natural neuroeffector ACh, an action known as the "nicotinic effect of ACh." However, larger amounts of nicotine bring about a ganglionic block, characterized initially by depolarization, followed by a typical competitive antagonism. To conduct nerve impulses, the cell must be able to carry out a polarization and depolarization process, and if the depolarized condition is maintained without repolarization, it is obvious that no conduction occurs. ACh itself, in high concentrations, will bring about an autoinhibition. Chemicals that cause this type of ganglionic block are not of therapeutic significance. The classes of ganglionic blocking agents that are described are therapeutically useful.

TABLE 17-7

RESULTS OF GANGLIONIC BLOCKERS ON ORGANS

Organ	*Predominant System*	*Results of Ganglionic Blockade*
Cardiovascular system		
Heart	Parasympathetic	Tachycardia
Arterioles	Sympathetic	Vasodilation
Veins	Sympathetic	Dilation
Eye		
Iris	Parasympathetic	Mydriasis
Ciliary muscle	Parasympathetic	Cycloplegia
GI tract	Parasympathetic	Relaxation
Urinary bladder	Parasympathetic	Urinary retention
Salivary glands	Parasympathetic	Dry mouth
Sweat glands	Sympathetic*	Anhidrosis

* Neurotransmitter is ACh.

Adapted from Goth, A.: Medical Pharmacology, 9th ed. St. Louis, C. V. Mosby Co., 1978.

NONDEPOLARIZING COMPETITIVE GANGLIONIC BLOCKING AGENTS

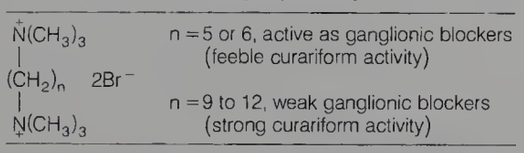
Compounds in this class possess the necessary affinity to attach to the nicotinic receptor sites that are specific for ACh but lack the intrinsic activity necessary for impulse transmission (i.e., they are unable to effect depolarization of the cell). Under experimental conditions, in the presence of a fixed concentration of blocking agent of this type, a large enough concentration of ACh can offset the blocking action by competing successfully for the specific receptors. When such a concentration of ACh is administered to a ganglion preparation, it appears that the intrinsic activity of the ACh is as great as it was when no antagonist was present, the only difference being in the larger concentration of ACh required. It is evident, then, that such blocking agents are "competitive" with ACh for the specific receptors involved and that either the agonist or the antagonist can displace the other if present in sufficient concentration. Drugs falling into this class are tetraethylammonium salts, hexamethonium, and trimethaphan. Mecamylamine possesses a competitive component in its action but is also noncompetitive, a so-called dual antagonist.

NONDEPOLARIZING NONCOMPETITIVE GANGLIONIC BLOCKING AGENTS

These blocking agents produce their effect, not at the specific ACh receptor site, but at some point further along the chain of events that is necessary for transmission of the nerve impulse. When the block has been imposed, increase of the concentration of ACh has no effect; thus, apparently, ACh does not act competitively with the blocking agent at the same receptors. Theoretically, a pure noncompetitive blocker should have a high specific affinity for the noncom-

petitive receptors in the ganglia, and a very low affinity for other cholinergic synapses, together with no intrinsic activity. Mecamylamine, as mentioned before, has a noncompetitive component but is also a competitive blocking agent.

The first ganglionic blocking agents employed in therapy were tetraethylammonium chloride and bromide. Although one might assume that curariform activity would be a deterrent to their use, it has been shown that the curariform activity of the tetraethyl compound is less than 1% of that of the corresponding tetramethylammonium compound. A few years after the introduction of the tetraethylammonium compounds, Paton and Zaimis[82] investigated the usefulness of the bistrimethylammonium polymethylene salts:



As shown, their findings indicate that there is a critical distance of about five to six carbon atoms between the onium centers for good ganglionic blocking action. Interestingly enough, the pentamethylene and hexamethylene compounds are effective antidotes for counteracting the curare effect of the decamethylene compound. Hexamethonium bromide and hexamethonium chloride emerged from this research as clinically useful products.

Trimethaphan camphorsulfonate, a monosulfonium compound, bears some degree of similarity to the quaternary ammonium types because it, too, is a completely ionic compound. Although it produces a prompt ganglion-blocking action on parenteral injection, its action is short, and it is used only for controlled hypotension during surgery. Almost simultaneously with the introduction of chlorisondamine (now long removed from the market), announcement was made of the powerful ganglionic blocking action of mecamylamine, a secondary amine *without* quarternary ammonium character. As expected, the latter compound showed uniform and predictable absorption from the GI tract as well as a longer duration of action. The action was similar to that of hexamethonium.

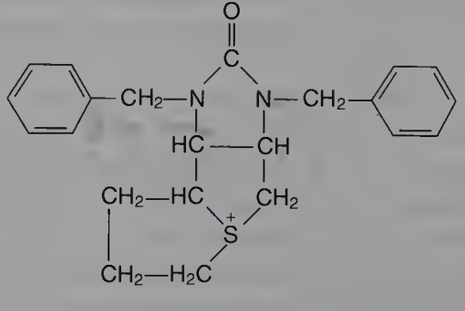
Drugs of this class have limited usefulness as diagnostic and therapeutic agents in the management of peripheral vascular diseases (e.g., thromboangiitis obliterans, Raynaud's disease, diabetic gangrene). However, the principal therapeutic application has been in the treatment of hypertension through blockade of the sympathetic pathways. Unfortunately, the action is nonspecific, and the parasympathetic ganglia, unavoidably, are blocked simultaneously to a greater or lesser extent, causing visual disturbances, dryness of the mouth, impotence, urinary retention, and constipation. Constipation, in particular, probably caused by unabsorbed drug in the intestine (poor absorption), has been a drawback because the condition can proceed to a paralytic ileus if extreme care is not exercised. For this reason, cathartics or a parasympathomimetic (e.g., pilocarpine nitrate) are frequently administered simultaneously. Another side effect is the production of orthostatic (postural) hypotension (i.e., dizziness when the patient stands up in an erect position). Prolonged administration of the ganglionic blocking agents results in diminished effectiveness because of a buildup of tolerance, though some are more prone to this than others. Because of the many serious side effects, this group of drugs has been replaced by more effective hypotensive agents.

In addition to these side effects, there are several limitations to the use of these drugs. For instance, they are contraindicated in disorders characterized by severe reduction of blood flow to a vital organ (e.g., severe coronary insufficiency, recent myocardial infarction, retinal and cerebral thrombosis) as well as situations in which there have been large reductions in blood volume. In the latter, the contraindication exists because the drugs block the normal vasoconstrictor compensatory mechanisms necessary for homeostasis. A potentially serious complication, especially in older male patients with prostatic hypertrophy, is urinary retention. These drugs should be used with care or not at all in the presence of renal insufficiency, glaucoma, uremia, and organic pyloric stenosis.

Trimethaphan Camsylate, USP

(+)-1, 3-Dibenzyldecahydro-2-oxoimidazo[4, 5-*c*]thieno [1,2-α]-thiolium 2-oxo-10-bornanesulfonate (1:1) (Arfonad). This drug consists of white crystals or is a crystalline powder with a bitter taste and a slight odor. It is soluble in water and alcohol but only slightly soluble in acetone and ether. The *p*H of a 1% aqueous solution is 5.0 to 6.0.

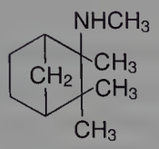
This ganglionic blocking agent is short-acting and is used for certain neurosurgical procedures for which excessive bleeding obscures the operative field. Certain craniotomies are included among these operations. The action of the drug is a direct vasodilation, and because of its transient action, it is subject to minute-by-minute control. However, this fleeting action makes it useless for hypertensive control. The drug is ineffective when given orally. The usual route of administration is intravenous. Trimethaphan camsylate is indicated in the treatment of hypertensive emergencies to rapidly reduce blood pressure. These emergencies may include pulmonary hypertension associated with systemic hypertension and acute dissecting aneurysm.



Trimethaphan Camsylate

Mecamylamine Hydrochloride

N,2,3,3-Tetramethyl-2-norbornanamine hydrochloride (Inversine). This secondary amine has a powerful ganglionic blocking effect that is almost identical with that of hexamethonium. It has an advantage over most of the ganglionic blocking agents in that it is absorbed readily and smoothly from the GI tract. It is rarely used, however, for the treatment of moderate to severe hypertension because of severe orthostatic hypotension that occurs when sympathetic ganglia are blocked by the drug.



Mecamylamine Hydrochloride

NEUROMUSCULAR BLOCKING AGENTS

Agents that block the transmission of ACh at the motor end-plate are called *neuromuscular blocking agents*. The therapeutic use of these compounds is primarily as adjuvants in surgical anesthesia to obtain relaxation of skeletal muscle. They also are used in various orthopedic procedures, such as alignment of fractures and correction of dislocations.

The therapeutically useful compounds in this group sometimes are referred to as possessing "curariform" or "curarimimetic" activity in reference to the original representatives of the class, which were obtained from curare. Since then, synthetic compounds have been prepared with a similar activity. Although all of the compounds falling into this category, natural and synthetic alike, bring about substantially the same end result (i.e., voluntary-muscle relaxation), there are some significant differences in mechanisms.

The possible existence of a junction between muscle and nerve was suggested as early as 1856, when Claude Bernard observed that the site of action of curare was neither the nerve nor the muscle. Since that time, it has been agreed that ACh mediates transmission at the neuromuscular junction by a sequence of events that has been described earlier in this chapter. The neuromuscular junction consists of the axon impinging onto a specialized area of the muscle known as the muscle end plate. The axon is covered with a myelin sheath, containing the nodes of Ranvier, but is bare at the ending. The nerve terminal is separated from the end plate by a gap of 200 Å. The subsynaptic membrane of the end plate contains the cholinergic receptor, the ion-conducting channels (which are opened under the influence of ACh), and AChE.

One of the anatomic differences between the neuromuscular junction and other ACh-responsive sites is the absence in the former of a membrane barrier or sheath that envelopes the ganglia or constitutes the blood–brain barrier. This is of importance in the accessibility of the site of action to drugs, particularly so for quaternary ammonium compounds because they pass through living membranes with considerably greater difficulty and selectivity than do compounds that can exist in a non-ionized species. The essentially bare nature (i.e., lack of lipophilic barriers) of the myoneural junction permits ready access by quaternary ammonium compounds. In addition, compounds with considerable molecular dimensions are accessible to the receptors in the myoneural junction. As a result of this property, variations in the chemical structure of quaternaries have little influence on the potential ability of the molecule to reach the cholinergic receptor in the neuromuscular junction. Thus, the following types of neuromuscular junction blockers have been noted.

NONDEPOLARIZING BLOCKING AGENTS

Traditionally, "*nondepolarizing blocking agents*" is a term applied to categorize drugs that compete with ACh for the recognition site on the nicotinic receptor by preventing depolarization of the end plate by the neurotransmitter. Thus, by decreasing the effective ACh–receptor combinations, the end-plate potential becomes too small to initiate the propagated action potential. This results in paralysis of neuromuscular transmission. The action of these drugs is quite analogous to that of atropine at the muscarinic receptor sites of ACh. Many experiments suggest that the agonist (ACh) and the antagonist compete on a one-to-one basis for the end-plate receptors. Drugs falling into this classification are tubocurarine, dimethyltubocurarine, pancuronium, and gallamine.

DEPOLARIZING BLOCKING AGENTS

Drugs in this category are known to bring about a depolarization of the membrane of the muscle end plate. This depolarization is quite similar to that produced by ACh itself at ganglia and neuromuscular junctions (i.e., its so-called nicotinic effect), with the result that the drug, if in sufficient concentration, eventually will produce a block. It has been known for years that either smooth or voluntary muscle, when challenged repeatedly with a depolarizing agent, eventually will become insensitive. This phenomenon is known as *tachyphylaxis* or *desensitization* and is demonstrated convincingly under suitable experimental conditions with repeated applications of ACh itself, the results indicating that within a few minutes the end plate becomes insensitive to ACh. The previous statements may imply that a blocking action of this type is clear-cut, but under experimental conditions, it is not quite so unambiguous because a block that begins with depolarization may regain the polarized state even before the block. Furthermore, a depolarization induced by increasing the potassium ion concentration does not prevent impulse transmission. For these and other reasons, it is probably best to consider the blocking action as a desensitization until a clearer picture emerges. Drugs falling into this classification are decamethonium and succinylcholine.

CURARE AND CURARE ALKALOIDS

Originally "*curare*" was a term used to describe collectively the very potent arrow poisons used since early times by the South American Indians. The arrow poisons were prepared from numerous botanic sources and often were mixtures of several different plant extracts. Some were poisonous by virtue of a convulsant action and others by a paralyzant action. Only the latter type is of value in therapeutics and is spoken of ordinarily as "curare."

Chemical investigations of the curares were not especially successful because of difficulties in obtaining of authentic samples with definite botanic origin. It was not until 1935, when a pure crystalline alkaloid, *d*-tubocurarine chloride, possessing in great measure the paralyzing action of the original curare, was isolated from a plant. Wintersteiner and Dutcher,[83] in 1943, also isolated the same alkaloid. However, they showed that the botanic source was *Chondodendron tomentosum* (Menispermaceae) and, thus, provided a known source of the drug.

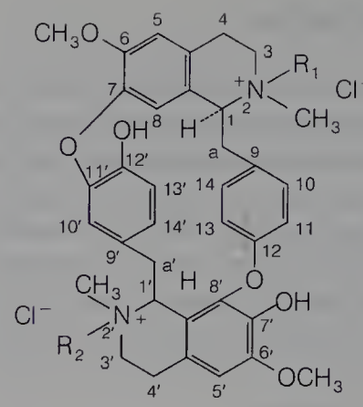
Following the development of quantitative bioassay methods for determining the potency of curare extracts, a purified and standardized curare was developed and marketed under the trade name Intocostrin (purified *C. tomentosum* extract), the solid content of which consisted of almost one-half (+)-tubocurarine solids. Following these essentially pioneering developments, (+)-tubocurarine chloride and dimethyltubocurarine iodide have appeared on the market as pure entities.

Tubocurarine Chloride, USP

(+)-Tubocurarine chloride hydrochloride pentahydrate. This alkaloid is prepared from crude curare by a process of purification and crystallization.

Tubocurarine chloride occurs as a white or yellowish white to grayish white, odorless, crystalline powder, which is soluble in water. Aqueous solutions of it are stable to heat sterilization.

The structural formula for (+)-tubocurarine was long thought to be represented as in Ia (see structure diagram). Through the work of Everett et al.,[84] the structure is now known to be that of Ib. The monoquaternary nature of Ib thus revealed has caused some reassessment of thinking concerning the theoretical basis for the blocking action because all previous assumptions had assumed a diquaternary structure (i.e., Ia). Nevertheless, this does not negate the earlier conclusions that a diquaternary nature of the molecule provides better blocking action than does a monoquaternary nature (e.g., Ib is approximately fourfold less potent than diemethyl tubocurarine iodide). It also may be of interest that (+)-isotubocurarine chloride (Ic) provides a compound with twice the activity of Ib in the particular test employed.



Tubocurarine is a nondepolarizing blocking agent used for its paralyzing action on voluntary muscles, the site of action being the neuromuscular junction. Its action is inhibited or reversed by the administration of AChE inhibitors, such as neostigmine, or by edrophonium chloride (Tensilon). Such inhibition of its action is necessitated in respiratory embarrassment caused by overdosage. Additionally, in somewhat higher concentrations, *d*-tubocurarine may enter the open ion channel and add a noncompetitive blockade. This latter action is not restored easily or fully by cholinesterase inhibitors. It often is necessary to use artificial respiration as an adjunct until the maximal curare action has passed. The drug is inactive orally because of inadequate absorption through lipoidal membranes in the GI tract and, when used therapeutically, usually is injected intravenously.

d-Tubocurarine binds for only 1 millisecond to the receptor, yet its pharmacologic effect of muscle paralysis, produced by administration of the drug intravenously during surgery, lasts for up to 2 hr. The basis of this action is the pharmacokinetics of the drug. *d*-Tubocurarine is given intravenously and, although 30% to 77% is bound to plasma proteins, the drug is distributed rapidly to central body compartments, including neuromuscular junctions. About 45% of *d*-tubocurarine is eliminated unchanged by the kidneys. Its half-life is 89 minutes.

Tubocurarine, in the form of a purified extract, was used first in 1943 as a muscle relaxant in shock therapy of mental disorders. By its use, the incidence of bone and spine fractures and dislocations resulting from convulsions owing to shock was reduced markedly. Following this, it was employed as an adjunct in general anesthesia to obtain complete muscle relaxation, a use that persists to this day. Before its use began, satisfactory muscle relaxation in various surgical procedures (e.g., abdominal operations) was obtainable only with "deep" anesthesia with the ordinary general anesthetics. Tubocurarine permits a lighter plane of anesthesia, with no sacrifice in the muscle relaxation so important to the surgeon. A reduced dose of tubocurarine is administered with ether because ether itself has a curare-like action.

Metocurine Iodide, USP

(+)-*O,O'*-Dimethylchondrocurarine diiodide (Metubine iodide). This drug is prepared from natural crude curare by

extracting the curare with methanolic potassium hydroxide. When the extract is treated with an excess of methyl iodide, the (+)-tubocurarine is converted to the diquaternary dimethyl ether and crystallizes out as the iodide (see "Tubocurarine Chloride," above). Other ethers besides the dimethyl ether have been made and tested. For example, the dibenzyl ether was one-third as active as tubocurarine chloride and the diisopropyl compound had only one-half the activity. This is compared with the dimethyl ether, which has approximately four times the activity of tubocurarine chloride.

The pharmacologic action of this compound is the same as that of tubocurarine chloride, namely, a nondepolarizing competitive blocking effect on the motor end plate of skeletal muscles. However, it is considerably more potent than *d*-tubocurarine, and it has the added advantage of exerting much less effect on the respiration. The effect on respiration is not a significant factor in therapeutic doses. Accidental overdosage is counteracted best by forced respiration.

SYNTHETIC COMPOUNDS WITH CURARIFORM ACTIVITY

Curare, until relatively recent times, remained the only useful curarizing agent; and it, too, suffered from a lack of standardization. The original pronouncement in 1935 of the structure of (+)-tubocurarine chloride, unchallenged for 35 years, led other workers to hope for activity in synthetic substances of less complexity. The quaternary ammonium character of the curare alkaloids coupled with the known activity of the various simple onium compounds hardly seemed to be coincidental, and it was natural for research to follow along these lines. One of the synthetic compounds discovered was marketed in 1951 as Flaxedil (gallamine triethiodide). A variety of other neuromuscular blocking agents has followed.

Atracurium Besylate

2-(2-Carboxyethyl)-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium benzensulfonate pentamethylene ester (Tracrium). Atracurium besylate is a nondepolarizing neuromuscular blocking agent that is approximately 2.5 times more potent than *d*-tubocurarine. Its duration of action (half-life 0.33 hr) is much shorter than that of *d*-tubocurarine. The drug is metabolized rapidly and nonenzymatically to yield laudanosine and a smaller quaternary compound (Fig. 17-20), which do not have activity as neuromuscular blockers. In vitro experiments show that atracurium besylate breaks down at *p*H 7.4 and 37°C by a Hoffman elimination reaction.[85] Atracurium besylate will undergo enzymatic decomposition of its ester function to

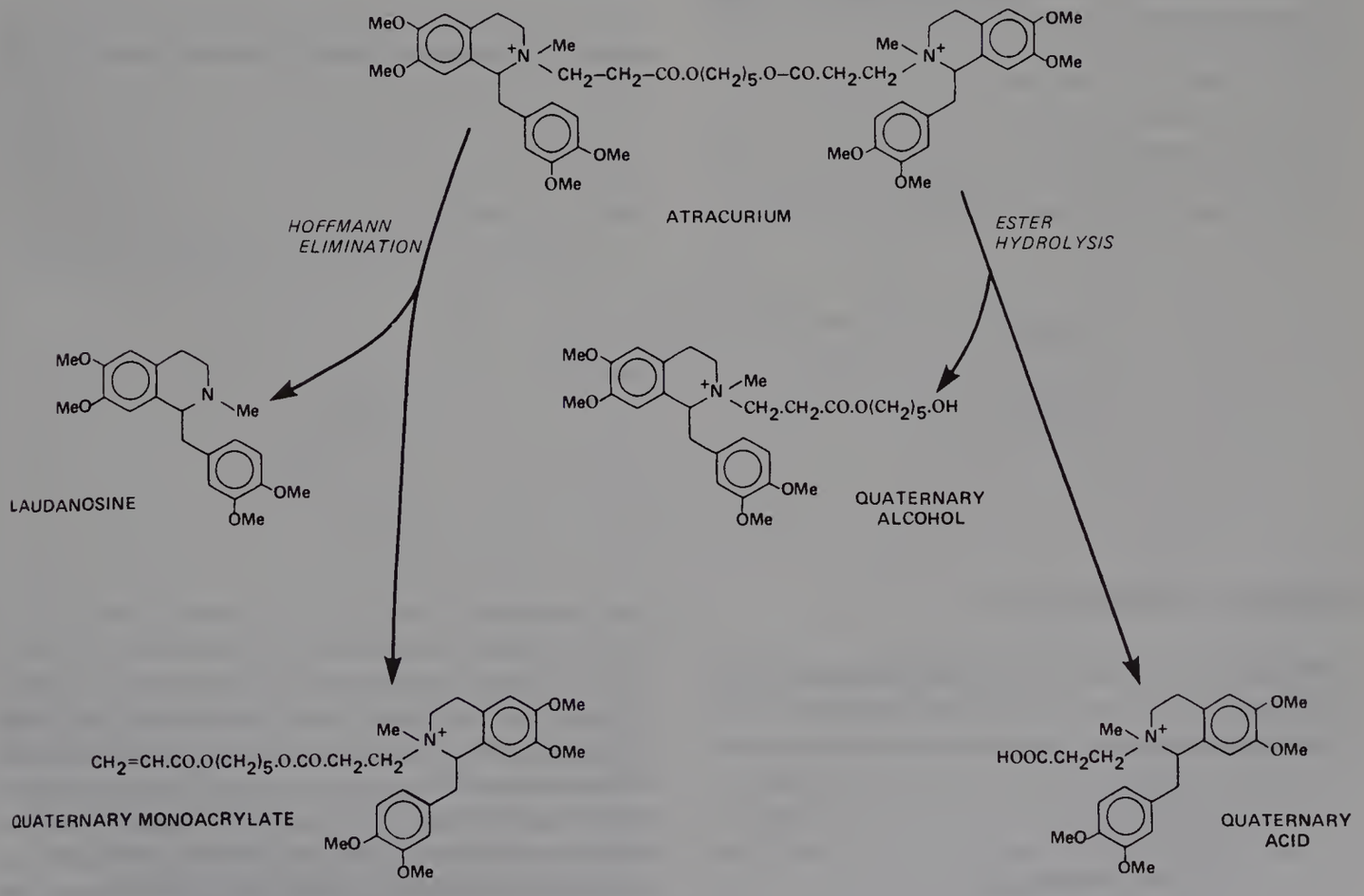
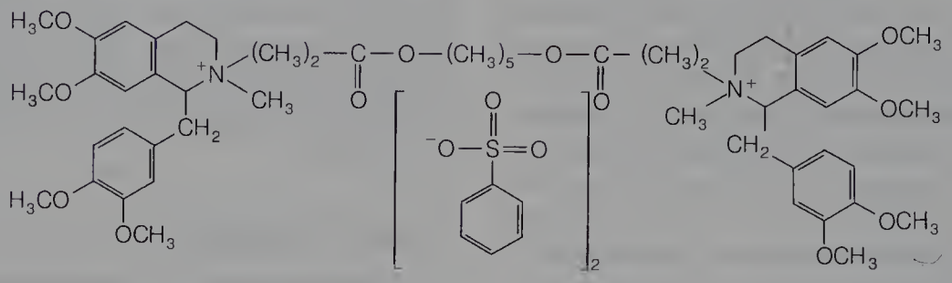


FIG. 17-20. Hoffman elimination and hydrolysis reactions of atracurium.

yield an inactive quaternary alcohol and quaternary acid. Paralysis by atracurium besylate is antagonized by AChE inhibitors such as neostigmine, edrophonium, and pyridostigmine.



Atracurium Besylate

Doxacurium Chloride

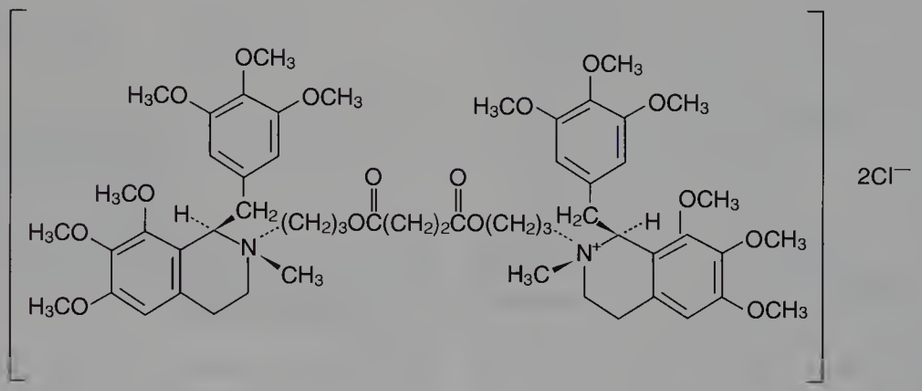
1, 2, 3, 4-tetrahydro-2-(3-hydroxypropyl)-6, 7, 8-trimethoxy-2-methyl-1-(3,4,5-trimethoxybenzyl) isoquinolinium chloride, succinate (Nuromax). The molecular structure of doxacurium chloride provides the possibility for ten stereoisomers: Four dl pairs and two *meso* forms. Of the ten stereoisomers, three are all *trans*-configuration, and these are the only active ones.[86] Doxacurium chloride is a long-acting nondepolarizing blocking agent. The drug differs from drugs such as gallium and pancuronium in that it has no vagolytic activity. It is used as a skeletal muscle relaxant in surgical procedures expected to last longer than 90 minutes.

sion in a manner similar to that of *d*-tubocurarine (i.e., a nondepolarizing blocking agent). It does have some differences, however. It has a strong vagolytic effect and a persistent decrease in neuromuscular function after successive doses that cannot be overcome by cholinesterase inhibitors. Gallamine triethiodide also has muscarinic antagonistic properties and binds with greater affinity to the M_2 receptors than to the M_1 receptor. This latter characteristic may be the cause of its strong vagolytic action.[86,87]

The drug is contraindicated in patients with myasthenia gravis, and it should be borne in mind that the drug action is cumulative, as with curare. The antidote for gallamine triethiodide is neostigmine.

Mivacurium Chloride

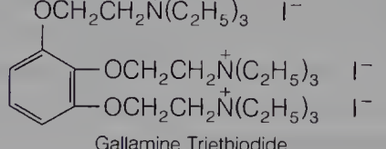
1,2,3,4-Tetrahydro-2-(3-hydroxypropyl)-6,7-dimethoxy-2-methyl-1-(3,4,5-trimethoxybenzyl) isoquinolinium chloride,



Doxacurium Chloride

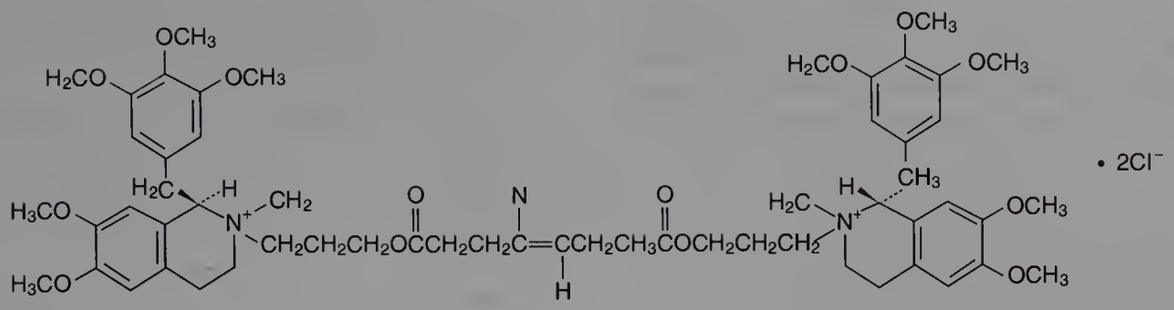
Gallamine Triethiodide, USP

[*v*-Phenenyl-tris(oxyethylene)]tris[triethylammonium] triiodide (Flaxedil). Gallamine triethiodide is a relaxant of skeletal muscle that works by blocking neuromuscular transmis-



Gallamine Triethiodide

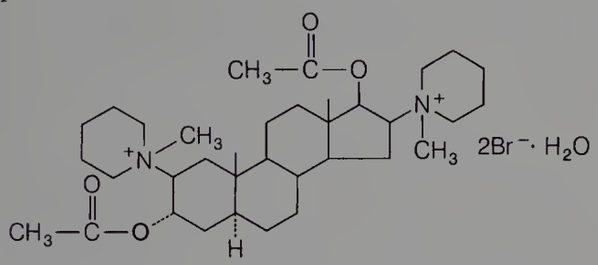
(E)-4-octandioate (Mivacron). Mivacurium chloride is a mixture of three stereoisomers, the *trans–trans, cis–trans*, and *cis–cis* diesters, each of which has neuromuscular blocking properties. The *cis–cis* isomer is about one-tenth as potent as the other isomers. Mivacurium chloride is a short-acting nondepolarizing drug used as an adjunct to anesthesia to relax skeletal muscle. The drug is hydrolyzed by plasma esterases, and it is likely that anticholinesterase agents, usually employed as antidotes, could prolong rather than reverse the effects of the drug.



Mivacurium Chloride

Pancuronium Bromide

2β,16β-Dipiperidino-5α-androstane-3α,17β-diol diacetate dimethobromide (Pavulon). Although pancuronium bromide is a synthetic product, it is based on the naturally occurring alkaloid malouetine, found in arrow poisons used by primitive Africans. Pancuronium bromide acts on the nicotinic receptor and in the ion channel, inhibiting normal ion fluxes.



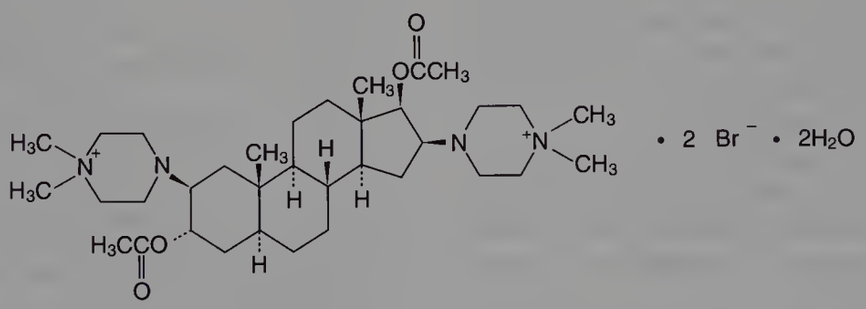
Pancuronium Bromide

This blocking agent is soluble in water and is marketed in concentrations of 1 mg/mL or 2 mg/mL for intravenous administration. It is a typical nondepolarizing blocker, with a potency approximately five times that of (+)-tubocurarine chloride and a duration of action approximately equal to the latter. Studies indicate that it has little or no histamine-releasing potential or ganglion-blocking activity and that it has little effect on the circulatory system, except for causing a slight rise in the pulse rate. As one might expect, it is competitively antagonized by ACh, anticholinesterases, and potassium ion, whereas its action is increased by inhalation anesthetics such as ether, halothane, enflurane, and methoxyflurane. The latter enhancement in activity is especially important to the anesthetist because the drug is frequently administered as an adjunct to the anesthetic procedure to relax the skeletal muscle. Perhaps the most frequent adverse reaction to this agent is the occasional prolongation of the neuromuscular block beyond the usual time course, a situation that can usually be controlled with neostigmine or by manual or mechanical ventilation, since respiratory difficulty is a prominent manifestation of the prolonged blocking action.

As indicated, the principal use of pancuronium bromide is as an adjunct to anesthesia, to induce relaxation of skeletal muscle, but it is also employed to facilitate the management of patients undergoing mechanical ventilation. It should be administered only by experienced clinicians equipped with facilities for applying artificial respiration, and the dosage should be adjusted and controlled carefully.

Pipecuronium Bromide

4,4′-(30(−17β-Dihydroxy-5α-androstan-2β, 16β-ylene)bis [1,1-dimethylpiperazinium]dibromide, diacetate (Arduan). Pipecuronium bromide is a nondepolarizing muscle relaxant similar, both chemically and clinically, to pancuronium bromide. It is a long-acting drug indicated as an adjunct to anesthesia and in patients undergoing mechanical ventilation.

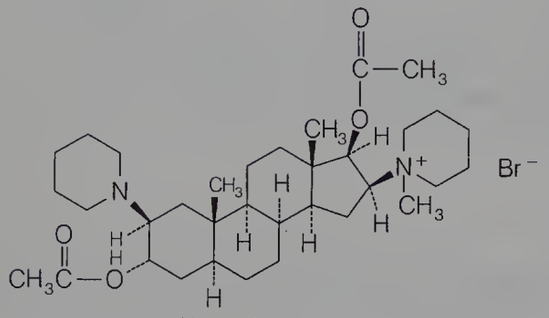


Pipecuronium Bromide

Vecuronium Bromide

1-(3α, 17β-Dihydroxy-2β-piperidino-5α-androstan-16β-yl)-1-methylpiperidinium bromide, diacetate (Norcuron). Vecuronium bromide is the monoquaternary analogue of pancuronium bromide. It belongs to the class of nondepolarizing neuromuscular blocking agents, producing similar effects to drugs in this class. It is unstable in the presence of acids and undergoes gradual hydrolysis of its ester functions

in aqueous solution. Aqueous solutions have a *p*H of about 4.0. This drug is used mainly to produce skeletal muscle relaxation during surgery and to assist in controlled respiration after general anesthesia has been induced.



Vecuronium Bromide

Succinylcholine Chloride, USP

Choline chloride succinate (2:1) (Anectine, Sucostrin). Succinylcholine chloride is a white odorless crystalline substance that is freely soluble in water to give solutions with a *p*H of about 4. It is stable in acidic solutions but unstable in alkali. Aqueous solutions should be refrigerated to ensure stability.

Succinylcholine chloride is characterized by a very short duration of action and a quick recovery because of its rapid hydrolysis after injection. It brings about the typical muscular paralysis caused by a blocking of nervous transmission at the myoneural junction. Large doses may cause a temporary respiratory depression, in common with other similar agents. Its action, in contrast with that of (+)-tubocurarine, is not antagonized by neostigmine, physostigmine, or edrophonium chloride. These anticholinesterase drugs actually prolong the action of succinylcholine chloride, and on this basis, it is believed that the drug is probably hydrolyzed by cholinesterases. The brief duration of action of this curare-like agent is said to render an antidote unnecessary if the proper supportive measures are available. However, succinylcholine chloride has a disadvantage in that its action cannot be terminated promptly by the usual antidotes.

It is used as a muscle relaxant for the same indications as other curare agents. It may be used for either short or long periods of relaxation, depending on whether one or several injections are given. In addition, it is suitable for continuous intravenous drip administration.

Succinylcholine chloride should not be used with thiopental sodium because of the high alkalinity of the latter, or if used together, they should be administered immediately after mixing. However, separate injection is preferable.

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CHAPTER **18**

Diuretics

Daniel A. Koechel

A *diuretic* is defined as a chemical that increases the rate of urine formation. The *primary action* of most diuretics is the direct inhibition of sodium transport at one or more of the four major anatomic sites along the nephron where sodium reabsorption takes place. Because the sodium-transport systems at each of these locations are unique, there is a different set of relatively rigid structural features that a diuretic must possess in order to inhibit sodium reabsorption at each site. Of additional importance are the *secondary (or indirect) events* that are triggered as a result of the diuretic's primary action. The nature and magnitude of many of the observed secondary effects are dependent on the locus of action of the diuretic and the response of nephron sites ''downstream'' to an enhanced delivery of fluid, sodium, or other solutes. The secondary events are quite characteristic for each class of diuretics and are often highly predictable if the reader has an understanding of normal renal physiologic processes. Collectively, the primary and secondary effects induced by a diuretic determine its electrolyte excretion pattern. A diuretic usually possesses some combination of *natriuretic, chloruretic, saluretic, kaliuretic, bicarbonaturetic, and calciuretic properties* depending on whether it enhances the renal excretion of sodium, chloride, sodium chloride, potassium, bicarbonate, or calcium, respectively.

In this chapter, the normal function of the nephron is presented, including the four major reabsorptive sites for sodium and other important solutes and the renal physiologic events that occur when sodium and water reabsorption are altered by the patient's state of hydration, disease, or intake of diuretics. This is followed by a discussion of each class of diuretics in current use. A knowledge of the important structural features and the site(s) of action of each class of diuretics should enable the reader to better understand the factors that dictate the nature and magnitude of the anticipated diuresis and the associated secondary effects.

ANATOMY AND PHYSIOLOGY OF THE NEPHRON

The functional unit of the kidney is the nephron with its accompanying glomerulus (Fig. 18-1). There are approximately one million nephrons in each kidney. The blood (or, more appropriately, the plasma), from which all urine is formed, is brought to each nephron within the glomerular capillary network (Fig. 18-2). Most of the plasma components are filtered into Bowman's space. During the process of urine formation, the resulting glomerular filtrate flows through the proximal tubule (convoluted and straight portions), the descending limb of Henle's loop, the thin and thick ascending limbs of Henle's loop, the area of the macula densa cells, the distal convoluted tubule, the connecting tubule (sometimes referred to as the late or terminal distal tubule), and the cortical and medullary collecting tubules. Each of these nephron segments consists of ultrastructurally and functionally unique cell types. The physiologic role of the glomerulus and each nephron segment is discussed as it relates to the handling of important solutes and water in normally hydrated (*normovolemic*) and dehydrated (*hypovolemic*) persons and in patients afflicted with various edematous disorders (e.g., congestive heart failure, cirrhosis of the liver with ascites, and the nephrotic syndrome).

FUNCTION

FUNCTION OF THE NEPHRON WHEN THE PLASMA VOLUME IS NORMAL (NORMOVOLEMIA OR EUVOLEMIA)

As blood is delivered to each glomerulus, many (but not all) of its components are filtered into Bowman's space through the pores in the glomerular capillary loops. Several physicochemical properties of each blood component dictate the

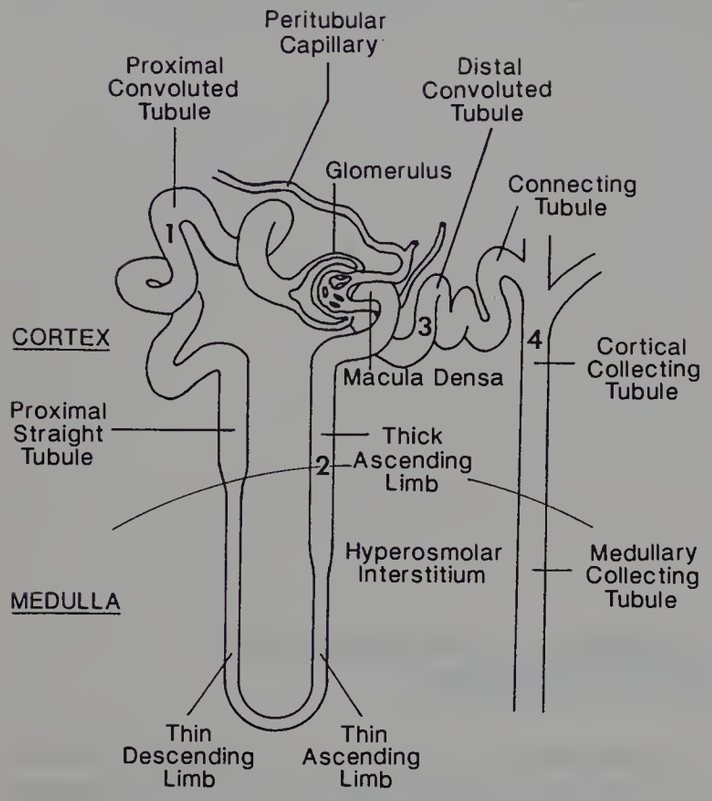


FIG. 18-1. *The anatomy of the nephron, with emphasis on the four major sites of sodium reabsorption.*

extent to which it is removed from the blood by glomerular filtration. These include the component's (1) relative molecular mass (M_r), (2) overall charge (applies primarily to large molecules), and (3) degree and nature of binding to plasma proteins. For example, plasma proteins with an M_r in excess of 50,000 Da and red blood cells are not readily filtered,

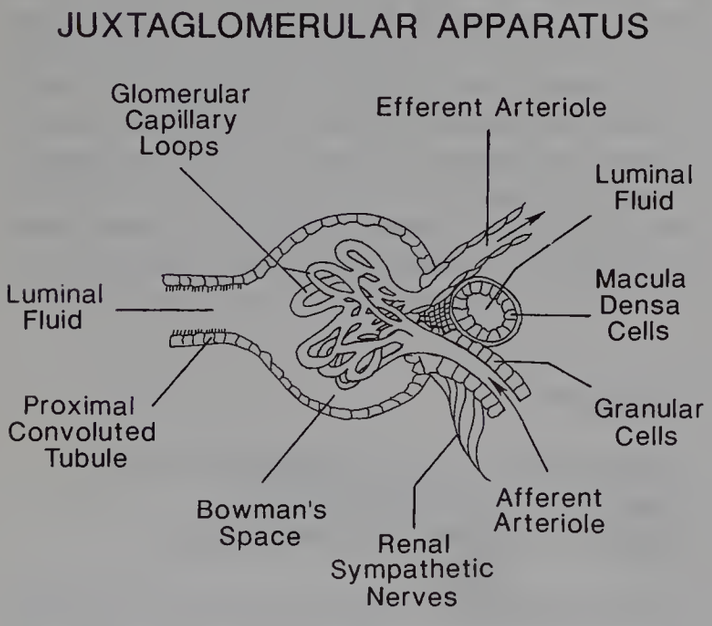


FIG. 18-2. *The juxtaglomerular apparatus (JGA). Urine is formed from the filtration of plasma through the glomerular capillary loops into Bowman's space. The JGA is of paramount importance for the operation of the tubuloglomerular feedback mechanism, which allows a nephron to regulate the glomerular filtration rate of its own glomerulus.*

whereas low-M_r, non-protein-bound components (e.g., sodium, potassium, chloride, bicarbonate, glucose, and amino acids) are readily filtered.[1]

The glomerular filtration *rate* (GFR) of plasma components that possess an M_r of less than 50,000 Da and are not bound to plasma proteins is (1) directly dependent on the hydraulic (hydrostatic) pressure in the renal vasculature (created by the pumping heart), which tends to drive water and solutes out of the glomerular capillaries into Bowman's space; (2) related inversely to the plasma oncotic pressure (the osmotic pressure created by the plasma proteins within the vasculature), which tends to hold or prevent the filtration of water and solutes across the glomerular capillaries into Bowman's space;[1] and (3) governed by the intrarenal signals that allow each nephron to adjust the filtration rate through its own glomerular capillary network (i.e., tubuloglomerular feedback).[2] Clearly, the cardiovascular and renal functional status of an individual will also affect the rate of filtration of plasma components through the glomeruli. In addition, neonates and the elderly usually have a reduced GFR, though for different reasons.[3,4]

The *fraction* of the total renal plasma flow that is filtered collectively by the glomeruli per unit time (i.e., the filtration fraction) is about one-fifth.[2] This means that only one-fifth (or 20%) of the plasma presented to the kidneys in a given period undergoes filtration at the glomeruli (i.e., about 650 mL of plasma flow through the kidneys each minute, approximately 125 mL/min of which are filtered through the glomerular capillaries). The remaining four-fifths (or 80%) of the renal plasma flow is directed into the peritubular capillaries (Fig. 18-1). Each minute only 1 mL of urine is formed from the 125 mL of glomerular filtrate.[5] Thus, approximately 99% of the glomerular filtrate is normally reabsorbed.

The *absolute quantity* of each filtrable plasma component that reaches Bowman's space—the filtered load of a substance—is directly dependent on the GFR and the plasma concentration of the filtrable substance. That is, the filtered load of a substance = GFR (mL/min) × $[\text{filtrable substance}]_{\text{plasma}}$ (amount/mL).[5] The glomerular filtrate that houses the filtered load of a given solute will hereafter be referred to below as the *luminal fluid* since it enters the lumen of each nephron immediately after leaving Bowman's space. In the following discussion, attention is focused on the percentage of the filtered load of sodium and other key solutes that are reabsorbed (i.e., transported from the luminal fluid into renal tubule cells with subsequent passage into the interstitium) at various nephron sites.

There are four major anatomic sites along the nephron that are responsible for the bulk of sodium reabsorption[6] (Fig. 18-1): *site 1,* the convoluted and straight portions of the proximal tubule; *site 2,* the thick ascending limb of Henle's loop; *site 3,* the distal convoluted tubule; and *site 4,* the connecting tubule (i.e., the terminal portion of the distal convoluted tubule) and the cortical collecting tubule. The actual transport processes involved in sodium reabsorp-

tion at each of these sites are highlighted in Figs. 18-3 through 18-6 and are discussed in order.

Site 1

The convoluted and straight portions of the proximal tubule are responsible for the reabsorption of about (1) 65% to 70% of the filtered loads of sodium, chloride, water, and calcium;[6,7] (2) 80% to 90% of the filtered loads of bicarbonate,[6,8] phosphate,[7] and urate;[9] and (3) essentially 100% of the filtered loads of glucose, amino acids, and low-M_r proteins.[10] Thus, under normal circumstances, the proximal tubule has a tremendous reabsorptive capacity. There are primarily two driving forces for this high degree of reabsorptive activity. First, because the plasma in the peritubular capillaries (Fig. 18-1) has a lower hydraulic pressure and a higher oncotic pressure than the luminal fluid or the plasma delivered to the glomerulus (owing to the removal of water, but not protein, from plasma during glomerular filtration), there is a net movement of the luminal fluid contents in a reabsorptive direction.[1] Second, the Na^+,K^+-ATPase, strategically located on the antiluminal membrane (sometimes referred to as the basolateral, peritubular, or contraluminal membrane) of the proximal tubule cells, catalyzes the countertransport of intracellular sodium ions into the interstitium and extracellular potassium ions into the proximal tubule cells[10] (Fig. 18-3). The stoichiometry for this countertransport is three sodium ions for two potassium ions. This activity creates a deficit of intracellular sodium, a surfeit of intracellular potassium, and a voltage-oriented negative inside proximal tubule cells.[10]

In response to the action of the Na^+,K^+-ATPase, sodium ions in the luminal fluid move down the concentration gradient into proximal tubule cells by a combination of at least three distinct processes, which are labeled A, B, and C in Fig. 18-3. The *first* mechanism of sodium reabsorption at site 1 involves carbonic anhydrase (CA), which is located in the cytoplasm and on the brush border of proximal tubule cells (Fig. 18-3A). Hydrogen ions generated as the result of the action of intracellular CA are exchanged (i.e., countertransported) for the filtered sodium in the luminal fluid. The sodium that enters proximal tubule cells during the exchange for hydrogen ions is then pumped into the interstitium by the Na^+,K^+-ATPase in the antiluminal membrane. The hydrogen ions that are secreted (i.e., transported uphill or against their gradient) into the luminal fluid react therein with the filtered bicarbonate ions to generate carbonic acid. The carbonic acid decomposes, either spontaneously or with the aid of the brush border-bound CA, to carbon dioxide and water. The carbon dioxide diffuses into the proximal tubule cells and is converted back into bicarbonate, which subsequently passes from the proximal tubule cells, across the antiluminal membrane, into the interstitium. CA is very plentiful in the convoluted portion of the proximal tubule of the human but nonexistent in the straight portion.[8] Thus, the processes just described occur primarily in the convoluted portion of the

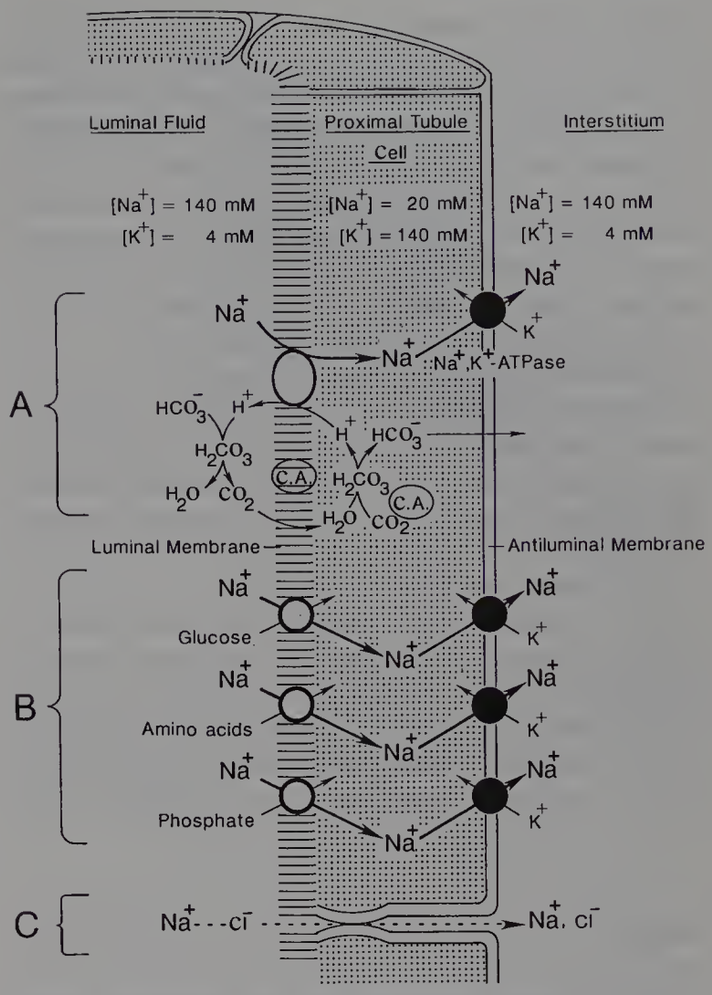


FIG. 18-3. *Site 1: The sodium transport systems that are responsible for the reabsorption of sodium and associated solutes in the proximal tubule.* **(A)** *Transcellular reabsorption of sodium/bicarbonate, which is controlled by carbonic anhydrase (CA). Acetazolamide and other CA inhibitors block sodium reabsorption by this route.* **(B)** *Transcellular reabsorption of sodium coupled to glucose, amino acids, and phosphate.* **(C)** *Paracellular transport of sodium/chloride. There are no commercially available agents that inhibit sodium reabsorption by routes B or C. The Na^+,K^+-ATPase is indicated by filled circles on the antiluminal membrane.*

proximal tubule and account for the reabsorption of about 20% to 25% of the filtered load of sodium (or about one-third of the filtered load of sodium that is reabsorbed at site 1) and about 80% to 90% of the filtered load of bicarbonate.[8,11]

The *second* mechanism by which sodium moves out of the luminal fluid at site 1 involves its cotransport into proximal tubule cells along with glucose, amino acids, or phosphate[10] (Fig. 18-3B). The latter three solutes enter proximal tubule cells against their concentration gradients. The reabsorption of the sodium that enters proximal tubular cells by these processes is completed when it is pumped into the interstitium and adjacent blood vessels by the antiluminal membrane-bound Na^+,K^+-ATPase. The amount of sodium reabsorbed by this type of cotransport is variable and dependent on the filtered loads of the three solutes. However, such cotransport is the mechanism by which 100% of the filtered loads of glucose and amino acids and 80% to 90% of the filtered load of phosphate are removed from the luminal fluid and subsequently reabsorbed.

Third, sodium is reabsorbed at site 1 along with chloride[10] (Fig. 18-3C). As the reabsorption of sodium occurs in the early proximal convoluted tubule accompanied by bicarbonate, glucose, amino acids, and phosphate, the concentration of chloride ions within the luminal fluid tends to rise. As a result, the concentration of chloride ions in the luminal fluid exceeds that in the interstitium, and chloride moves paracellularly (i.e., between the proximal tubular cells) into the interstitium; sodium follows. This process occurs in the proximal tubule beyond the very early portion.

Collectively, these site 1 sodium-transporting processes remove 65% to 70% of the filtered load of sodium from the luminal fluid, and they do so in an isosmotic fashion (i.e., the osmolality of the luminal fluid entering the descending limb of Henle's loop is similar to that of the initial glomerular filtrate).

As the luminal fluid moves through the descending limb of Henle's loop, the high osmolality (or concentration of solutes) in the surrounding medullary interstitium draws approximately 15% of the filtered load of water out of the luminal fluid by osmosis and allows a small amount of sodium from the interstitium to be added to the luminal fluid. In other words, the luminal fluid is concentrated as it flows through the descending limb of Henle's loop.[12]

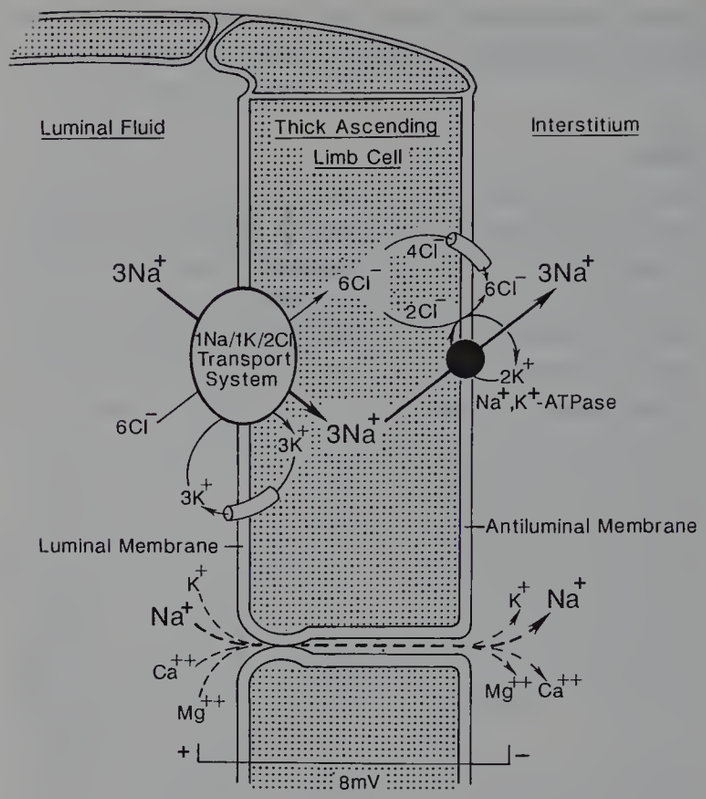


FIG. 18-4. *Site 2: The sodium-transport systems that are responsible for the reabsorption of sodium and associated solutes in the water-impermeable cortical and medullary portions of the thick ascending limb of Henle's loop. The collective actions of the antiluminal membrane-bound Na^+,K^+-ATPase and the luminal membrane-bound $1Na^+/1K^+/2Cl^-$ cotransport system account for* transcellular *reabsorption of sodium chloride, in a ratio of 3 sodium/6 chloride, and the generation of a lumen-positive potential that drives the reabsorption of sodium and other cations via the* paracellular *pathway (dashed line). Diuretic agents that block sodium reabsorption in the thick ascending limb by inhibition of the luminal membrane-bound $1Na^+/1K^+/2Cl^-$ cotransport system include furosemide, bumetanide, torsemide, ethacrynic acid, and a number of miscellaneous agents cited in Fig. 18-13.*

Site 2

When the luminal fluid enters the thick ascending limb of Henle's loop, it comes into contact with tubule cells that are impermeable to water and possess a capacious luminal membrane-bound transport system for sodium (Fig. 18-4). Here, as occurred at site 1, the major driving force for the reabsorption of sodium is the creation of an intracellular deficit of sodium by the antiluminal membrane-bound Na^+,K^+-ATPase. The electroneutral sodium/potassium/chloride cotransport system located on the luminal membrane of thick ascending limb cells then transports sodium, along with potassium and chloride, from the luminal fluid into the cells of the thick ascending limb in a ratio of $1Na^+/1K^+/2Cl^-$.[10] Reabsorption of the sodium that enters thick ascending limb cells by this mechanism is completed when it is pumped actively into the interstitium and surrounding vasculature by the antiluminal membrane-bound Na^+,K^+-ATPase; the chloride ions enter the interstitium through chloride channels in the antiluminal membrane and by cotransport with potassium ions. The luminal potassium that accompanies sodium and chloride into the thick ascending limb cells recycles passively downhill back into the luminal fluid. The potassium that enters the thick ascending limb cells by way of the antiluminal membrane-bound Na^+,K^+-ATPase likewise recycles back into the intersitium via cotransport with chloride. Hence, the net result is the transport of three positively charged sodium ions and six negatively charged chloride ions from the luminal fluid into the interstitium. This results in the generation of a lumen-positive transepithelial voltage. It is this positive luminal environment that drives more cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) from the lumen into the interstitium paracellularly (i.e., between the thick ascending limb cells).[10,13] The combined activities of the Na^+,K^+-ATPase countertransport system on the antiluminal membrane and the $1Na^+/1K^+/2Cl^-$ contransport system on the luminal membrane of the thick ascending limb cells normally account for the reabsorption of 20% to 25% of the filtered load of sodium;[6] the reabsorption of up to 20% to 30% of the filtered load of calcium;[10] the maintenance of the high osmolality of the medullary interstitium, which is absolutely critical for the normal functioning of the human nephron;[12] and the ability of this nephron segment to reabsorb more sodium and other solutes than usual when proximal tubule sodium transport has been inhibited.[6,14] This latter compensatory phenomenon explains why diuretics that act primarily at site 1 are not particularly efficacious.

The descending limb of Henle's loop is responsible for the concentration of luminal fluid (i.e., removal of water and addition of sodium), while the thick ascending limb is

responsible for the dilution of luminal fluid (i.e., removal of solute from the luminal fluid without the concomitant removal of water). Hence, collectively, these two nephron segments produce a massive overall reduction of luminal fluid volume and solute content. Interestingly, the osmolality of the luminal fluid in the terminal portion of the thick ascending limb of Henle's loop is not much different from that which enters the descending portion of the loop (though drastic changes take place in between).

As the luminal fluid leaves the thick ascending limb of Henle's loop, it comes into contact with the *macula densa cells*, a specialized group of tubule cells that communicate with the granular cells of the afferent arteriole belonging to the same nephron[15] (Fig. 18-2). The macula densa cells are like the thick ascending limb cells in that they house the luminal membrane-bound $1Na^+/1K^+/2Cl^-$ cotransport system, but their uniqueness lies in their ability to detect changes in the rate of luminal fluid flow (or some other factor such as solute composition of the luminal fluid) and to generate appropriate signals, which are transmitted by way of the granular cells to the afferent arteriole associated with that nephron.[2] Thus, the activities of the macula densa cells can regulate the filtration rate through the associated glomerulus (commonly referred to as tubuloglomerular feedback). When there is an increase in the luminal fluid flow rate past the macula densa cells in a normovolemic individual, as occurs with certain diuretics, a vasoconstrictive substance is released from the granular cells of the juxtaglomerular apparatus that constricts the afferent arteriole and decreases GFR. Thus, it is not surprising that some of the diuretics discussed herein will reduce the GFR while enhancing the urinary loss of sodium. If the GFR is depressed too much, the filtered loads of sodium and the diuretic will be reduced and the magnitude of the diuresis will be blunted. When there is a decrease in the luminal fluid flow rate past the macula densa cells due to reduced vascular volume and associated reductions in renal arterial pressure and increased sympathetic tone, renin is released from the granular cells. This is followed by the formation of angiotensin II, a potent vasoconstrictor, which constricts the renal vasculature and depresses both renal blood flow and GFR. Fluids and electrolytes are conserved so that vascular volume can be replenished.[2]

Site 3

Following its sojourn past the macula densa cells, the luminal fluid comes into contact with the third major site for the reabsorption of sodium, the relatively short, water-impermeable, distal convoluted tubule (Fig. 18-5). Again, the major driving force for sodium reabsorption from the luminal fluid at site 3 involves the deficit of intracellular sodium produced by the action of the antiluminal membrane-bound Na^+,K^+-ATPase. In this instance, the luminal membrane-bound Na^+/Cl^- cotransport system moves luminal fluid sodium downhill and luminal fluid chloride uphill into distal convoluted tubule cells. The reabsorption of sodium is completed when the antiluminal membrane-bound Na^+,K^+-ATPase actively pumps it into the interstitium and surrounding vasculature; the intracellular chloride ions enter the interstitium through channels in the antiluminal membrane. Approximately 5% to 8% of the filtered load of sodium is reabsorbed at site 3.[6,11]

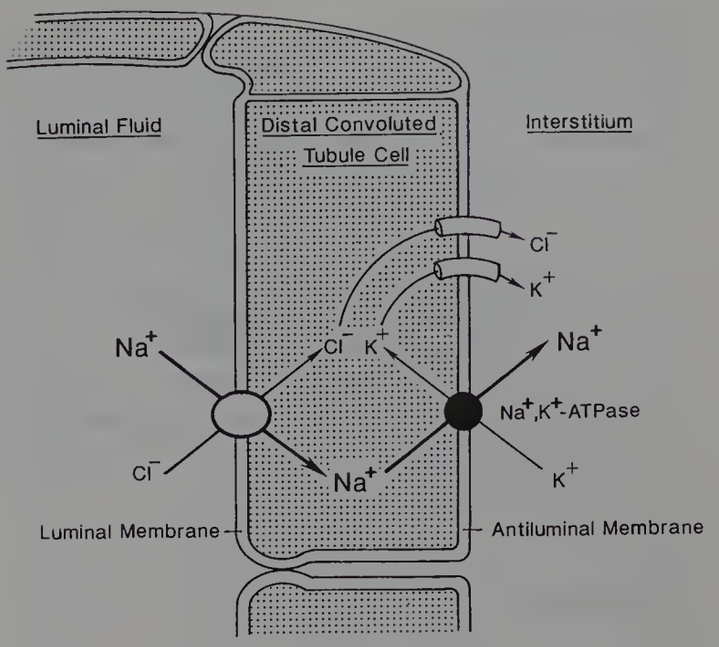


FIG. 18-5. *Site 3: The sodium-transport systems that are responsible for the reabsorption of sodium and chloride in the water-impermeable distal convoluted tubule. Inhibitors of the luminal membrane-bound Na^+/Cl^-cotransport system include the thiazide and thiazide-like diuretics.*

Site 4

The connecting tubule (i.e., late portion of the distal convoluted tubule) and the cortical collecting tubule house the fourth and final major site for the reabsorption of sodium from the luminal fluid[6] (Fig. 18-6). This portion of the nephron is composed of two distinct cell types, the principal cells and the intercalated cells. The principal cells are most important for sodium reabsorption and potassium secretion, whereas the intercalated cells are most important for the generation and secretion of hydrogen ions. The intercalated cells do not possess Na^+,K^+-ATPase on their antiluminal membranes, but they do contain intracellular CA, which catalyzes the formation of carbonic acid from CO_2 and water. The carbonic acid ionizes to hydrogen ions and bicarbonate ions. The hydrogen ions can be pumped actively into the luminal fluid by the luminal membrane-bound H^+-ATPase. The driving force for the reabsorption of sodium in the principal cells is once again the deficit of intracellular sodium created by the Na^+,K^+-ATPase on the antiluminal membrane, which pumps three sodium ions uphill from the princi-

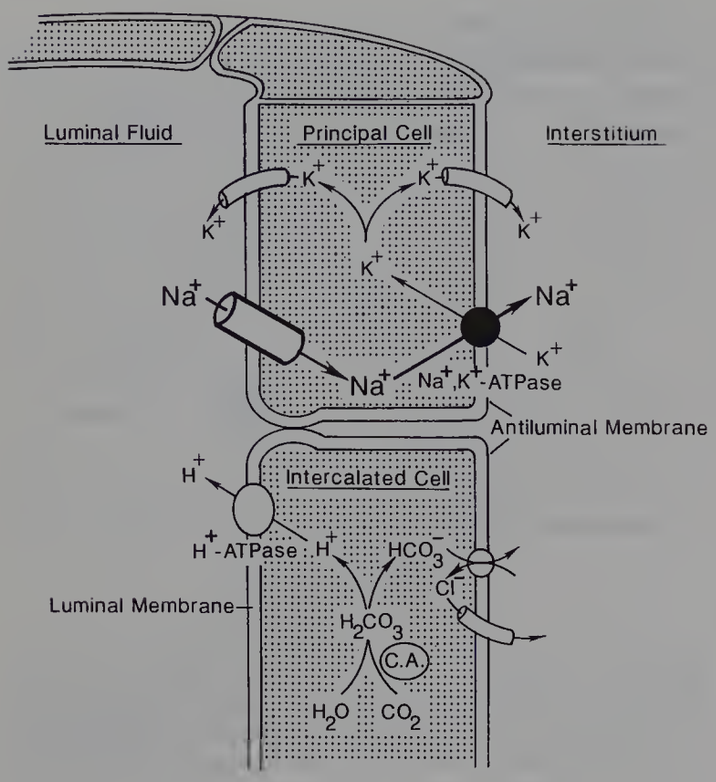


FIG. 18-6. *Site 4: The sodium-transport systems that are responsible for the reabsorption of sodium in the connecting and cortical collecting tubules. Sodium reabsorption and potassium secretion take place in the principal cells, whereas hydrogen ion formation and secretion occur in the intercalated cells. Spironolactone inhibits sodium reabsorption by competitively antagonizing the effects of aldosterone on the principal cells. Triamterene and amiloride "plug" the sodium channels in the luminal membrane of the principal cells, thereby preventing sodium reabsorption and potassium and hydrogen ion secretion. Hence, while producing a modest natriuresis, these drugs prevent potassium loss and are commonly referred to as potassium-sparing diuretics.*

pal cells into the interstitium and two potassium ions uphill from the interstitium into the principal cells. In response to the deficit of sodium in the principal cells, the sodium in the luminal fluid moves downhill into the principal cells through sodium channels in the luminal membrane and subsequently is pumped actively into the interstitium by the antiluminal membrane-bound Na^+,K^+-ATPase. This creates a lumen-negative transepithelial voltage. In response to this voltage difference, some combination of the following three processes occurs: (1) chloride ions move paracellularly from the lumen into the interstitium, (2) potassium in the principal cells moves downhill into the luminal fluid through potassium channels in the luminal membrane, and (3) hydrogen ions generated in the intercalated cells move into the luminal fluid by way of the H^+-ATPase.[10] Because the latter two processes dominate, one may view the activities at site 4 as an exchange of luminal fluid sodium ions for principal cell potassium ions and intercalated cell hydrogen ions. The exchange of luminal fluid sodium for intracellular hydrogen or potassium ions normally is associated with the reabsorption of only 2% to 3% of the filtered load of sodium,[6] and the distal location of this exchange system dictates the final acidity and potassium content of the urine.

The amount of sodium reabsorbed at site 4, and therefore the amount of hydrogen ions and potassium ions present in the final urine, is modulated by the (1) plasma and renal levels of mineralocorticoids like aldosterone - the higher the levels of circulating aldosterone, the greater the degree of sodium reabsorption and potassium and hydrogen ion excretion—(2) luminal fluid flow rate and the percentage of the filtered load of sodium presented to the exchange sites—the greater the flow rate and the load of sodium, the greater the amount of exchange—and (3) acid-base status of the individual—acidosis favors exchange of sodium and hydrogen ions, whereas alkalosis favors exchange of sodium and potassium ions.[6,16] The classes of diuretics that inhibit the reabsorption of sodium at sites 1, 2, or 3 (i.e., sites proximal to site 4) ultimately increase the luminal fluid flow rate and the percentage of the filtered load of sodium delivered to site 4. Thus, many diuretics acutely enhance the urinary loss of potassium ions and may be associated with the induction of hypokalemia.

FUNCTION OF THE NEPHRON DURING REDUCED PLASMA VOLUME (HYPOVOLEMIA)

An individual's plasma volume may be reduced by hemorrhage, diarrhea, vomiting, excessive sweating, or the overzealous use of diuretic agents. When this occurs, the renal processes previously discussed shift into a "conserve" mode to prevent further loss of vital body fluids and solutes.[2,17] The reduction in arterial pressure that accompanies a reduction in plasma volume triggers several crucial events. First, there is an increase in sympathetic tone that stimulates the release of catecholamines, which in turn stimulate the intrarenal release of renin, an enzyme that catalyzes the formation of angiotensin I. Angiotensin I is then converted to angiotensin II, a potent renovasoconstrictor. The catecholamines and angiotensin II work in concert to constrict the renal vasculature and to reduce renal blood flow and the GFR. Angiotensin II also stimulates the production of aldosterone, which ultimately enhances reabsorption of sodium at site 4 and, together with other factors, reabsorption of all substances normally reabsorbed in the proximal tubule.[14,17] Second, antidiuretic hormone (ADH) is released into the bloodstream from the posterior pituitary gland in response to the reduced arterial blood pressure and elevated plasma osmolality. ADH conserves water by increasing the permeability of the cortical and medullary collecting ducts to water. In the presence of ADH, the high osmolality of the medullary interstitium (created in part by the collective actions of the luminal membrane-bound $1Na^+/1K^+/2Cl^-$ transport system and the antiluminal membrane-bound Na^+,K^+-ATPase of the thick ascending limb cells) draws the water out of the lumens of the collecting tubules by osmosis. Water is conserved and vascular volume is restored.

FUNCTION OF THE NEPHRON DURING DISEASE STATES ASSOCIATED WITH RETENTION OF BODY FLUIDS (EDEMATOUS STATES)

Frequently, the kidneys of individuals with congestive heart failure, cirrhosis of the liver with ascites, or the nephrotic syndrome receive messages that are interpreted to mean that they are being hypoperfused. This may occur whether or not there is an actual plasma volume reduction. The kidneys attempt to retain body fluids and solutes by any one or a combination of the processes discussed above. Ultimately, edema ensues.[18]

INTRODUCTION TO THE DIURETICS

Several issues must be addressed before embarking on a discussion of the various classes of diuretics. It is important to understand (1) the difference between potency and efficacy as they relate to diuretics and (2) the major determinants of diuretic efficacy. In addition, it is important to appreciate the shortcomings of many of the previous structure–activity relationship (SAR) studies that have involved diuretics.

There is a clear distinction to be made between the terms "potency" and "efficacy."[19] The *potency* of a diuretic is related to the absolute amount of drug (e.g., milligrams, milligrams per kilogram) required to produce an effect. The *relative potency* is a convenient means of comparing two diuretics and is expressed as a ratio of equieffective doses. The potency of a diuretic is influenced by its absorption, distribution, biotransformation, excretion, and inherent ability to combine with its receptor (i.e., its intrinsic activity). The potency of a diuretic is important for establishing its dosage but is otherwise a relatively unimportant characteristic. *Efficacy* relates to the maximal diuretic effect attainable (usually measured in terms of urine volume/time or urinary loss of sodium or sodium chloride/time).

There are a number of factors that contribute to the efficacy of a diuretic. First, the anatomic site of action and the capacity of the sodium-reabsorbing sites downstream play a major role in determining overall efficacy. That is, a diuretic's efficacy is determined primarily by whether it acts at site 1, 2, 3, or 4. Diuretics that inhibit the reabsorption of sodium at the same anatomic site are usually equiefficacious (i.e., evoke similar maximal responses) but may vary in potency (i.e., the amount of diuretic necessary to produce similar effects). Diuretics that act at site 1 may inhibit the reabsorption of 20% to 25% of the filtered load of sodium but are not as efficacious as one might think because the three major sites of sodium reabsorption downstream (i.e., sites 2, 3, and 4) compensate by reabsorbing most of the extra sodium presented to them. Diuretics that inhibit the reabsorption of sodium at site 2 are the most efficacious of all because site 2 normally is responsible for the reabsorption of 20% to 25% of the filtered load of sodium and the two sodium-reabsorptive sites downstream (i.e., sites 3 and 4) are relatively low-capacity sites. Diuretics that act at site 2 frequently are referred to as "high-ceiling" or "loop" diuretics. Diuretics that act at site 3 or site 4 are less efficacious because these two sites are responsible for the reabsorption of only 5% to 8% and 2% to 3% of the filtered load of sodium, respectively.

Second, the efficacy of a diuretic is dependent on its concentration at the site where it inhibits sodium transport. In all but a few cases, diuretics interfere with the processes responsible for the reabsorption of sodium that are located on the luminal membrane, and hence, their intraluminal concentration is of critical importance. The concentration of a diuretic agent that ultimately is presented to a luminal site is determined by how well it is filtered at the glomerulus, whether it undergoes active tubular secretion in proximal tubules, and whether it undergoes non-ionic back diffusion in the distal nephron segments. All diuretics enter luminal fluid by the process of glomerular filtration but to varying degrees. The amount that enters the luminal fluid by the filtration process is dependent on the GFR, the plasma concentration of the diuretic agent, and the extent that the diuretic is bound to the predominant unfiltrable plasma protein, albumin. In addition, all but a few of the diuretics attain relatively high concentrations in the luminal fluid of the proximal tubule by a two-step process commonly referred to as *active tubular secretion*[20] (Fig. 18-7). The antiluminal membrane of the proximal tubule houses a set of bidirectional active-transport systems that participate in the first step of active tubular secretion of a diuretic. The organic anion transport system (OATS) transports endogenous and exogenous organic anions, whereas the organic cation transport system (OCTS) handles endogenous and exogenous organic cations. Because most diuretics are weak organic acids (e.g., carboxylic acids or sulfonamides) or weak organic bases (e.g., amines), they exist as organic anions and cations, respectively, and are likely to be handled by the OATS or the OCTS. Although the OATS and OCTS are bidirectional, they transport diuretics primarily in a secretory direction (i.e., from the interstitium into proximal tubule cells). Even diuretics that are bound extensively to plasma proteins may be secreted avidly. Importantly, neither the OATS nor the OCTS possesses rigid structural requirements for the respective organic anion or cation being transported. The second step of active tubular secretion of a diuretic involves its passage from proximal tubule cells into the luminal fluid, probably by a combination of passive diffusion and active transport.

In addition to the filtration and secretion processes, the concentration of a diuretic in the luminal fluid of the more distal segments of the tubule is determined by the agent's lipid/water partition coefficient and pK_a, as well as the *p*H of the distal luminal fluid. These factors will modulate the concentration of diuretic at sites 3 and 4. Weakly acidic diuretics, the undissociated forms of which possess a favorable balance of lipid and water solubility, may undergo *p*H-

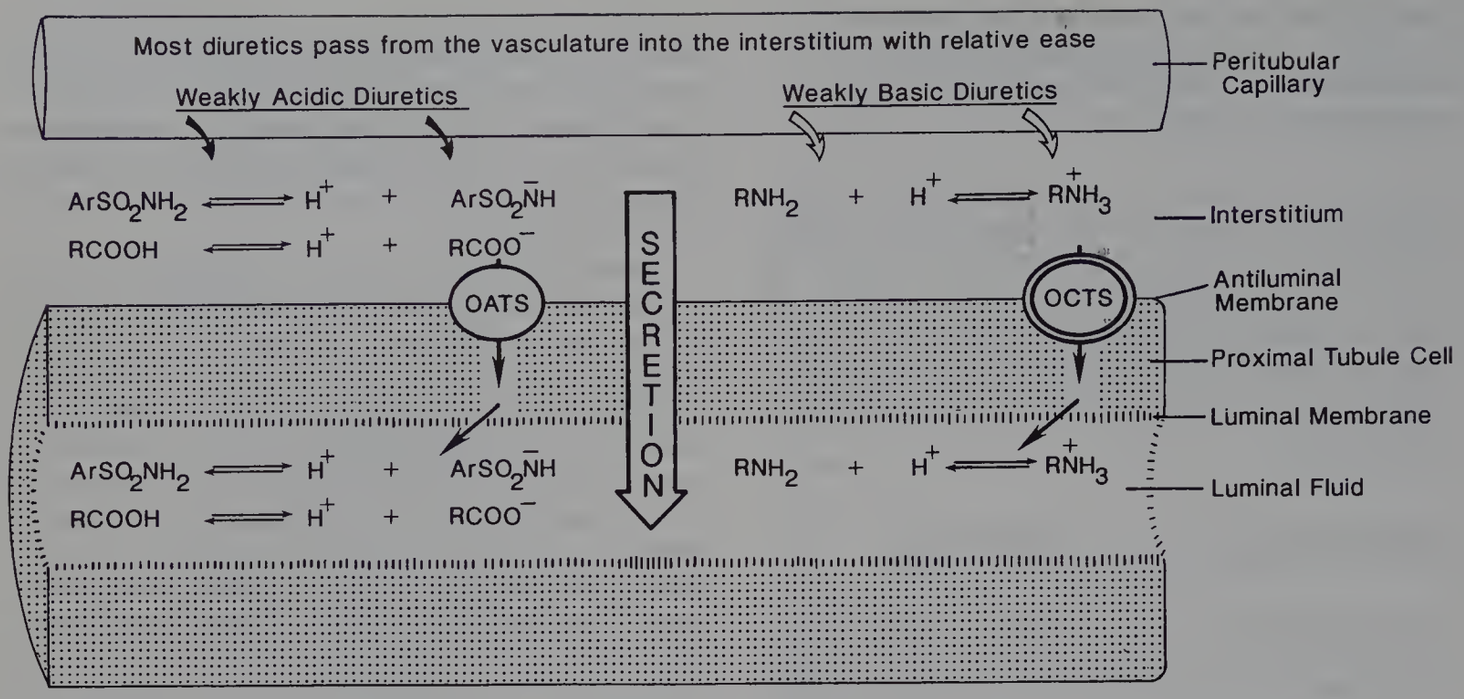


FIG. 18-7. *Active tubular secretion of a drug is a two-step process that is localized only in the proximal tubule area of the nephron. The first step involves the active transport of a drug that can exist as an organic anion or an organic cation from the interstitium into proximal tubule cells by way of the organic anion transport system (OATS) or the organic cation transport system (OCTS), respectively. These systems are located on the antiluminal membrane of proximal tubule cells. The second step involves some combination of passive diffusion of the uncharged drug species or active transport of the charged drug species from the proximal tubule cells into the luminal fluid. Active tubular secretion contributes in a major way to the high luminal fluid levels of most diuretics.*

dependent diffusion (referred to as *non-ionic back diffusion*) from the distal tubular luminal fluid back into the bloodstream. This frequently decreases the luminal fluid concentration and the renal excretion rate of the diuretic but prolongs its plasma half-life. Diuretics that are weak bases will follow a similar course if the urinary *p*H is on the alkaline side, which favors the presence of the uncharged drug species. Weak organic acids or bases, the uncharged forms of which possess an unfavorable lipid/water partition coefficient, will not undergo non-ionic back diffusion. These diuretics will be retained within the luminal fluid and, ultimately, excreted. Thus, diuretic agents may reach high concentrations in luminal fluid following glomerular filtration, active tubular secretion, and little or no subsequent nonionic back diffusion. Diuretics that act at sites 2 and 3 as well as some that act at site 4 inhibit sodium-transport processes on the luminal membrane and must attain relatively high luminal fluid concentrations. The CA-inhibiting diuretics that act at site 1 must attain adequate concentrations within luminal fluid as well as intracellularly, and the aldosterone antagonist spironolactone must attain adequate intracellular concentrations at site 4.

Other factors that determine the efficacy of a diuretic include extracellular fluid volume and renal functional status. Any underlying condition or drug that reduces the GFR will decrease not only the filtered load of the diuretic, and hence its intraluminal concentration, but also the filtered load of sodium. In such situations, a diuretic is less able to produce a maximal diuresis and usually will be less efficacious.

Many of the past SAR studies involving diuretics have been conducted in whole animals, and the results may be misinterpreted unless caution is exercised. Generally speaking, compounds of varied chemical structure are administered to animals and ranked according to their ability to produce changes in urine or sodium output over a prescribed period. Conclusions are then drawn about which functional groups are the most important for optimal diuretic activity. It is extremely important for the novice to remember that the results from such studies are not necessarily to be interpreted as a ranking of the intrinsic activity of the agents under study. Diuretic SAR studies conducted in whole animals yield results that are a composite of differences in the absorption, plasma protein binding, distribution, biotransformation, excretion, active tubular secretion, intrinsic activity, and secondary effects (e.g., changes in the GFR) of the various agents. Unfortunately, most, if not all, of these variables are neglected during initial diuretic screening procedures; therefore, it may be assumed erroneously that differences in diuretic activity are due to differences in intrinsic activity. If one is interested in the intrinsic activity of the members of a group of diuretics, a closer approximation can be achieved by examining the agents at isolated nephron segments upon which related or prototypic diuretics are known to act. Several such studies have been conducted.[21,22] It should not be a surprise when results from in vivo and in vitro SAR studies differ. This occurs because of the interplay between numerous parameters in the in vivo studies (i.e., absorption, distribution, and such) that can be eliminated in

a properly designed in vitro study. Almost all structure–activity data cited in the upcoming portion of this chapter were obtained from whole-animal and human investigations.

SITE 1 DIURETICS: CARBONIC ANHYDRASE INHIBITORS

Although the available CA inhibitors are employed only infrequently as diuretics, they played an important role in the development of other major classes of diuretics that are currently in widespread use and aided in our understanding of basic renal physiology.

Shortly after its introduction for the treatment of bacterial infections, sulfanilamide (Fig. 18-8) was observed to produce a mild diuresis characterized by the presence of urinary sodium and a substantial amount of bicarbonate.[23] It was subsequently shown that it induced this effect through inhibition of renal CA.[24,25] However, it was a relatively weak inhibitor of renal CA, and the dose needed to exert an adequate diuresis was associated with severe adverse effects. To improve upon the CA-inhibitory property of sulfanilamide,

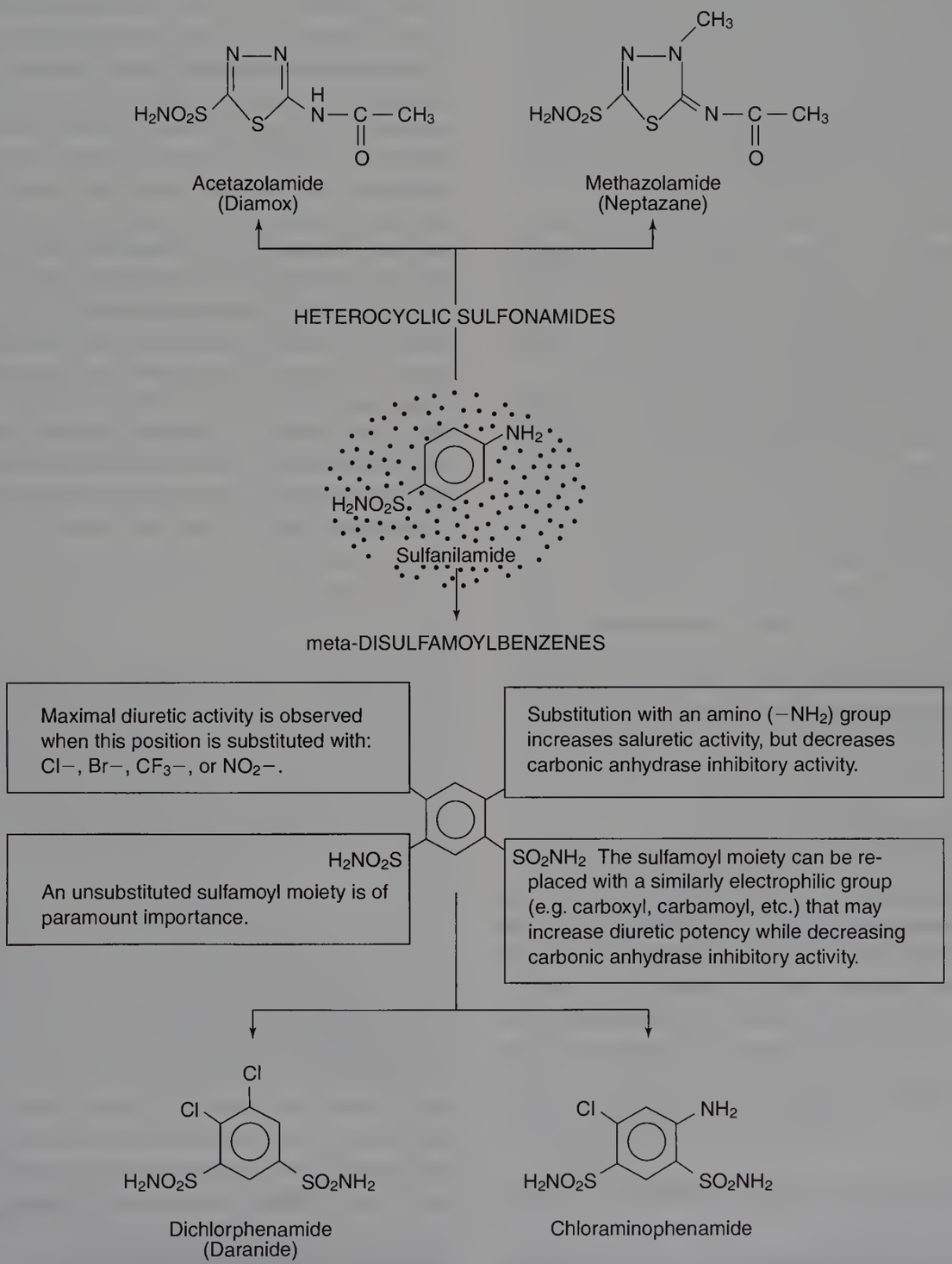


FIG. 18-8. *Development of two classes of carbonic anhydrase inhibitors based on the actions of sulfanilamide.*

many sulfamoyl-containing compounds ($-SO_2NH_2$) were synthesized and screened for their diuretic activity in vivo and their ability to inhibit CA in vitro. Two groups of CA inhibitors emerged: simple heterocyclic sulfonamides and metadisulfamoylbenzene derivatives (Fig. 18-8).

STRUCTURE–ACTIVITY RELATIONSHIPS

SAR studies involving the simple heterocyclic sulfonamides yielded the prototypic CA inhibitor acetazolamide[26–30] (Fig. 18-8). The sulfamoyl group is essential for the in vitro CA-inhibitory activity and for the production of diuresis in vivo. The sulfamoyl nitrogen atom must remain unsubstituted to retain both in vivo and in vitro activities. This feature explains why all of the antibacterial sulfonamides, except sulfanilamide, are incapable of inhibiting CA or exerting diuresis. In contrast, substitution of a methyl group on one of acetazolamide's ring nitrogens yields methazolamide (Fig. 18-8), a product that retains CA-inhibitory activity. The moiety to which the sulfamoyl group is attached must possess aromatic character. In addition, within a given series of heterocyclic sulfonamides, the derivatives with the highest lipid/water partition coefficients and the lowest pK_a values have the greatest CA-inhibitory and diuretic activities.

SAR studies involving the metadisulfamoylbenzenes revealed that the parent 1,3-disulfamoylbenzene lacked diuretic activity, but key substitutions (summarized in Fig. 18-8) led to compounds with diuretic activity.[31] The first commercially available analog, dichlorphenamide (Fig. 18-8), is similar to acetazolamide in its CA-inhibitory activity, but it is also a chloruretic agent. Subsequently, chloraminophenamide (Fig. 18-8) was shown to possess less CA-inhibitory activity but greater chloruretic activity when given by the intravenous route. Poor diuretic activity following the oral administration of chloraminophenamide precluded its marketing.

PHARMACOKINETICS

The clinically available CA inhibitors are absorbed well from the gastrointestinal tract, are distributed to the sites of major importance for CA inhibition, undergo little if any biotransformation, and are excreted primarily by the kidneys. All CA inhibitors attain relatively high concentrations in renal luminal fluid (by a combination of glomerular filtration and active tubular secretion) and in proximal tubule cells.

SITE AND MECHANISM OF ACTION

CA is located both intracellularly and in the luminal brush border membrane of proximal convoluted tubule cells (Fig. 18-3A). Both of these site 1 locations are major targets of the CA inhibitors.[8] This group of diuretics also inhibits intracellular CA in the intercalated cells of the connecting and cortical collecting tubules (i.e., site 4; Fig. 18-6).

During the first 4 to 7 days of continuous therapy with a CA inhibitor, several noteworthy events occur that lead to an increase in sodium and bicarbonate excretion: (1) inhibition of intracellular CA in proximal tubule cells results in a decrease in the available hydrogen ions that normally are exchanged for luminal fluid sodium ions, thus, decreasing proximal tubule reabsorption of sodium (Fig. 18-3), and (2) inhibition of CA on the luminal brush border membrane of proximal tubule cells causes a decrease in the production of carbon dioxide within the luminal fluid and a decrease in the proximal tubule uptake of carbon dioxide. The net result is a decrease in the reabsorption of bicarbonate. It might be assumed that a massive diuresis would follow inhibition of the portion of proximal tubule sodium reabsorption under the control of CA (i.e., one-third of the 65% to 70% of the filtered load of sodium normally reabsorbed from the proximal luminal fluid or about 22% of the filtered load of sodium). However, sodium reabsorption sites downstream (especially site 2) can compensate for such an action by reabsorbing much of the additional sodium presented to them.[6,11,13] Some of the luminal fluid bicarbonate is reabsorbed downstream by a non-CA-mediated system.[32] Thus, the actions of the CA inhibitors ultimately result in the urinary loss of only 2% to 5% of the filtered load of sodium and up to 30% of the filtered load of bicarbonate.

Secondarily, the CA inhibitors enhance the urinary excretion of a substantial amount of potassium.[11,33] The urinary loss of potassium increases because the proximal tubule actions of CA inhibitors present a greater percentage of the filtered load of sodium to site 4, increase the flow rate of luminal fluid through the distal convoluted tubule and collecting tubule, and decrease the availability of intracellular hydrogen ions at site 4. All three changes favor an enhanced exchange of luminal fluid sodium for intracellular potassium at site 4. The urinary concentration of chloride actually decreases after administration of CA inhibitors.[32] Hence, CA inhibitors are natriuretic, bicarbonaturetic, and kaliuretic agents.

Toward the end of the first week of continuous therapy with a CA inhibitor, resistance to its diuretic effect develops.[34] This is due primarily to two factors. First, there is a marked reduction in the filtered load of bicarbonate because the CA inhibitors produce both a reduction in the plasma concentration of bicarbonate and a 20% reduction in the GFR. When there is less bicarbonate present in the luminal fluid, there is less bicarbonate reabsorption to inhibit. Second, the metabolic acidosis created by these diuretics provides a sufficient amount of non-CA-generated intracellular hydrogen ions to exchange for the luminal fluid sodium. Sodium reabsorption at site 1 progressively returns to a near normal rate, and diuresis wanes.

ADVERSE EFFECTS

Several highly predictable adverse effects are associated with the CA inhibitors.

1. Development of metabolic acidosis due to the renal loss of bicarbonate
2. Hypokalemia due to the renal loss of potassium
3. Up to a 20% reduction in the GFR, which appears to be mediated via the juxtaglomerular apparatus because of the increased flow rate of luminal fluid past the macula densa cells (i.e., tubuloglomerular feedback)[33,35]
4. Typical sulfonamide-associated hypersensitivity reactions, such as urticaria, drug fever, blood dyscrasias, and interstitial nephritis

CA inhibitors may also be associated with the production of *paresthesias* (tingling in the extremities), drowsiness, fatigue, anorexia, gastrointestinal disturbances, and urinary calculi. The latter occurs because of a reduction in the urinary excretion rate of citrate, a normal urinary component that assists in maintaining urinary calcium salts in a solubilized form.[32,33]

CA inhibitors can exacerbate the symptoms associated with cirrhosis of the liver.[32,36] Consequently, their use should be avoided in patients with this disorder. CA inhibitor-induced alkalinization of the urine decreases the normal luminal fluid trapping of ammonia (NH_3) in the form of ammonium ions (NH_4^+). This leads to a subsequent reduction in the urinary excretion of ammonium ions. Under these circumstances, the highly diffusible ammonia is diverted from the luminal fluid into the systemic circulation, where it may contribute to the development of hepatic encephalopathy.

USES

The major use of the CA inhibitors is in the treatment of glaucoma.[36] CA is a functionally important enzyme in the eye, where it plays a key role in the formation of aqueous humor. Inhibition of this ocular enzyme reduces the rate of formation of the aqueous humor, thereby reducing the intraocular pressure associated with glaucoma. Interestingly, a reduction in the intraocular pressure usually persists at a time when resistance has developed to the renal effects of the CA inhibitors.[33] CA inhibitors have been used prophylactically to counteract acute mountain sickness,[36] as adjuvants for the treatment of epilepsy, and to create an alkaline urine in an attempt to hasten the renal excretion of certain noxious weak acids or to maintain the urinary solubility of certain poorly water-soluble, endogenous, weak acids (e.g., uric acid).[36]

PRODUCTS

Acetazolamide, USP

N-[5-(Aminosulfonyl)-1,3,4-thiadiazol-2-yl] acetamide (Diamox) (Fig. 18-8). Acetazolamide was introduced in 1953 as the first orally effective, nonmercurial diuretic available to the physician. It has a relatively restricted use today because of its limited efficacy and the refractoriness that develops to its diuretic action within the first week of continuous therapy. However, it remains the most important CA inhibitor available and serves as the prototypic agent in its class. Acetazolamide is absorbed extremely well from the gastrointestinal tract, bound extensively to plasma proteins, and not biotransformed. Peak plasma levels are attained within 2 to 4 hr. Its onset of action is about 1 hr, and its duration of action ranges from 6 to 12 hr. Acetazolamide is removed totally from the plasma by the kidneys within 24 hr. The renal handling of acetazolamide involves its filtration at the glomeruli, active tubular secretion in the proximal tubule, and a varying degree of *p*H-dependent non-ionic back diffusion in the distal segments of the nephron. Acetazolamide is available as 500-mg extended-release capsules, 125- and 250-mg tablets, and a sterile solution for parenteral use containing 500 mg of acetazolamide sodium.

Methazolamide, USP

N-[5-(Aminosulfonyl)-3-methyl-1,3,4-thiadiazol-2(3*H*)-ylidene]acetamide (Neptazane) (Fig. 18-8). Although in vitro studies have shown methazolamide to be a more potent CA inhibitor than the prototypic acetazolamide, it is used as a diuretic (for the same reasons as stated for acetazolamide). It displays improved penetration into the eye,[28] a property that contributes to its usefulness in the treatment of glaucoma. It is available as 25- and 50-mg tablets.

Dichlorphenamide, USP

4,5-Dichloro-1,3-benzenedisulfonamide (Daranide) (Fig. 18-8). Like the other CA inhibitors, dichlorphenamide is seldom used as a diuretic. Little is known about its pharmacokinetics. It reduces intraocular pressure like other CA inhibitors and may be useful in the treatment of glaucoma. The importance of dichlorphenamide and chloraminophenamide is that they ultimately served as stepping stones away from the "pure" CA-inhibiting diuretics and toward the development of the thiazide and thiazide-like diuretics, which are effective natriuretic and chloruretic agents with minimal CA-inhibitory activity.[28,30] Dichlorphenamide is available as 50-mg tablets.

SITE 3 DIURETICS: THIAZIDE AND THIAZIDE-LIKE DIURETICS

Chloraminophenamide became a logical key intermediate in the development of diuretics that lacked the undesirable properties of the CA inhibitors. When chloraminophenamide was treated with acylating reagents, cyclization resulted in

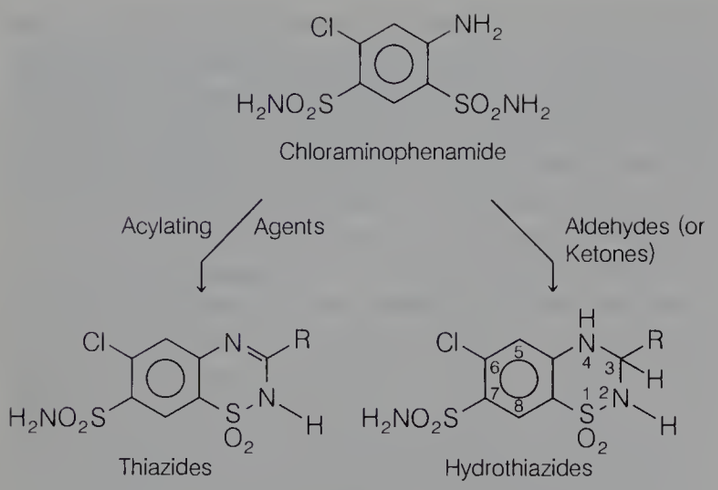


FIG. 18-9. *Development of thiazide and hydrothiazide diuretics from chloraminophenamide.*

the formation of 1,2,4-benzothiadiazine-1,1-dioxides[28] (Fig. 18-9). The use of aldehydes or ketones in place of the acylating reagents yielded the corresponding dihydro derivatives. The products of these reactions became known as thiazides and hydrothiazides, respectively. Hereafter, they are referred to collectively as the *thiazide diuretics*. The thiazides were the first orally effective saluretic agents, the diuretic activity of which was not influenced by the acid-base status of the individual.

STRUCTURE–ACTIVITY RELATIONSHIPS

Exhaustive SAR studies have been conducted with the thiazide diuretics.[28,30] Refer to Figure 18-9 for the numbering of the thiazide ring positions. Briefly, the 2-position can tolerate the presence of relatively small alkyl groups, such as CH_3–. The 3-position is an extremely important site of molecular modification. Substituents in the 3-position play a dominant role in determining the potency and duration of action of the thiazide diuretics. In addition, certain substituents in the 3-position have resulted in compounds that are relatively specific inhibitors of the diuretic action of the thiazides.[37–40] Loss of the carbon–carbon double bond between the 3- and 4-positions of the benzothiadiazine-1, 1-dioxide nucleus increases the potency of this class of diuretics approximately three- to tenfold. Direct substitution of the 4-, 5-, or 8-position with an alkyl group usually results in diminished diuretic activity. Substitution at the 6-position with an ''activating'' group is essential for diuretic activity. The best substituents include Cl–, Br–, CF_3–, and NO_2– groups. The sulfamoyl group in the 7-position is a prerequisite for diuretic activity. Table 18-1 depicts the commercially available diuretics that have evolved from the many alterations performed on the benzothiadiazine-1,1-dioxide nucleus.

When it was discovered that the sulfamoyl group *para* to the activating group in the metadisulfamoylbenzenes could be replaced by several other electronegative groups with retention of diuretic activity (Fig. 18-8), a host of diuretics emerged that have become known as *thiazide-like diuretics*. The diuretics shown in Figure 18-10 represent the most active member(s) of each series. Clearly, these diuretics are not benzothiadiazines, but their sites of action, efficacy, electrolyte excretion patterns, and adverse effects resemble the thiazides. For these reasons, the thiazide and thiazide-like diuretics are discussed as a group.

PHARMACOKINETICS

Most of the thiazide and thiazide-like diuretics are absorbed well after oral administration, except chlorothiazide (only about 10% of which is absorbed).[32] Their onset of action usually occurs within 1 to 2 hr, and their peak diuretic effect is expressed within 3 to 6 hr.[41] However, these diuretics differ drastically in their durations of action.[32,41–43] Most diuretics in this class are bound extensively to plasma proteins (or to red blood cell CA for chlorthalidone and metolazone[32,44]), undergo little if any biotransformation (except mefruside and metolazone[44]), and are excreted primarily by the kidneys.[32,42,44] Relatively high luminal fluid concentrations of these diuretics are attained usually by a combination of glomerular filtration and active tubular secretion by the OATS in the proximal tubule (Fig. 18-7). The luminal fluid concentration of these diuretics is critical for elicitation of diuresis.[45]

The diuretics in this class differ primarily in potency and duration of action. The differences in potency (which are reflected in their dosages[32,46]) are determined mainly by the chemical nature of the moiety attached to the 3-position of the benzothiadiazine nucleus, which modulates the overall lipophilicity of the diuretic.[28,30] The differences in duration of action are dictated primarily by the degree of plasma protein binding (or red blood cell binding) and the lipid/water partition coefficients.[43,47] The latter values along with the *pKa of the drug and the* pH of the luminal fluid determine the extent to which each member of the class undergoes reabsorption in the distal convoluted tubule by non-ionic back diffusion. Many of the diuretics in this class have long half-lives in part because they undergo a significant degree of non-ionic back diffusion. Pertinent pharmacologic data on the thiazide and thiazide-like diuretics are presented in Tables 18-2 and 18-3, respectively.

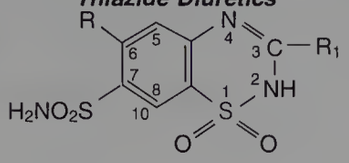
SITE AND MECHANISM OF ACTION

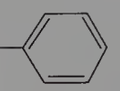
The site of action of the thiazide and thiazide-like diuretics differs slightly from one species to another. However, in humans, it appears safe to conclude that all of these diuretics block the reabsorption of sodium (and thereby the reabsorption of chloride) in the distal convoluted tubules by inhibiting the luminal membrane-bound Na^+/Cl^- cotransport sys-

TABLE 18-1

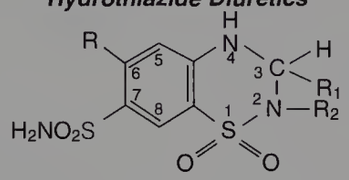
THIAZIDE AND HYDROTHIAZIDE DIURETICS

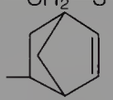
Thiazide Diuretics



Generic Name	*Proprietary Name*	*R*	*R_1*	*R_2*
Chlorothiazide USP	Diuril	—Cl	—H	
Benzthiazide USP	Exna, Hydrex	—Cl	$-CH_2-S-CH_2-$ 	

Hydrothiazide Diuretics



Generic Name	*Proprietary Name*	*R*	*R_1*	*R_2*
Hydrochlorothiazide USP	HydroDIURIL, Esidrix, Oretic	—Cl	—H	—H
Hydroflumethiazide USP	Saluron, Diucardin	$-CF_3$	—H	—H
Bendroflumethiazide USP	Naturetin	$-CF_3$	$-CH_2-$ 	—H
Trichlormethiazide USP	Naqua, Metahydrin	—Cl	$-CHCl_2$	—H
Methyclothiazide USP	Enduron, Aquatensen	—Cl	$-CH_2Cl$	$-CH_3$
Polythiazide USP	Renese	—Cl	$-CH_2-S-CH_2-CF_3$	$-CH_3$
Cyclothiazide USP	Anhydron	—Cl		—H

tem[6,45,48,49] (Fig. 18-5). Thus, all diuretics in this class are responsible for the urinary loss of about 5% to 8% of the filtered load of sodium. Although they differ in their potencies (i.e., the amount of drug needed to produce a given diuretic response), it is important to note that they are equally efficacious (i.e., they are all capable of exerting a similar maximal diuretic response).[28,30,50]

As a result of their action at site 3, the thiazide and thiazide-like diuretics secondarily alter the renal excretion rate of important ions other than sodium. Inhibition of sodium reabsorption at site 3 ultimately results in the delivery of more of the filtered load of sodium, at a faster rate, to site 4. At this latter site there is an enhanced exchange of the luminal fluid sodium for the principal cell potassium, and an increase in the urinary excretion rate of potassium follows. Most of the thiazide and thiazide-like diuretics possess a residual degree of CA-inhibitory activity that can be associated with a slight increase in the renal excretion rate of bicarbonate. Unlike the "pure" CA inhibitors, resistance usually does not develop to the thiazide and thiazide-like diuretics as a result of drug-induced derangements in acid-base balance. Hence, diuretics in this class may be referred to as natriuretic, chloruretic, saluretic, kaliuretic, and extremely weak bicarbonaturetic agents. Importantly, short-term administration of thiazide and thiazide-like diuretics results in little or no change in the excretion of calcium; however, long-term use of these agents leads to a reduction in calcium excretion.[36]

ADVERSE EFFECTS

Some of the adverse effects associated with the thiazide and thiazide-like diuretics are highly predictable because of their chemical makeup or their site of action along the nephron.

1. All of these diuretics possess a sulfamoyl moiety, which has been associated with hypersensitivity reactions such as urticaria, drug fever, blood dyscrasias, and interstitial nephritis. If an individual is hypersensitive to one of the agents in this class, he or she probably will be hypersensitive to all of them. Cross-hypersensitivity also may

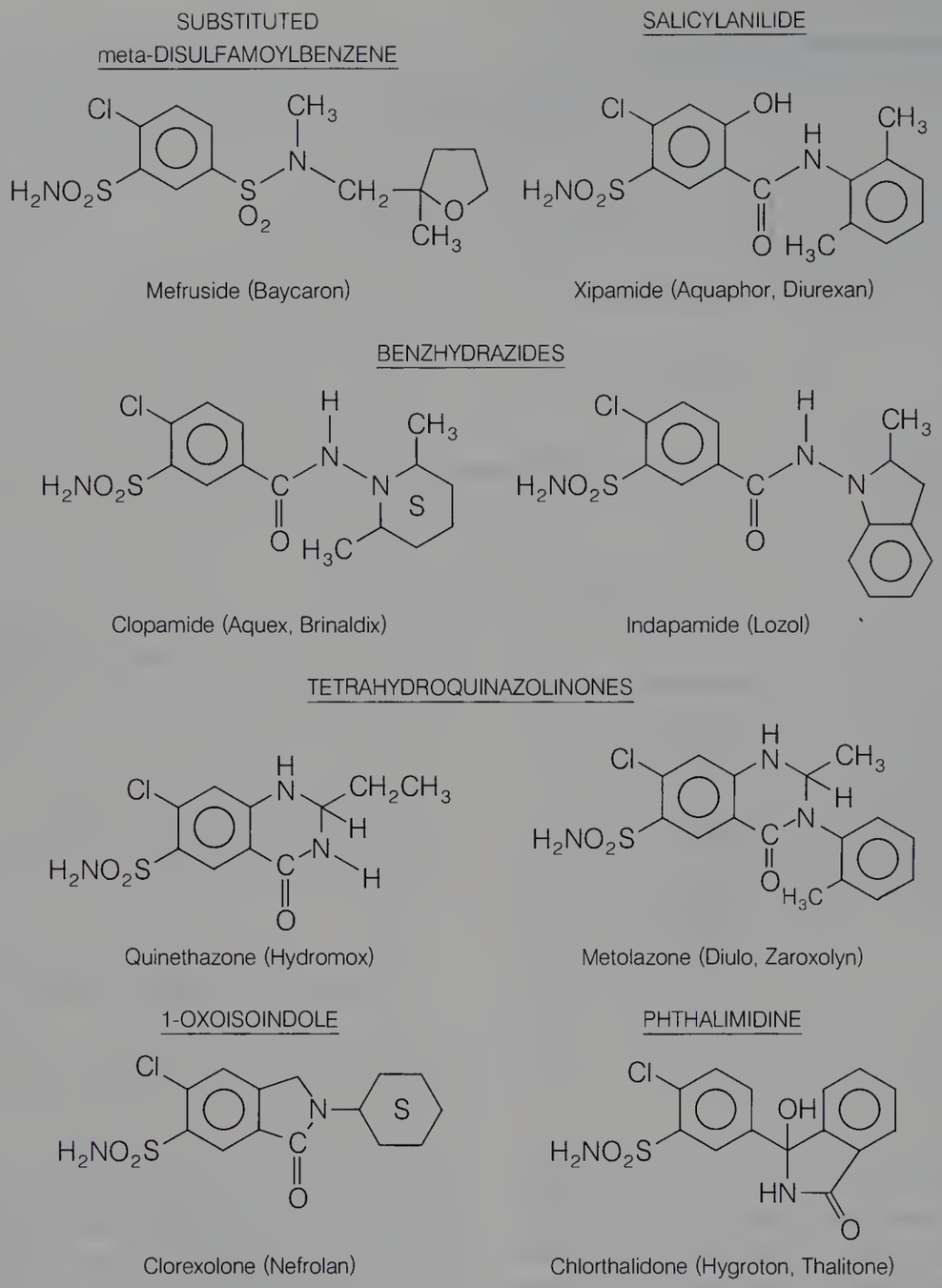


FIG. 18-10. *Representatives from six classes of thiazide-like diuretics. These diuretics were developed as an outgrowth of the thiazide research that involved molecular modification of aromatic sulfamoyl-containing compounds.*

occur between the thiazide and thiazide-like diuretics, CA inhibitors, and the sulfamoyl-containing loop diuretics such as furosemide and bumetanide.

2. Hypokalemia is a product of the diuretic-induced increase in the renal excretion of potassium.
3. Initially, these diuretics produce a slight reduction in cardiac output. Slight reductions in plasma volume and blood pressure occur upon continued use. These latter changes are frequently associated with an increase in the proximal tubule reabsorption of water and solutes, aldosterone secretion, and the renal release of renin. These changes usually assist in mitigating the diuretic effect, but the reduction of blood pressure persists.[51]
4. Occasionally, a patient may experience hypercalcemia or hyperuricemia after chronic use of a thiazide or thiazide-like diuretic. This occurs as a result of diuretic-induced reduction of the patient's plasma volume and a concomitant compensatory increase in the proximal tubule reabsorption of luminal fluid and solutes. In such a situation, more than the usual quantity of calcium and uric acid will be reabsorbed proximally.[36] The seriousness of these two adverse effects is, in part, dependent on the duration and degree of the plasma volume reduction.

The precise mechanisms behind some of the adverse effects of the thiazide and thiazide-like diuretics are not well

TABLE 18-2

IMPORTANT PHARMACOLOGIC PARAMETERS OF THE THIAZIDE DIURETICS

Thiazide or Hydrothiazide (Trade Name)	Partition Coefficient (ether/H_2O)[47]	Plasma Protein Binding (%)[32,44]	Usual Daily Adult Oral Dosage Range (mg)		Diuretic Effect			% Parent Drug Excreted in Urine[32,44]
			Optimal Diuresis[32]	Hypertension[46]	Onset (hours)[41]	Peak (hours)[41]	Duration (hours)[41–43]	
Chlorothiazide USP (*Diuril*)	0.08	88–96	500–2000	125–500	2	4	6–12	92
Benzthiazide USP (*Exna, Hydrex*)			50–200	12.5–50	2	4–6	12–18	
Hydrochlorothiazide USP (*HydroDIURIL, Esidrix, Oretic*)	0.37	64	25–100	12.5–50	2	4	6–12	95 (IV)
Hydroflumethiazide USP (*Diucardin, Saluron*)		95	25–200	12.5–50	1–2	3–4	18–24	65 (oral) 85 (IV)
Trichlormethiazide USP (*Metahydrin, Naqua*)	1.53		1–4	1–4	2	6	up to 24	62–70
Bendroflumethiazide USP (*Naturetin*)		93	2.5–15	2.5–25	1–2	4	6–12	
Methyclothiazide USP (*Aquatensen, Enduron*)			2.5–10	2.5–5	2	6	>24	
Polythiazide USP (*Renese*)		83.5	1–4	1–4	2	6	24–48	25 (oral) 83 (IV)
Cyclothiazide USP (*Anhydron*)			1–2	1–2	2–4	7–12	18–24	

understood. These include an acute reduction in the GFR (especially after intravenous administration[32]) and hyperglycemia. It is unlikely that the reduction in GFR is related to the tubuloglomerular feedback mechanism because the major site of action of these diuretics is distal to the macula densa cells. Some investigators have suggested that the thiazide and thiazide-like diuretics act directly on the renal vasculature to depress the GFR.[32,42] Nonetheless, the acute reduction in GFR involves all diuretics in this class, with the possible exceptions of metolazone and indapamide.[52] This is particularly important to individuals who must commence diuretic therapy but who have preexisting impairment of renal function. Thiazide and thiazide-like drugs are frequently ineffective in individuals who have a GFR of less than 15 to 25 mL/min. Metolazone[42,43,46,53,54] and indapamide[52] may be useful in such circumstances.

TABLE 18-3

IMPORTANT PHARMACOLOGIC PARAMETERS OF THE THIAZIDE-LIKE DIURETICS

Thiazide-like Diuretic (Trade Name)	Partition Coefficient (Octanol/ H_2O PO_4 Buffer)[30]	Plasma Protein Binding (%)[44]	Usual Daily Adult Oral Dosage Range (mg)		Diuretic Effect			% Parent Drug Excreted in Urine[44]
			Optimal Diuresis[32]	Hypertension[46]	Onset (hours)[41]	Peak (hours)[41]	Duration (hours)[32,41–43]	
Mefruside (*Baycaron*)								0.3–1.1
Xipamide (*Aquaphor, Diurexan*)		99						
Clopamide (*Aquex, Brinaldix*)	6.8	46						27
Indapamide (*Lozol*)	31.7	71–79	2.5–5	2.5–5			24–36	5–7
Quinethazone USP (*Hydromox*)			50–200	25–100	2	6	18–24	
Metolazone (*Diulo, Zaroxolyn, Mykrox*)		50–70 (RBC)	2.5–20	1.25–5*	1–2	2	12–24	80–95
Clorexolone (*Nefrolan*)				0.5–1				
Chlorthalidone USP (*Hygroton, Thalitone*)	5.0	94–99 (RBC)	25–200	12.5–50	2	2	48–72	44 (oral) 65 (IV)

* Mykrox dose is 0.5–1 mg.

Thiazide and thiazide-like diuretics can be involved in several potentially serious drug interactions. The first of these may occur if lithium is administered to individuals on long-term thiazide or thiazide-like diuretic therapy. The proximal tubule handling of lithium is similar to that of sodium. During long-term thiazide treatment, the resulting reduction in plasma volume triggers a compensatory increase in the proximal tubule reabsorption of fluid and solutes. Thus, more lithium is reabsorbed than would occur in normovolemic individuals. The resulting elevated plasma levels of lithium may provoke serious lithium toxicity.[43] Second, concurrent administration of a thiazide or thiazide-like diuretic with large doses of calcium-containing substances may result in hypercalcemia because of the calcium-retaining property of these diuretics. Third, concurrent use of thiazide and thiazide-like diuretics and nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit prostaglandin synthesis, can result in the latter antagonizing the diuresis produced by the former. In addition, NSAIDs can increase the risk of renal failure in patients whose marginal renal function is being maintained by the intrarenal release of prostaglandins. Fourth, when thiazide and thiazide-like diuretics are used along with cardiac glycosides (e.g., digoxin or digitoxin) in the treatment of congestive heart failure, serious toxicity can result if hypokalemia occurs (see discussion below).

USES

Thiazide and thiazide-like diuretics are extremely useful in the treatment of edema associated with mild to moderate congestive heart failure, cirrhosis of the liver, or nephrotic syndrome. Because edema is a symptom of an underlying disease and not a disease itself, the underlying disease should be treated first if possible. If treatment of the underlying disease does not result in the removal of the edema fluid, then diuretic therapy may be indicated. Caution should always be exercised when thiazide or thiazide-like diuretics are coadministered with cardiac glycosides for the treatment of edema associated with congestive heart failure. These diuretics have a tendency to promote hypokalemia, a condition that enhances the general toxicity of the cardiac glycosides.[36,43] Combination diuretic therapy (i.e., a thiazide or thiazide-like diuretic plus a potassium-sparing diuretic) may prevent potassium loss under these circumstances. If combination diuretic therapy is instituted, the recipient should be advised not to take potassium supplements, in order to avoid serious hyperkalemia.[32]

Thiazide and thiazide-like diuretics are also useful in the treatment of certain nonedematous disorders. These include hypertension, diabetes insipidus (either the nephrogenic or the neurohypophyseal form), type II renal tubular acidosis, and hypercalciuria. These diuretics are primary agents in the control of hypertension, either alone or in combination with other drugs depending on the severity of the condition. Thiazides generally lower blood pressure 10 to 15 mm Hg within the first 3 to 4 days of continuous treatment.[55] After approximately a week of continuous treatment (about the time that there is a concomitant reduction in plasma volume), the kidneys readjust to the initial effects of the diuretic and a waning of the diuretic effect is observed, whereas the reduction of blood pressure is maintained.[51] This readjustment occurs provided that sodium intake is not increased.

Some individuals with hypercalciuria (i.e., an elevated urinary concentration of calcium) are prone to the formation of calcium-containing stones within the urinary tract. Because the chronic use of thiazide and thiazide-like diuretics decreases the urinary excretion rate of calcium, they may be helpful in preventing calcium-containing stone formation.[36,56]

PRODUCTS

Pertinent pharmacologic data on the thiazide and thiazide-like diuretics are cited in Tables 18-2 and 18-3.

Chlorothiazide, USP

6-Chloro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide (Diuril). Chlorothiazide is usually supplied as 250- and 500-mg tablets, an oral suspension containing 50 mg/mL, and the sodium salt for injection (500 mg [base]).

Benzthiazide, USP

6-Chloro-3[[(phenylmethyl)thio]-methyl]-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide (Exna, Hydrex). Benzthiazide usually is supplied as 50-mg tablets.

Hydrochlorothiazide, USP

6-Chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide (Esidrix, HydroDIURIL, Oretic). Hydrochlorothiazide usually is supplied as 25-, 50-, or 100- mg tablets and as an oral solution (10 and 100 mg/mL). It is also available in fixed combinations with the potassium-sparing diuretics.

Hydroflumethiazide, USP

3,4-Dihydro-6-(trifluoromethyl)-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide (Diucardin, Saluron). Hydroflumethiazide usually is supplied as 50-mg tablets.

Trichlormethiazide, USP

6-Chloro-3-(dichloromethyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide (Metahydrin, Naqua). Trichlormethiazide usually is supplied as 2- and 4-mg tablets.

Bendroflumethiazide, USP

3-Benzyl-3,4-dihydro-6-(trifluoromethyl)-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide (Naturetin). Bendroflumethiazide usually is supplied as 5- and 10-mg tablets.

Methyclothiazide, USP

6-Chloro-3-(chloromethyl)-3,4-dihydro-2-methyl-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide (Aquatensen, Enduron). Methyclothiazide usually is supplied as 2.5- and 5-mg tablets.

Polythiazide, USP

6-Chloro-3,4-dihydro-2-methyl-3-[[(2,2,2-trifluoroethyl) thio]methyl]-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide (Renese). Polythiazide usually is supplied as 1-, 2-, and 4-mg tablets.

Cyclothiazide, USP

3-Bicyclo[2.2.1]hept-5-en-2-yl-6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide (Anhydron). Cyclothiazide is usually available as 2-mg tablets.

Mefruside

4-Chloro-N^1-methyl-N^1-[(tetrahydro-2-methyl-2-furanyl) methyl]-1,3-benzenedisulfonamide (Baycaron). Not available in the United States.

Xipamide

4-Chloro-5-sulfamoyl-2′,6′-salicyloxylidide (Aquaphor, Diurexan). Not available in the United States.

Clopamide

3-Aminosulfonyl-4-chloro-*N*-(2,6-dimethyl-1-piperidinyl) benzamide (Aquex, Brinaldix). Not available in the United States.

Indapamide

1-(4-Chloro-3-sulfamoylbenzamido)-2-methylindoline (Lozol). Indapamide usually is available as 2.5-mg tablets.

Quinethazone, USP

7-Chloro-2-ethyl-1,2,3,4-tetrahydro-4-oxo-6-quinazolinesulfonamide (Hydromox). Quinethazone usually is supplied as 50-mg tablets.

Metolazone

7-Chloro-1,2,3,4-tetrahydro-2-methyl-4-oxo-3-o-tolyl-6-quinazolinesulfonamide (Diulo, Zaroxolyn). Metolazone usually is supplied as 2.5-, 5-, and 10-mg extended tablets and as 0.5-mg prompt tablets.

Clorexolone

6-Chloro-2-cyclohexyl-2,3-dihydro-3-oxo-1*H*-isoindole-5-sulfonamide (Nefrolan). Not available in the United States.

Chlorthalidone, USP.

2-Chloro-5-(1-hydroxy-3-oxo-1-isoindolinyl)benzene- sulfonamide (Hygroton, Thalitone). Chlorthalidone usually is supplied as 25-, 50-, and 100-mg tablets.

SITE 2 DIURETICS: HIGH-CEILING OR LOOP DIURETICS

The diuretics that belong to this class are of extremely diverse chemical structure.[57] Although brief mention is made of the organomercurial diuretics, primary attention is focused on the agents that currently enjoy widespread use: for example, furosemide (a 5-sulfamoyl-2-aminobenzoic acid or anthranilic acid derivative), bumetanide (a 5-sulfamoyl-3-aminobenzoic acid or metanilic acid derivative), torsemide (a 4-amino-3-pyridinesulfonylurea), and ethacrynic acid (a phenoxyacetic acid derivative).

ORGANOMERCURIALS

The organomercurials were the mainstay of diuretic therapy from 1920 to the early 1950s.[28] They elicit diuresis by an inhibition of sodium reabsorption at site 2,[58] and they block the subsequent exchange of sodium for potassium at site 4.[59] Thus, they are natriuretic and chloruretic without being kaliuretic. These properties are true attributes for any class of diuretics; however, the organomercurials have several serious limitations. First, they cannot be relied upon to elicit diuresis when given orally because of poor and erratic absorption. Second, after parenteral administration there is a 1- to 2-hr lag in onset of diuresis.[60] Third, their ability to trigger a diuretic response is dependent on the acid-base

status of the individual (i.e., they are ineffective when the urine is alkaline).[61,62] Fourth, they are cardio- and nephrotoxic. The organomercurials became obsolete with the introduction of the thiazide and thiazide-like diuretics, furosemide, bumetanide, and ethacrynic acid. All of the latter agents are orally effective, equally effective in acidotic and alkalotic conditions, capable of inducing a relatively rapid diuresis when given parenterally, and relatively nontoxic. The reader is encouraged to consult the eighth edition of this textbook for an in-depth discussion of the organomercurials.

5-SULFAMOYL-2- AND -3-AMINOBENZOIC ACID DERIVATIVES

Bumetanide, USP

3-(Butylamino)-4-phenoxy-5-sulfamoylbenzoic acid (Bumex) (Fig. 18-11).

Furosemide, USP

4-Chloro-*N*-furfuryl-5-sulfamoylanthranilic acid (Lasix) (Fig. 18-11).

Structure–Activity Relationships

The development of these diuretics is an outgrowth of the research involving the thiazide and thiazide-like diuretics.[30] There are important structural requirements that are common to the 5-sulfamoyl-2-aminobenzoic acid derivatives and the 5-sulfamoyl-3-aminobenzoic acid derivatives (Fig. 18-11). First, the substituent at the 1-position must be acidic. The carboxyl group provides optimal diuretic activity, but other groups, such as a tetrazole, may impart respectable diuretic activity. Second, a sulfamoyl group in the 5-position is a prerequisite for optimal high-ceiling diuretic activity. Third, the "activating" group (–X) in the 4-position can be Cl– or CF_3–, as was the case with the thiazide and thiazide-like diuretics, or, better yet, a phenoxy, alkoxy, anilino, benzyl, or benzoyl group. Interestingly, when the latter five functional groups are substituted for the Cl– or CF_3– group in the thiazide or thiazide-like diuretics, their diuretic activity is decreased.

Major differences exist between these two series of 5-sulfamoylbenzoic acids in the nature of the functional groups that can be substituted into the 2- and 3-positions with the retention of maximal diuretic activity (Fig. 18-11). The substituents that can be tolerated on the 2-amino group of the 5-sulfamoyl-2-aminobenzoic acids are extremely limited, and no deviations are allowed on the few moieties that are acceptable. For example, only furfuryl-, benzyl-, and thienylmethyl (in decreasing order) yield derivatives with maximal diuretic activity. However, the substituents allowable on the 3-amino group of the 5-sulfamoyl-3-aminobenzoic acids can vary widely without jeopardizing optimal diuretic activity. High-ceiling diuretics that have emerged from the 5-sulfamoyl-2-aminobenzoic acid series include furosemide and azosemide and, from the 5-sulfamoyl-3-aminobenzoic acid series, bumetanide and piretanide. Only furosemide and bumetanide are commercially available in the United States.

Pharmacokinetics

Furosemide and bumetanide differ pharmacologically primarily in their potencies and bioavailabilities. When admin-

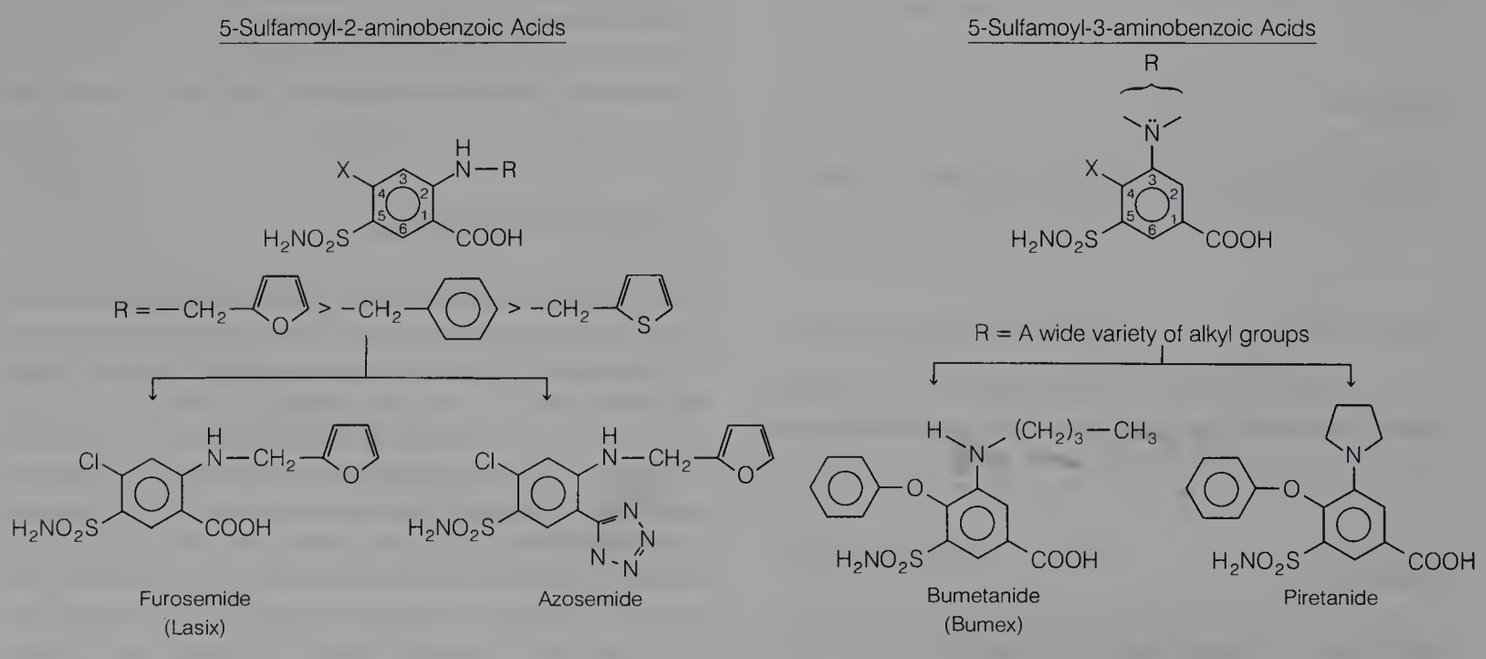


FIG. 18-11. Results from structure–activity relationship studies that led to the development of furosemide and bumetanide.

istered orally, the bioavailability of furosemide is about 60% to 69% in normal subjects but only 43% to 46% in individuals with end-stage renal disease.[43] The bioavailability of bumetanide in normal individuals is 80% to 90%.[63] Bumetanide is also more potent than furosemide; it will produce an equieffective diuresis at about one-fortieth the dose.[63,64]

Following parenteral administration, both furosemide and bumetanide have an extremely rapid onset of action (3 to 5 minutes). Duration of action following parenteral therapy is 2 hr for furosemide and 3.5 to 4 hr for bumetanide. Both diuretics have an onset of action of approximately 30 to 60 minutes after oral therapy, but furosemide has a slightly longer duration of action than bumetanide (6 to 8 hr compared to 4 to 6 hr).[41] Once these agents reach the bloodstream, they are bound extensively to plasma proteins (93% to 95%).[63] This degree of plasma protein binding severely limits the amount of each drug that can be removed from the plasma by glomerular filtration, but it does not prevent either drug from attaining high renal luminal fluid concentrations by the process of active tubular secretion. Both diuretics are weak organic acids and are secreted avidly into the luminal fluid of the proximal tubule (Fig. 18-7).[20,65] This is important for two reasons: first, it permits the relatively rapid renal excretion of both diuretics; second, it provides for the delivery of substantial amounts of each diuretic to their luminal site of action.

The previously discussed factors that determine the luminal concentration of diuretics are critical when employing these agents in individuals with uremia. Uremic individuals frequently have a depressed GFR and high circulating levels of endogenous weak organic acids, both of which lower the luminal fluid concentrations of the loop diuretics. The endogenous weak organic acids compete with the weakly acidic diuretics for active tubular secretion into proximal tubule luminal fluid. Often, the effects of the endogenous weak acids can be overridden by increasing the dose of these diuretics. Caution must be exercised, however, because an increased incidence of adverse effects is likely to accompany increased doses.

A small percentage of furosemide is converted to the corresponding glucuronide, and 88% of the administered drug is excreted by the kidneys. Bumetanide undergoes more extensive biotransformation in humans, and 81% of it is excreted in the urine (45% as unchanged drug).[41]

Site and Mechanism of Action

The events that contribute to the tremendous efficacy of furosemide and bumetanide are multifaceted. First, these diuretics inhibit the $1Na^+/1K^+/2Cl^-$ cotransport system located on the luminal membrane of cells of the thick ascending limb of Henle's loop[21,57] (Fig. 18-4). Importantly, the carboxylate moieties of furosemide and bumetanide are thought to be responsible for their competing with Cl^- for the Cl^- binding site on the $1Na^+/1K^+/2Cl^-$ cotransport system. Because site 2 is such a high-capacity site for sodium reabsorption, up to 20% to 25% of the filtered load of sodium that normally is reabsorbed in this nephron segment may be excreted in the urine. In addition, it is the reabsorption of this 20% to 25% of the filtered load of sodium (and chloride) that is required to maintain the hypertonicity of the medullary interstitium.[12] The hypertonic medullary interstitium allows us to produce concentrated urine by drawing water out of the descending limb of the loop of Henle by osmosis and out of the collecting duct by osmosis when ADH is present. Thus, when these diuretics inhibit the reabsorption of 20% to 25% of the filtered load of sodium at site 2, within minutes they also destroy the hypertonicity of the medullary interstitium.[6] The net result is that when sodium and chloride are not reabsorbed at site 2, water is no longer removed from the luminal fluid in the descending limb of Henle's loop or from the collecting tubule by osmosis. Large amounts of water, sodium, and chloride are excreted. Second, high concentrations of furosemide and bumetanide are attained in proximal luminal fluid by way of the OATS and delivered to the $1Na^+/1K^+/2Cl^-$ cotransport system at site 2. Third, although these diuretics increase the flow rate of luminal fluid past the macula densa cells, the expected reduction in GFR (which normally would mitigate the diuresis) does not occur. This is because these efficacious diuretics block the tubuloglomerular feedback mechanism.[66] Fourth, these diuretics also transiently increase total renal blood flow by enhancing the intrarenal release of vasodilatory prostaglandins. Fifth, they induce a redistribution of intrarenal blood flow that is thought to participate in a positive way toward the magnitude of the diuresis.[57]

All diuretics that act at site 2 are equally efficacious and far more efficacious than diuretics that act at sites 1, 3, or 4. Because of their site of action and efficacy, these agents are commonly referred to as "*loop*" or "*high-ceiling*" *diuretics*.[57]

The high-ceiling diuretics secondarily enhance the urinary loss of potassium and hydrogen ions. First, they block the reabsorption of potassium at site 2 by inhibiting the $1Na^+/1K^+/2Cl^-$ cotransport complex. Second, inhibition of sodium reabsorption at site 2 ultimately delivers more of the filtered load of sodium, at a faster rate, to site 4. This leads to an enhanced exchange of luminal fluid sodium ions for the potassium ions in the principal cells and the hydrogen ions in the intercalated cells at site 4 (Fig. 18-6).

When the loop diuretics are used in "submaximal" doses for the treatment of hypertension, it is intended that they create a diuresis similar in magnitude to that produced by the thiazide and thiazide-like diuretics. Under these circumstances, loop diuretics usually are associated with a lower frequency of hypokalemia than the thiazide and thiazide-like diuretics because their duration of action is not as prolonged and the kidneys have a greater portion of the day to readjust.[30,67] However, when loop diuretics are used to treat acute edema, higher dosages frequently are employed and the sodium and potassium losses exceed those accompanying thiazide therapy.[30]

When loop diuretics inhibit the $1Na^+/1K^+/2Cl^-$ cotransport system in the luminal membrane of thick ascending limb cells, they in turn decrease the lumen-positive transepithelial voltage that promotes the paracellular movement of luminal fluid cations, such as calcium, into the interstitium (Fig. 18-4). Hence, loop diuretics may induce the renal excretion of up to 20% to 30% of the filtered load of calcium, provided the plasma volume is not allowed to decrease.[10] If plasma volume decreases as a result of diuresis, there is an accompanying compensatory increase in the proximal tubule reabsorption of fluid and solutes. About 60% of the filtered load of calcium is reabsorbed in the proximal tubule during normovolemia, and the percentage of proximally reabsorbed calcium will increase in a state of plasma volume reduction. Thus, there will be less calcium delivered to the thick ascending limb for the loop diuretics to inhibit; this will blunt the loop diuretic's calciuretic effect.

Adverse Effects

Highly predictable adverse effects are associated with furosemide and bumetanide.

1. Hypokalemic alkalosis occurs as a result of the enhanced exchange of luminal fluid sodium for intracellular potassium or hydrogen ions at site 4. Caution should be exercised when concurrent therapy with loop diuretics and cardiac glycosides is instituted because hypokalemia intensifies the toxicity of the cardiac glycosides.[41]
2. Initially, fluid and electrolyte losses may not be accompanied by changes in GFR because of the effect of these agents on the tubuloglomerular feedback mechanism. However, long-term use of these diuretics may induce a reduction in plasma volume. If this condition is allowed to persist, the previously mentioned compensatory changes take place, one of which is a reduction in GFR.
3. Because diuretic-induced plasma volume reduction leads to an increase in the reabsorption of solutes normally handled by the proximal tubule, it should not be surprising to find that some individuals develop hyperuricemia when placed on long-term loop diuretic therapy. For similar reasons, coadministration of loop diuretics and lithium may lead to severe lithium toxicity.[41]
4. Furosemide and bumetanide are similar to the CA inhibitors, thiazides, and thiazide-like diuretics in that they possess a sulfamoyl moiety. This functional group has been associated with hypersensitivity reactions such as urticaria, drug fever, blood dyscrasias, and interstitial nephritis.

Several adverse effects, which were unforeseen, are associated with the loop diuretics. For example, they are unique among diuretics in that they can produce ototoxicity. Usually, hearing loss is temporary, but on occasion it may be permanent. Ototoxicity may be associated directly with rising plasma concentrations of the loop diuretics. Accordingly, individuals with impaired renal function appear to be at risk because they have a reduced ability to excrete these efficacious diuretics.[43] Although the milligram dose of bumetanide is one-fortieth that of furosemide, both agents appear to be quite similar in their ototoxic potential. Caution must be exercised if loop diuretics are administered to patients who are receiving any of the available aminoglycoside antibiotics. The ototoxicity of these two classes of drugs may be additive.[41] Other adverse effects of furosemide and bumetanide include hyperglycemia, nausea, vomiting, and myalgia.

NSAIDs, which inhibit prostaglandin synthesis, may blunt the natriuresis produced by loop diuretics. In patients with preexisting impairment of renal function who are on diuretic therapy, NSAIDs may increase the risk of renal failure by blocking the intrarenal synthesis of vasodilatory prostaglandins, the one thing that may be sustaining renal blood flow in these patients.[41]

Uses

High-ceiling diuretics are effective for the treatment of edema that may accompany congestive heart failure, cirrhosis of the liver, and the nephrotic syndrome. A most important use of furosemide or bumetanide is in the treatment of pulmonary edema. No other group of diuretics is more effective than the loop diuretics in this situation. When loop diuretics are employed in the treatment of pulmonary edema that may accompany congestive heart failure, it is of paramount importance to avoid their overzealous use. Such use may lead to an acute reduction in plasma volume, decreased venous return and cardiac output, and an exacerbation of heart failure.[68]

Loop diuretics may be employed for the treatment of certain nonedematous disorders. Symptomatic hypercalcemia may be treated with a loop diuretic, provided that a reduction in plasma volume is not allowed to occur and the fluid used for replacement of the urinary loss is calcium-free.[69] In addition, furosemide has been used for the treatment of hypertension. However, some investigators believe that because of its relatively short duration of action, it may be less effective than the thiazide or thiazide-like diuretics. It has been suggested that furosemide be reserved for hypertensive patients with fluid retention refractory to thiazides or for patients with impaired renal function.[46]

In general, furosemide or bumetanide is preferred over ethacrynic acid (another site 2 diuretic) because they have a broader dose-response curve, less ototoxicity, and less gastrointestinal toxicity.[43]

Products

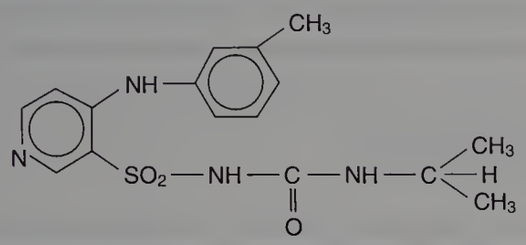
Bumetanide is available as 0.5-, 1.0-, and 2.0-mg tablets and as a powder to be made into a solution for parenteral use (0.25 mg/mL).

Furosemide is available as an oral solution, containing either 8 or 10 mg/mL; as 20-, 40-, or 80-mg tablets; and as an injectable form, containing 10 mg/mL.

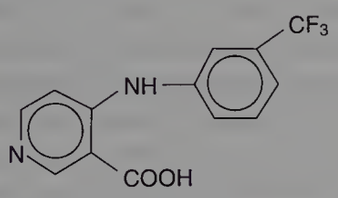
4-AMINO-3-PYRIDINESULFONYLUREAS

Torsemide

1-Isopropyl-3-{[4-(3-methylphenylamino)pyridine]-3-sulfonyl}urea (Demadex).



Torsemide



Triflocin

Structure–Activity Relationships

Initial screening of many 4-amino-3-pyridinesulfonylureas revealed that maximal diuretic activity was attainable in torsemide.[70] Torsemide is closely related structurally to triflocin, a loop diuretic that was studied extensively in the late 1960s and early 1970s but abandoned when it was shown to produce transitional cell carcinoma in the urinary bladders of over 50% of the rats that had received high doses over an 18 to 22-month period.

Pharmacokinetics

Torsemide is approximately 80% bioavailable after oral administration. Its extensive plasma protein binding (98% to 99%) is similar to that of other loop diuretics. Peak serum concentrations are generally attained within 1 hr, and the half-life is 3 to 4 hr, somewhat longer than that of furosemide (2 hr) and bumetanide (1 to 1.5 hr). Torsemide is metabolized by the hepatic cytochrome P_{450} system. The primary products result from oxidation of the aromatic methyl group to hydroxy and carboxyl derivatives and *para* hydroxylation of the methylphenylamino moiety. Approximately 20% of the dose is excreted unchanged in the urine.[71,72]

Site and Mechanism of Action

Like furosemide and bumetanide, torsemide induces diuresis by inhibition of the $1Na^+/1K^+/2Cl^-$ cotransport system on the luminal membrane of thick ascending limb cells (Fig. 18-4). Thus, it is essential that torsemide reach adequate levels in the luminal fluid. Higher doses, which have been studied in isolated nephron segments, also inhibit the efflux of chloride ions out of the thick ascending limb cells by the chloride channels on the basolateral membrane.[73] Whether this second site of action is clinically relevant remains to be determined.

Adverse Effects

Torsemide may produce fatigue, dizziness, muscle cramps, nausea, and orthostatic hypotension. At this early date, no evidence of ototoxicity has appeared in humans, but studies in cats have shown torsemide to be similar to furosemide in ototoxic potential.[74]

Products

Torsemide is usually available as 5-, 10-, 20-, and 100-mg tablets and as an injectable form containing 10 mg/mL.

Uses

Torsemide is useful in the treatment of mild to moderate hypertension in doses of 2.5 to 5 mg given once daily. These doses will lower blood pressure as effectively as 25 mg of hydrochlorothiazide but without producing diuresis. Higher doses (10 to 20 mg) are associated with significant diuresis and are effective in treating edema associated with congestive heart failure and cirrhosis of the liver.[71,75]

PHENOXYACETIC ACIDS

The phenoxyacetic acid group of high-ceiling diuretics was developed and introduced into clinical use about the same time as furosemide.

Ethacrynic Acid, USP

[2,3-Dichloro-4-(2-methylene-1-oxobutyl)phenoxy]acetic acid (Edecrin) (Fig. 18-12).

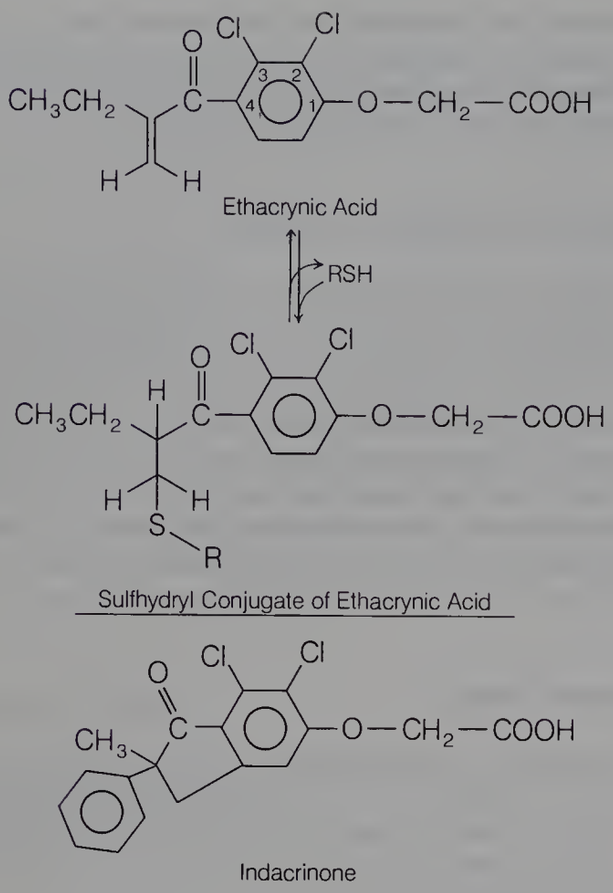


FIG. 18-12. *Ethacrynic acid is a high-ceiling diuretic that readily reacts with many sulfhydryl-containing nucleophiles (upper portion of the figure). Indacrinone, a structurally related high-ceiling diuretic, lacks sulfhydryl reactivity (lower portion of the figure).*

Structure-Activity Relationships

As mentioned previously, certain organomercurials are capable of eliciting a diuretic response, but because of their heavy metal content they are too toxic for widespread use. Consequently, a search was commenced for a non-mercury-containing compound that would react with sulfhydryl-containing receptors in renal tissue, like the organomercurials, but be devoid of heavy metal-type toxicity. Because one of the commercially available organomercurials (i.e., mersalyl, Salyrgan) possessed a phenoxyacetic acid moiety, the phenoxyacetic acids served as the chemical root for new non-mercury-containing diuretics. Hundreds of phenoxyacetic acids were examined.[76,77]

Within the phenoxyacetic acid series (Fig. 18-12), optimal diuretic activity is achieved when (1) an oxyacetic acid moiety is placed in the 1-position on the benzene ring, (2) a sulfhydryl-reactive acryloyl moiety is located *para* to the oxyacetic acid group, (3) activating groups (i.e., Cl– or CH_3–) occupy either the 3-position or the 2- and 3-positions, (4) alkyl substituents of two- to four-carbon atoms in length occupy the position α to the carbonyl on the acryloyl moiety, and (5) hydrogen atoms occupy the terminal position of the carbon–carbon double bond of the acryloyl moiety. These structural features seemed to maximize both the diuretic activity and the in vitro rate of reactivity with various sulfhydryl-containing nucleophiles. The correlation between diuretic activity and chemical reactivity within this series of diuretics was strengthened when it was found that reduction or epoxidation of the carbon–carbon double bond in the acryloyl moiety yielded compounds with little or no diuretic activity or chemical reactivity.[78,79] The design and synthesis of ethacrynic acid appeared to be the ultimate in terms of "the rational approach to drug design." However, the need for designing a diuretic with a high degree of sulfhydryl reactivity was foiled when indacrinone was found to be highly efficacious but incapable of reacting with sulfhydryl-containing nucleophiles (Fig. 18-12).[80] Indacrinone was withdrawn from clinical trials because of the appearance of abnormal liver function tests in certain individuals.

Pharmacokinetics

In spite of ethacrynic acid's unique chemical structure and avid reactivity toward various nucleophiles, it has many pharmacologic features in common with the sulfamoyl-containing loop diuretics. First, after oral administration, onset of action is about 30 minutes and duration of action, 6 to 8 hr. Second, after parenteral administration, onset and duration of action are 3 to 5 minutes and 2 to 3 hr, respectively. Third, ethacrynic acid is highly bound to plasma proteins (i.e., >95%). Fourth, ethacrynic acid is handled and excreted predominantly by the kidneys. Very little of the drug is removed from the plasma by glomerular filtration because of its extensive binding to unfiltrable plasma proteins such as albumin. However, the drug is secreted avidly into the luminal fluid of the proximal tubule with the assistance of the OATS (Fig. 18-7).[65,81,82] High luminal fluid concentrations of ethacrynic acid are essential for its diuretic action and ultimate excretion.

Ethacrynic acid is biotransformed by a pathway completely different from that of furosemide or bumetanide. Ethacrynic acid alkylates the thiol group of glutathione in vivo (Fig. 18-12; RSH = glutathione), and the resulting conjugate subsequently is converted to the ethacrynic acid–cysteine and ethacrynic acid–*N*-acetyl-cysteine (the mercapturic acid) conjugates. Ethacrynic acid–cysteine is quite unstable in vivo and in vitro; it readily releases cysteine and ethacrynic acid. Ethacrynic acid, ethacrynic acid–glutathione, and ethacrynic acid–cysteine are equiefficacious diuretics because of the aforementioned interconversions.[76] Approximately two-thirds of the ethacrynic acid appears in the urine in the various forms cited, whereas the remaining one-third is found in the bile.

Site and Mechanism of Action

Similar to furosemide and bumetanide, ethacrynic acid (1) blocks reabsorption of 20% to 25% of the filtered load of sodium at site 2 by inhibiting the $1Na^+/1K^+/2Cl^-$ cotransport system located on the luminal membrane of cells in the thick ascending limb of Henle's loop[21] (Fig. 18-4); (2) reaches high levels in luminal fluid due to its active tubular secretion by the OATS in proximal tubular cells; (3) blocks the tubuloglomerular feedback mechanism that normally would result in an acute reduction of the GFR when the flow of luminal fluid is increased through the nephron segment possessing the macula densa cells;[83] (4) transiently increases total renal blood flow by enhancing the intrarenal release of vasodilatory prostaglandins,[84] and (5) transiently induces a redistribution of intrarenal blood flow, which contributes in a positive way toward the magnitude of the diuresis.[85] Because ethacrynic acid induces an acute increase in the renal excretion rate of sodium, chloride, potassium, and calcium, it is a natriuretic, chloruretic, saluretic, kaliuretic, and calciuretic agent.

Adverse Effects

Ethacrynic acid may produce all of the adverse effects noted with furosemide and bumetanide except those related to the presence of a sulfamoyl group. The use of ethacrynic acid has been curtailed because it is more ototoxic than furosemide and bumetanide and it produces more serious gastrointestinal effects (i.e., gastrointestinal hemorrhage) than observed with the sulfamoyl-containing loop diuretics.

In addition, as with furosemide and bumetanide, serious drug interactions may occur when ethacrynic acid is used concurrently with lithium, cardiac glycosides, aminoglycoside antibiotics, or NSAIDs (see discussion under adverse effects of furosemide and bumetanide).

Uses

Ethacrynic acid has the same indications as cited for furosemide and bumetanide. However, when a high-ceiling diuretic is indicated in the treatment of an individual who has a known hypersensitivity to sulfamoyl-containing drugs, ethacrynic acid may be an appropriate substitute.

Products

Ethacrynic acid is available as 25- and 50-mg tablets and as ethacrynate sodium for injection, which contains 50 mg (base).

MISCELLANEOUS SITE 2 DIURETICS

There are three nondiuretic agents that are biotransformed to high-ceiling diuretics in vivo by sulfation of their –OH moieties (Fig. 18-13). The sulfated metabolites exert a diuresis by inhibition of the $1Na^+/1K^+/2Cl^-$ cotransport system on the luminal membrane of thick ascending limb cells (Fig. 18-4). These agents include: 2-(*p*-fluorophenoxy), 1-(*o*-hydroxyphenyl)ethane (CRE 10904)[86] 2-(aminomethyl)-4-(1,1-dimethylethyl)-4-iodophenol (MK-447):[87] and 6-chloro-2,3-dihydro-1-(1-oxopropyl)-4(H)-quinolinone4-oxime(M12285).[88]

In each case, the sulfated metabolite undergoes active tubular secretion by the OATS in proximal tubular cells and, hence, attains high levels in luminal fluid. The negatively charged sulfate moiety probably binds to the Cl^--binding site on the luminal membrane-bound $1Na^+/1K^+/2Cl^-$ cotransport system of thick ascending limb cells.

In addition, etozoline (after being hydrolyzed) and muzolimine possess diuretic activity by virtue of their actions on the transport processes in the cells of the thick ascending limb of Henle's loop. Etozoline is hydrolyzed in vivo to ozolinone (a carboxylic acid), which is the active diuretic species. Ozolinone is secreted actively into proximal tubule luminal fluid by the OATS. The high concentrations of ozolinone delivered to the thick ascending limb cells of Henle's loop inhibit the luminal membrane-bound $1Na^+/1K^+/2Cl^-$ cotransport system[89,90] (Fig. 18-4). The precise mechanism(s) by which muzolimine exerts a diuresis remains to be determined.[91] However, it has been suggested that muzolimine inhibits the potassium/chloride cotransport system on the basolateral membrane of the thick ascending limb cells, which in turn inhibits the $1Na^+/1K^+/2Cl^-$ cotransport system.[92] None of these miscellaneous site 2 agents has been marketed in the United States.

SITE 4 DIURETICS: POTASSIUM-SPARING DIURETICS

A negative feature of all of the previously discussed classes of diuretics (except the organomercurials) is that they induce an increase in the renal excretion rate of potassium. Over the years, three chemically distinct diuretics have emerged that increase sodium and chloride excretion, without a concomitant increase in the urinary excretion rate of potassium. These agents are known as *potassium-sparing diuretics* or *antikaliuretic agents*. Although the potassium-sparing diuretics are derived from completely different chemical roots, they have a similar anatomic site of action in the nephron, efficacy, and electrolyte excretion pattern. They even share certain adverse effects. The potassium-sparing diuretics include the spirolactone spironolactone, the 2,4,7-triamino-6-arylpteridine triamterene, and the pyrazinoylguanidine amiloride.

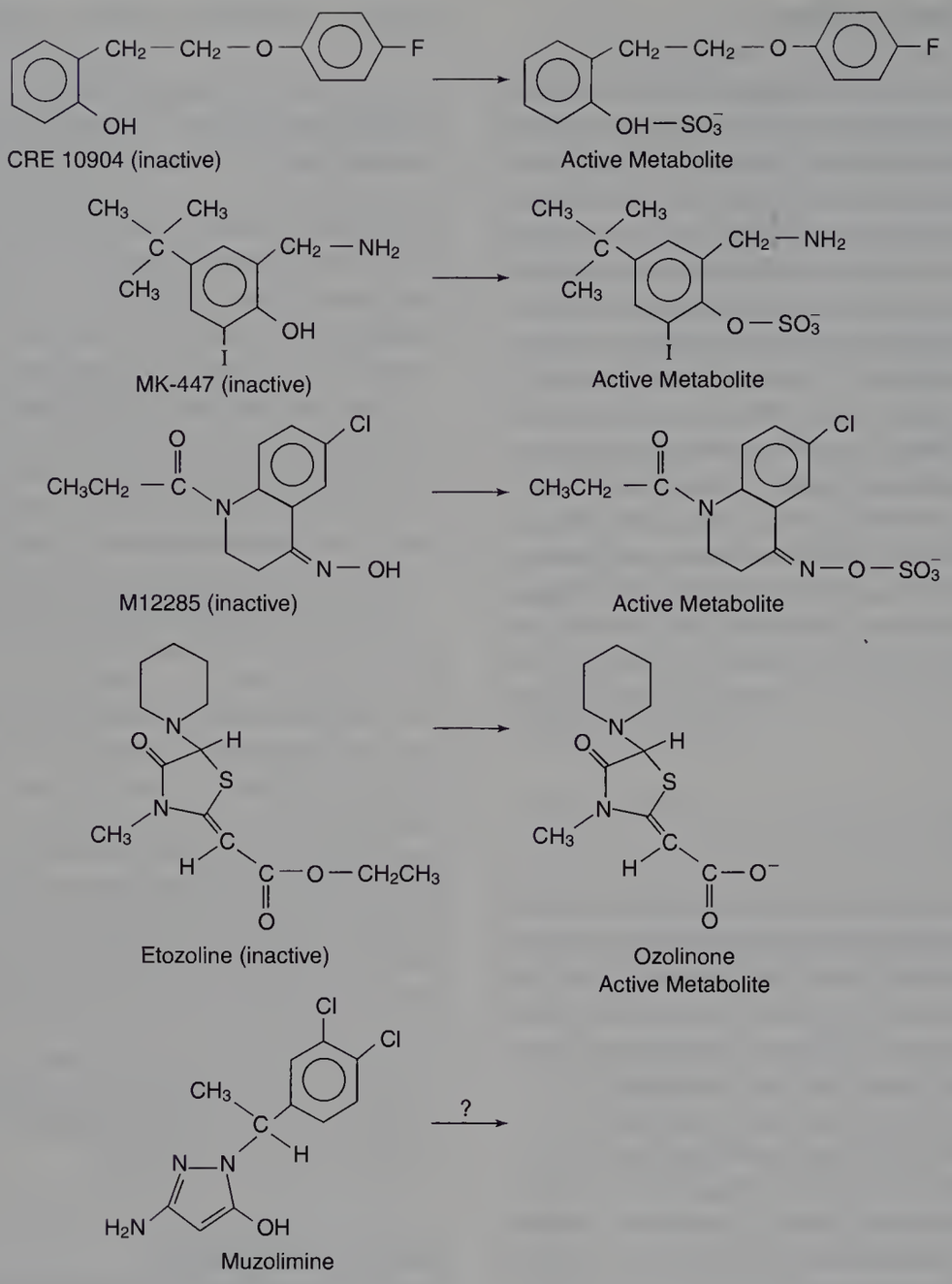


FIG. 18-13. *Various seemingly unrelated agents that block sodium reabsorption in the thick ascending limb of Henle's loop. The precise mechanism of action of muzolimine is unknown. However, all of the other agents are inactive as such and must be biotransformed to active metabolites before their diuretic activity can be expressed. In each case, it is important to note that, like furosemide, bumetanide, torsemide, and ethacrynic acid, the active metabolites have an anionic moiety that may permit binding to the Cl^- binding site on the $1Na^+/1K^+/2Cl^-$ cotransport system in thick ascending limb cells.*

SPIROLACTONES, ALDOSTERONE ANTAGONISTS

Spironolactone, USP

7α-(Acetylthio)-17β-hydroxy-3-oxopregn-4-ene-21-carboxylic acid γ-lactone (Aldactone) (Fig. 18-14).

Structure–Activity Relationships

In the mid 1950s, it was observed that progesterone inhibited the antinatriuretic and kaliuretic effects of aldosterone, the primary mineralocorticoid in humans.[93,94] An intensive effort was launched to develop steroidal derivatives that possessed only the antimineralocorticoid activity of progesterone.[95–98] Spironolactone was selected from a host of derivatives for further examination.[97]

Pharmacokinetics

Spironolactone is absorbed well after oral administration (bioavailability is >90%); biotransformed rapidly and extensively by the liver (about 80%) to canrenone, an active

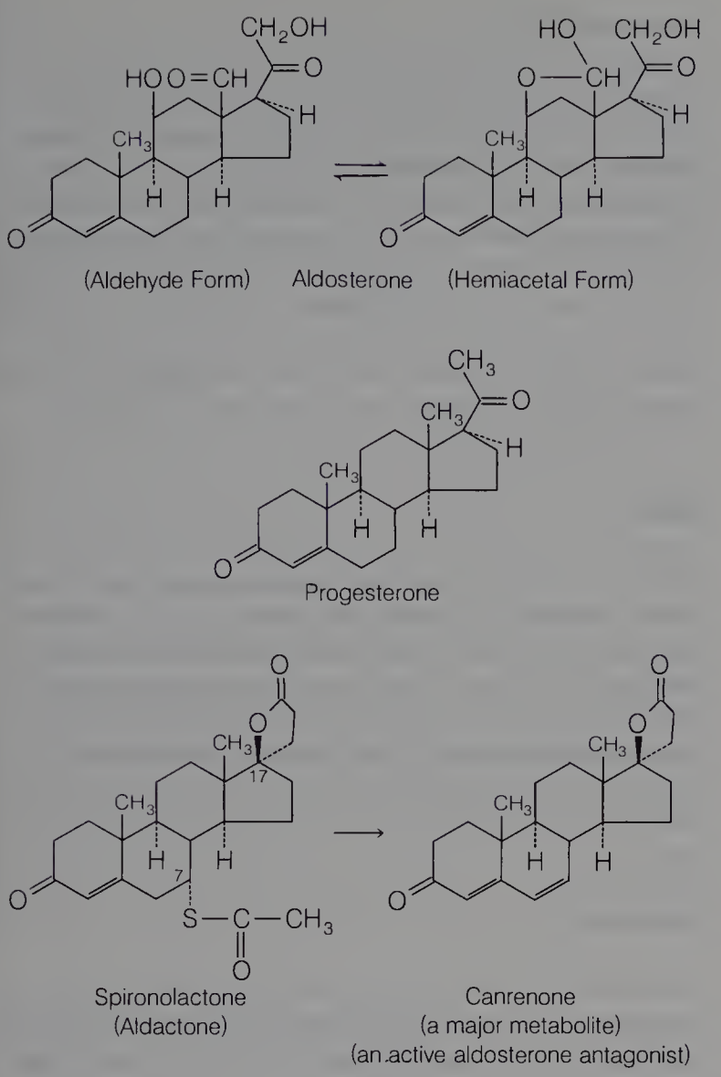


FIG. 18-14. *Aldosterone enhances the passage of sodium from the luminal fluid into tubular cells and the passage of intracellular potassium into the luminal fluid at site 4. Progesterone inhibits these actions of aldosterone but has undesirable hormonal side effects. Spironolactone and canrenone also competitively inhibit the actions of aldosterone at site 4 and are associated with a much lower frequency of hormonal side effects.*

metabolite (Fig. 18-14); bound extensively to plasma proteins (most likely as canrenone); and excreted primarily as metabolites in the urine. Some biliary excretion of metabolites also occurs. Its onset of action is slow (12 to 72 hr),[99] and its duration of action is quite long (2 to 3 days).[41]

Site and Mechanism of Action

Spironolactone inhibits the reabsorption of 2% to 3% of the filtered load of sodium at site 4 by competitively inhibiting the actions of aldosterone[6,36] (Fig. 18-6). Under normal circumstances, aldosterone enters the principal cells of the connecting tubule and cortical collecting tubule and combines with a cytosolic receptor, and the complex moves into the nucleus and turns on the synthesis of transport proteins, such as the Na^+,K^+-ATPase,[100,101] luminal membrane channels in principal cells that are involved in the exchange of sodium for potassium, and the H^+-ATPase that actively pumps hydrogen ions into the luminal fluid at site 4. Thus, passage of luminal fluid sodium into, and potassium and hydrogen ions out of, the late distal convoluted tubule and the early collecting tubule cells is enhanced. Increased intracellular levels of sodium stimulate the basolateral membrane-bound Na^+,K^+-ATPase.[11] Because spironolactone competitively inhibits these actions of aldosterone,[101] it enhances water, sodium, and chloride excretion. Therefore, spironolactone is a natriuretic, chloruretic, saluretic, and antikaliuretic agent. Unlike the other potassium-sparing diuretics, spironolactone requires the presence of endogenous aldosterone to exert its diuretic action. Because it inhibits the reabsorption of only 2% to 3% of the filtered load of sodium, it (and the other members of the site 4 potassium-sparing diuretics) has an extremely low efficacy.

Adverse Effects

It might be anticipated that inhibition of the exchange of luminal fluid sodium for intracellular potassium and hydrogen ions would lead to retention of the latter two ions in certain individuals. The major adverse effects of spironolactone are hyperkalemia and mild metabolic acidosis, especially in individuals with poor renal function.[6,36] In addition, spironolactone may produce gynecomastia in men and breast tenderness and menstrual disturbances in women because of its residual hormonal activity.[102] Other adverse effects include minor gastrointestinal symptoms and skin rashes.[32,43]

Uses

Spironolactone may be used alone as an extremely mild diuretic to remove edema fluid in individuals with congestive heart failure, cirrhosis, or the nephrotic syndrome or as an antihypertensive agent. However, its primary use has been in combination with diuretics that act at site 2 or 3 in an attempt to reduce the urinary potassium loss associated with these latter groups of diuretics.

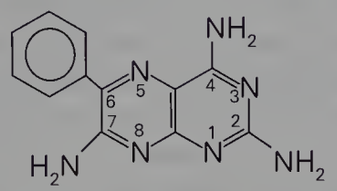
Products

Spironolactone is available in tablets of 25, 50, and 100 mg. It is also available as a fixed combination with hydrochlorothiazide (Aldactazide, generic). Tablets are available that contain either 25 mg of spironolactone and 25 mg of hydrochlorothiazide or 50 mg of each.

2,4,7-TRIAMINO-6-ARYLPTERIDINES

Triamterene, USP

2,4,7-Triamino-6-phenylpteridine (Dyrenium).



Triamterene

Structure–Activity Relationships

Triamterene is the primary compound selected from a host of synthetic pteridine analogs.[103] Although it bears a structural resemblance to folic acid and certain dihydrofolate reductase inhibitors, it has little, if any, of their activities.[32]

Pharmacokinetics

Triamterene is absorbed rapidly but incompletely (30% to 70%) from the gastrointestinal tract,[41] bound to plasma proteins to the extent of about 60%, biotransformed extensively in the liver, and excreted primarily by the biliary route and secondarily via the renal route as unchanged drug (20%) and metabolites (80%). It enters the luminal fluid of the nephrons by glomerular filtration and active tubular secretion in the proximal tubule. Because it is a weak organic base, it is assumed to be handled by the proximal tubule OCTS[32] (Fig. 18-7).

Its onset of action following a single oral dose is 2 to 4 hr, and its duration of action is 7 to 9 hr.[41]

Site and Mechanism of Action

Triamterene "plugs" the sodium channels in the luminal membrane of the principal cells at site 4 and thereby inhibits the electrogenic entry of 2% to 3% of the filtered load of sodium into these cells[6,11] (Fig. 18-6). As the principal cell concentration of sodium decreases as a result of triamterene's action, there is a decrease in the antiluminal membrane-bound Na^+,K^+-ATPase activity. This leads to a decrease in the cellular extrusion of sodium and in the cellular uptake of potassium. Because the secretion of potassium and hydrogen ions at site 4 is linked to sodium reabsorption, a concomitant reduction in the excretion rate of potassium and hydrogen ions occurs. The presence of aldosterone is not a prerequisite for triamterene's diuretic action. Triamterene, like the other potassium-sparing diuretics, has a low efficacy and may be referred to as a mild natriuretic, chloruretic, saluretic, and antikaliuretic agent.

Adverse Effects

As with the other potassium-sparing diuretics, the primary actions of which are elicited at site 4, triamterene's major adverse effect is hyperkalemia.[36] In addition, it appears to be unique among the potassium-sparing diuretics in being associated with the formation of renal stones. Approximately 1 out of 1500 individuals taking a triamterene-containing diuretic experiences nephrolithiasis.[104,105] The stones consist of triamterene (with or without its metabolite) or triamterene along with calcium oxalate or uric acid. It also may produce nausea, vomiting, leg cramps, and dizziness.[32]

Uses

Triamterene may be used alone in the treatment of mild edema associated with congestive heart failure or cirrhosis, but it should not be given to patients with impaired renal function.[106] It is not to be used alone in the treatment of hypertension.[46,106] Its primary use is in combination with hydrochlorothiazide (or other diuretics that act at site 2 or 3) to prevent the hypokalemia associated with the latter diuretics.

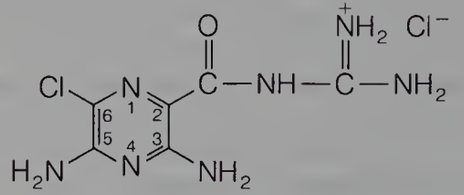
Products

Triamterene is available as 50 and 100 mg capsules. Fixed combinations of triamterene and hydrochlorothiazide are also available in capsule (Dyazide) or tablet (Maxzide) form: triamterene (37.5 mg)/hydrochlorothiazide (25 mg), triamterene (50 mg)/hydrochlorothiazide (25 mg), and triamterene (75 mg)/hydrochlorothiazide (50 mg).

PYRAZINOYLGUANIDINES

Amiloride Hydrochloride, USP

3,5-Diamino-*N*-(aminoiminomethyl)-6-chloropyrazinecarboxamide monohydrochloride dihydrate (Midamor).



Amiloride Hydrochloride

Structure–Activity Relationships

An extensive screening procedure that examined over 25,000 agents was undertaken in an attempt to discover an antikaliuretic agent that did not have overlapping hormonal activity,

such as that of spironolactone.[107] Promising activity was noted with appropriately substituted pyrazinoylguanidines. Optimal diuretic activity in this series is observed when the 6-position is substituted with chlorine, the amino groups in the 3- and 5-positions are unsubstituted, and the guanidino nitrogens are not multiply substituted with alkyl groups. Amiloride emerged as the most active compound in the series.

Pharmacokinetics

Amiloride contains the strongly basic guanidine moiety and possesses a pK_a of 8.7. Hence, it exists predominantly as the charged guanidinium ion in the *p*H range of most body tissues and fluids. Therefore, it is not surprising that amiloride is absorbed incompletely and erratically (i.e., 15% to 20%) from the gastrointestinal tract, an event that occurs by passive diffusion of the uncharged form of most drugs. Amiloride is bound to plasma proteins to a moderate degree, is not biotransformed, and is excreted in the urine (20% to 50%) and in the feces (40%). The fecal content may represent unabsorbed drug. Amiloride reaches the luminal fluid by glomerular filtration and active tubular secretion. The proximal tubule OCTS (Fig. 18-7) is involved in the latter process.[32] Onset of action occurs within 2 hr after oral administration, and duration of action may extend to 24 hr.[41]

Site and Mechanism of Action

Like triamterene, amiloride inhibits the electrogenic entry of 2% to 3% of the filtered load of sodium into the principal cells of the connecting and cortical collecting tubules (i.e., site 4) by "plugging" the sodium channels in the luminal membrane (Fig. 18-6). In turn, the driving force for potassium secretion is reduced or eliminated.[6,11,36] Similar to triamterene, amiloride does not require the presence of aldosterone in order to produce diuresis. It induces the urinary loss of sodium, chloride, and water and, therefore, is a natriuretic, chloruretic, saluretic, and antikaliuretic agent, though with low efficacy.

Adverse Effects

The major adverse effect of amiloride is hyperkalemia, which also may be observed with the other potassium-sparing diuretics that act at site 4. Nausea, vomiting, diarrhea, and headache also have been noted after the use of amiloride.[32]

Uses

Amiloride may be used alone in the treatment of mild edema associated with congestive heart failure, cirrhosis, or the nephrotic syndrome or in the treatment of hypertension. Its most common use is in combination with diuretics that act at site 2 or 3, to circumvent the renal loss of potassium commonly associated with the latter agents.

Products

Amiloride hydrochloride (Midamor) is available for oral use as 5-mg tablets. In addition, a fixed combination of amiloride hydrochloride (5 mg) and hydrochlorothiazide (50 mg) (Moduretic) is available in tablet form.

MISCELLANEOUS DIURETICS

Mannitol, USP (Osmitrol)

The prototypic osmotic diuretic D-mannitol is a water-soluble, lipid-insoluble, hexahydroxy alcohol. Because of its lack of lipid solubility, mannitol does not diffuse across the gastrointestinal epithelium and must be given by the intravenous route in order to obtain systemic effects. Once it enters the bloodstream, little, if any, is bound to plasma albumin; its distribution is confined to extracellular fluids, and it is not biotransformed. It enters renal luminal fluid only by glomerular filtration; it is neither secreted nor reabsorbed. The net result of its renal handling is twofold. First, it is excreted primarily by the kidneys; up to 80% of a 100 g intravenous dose appears in the urine within a 3-hr period.[41] Second, high luminal fluid concentrations of mannitol create an osmotic effect, and a great deal of the water in the luminal fluid is retained within the lumens of the nephrons. This osmotic effect prevents the reabsorption of up to 28% of the filtered load of water.[99] Mannitol, therefore, may be employed prophylactically in a hospital setting to keep the nephrons open and to avoid acute renal failure in certain circumstances. It has also been useful for the reduction of cerebrospinal fluid volume and pressure. Because solutions of mannitol may expand the extracellular fluid volume, they should not be used in patients with severe renal disease or cardiac decompensation. Aqueous solutions are available in a range of concentrations for intravenous use. The adult dosage range for the induction of diuresis is from 50 to 200 g/24 hr.

Theophylline

The prototypic xanthine, theophylline is known to promote a weak diuresis by stimulation of cardiac function and by a direct action on the nephron. Although it is used infrequently as a diuretic, diuresis may be an observed side effect when it is used as a bronchodilator.

SUMMARY

The major driving force for the reabsorption of sodium at all four sodium reabsorption sites is the deficit of intracellular

sodium created by the activity of the basolateral membrane-bound Na^+,K^+-ATPase. In response, the luminal fluid sodium moves into the sodium-deficient cells by a luminal membrane-bound sodium-transporting system that is unique to each of the four sites. It is important for most diuretics to attain sufficient concentration in luminal fluid to inhibit a luminal membrane-bound sodium-transporting system; this usually is accomplished by a combination of glomerular filtration and active tubular secretion. The chemical structure of a diuretic dictates which of the four sodium-transporting sites will be inhibited. The site that is inhibited is one of the major determinants of the efficacy of the diuretic. The historical development of many diuretics has involved molecular modification of the chemical structure of sulfamoyl-containing compounds. This has yielded CA inhibitors, which inhibit the reabsorption of sodium bicarbonate at site 1; the thiazide and thiazide-like diuretics, which act at site 3; and the high-ceiling diuretics, which block sodium reabsorption at site 2. Increasing diuretic efficacy has occurred with the corresponding changes in the site of action of each of the three classes of diuretics. Predictable secondary effects that are dependent on a diuretic's site of action have also surfaced.

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CHAPTER 19

Cardiovascular Agents

George H. Cocolas

The treatment and therapy of cardiovascular disease have undergone dramatic changes since the 1950s. Data show that since 1968 and continuing through the 1990s, there has been a noticeable decline in mortality from cardiovascular disease. The basis for advances in the control of heart disease have been (1) a better understanding of the disease state, (2) the development of effective therapeutic agents, and (3) innovative medical intervention techniques to treat problems of the cardiovascular system.

The drugs discussed in this chapter are used for their action on the heart or other parts of the vascular system, to modify the total output of the heart or the distribution of blood to the circulatory system. These drugs are employed in the treatment of (1) angina, (2) cardiac arrhythmias, (3) hypertension, (4) hyperlipidemias, and (5) disorders of blood coagulation. This chapter also includes a discussion of hypoglycemic agents, thyroid hormones, and antithyroid drugs.

ANTIANGINAL AGENTS AND VASODILATORS

Most coronary artery disease conditions are due to deposits of atheromas in the intima of large and medium-sized arteries serving the heart. The process is characterized by an insidious onset of episodes of cardiac discomfort caused by ischemia from inadequate blood supply to the tissues. Angina pectoris (angina), the principal symptom of ischemic heart disease, is characterized by a severe constricting pain in the chest, often radiating from the precordium to the left shoulder and down the arm. The syndrome has been described since 1772, but it was not until 1867 that amyl nitrite was introduced for the symptomatic relief of angina pectoris.[1] It was believed at that time that anginal pain was precipitated by an increase in blood pressure and that the use of amyl nitrite reduced both blood pressure and, concomitantly, the work required of the heart. Later, it generally became accepted that nitrites relieved angina pectoris by dilating the coronary arteries and that changes in the work of the heart were of only secondary importance. It is now known that the coronary blood vessels in the atherosclerotic heart already are dilated and that ordinary doses of dilator drugs do not significantly increase blood supply to the heart; instead, relief from anginal pain is by a reduction of cardiac consumption of oxygen.[2]

Although vasodilators are used in the treatment of angina, a more sophisticated understanding of the hemodynamic response to these agents has broadened their clinical usefulness to other cardiovascular conditions. Because of their ability to reduce peripheral vascular resistance, vasodilators, including organonitrates, angiotensin-converting enzyme (ACE), inhibitors, are used to improve cardiac output in some patients with congestive heart failure.

The coronary circulation supplies blood to the myocardial tissues to maintain cardiac function. It is capable of reacting to the changing demands of the heart by dilation of its blood vessels to provide sufficient oxygen and other nutrients and to remove metabolites. Myocardial metabolism is almost exclusively aerobic, which makes blood flow critical to the support of metabolic processes of the heart. This demand is met effectively by the normal heart because it extracts a relatively large proportion of the oxygen delivered to it by the coronary circulation. The coronary blood flow is strongly dependent upon myocardial metabolism, which in turn is affected by work done by the heart and the efficiency of the heart. The coronary system normally has a reserve capacity that allows it to respond by vasodilatation to satisfy the needs of the heart during strenuous activity by the body.

Coronary atherosclerosis, one of the more prevalent cardiovascular diseases, develops with increasing age and may lead to a reduction of the reserve capacity of the coronary system. It most often results in multiple stenoses and makes it difficult for the coronary system to meet adequately the oxygen needs of the heart that occur during physical exercise or emotional duress. The insufficiency of the coronary blood flow (*myocardial ischemia*) in the face of increased oxygen demand produces angina pectoris.

The principal goal in the prevention and relief of angina is to limit the oxygen requirement of the heart so that the amount of blood supplied by the stenosed arteries is adequate. Nitrate esters, such as nitroglycerin, lower arterial blood pressure and, in turn, reduce the work of the left ventricle. This action is produced by the powerful vasodilating effect of the nitrates on the arterial system and, to an even greater extent, on the venous system. The result is a reduction of cardiac filling pressure and ventricular size. This reduces the work required of the ventricle and decreases the oxygen requirements, allowing the coronary system to satisfy the oxygen demands of myocardial tissue and relieve anginal pain.

INTERMEDIARY MYOCARDIAL METABOLISM

Energy metabolism by heart tissue provides an adequate supply of high-energy phosphate compounds to replace the adenosine triphosphate (ATP) that is continually being consumed in contraction, ion exchange across membranes, and other energy-demanding processes. Because of the high turnover rate of ATP in heart muscle, a correspondingly high rate of ATP production in the mitochondria is required.

Normal myocardial metabolism is aerobic, and the rate of oxygen utilization parallels the amount of ATP synthesized by the cells.[3] Free fatty acids are the principal fuel for myocardial tissue, but lactate, acetate, acetoacetate, and glucose are also oxidized to CO_2 and water. A large volume of the myocardial cell consists of mitochondria in which two-carbon fragments from free fatty acid breakdown are metabolized through the Krebs cycle. The reduced flavin and nicotinamide dinucleotides formed by this metabolism are reoxidized by the electron-transport chain because of the presence of oxygen (Fig. 19-1). In the hypoxic or ischemic heart, the lack of oxygen inhibits the electron-transport chain function and causes an accumulation of reduced flavin and nicotinamide coenzymes. As a result, fatty acids are converted to lipids rather than being oxidized. To compensate for this, glucose utilization and glycogenolysis increase, but the resulting pyruvate cannot be oxidized; instead, it is converted to lactate. A great loss of efficiency occurs as a result of the change of myocardial metabolism from aerobic to anaerobic pathways. Normally, 36 moles of ATP are formed from the oxidation of 1 mole of glucose, but only 2 moles are formed from its glycolysis. This great loss of high-energy stores during hypoxia thus limits the functional capacity of the heart during stressful conditions and is reflected by the production of anginal pain.

NITROVASODILATORS

Smooth Muscle Relaxation

The contractile activity of all types of muscle (smooth, skeletal) is regulated primarily by the reversible phosphorylation of myosin. Myosin of smooth muscle consists of two heavy chains (MW 200,000 each) that are coiled to produce a filamentous tail. Each heavy chain is associated with two pairs

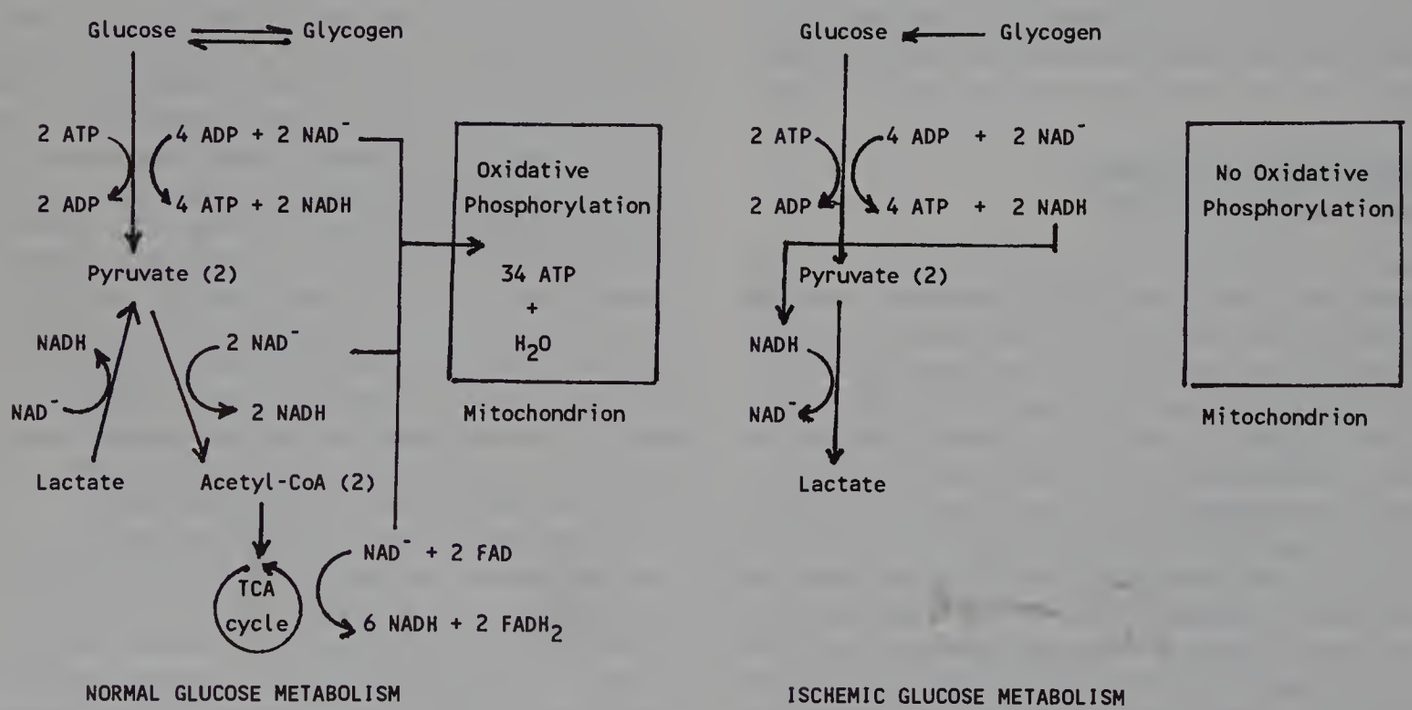


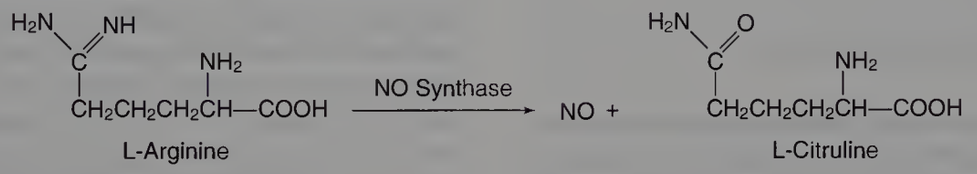
FIG. 19-1. Normal and ischemic myocardial metabolism of glucose. A total production of 36 moles of ATP results from the aerobic catabolism of 1 mole of glucose and utilization of NADH and $FADH_2$ in the oxidative phosphorylation process in mitochondria. When oxygen is not available, NADH and $FADH_2$ levels rise and shut off the tricarboxylic acid (TCA) cycle. Pyruvate is converted to lactate. Only 2 moles of ATP are formed from anaerobic catabolism of 1 mole of glucose. (Adapted from Giuliani, E. R., et al.: Cardiology: Fundamentals and Practice, 2nd ed. by permission of the Mayo Foundation, Rochester, MN.)

of light chains (MW 20,000 and 16,000) that serve as substrates for calcium/calmodulin-dependent protein kinases in the contraction process. Together with actin (MW 43,000) they participate in a cascade of biochemical events that are part of the processes of muscle contraction and relaxation (Fig. 19-2).

Cyclic nucleotides, cyclic adenosine monophosphate (cAMP) and, especially cyclic guanosine monophosphate (cGMP) play important roles in the regulation of smooth muscle tension. Cyclic AMP is the mediator associated with the smooth muscle relaxant properties of drugs such as β-adrenergic agonists. It activates the protein kinases that phosphorylate myosin light chain kinase (MLCK). Phosphorylation of MLCK inactivates this kinase and prevents its action with Ca^{2+} and calmodulin to phosphorylate myosin, which interacts with actin to cause contraction of smooth muscle (Fig. 19-2).

The activity of cGMP in smooth muscle relaxation is affected by exogenous and endogenous agents. It is suggested[4] that nitrovasodilators undergo metabolic transformation in vascular smooth muscle cells to form nitric oxide (NO). NO mediates smooth muscle relaxation by activating guanylate cyclase to increase intracellular concentrations of cGMP. Cyclic GMP activates protein kinases that can regulate free Ca^{2+} levels in the muscle cell and cause relaxation of smooth muscle by phosphorylating MLCK.

A short-lived free radical gas, NO is widely distributed in the body and plays an important role by its effect through cGMP on the smooth muscle vasculature. It is synthesized in the vascular endothelial cell from the semiessential amino acid L-arginine by NO synthase. After production in the cell, it diffuses to the smooth muscle cell, where it activates the enzyme guanylate cyclase, which leads to an increase in cGMP and then muscle relaxation (Fig. 19-3). Endothelium-derived relaxing factor (EDRF), released from the endothelial cell to mediate its smooth muscle-relaxing properties through cGMP, has been found to be identical with NO.



Inhibitors of phosphodiesterases of cAMP and cGMP also cause smooth muscle relaxation. These inhibitors increase cellular levels of cAMP and cGMP by preventing their hydrolysis to AMP and GMP, respectively. Drugs such as papaverine (see Chap. 17) and theophylline (see Chap. 18), which relax smooth muscle, do so in part by inhibiting phosphodiesterases.

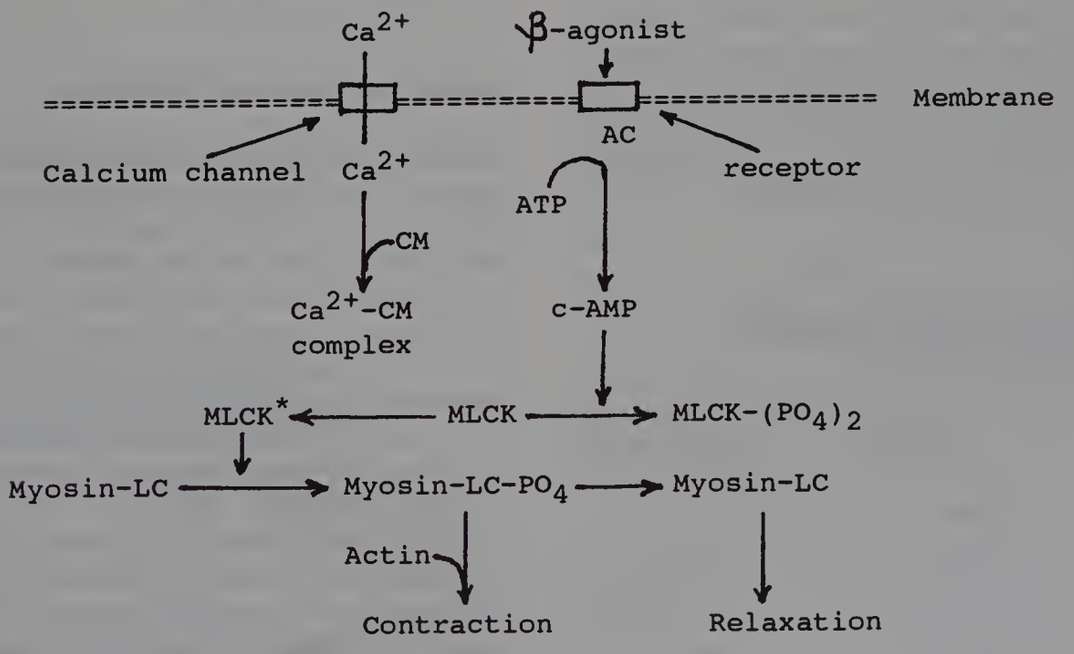


FIG. 19-2. *Regulation of smooth muscle contraction. Contraction is triggered by an influx of Ca^{2+}. The increase of free Ca^{2+} causes binding to calmodulin (CM). The Ca^{2+}–CM complex binds to myosin light chain kinase (MLCK) to cause its activation (MLCK*). MLCK* phosphorylates myosin, which combines with actin to produce contraction of smooth muscle. Myosin is dephosphorylated in the presence of myosin phosphatase to cause muscle relaxation.* β-agonists activate adenylate cyclase (AC) to raise levels of cAMP, which in turn activate kinases that phosphorylate MLCK, inactivating it to prevent muscle contraction.

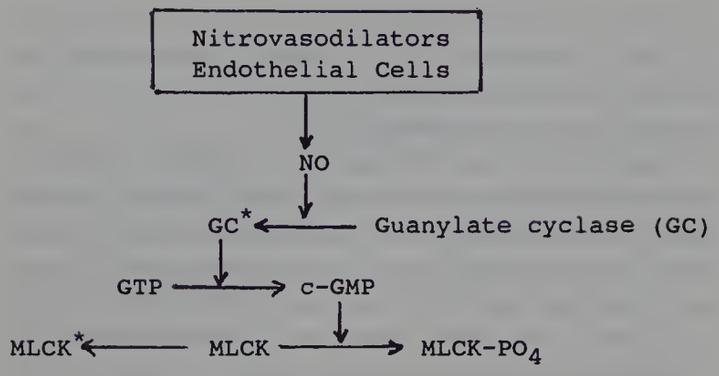


FIG. 19-3. Mechanism of nitrovasodilators. Nitric oxide (NO) formed in smooth muscle from nitrovasodilators or from endothelial cells (EDRF) activates guanylate cyclase (GC). GC* activates cGMP-dependent protein kinases that phosphorylate myosin light chain kinase, causing its inactivation and subsequent muscle relaxation (see also Fig. 19-2).*

Metabolism of Nitrovasodilators

Organic nitrates are metabolized rapidly after oral administration by the liver, kidney, lungs, intestinal mucosa, and vascular tissue. Buccal absorption reduces the immediate hepatic destruction of the organic nitrates because only 15% of the cardiac output is delivered to the liver; this allows a transient but effective circulating level of the intact organic nitrate before it is inactivated.[5]

Organic nitrates, nitrites, nitroso compounds, and a variety of other nitrogen-containing substances, such as sodium nitroprusside, for the most part cause their pharmacological effects by generating or releasing NO in situ. In some ways, these drugs are viewed as "replacement agents" for the endogenous NO generated by the NO synthase pathway from arginine. The mechanisms by which vasodilatory drugs release NO have become better understood recently. Table 19-1 shows the oxidation state of various nitrosyl compounds that would be common in nitrovasodilatory drugs. A common feature of these drugs is that they release nitrogen in the form of NO and contain nitrogen in an oxidation state higher than −3 (as would occur in ammonia, amines, amides, and most biological nitrogen compounds). The nitrogen in NO has an oxidation state of +2. Compounds such as nitroprusside, nitrosoamines, and nitrothiols with oxidation states of +3 release NO nonenzymatically. Although their spontaneous liberation of NO is by an unknown mechanism, it involves only a one-electron reduction, which may occur upon exposure of these chemicals to the variety of reducing agents in the tissue of vascular smooth muscle membranes. Organic nitrites such as amyl nitrite react with available thiol groups to form unstable S-nitrosothiols, which rapidly decompose to NO by homolytic cleavage of their S–N bond. In mammalian smooth muscle, this will occur almost exclusively with glutathione as the most abundant thiol compound.[6]

TABLE 19-1

NITROSYL VASODILATORY SUBSTANCES AND THEIR OXIDATION STATE

Nitrosyl Compound	*Structure*	*Nitrogen Oxidation State*
Nitric oxide	N=O$^{\bullet}$	+2
Nitrite	—ONO	+3
Nitrate	—ONO_2	+5
Organic nitrite	R—O—N=O	+3
Nitrosothiol	R—S—N=O	+3
Organic nitrate	R—O—NO_2	+5
Thionitrate	R—S—NO_2	+5
Nitroprusside	$[(CN)_5Fe—N=O]^{2-}$	+3

(Reprinted from Harrison, D. G., and Bates, J. M.: Circulation 87: 1462, 1993, with permission from the American Heart Association.)

The pharmacodynamic action of nitroglycerin is preceded by metabolic changes that follow a variety of paths. Biotransformation of nitroglycerin to the dinitrates and the increase of intracellular cGMP precede vascular relaxation. Sulfhydryl-containing compounds, such as cysteine, react chemically with organic nitrates to form inorganic nitrite ions. The release of NO from an organic nitrate, such as nitroglycerin, appears to occur in a stepwise fashion involving nonenzymatic and enzymatic steps. Because nitroglycerin requires a three-electron reduction to release NO, thiols may be involved in the process. Nitroglycerin may decompose nonenzymatically by interaction with a variety of thiols, such as cysteine or *N*-acetylcysteine, that may be present in tissue to form a nitrosothiol intermediate before undergoing enzymatic transformation to release NO. Nitroglycerin also readily releases NO by acting on an enzyme system attached to the cellular surface membrane of smooth muscle. The process may include glutathione-*s*-transferases, which convert nitroglycerin to a vasoinactive nitrite, which then may release NO nonenzymatically.[7]

Esters of Nitrous and Nitric Acids

Inorganic acids, like organic acids, will form esters with an alcohol. Pharmaceutically, the important ones are sulfate, nitrite, and nitrate. Sulfuric acid forms organic sulfates, of which methyl sulfate and ethyl sulfate are examples.

Nitrous acid (HNO_2) esters may be formed readily from an alcohol and nitrous acid. The usual procedure is to mix sodium nitrite, sulfuric acid, and the alcohol. Organic nitrites are generally very volatile liquids that are only slightly soluble in water but soluble in alcohol. Preparations containing water are very unstable because of hydrolysis.

The organic nitrates and nitrites and the inorganic nitrites have their primary utility in the prophylaxis and treatment of angina pectoris. They have a more limited application in treating asthma, gastrointestinal spasm, and certain cases of migraine headache. Their application may be regarded as causal therapy since they act by substituting an endogenous factor, the production or release of NO, which may be impaired under pathophysiological circumstances associated

with dysfunction of the endothelial tissue. Nitroglycerin (glyceryl trinitrate) was one of the first members of this group to be introduced into medicine and remains an important member of the group. By varying the chemical structure of the organic nitrates, differences in speed of onset, duration of action, and potency can be obtained (Table 19-2). Although the number of nitrate ester groups may vary from two to six or more, depending on the compound, there is no direct relationship between the number of nitrate groups and the level of activity.

It appears that the higher the oil/water partition coefficient of the drug, the greater the potency. The orientation of the groups within the molecule also may affect potency. Lipophilicity of the nitrogen oxide-containing compound produces a much longer response of vasodilatory action. The highly lipophilic ester nitroglycerin permeates the cell membrane, allowing continual formation of NO within the cell. The same effect appears to occur for sodium nitroprusside, nitroso compounds, and other organic nitrate and nitrite esters.[4]

Antianginal Action of Nitrovasodilators

The action of short-acting sublingual nitrates in the relief of angina pectoris is complex. Although the sublingual nitrates relax vascular smooth muscle and dilate the coronary arteries of normal humans, there is little improvement of coronary blood flow when these chemicals are administered to individuals with coronary artery disease. Nitroglycerin is an effective antianginal agent because it causes redistribution of coronary blood flow to the ischemic regions of the heart and reduces myocardial oxygen demand. This latter effect is produced by a reduction of venous tone owing to the nitrate vasodilating effect and a pooling of blood in the peripheral veins that results in a reduction in ventricular volume, stroke volume, and cardiac output. It also causes reduction of peripheral resistance during myocardial contractions. The combined vasodilatory effects cause a decrease in cardiac work and reduce oxygen demand.

TABLE 19-2

RELATIONSHIP BETWEEN SPEED AND DURATION OF ACTION OF SODIUM NITRITE AND CERTAIN INORGANIC ESTERS

Compound	Action Begins (min)	Maximum Effect (min)	Duration of Action (min)
Amyl nitrite	0.25	0.5	1
Nitroglycerin	2	8	30
Isosobide dinitrate	3	15	60
Sodium nitrite	10	25	60
Erythrityl tetranitrate	15	32	180
Pentaerythritol tetranitrate	20	70	330

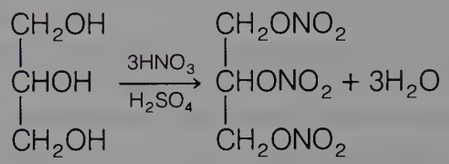
Products

Amyl Nitrite, USP. Isopentyl nitrite. Amyl nitrite [$(CH_3)_2CHCH_2CH_2ONO$] is a mixture of isomeric amyl nitrites but is principally isoamyl nitrite. It may be prepared from amyl alcohol and nitrous acid by several procedures. Usually, amyl nitrite is dispensed in ampul form and used by inhalation or orally in alcohol solution. Currently, it is recommended for treating cyanide poisoning; although not the best antidote, it does not require intravenous injections.

Amyl nitrite is a yellowish liquid having an ethereal odor and a pungent taste. It is volatile and inflammable at room temperature. Amyl nitrite vapor forms an explosive mixture in air or oxygen. Inhalation of the vapor may involve definite explosion hazards if a source of ignition is present, as both room and body temperatures are within the flammability range of amyl nitrite mixtures with either air or oxygen. It is nearly insoluble in water but is miscible with organic solvents. The nitrite also will decompose into valeric acid and nitric acid.

Nitroglycerin. Glyceryl trinitrate is the trinitrate ester of glycerol and is listed as available in tablet form in the *USP*. It is prepared by carefully adding glycerin to a mixture of nitric and fuming sulfuric acids. This reaction is exothermic and the reaction mixture must be cooled to between 10° and 20°C.

The ester is a colorless oil, with a sweet, burning taste. It is only slightly soluble in water, but it is soluble in organic solvents.



Nitroglycerin

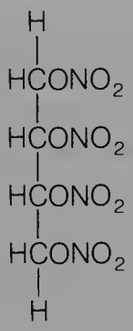
Nitroglycerin is used extensively as an explosive in dynamite. A solution of the ester, if spilled or allowed to evaporate, will leave a residue of nitroglycerin. To prevent an explosion of the residue, the ester must be decomposed by the addition of alkali. It has a strong vasodilating action and, because it is absorbed through the skin, is prone to cause headaches among workers associated with its manufacture. In medicine, it has the action typical of nitrites, but its action develops more slowly and is of longer duration. Of all the known coronary vasodilatory drugs, nitroglycerin is the only one capable of stimulating the production of coronary collateral circulation and the only one able to prevent experimental myocardial infarction by coronary occlusion.

Previously, the nitrates were thought to be hydrolyzed and reduced in the body to nitrites, which then lowered the blood pressure. However, this is not true. The mechanism of vasodilation of nitroglycerin through its formation of NO was described above.

Nitroglycerin tablet instability was reported in molded sublingual tablets.[8] The tablets, although uniform when manufactured, lost potency both because of volatilization of ni-

troglycerin into the surrounding materials in the container and intertablet migration of the active ingredient. Nitroglycerin may be stabilized in molded tablets by incorporating a "fixing" agent such as polyethylene glycol 400 or polyethylene glycol 4000.[9] In addition to sublingual tablets, the drug has been formulated into an equally effective lingual aerosol for patients who have problems with dissolution of sublingual preparations because of dry mucous membranes. Transdermal nitroglycerin preparations appear to be less effective than other long-acting nitrates, as absorption from the skin is variable.

Diluted Erythrityl Tetranitrate, USP. Erythritol tetranitrate, 1,2,3,4-butanetetrol, tetranitrate (*R**, *S**)- (Cardilate) is the tetranitrate ester of erythritol and nitric acid. It is prepared in a manner analogous to that used for nitroglycerin. The result is a solid, crystalline material. This ester is also very explosive and is diluted with lactose or other suitable inert diluents to permit safe handling; it is slightly soluble in water and soluble in organic solvents.

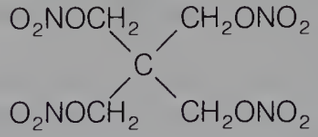


Erythrityl Tetranitrate

Erythrityl tetranitrate requires slightly more time than nitroglycerin to produce its effect, which is of longer duration. It is useful where mild, gradual, and prolonged vascular dilation is warranted. The drug is used in the treatment of, and as a prophylaxis against, attacks of angina pectoris and to reduce blood pressure in arterial hypertonia.

Erythrityl tetranitrate produces a reduction of cardiac preload as a result of pooling of blood on the venous side of the circulatory system by its vasodilating action. This action results in a reduction of blood pressure on the arterial side during stressful situations and is an important factor in preventing the precipitation of anginal attacks.

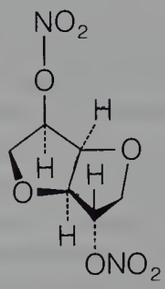
Diluted Pentaerythritol Tetranitrate, USP. 2,2-Bis (hydroxymethyl)-1,3-propanediol tetranitrate (Peritrate, Pentritol). This compound is a white, crystalline material with a melting point of 140°C. It is insoluble in water, slightly soluble in alcohol, and readily soluble in acetone. The drug is a nitric acid ester of the tetrahydric alcohol pentaerythritol and is a powerful explosive. Accordingly, it is diluted with lactose or mannitol or other suitable inert diluents to permit safe handling.



Pentaerythritol Tetranitrate

It relaxes smooth muscle of smaller vessels in the coronary vascular tree. Pentaerythritol tetranitrate is used prophylactically to reduce the severity and frequency of anginal attacks and usually is administered in sustained-release preparations to increase its duration of action.

Diluted Isosorbide Dinitrate, USP. 1,4:3,6-Dianhydro-D-glucitol dinitrate (Isordil, Sorbitrate) occurs as a white, crystalline powder. Its water solubility is about 1 mg/mL.



Isosorbide Dinitrate

Isosorbide dinitrate, as a sublingual or chewable tablet, is effective in the treatment or prophylaxis of acute anginal attacks. When given sublingually, the effect begins in about 2 minutes, with a shorter duration of action than when given orally. Oral tablets are not effective in acute anginal episodes; the onset of action ranges from 15 to 30 minutes.

CALCIUM ANTAGONISTS

Excitation–Contraction Coupling Muscle

Stimulation of the cardiac cell initiates the process of excitation, which has been related to ion fluxes through the cell membrane. Depolarization of the tissue in the atria of the heart is mediated by two inwardly directed ionic currents. When the cardiac cell potential reaches its threshold, ion channels in the membrane are opened and Na^+ enters the cell through ion channels. These channels give rise to the fast sodium current that is responsible for the rapidly rising phase, phase 0, of the ventricular action potential (Fig. 19-4). The second current is caused by the slow activation of an L-type Ca^{2+} ion channel that allows the movement of Ca^{2+} into the cell. This "slow channel" contributes to the maintenance of the plateau phase (phase 2) of the cardiac action potential. It is now understood that the Ca^{2+} that enters with the action potential initiates a second and larger release of Ca^{2+} from the sarcoplasmic reticulum in the cell. This secondary release of Ca^{2+} is sufficient to initiate the contractile process of cardiac muscle.

Contraction of cardiac and other muscle occurs from a reaction between actin and myosin. In contrast to smooth vascular muscle, the contractile process in cardiac muscle involves a complex of proteins (troponin I, C, and T and tropomyosin) attached to myosin that modulate the interaction between actin and myosin. Free Ca^{2+} ions bind to troponin C, uncovering binding sites on the actin molecule and

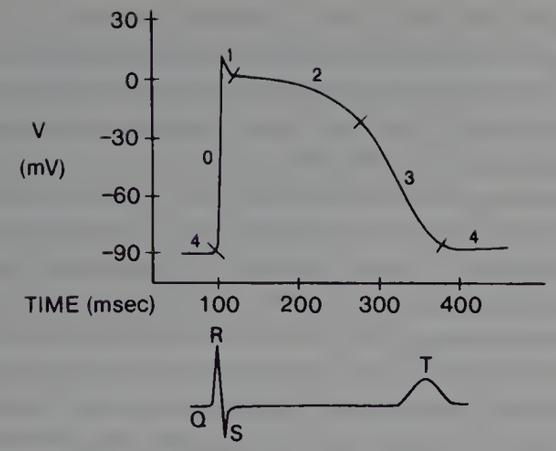


FIG. 19-4. Diagrammatic representation of the membrane action potential, as recorded from a Purkinje fiber, and an electrogram recorded from an isolated ventricular fiber. The membrane resting potential is 90 mV relative to the exterior of the fiber. At the point of depolarization, there is a rapid change (phase 0) to a more positive value. The phases of depolarization and repolarization are indicated by the numbers 0, 1, 2, 3, 4. Note that phases 0 and 3 of the membrane action potential correspond in time to the inscription of the QRS and T waves, respectively, of the local electrogram.

allowing interaction with myosin, causing contraction of the muscle. The schematic diagram in Figure 19-5 shows the sequence of events.[10] Contraction of vascular smooth, like cardiac, muscle is regulated by the concentration of cytoplasmic Ca^{2+} ions. However, the mechanism by which the contraction is effected includes a calcium/calmodulin-dependent kinase instead of a Ca^{2+}-sensitive troponin–tropomyosin complex (Fig. 19-2). The activating effect is dependent on a different type of reaction. The elevated free cytosolic Ca^{2+} in vascular smooth muscle cells binds to a high-affinity binding protein, calmodulin.

Ion Channels and Calcium

Calcium ions play an important role in the regulation of many cellular processes, such as synaptic transmission and muscle contraction. The role of calcium in these cellular

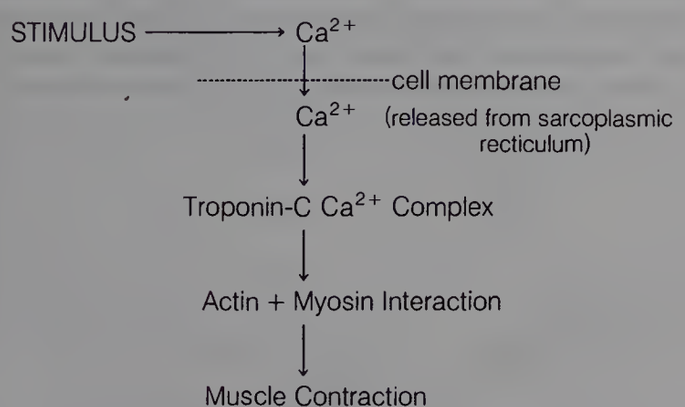


FIG. 19-5. Sequence of events showing excitation-contraction coupling in cardiac muscle.

functions is as a second messenger, for example, regulating enzymes and ion channels. The entry of extracellular Ca^{2+} into the cytosol of myocardial cells and the release of Ca^{2+} from intracellular storage sites is important for initiating contractions of the myocardium. Normally, the concentration of Ca^{2+} in the extracellular fluid is in the millimolar range, whereas the intracellular concentration of free Ca^{2+} is less than 10^{-7} *M* even though the total cellular concentration may be 10^{-3} *M* or higher. Most of the Ca^{2+} is stored within intracellular organelles or tightly bound to intracellular proteins. The free Ca^{2+} needed to satisfy the requirements of a contraction resulting from a stimulus may result from activation of calcium channels on the cell membrane and/or the release of calcium from bound internal stores. Each of these methods of increasing free cytosolic Ca^{2+} involves channels that are selective for the calcium ion. Calcium channel blockers reduce or prevent the increase of free cytosolic calcium ions by interfering with the transport of calcium ions through these pores.

Calcium is one of the most common elements on earth. The majority of calcium involved in biological systems occurs as hydroxyapatite, a static, stabilizing structure like that found in bone. The remaining calcium is ionic (Ca^{2+}). Ionic calcium functions as a biochemical regulator, more often within the cell. The importance of calcium ions to physiological functions was realized first by Ringer, who observed in 1883 the role of Ca^{2+} in cardiac contractility.

It is well established that the ionic composition of the cytosol in excitable cells, including cardiac and smooth muscle cells, is controlled to a large extent by the plasma membrane, which prevents the free movement of ions across this barrier. Present in the membranes are ion-carrying channels that open in response to either a change in membrane potential or the binding of a ligand. Calcium-sensitive channels include (1) Na^+:Ca^{2+} exchanger, which transports three Na^+ ions in return for one Ca^{2+}; (2) a voltage-dependent Ca^{2+} channel, which provides the route for entry of Ca^{2+} for excitation and contraction in cardiac and smooth muscle cells and is the focus of the channel-blocking agents used in medicine; and (3) receptor-operated Ca^{2+} channels mediated by ligand binding to membrane receptors as in the action of epinephrine on the α-adrenergic receptor. The membrane of the sarcolemma within the cell also has ion-conducting channels that facilitate movement of Ca^{2+} ions from storage loci in the sarcoplasmic recticulum.

Four types of calcium channels, differing in location and function, have been identified: (1) L-type, located in skeletal, cardiac, and smooth muscles, causing contraction of muscle cells; (2) T-type, found in pacemaker cells, causing Ca^{2+} entry, inactivated at more negative potentials and more rapidly than the L-type; (3) N-type, found in neurons and acting in transmitter release; and (4) P-type, located in Purkinje cells, function is unknown at this time.

Calcium antagonists act only on the L-type channel to produce their pharmacological effects. The L-channels are so called because their action is long-lasting once the mem-

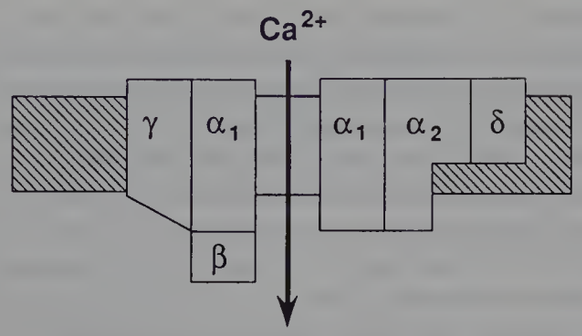


FIG. 19-6. Schematic representation of an L-type Ca^{2+} channel.

brane has been depolarized. L-channels, in order to open once the membrane has been depolarized, must be phosphorylated. Although there are similarities between L-type calcium channels that exist in cardiac and smooth muscle, there are differences between the two. Cardiac L-channels are activated through β-adrenergic stimulation via a cAMP-dependent phosphorylation process,[11] while L-channels in smooth muscle may be regulated by the inositol phosphate system linked to G-protein-coupled, receptor-linked phospholipase C activation.[12]

Calcium Channel Blockers. The L-type calcium channel, acted on by calcium channel blockers, consists of five different subunits, designated α_1, α_2, β, γ, and δ. The α_1 subunit provides the central pore of the channel (Fig. 19-6). Calcium channel blockers can be divided conveniently into the three different chemical classes of the prototype drugs that have been used: phenylalkylamines (verapamil); 1,4-dihydropyridines (nifedipine); and benzothiazepines (diltiazem). These prototype compounds sometimes are termed the ''first generation'' of calcium channel blockers as two of the groups of drug classes have been expanded by the introduction of a ''second'' generation of more potent analogues (Table 19-3).

The specific Ca^{2+} channel antagonists verapamil, nifedipine, and diltiazem interact at specific sites on the calcium channel protein. These blockers do not occlude the channel physically but bind to sites in the channel as they can promote both channel activation and antagonism. Affinity for binding sites on the channel varies depending on the status of the channel. The channel can exist in either the open (O), resting (R), or inactivated (I) state, the equilibrium between which is determined by stimulus frequency and membrane potential (Fig. 19-7). Verapamil and diltiazem do not bind to the channel when in the resting state, only after the channel has been opened. They are ionized, water-soluble Ca^{2+}-entry blockers that reach their binding sites by the hydrophilic pathway when the channel is open. Verapamil and diltiazem are use-dependent (i.e., their Ca^{2+}-blocking activity is a function of the frequency of contractions). An increase in contraction frequency causes a reduction, rather than an augmentation, of contractions. Nifedipine is a neutral molecule at physiological *p*H and can cause interference with the Ca^{2+} in the open or closed state. In the closed state, nifedipine can traverse the phospholipid bilayer to reach its binding site because of its lipid solubility.

TABLE 19-3

FIRST- AND SECOND-GENERATION CALCIUM CHANNEL BLOCKERS

Chemical Classification	*First-Generation*	*Second-Generation*
Phenylalkylamines	Verapamil	Anipamil Bepridil
1,4-Dihydropyridine	Nifedipine	Amlodipine Felodipine Isradipine Nicardipine Nimodipine
Benzothiazepine	Diltiazem	—

Cardiovascular Effects of Calcium Ion Channel Blockers

All Ca^{2+} antagonists yet developed are vasodilators. Vasodilation is due to the uncoupling of the contractile mechanism of vascular smooth muscle, which requires Ca^{2+}. Coronary artery muscle tone is reduced in healthy humans but is particularly pronounced in a condition of coronary spasm. Peripheral arteriole resistance is reduced more than venous beds. The vasodilatory effect of these drugs is the basis for their use in the control of angina and hypertension.[13]

Although verapamil, nifedipine, and diltiazem are able to cause vasodilation, they are not equally effective at blocking the Ca^{2+} channels found in various tissues. The phenylalkylamine verapamil and the benzothiazepine diltiazem have both cardiac and vascular actions. These drugs have antiarrhythmic, antianginal, and antihypertensive activity. They depress the cardiac neural network, resulting in a slowing of sinus node automaticity, a prolongation of atrioventricular (AV) nodal conductance, and a depressing of myocardial contractility, as well as reducing peripheral vascular resistance to prevent a coronary vascular spasm. Nifedipine and other 1,4-dihydropyridines are more effective at causing vasodilation than affecting pacemaker and tension responses in the heart. This is especially important as selectivity occurs as a consequence of disease states. Hypertensive smooth muscle is more sensitive to Ca^{2+} channel blockers than nor-

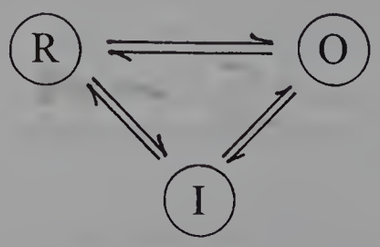


FIG. 19-7. Schematic representation of an ion channel existing in an equilibrium of resting (R), open (O), and inactivated (I) states.

motensive tissue.[14] This makes verapamil and diltiazem more useful in ischemic conditions as they have a more profound effect on cardiac muscle calcium channels.[15]

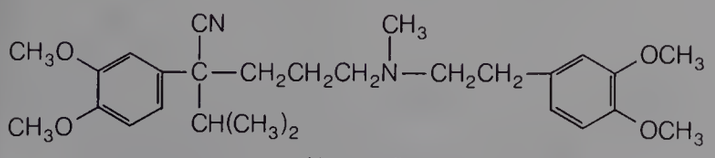
The inhibition of Ca^{2+} influx into cardiac tissue by Ca^{2+} antagonists is also the basis of the use of these drugs as antiarrhythmic agents. The Ca^{2+} channel blockers have a dampening effect on Ca^{2+}-dependent automaticity in the regular pacemaker cells in the sinoatrial (SA) node and depress the origination of ectopic foci. Calcium antagonists can block reentry pathways in myocardial tissue, an integral component of arrhythmias. Numerous side effects in the heart, such as bradycardia, decrease in cardiac contractility, and reduction of AV conductance, are traced to Ca^{2+} channel-blocking activity.

Products

Verapamil. 5-[3,4-Dimethoxyphenethyl)methylamino]-2-(3,4-dimethoxyphenyl)-2-isopropylvaleronitrile (Calan, Isoptin). Verapamil was introduced in 1962 as a coronary vasodilator and is the prototype of the Ca^{2+} antagonists used in cardiovascular diseases. It is employed in the treatment of angina pectoris, arrhythmias from ischemic myocardial syndromes, and supraventricular arrhythmias.

Verapamil's major effect is on the slow Ca^{2+} channel. The result is a slowing down of AV conduction and the sinus rate. This inhibition of the action potential inhibits one limb of the reentry circuit believed to underlie most paroxysmal supraventricular tachycardias that use the AV node as a reentry point. It is categorized as a class IV antiarrhythmic drug (see "Classes of Antiarrhythmic Drugs" below). Hemodynamically, verapamil causes a change in the preload, afterload, contractility, heart rate, and coronary blood flow. The drug reduces systemic vascular resistance and mean blood pressure, with minor effects on cardiac output.

Verapamil is a synthetic compound possessing slight structural similarity to papaverine. It can be separated into its optically active isomers, of which the levorotatory enantiomer is the most potent. It is absorbed rapidly after oral administration. The drug is metabolized quickly and, as a result, has low bioavailability. The liver is the main site of first-pass metabolism, forming several products. The preferential metabolic step involves *N*-dealkylation, followed by *O*-demethylation, and subsequent conjugation of the product before elimination. The metabolites have no significant biological activity. Verapamil has an elimination half-life of approximately 5 hr.



Verapamil

The route traveled by a Ca^{2+} channel blocker, such as verapamil, to its receptor site parallels that observed with many local anesthetic-like antiarrhythmic agents. It is believed that verapamil, as do most of the Ca^{2+} channel blockers, crosses the cell membrane in an uncharged form to gain access to its site of action on the intracellular side of the membrane. Data show a greater affinity of verapamil and other Ca^{2+} channel blockers to the inactivated state of the channel.[16]

Diltiazem Hydrochloride. (+)-*cis*-3-(Acetoxy)-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)one hydrochloride (Cardizem). Diltiazem hydrochloride was developed and introduced in Japan as a cardiovascular agent to treat angina pectoris. It was observed to dilate peripheral arteries and arterioles. The drug increases myocardial oxygen supply by relieving coronary artery spasm and reduces myocardial oxygen demand by decreasing heart rate and reducing overload. Diltiazem hydrochloride is used in patients with variant angina. The drug has electrophysiological properties similar to verapamil, being also employed in clinically similar treatment conditions as an antiarrhythmic agent, but it is less potent.

The drug is absorbed rapidly and almost completely from the digestive tract. It reaches peak plasma levels within 1 hr after administration in gelatin capsules. Oral formulations on the market are sustained-release preparations providing peak plasma levels 3 to 4 hr after administration.

Diltiazem hydrochloride is metabolized extensively after oral dosing by first-pass metabolism. As a result, the bioavailability is about 40% of the administered dose. The drug undergoes several biotransformations, including deacetylation, oxidative *O*- and *N*-demethylations, and conjugation of the phenolic metabolites. Of the various metabolites (Fig. 19-8), only the primary metabolite, deacetyldiltiazem, is pharmacologically active. Deacetyldiltiazem has about 40% to 50% of the potency of the parent compound.

Nifedipine. Dimethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylate (Adalat, Procardia). Nifedipine is a dihydropyridine derivative that bears no structural resemblance to the other calcium antagonists. It is not a nitrate, but its nitro group is essential for its antianginal effect.[17] The drug has potent peripheral vasodilatory properties. It inhibits the voltage-dependent calcium channel in the vascular smooth muscle but has little or no direct depressant effect on the SA or AV nodes, even though it inhibits calcium current in normal and isolated cardiac tissues. Nifedipine is more effective in patients whose anginal episodes are due to coronary vasospasm and is used in the treatment of vasospastic angina as well as classic angina pectoris. Because of its strong vasodilatory properties, it is used in selected patients to treat hypertension.

Nifedipine is absorbed efficiently on oral or buccal administration. A substantial amount (90%) is protein-bound. Systemic availability of an oral dose of the drug may be approximately 65%. Two inactive metabolites are the major products of nifedipine metabolism and are found in equilibrium with each other (Fig. 19-9). Only a trace of unchanged nifedipine is found in the urine.[18]

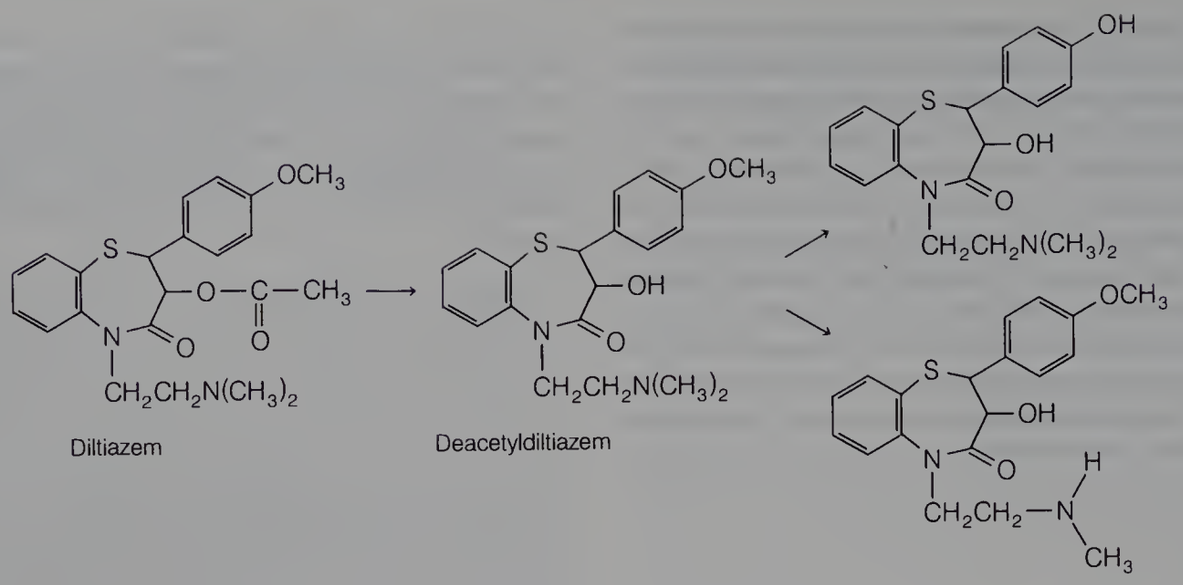
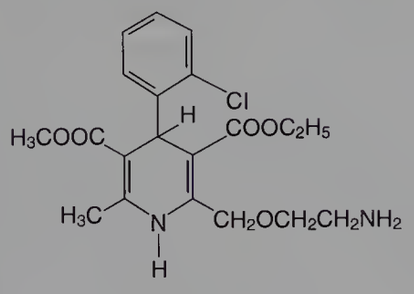


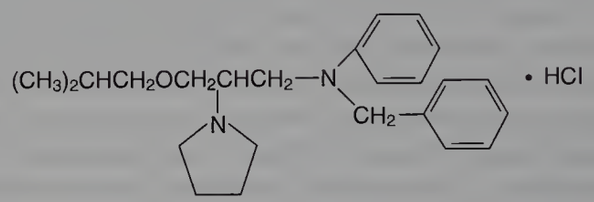
FIG. 19-8. *Biotransformations of diltiazem.*

Amlodipine. 3,5-Pyridinedicarboxylic acid, 2-[(2-aminoethoxy)methyl] - 4 - (2-chlorophenyl)-1,4-dihydro-6-methyl-3-ethyl 5-methyl ester (Norvasc). Amlodipine is a second-generation 1,4-dihyropyridine derivative of the prototypical molecule nifedipine. Like most of the second-generation dihydropyridine derivatives, it has a greater selectivity for the vascular smooth muscle than myocardial tissue, a longer half-life (34 hr), and less negative inotropy relative to the prototypical nifedipine. Amlodipine is used in the treatment of chronic stable angina and in the management of mild to moderate essential hypertension. It is marked as the benzene sulfonic acid salt (besylate).



Amlodipine

Bepridil Hydrochloride. 1-Pyrrolidineethylamine, β-[(methylpropoxy)methyl]-*N*-phenyl-*N*-(phenylmethyl) hydrochloride (Bepadin, Vascor). Bepridil hydrochloride is a second-generation alkylamine-type channel blocker. Its actions are less specific than those of the three prototypical channel blockers, verapamil, diltiazem, and nifedipine. In addition to being a Ca^{2+} channel blocker, it inhibits sodium flow into the heart tissue and lengthens cardiac repolarization, causing bradycardia. Caution should be used if given to a patient with hypokalemia. Bepridil hydrochloride is used for stable angina. The drug has a half-life of 33 hr and is highly bound to protein (99%).



Bepridil Hydrochloride

Felodipine. 3,5-Pyridinedicarboxylic acid, 4-(2,3-dichlorophenyl)1,4-dihydro-2,6-dimethyl-, ethyl methyl ester (Plendil). Felodipine is a second-generation dihydropyridine channel blocker of the nifedipine type. It is more selective for vascular smooth muscle than myocardial tissue and serves as an effective vasodilator. The drug is used in the treatment

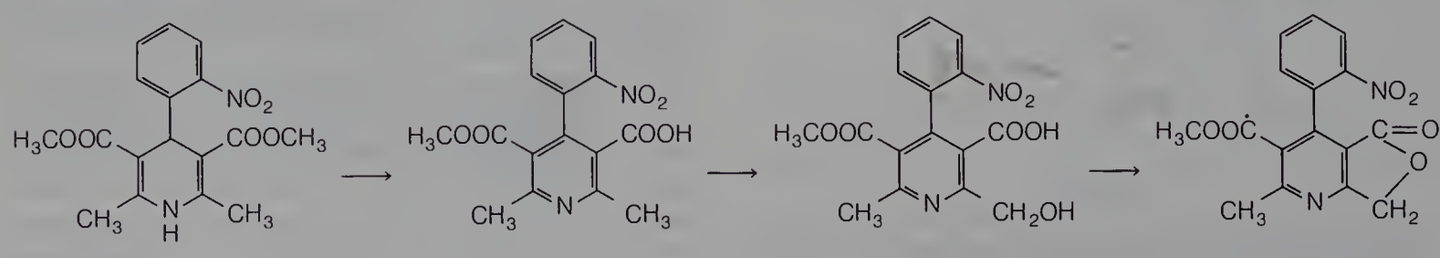
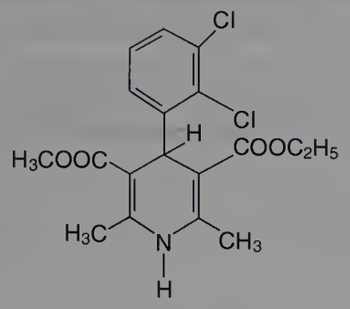


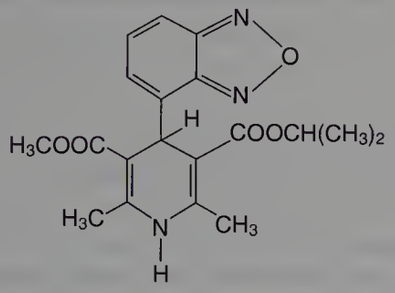
FIG. 19-9. *Nifedipine metabolism.*

of angina and mild to moderate essential hypertension. Felodipine, like most of the dihydropyridines, exhibits a high degree of protein binding and has a half-life ranging from 10 to 18 hr.



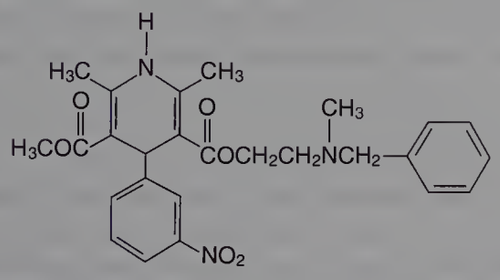
Felodipine

Isradipine. 3,5-Pyridinecarboxylic acid, 4-(4-benzofuranazyl)-1,4-dihydro-2,6-dimethyl-, methyl 1-methylethyl ester (DynaCirc). Isradipine is another second-generation dihydropyridine-type channel blocker. This drug, like the other second-generation analogues, is more selective for vascular smooth muscle than for myocardial tissue. It is effective in the treatment of stable angina, reducing the frequency of anginal attacks and the need to use nitroglycerin.



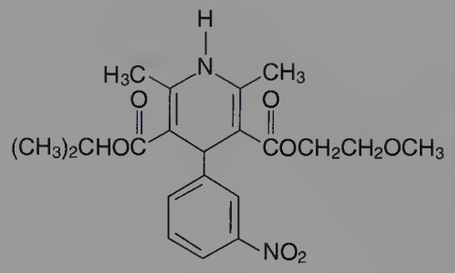
Isradipine

Nicardipine. 3,5-Pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl) methyl 2-[methyl(phenylmethyl)amino] ethyl ester hydrochloride (Cardene). Nicardipine is a more potent vasodilator of the systemic, coronary, cerebral, and renal vasculature and has been used in the treatment of mild, moderate, and severe hypertension. The drug is also used in the management of stable angina.



Nicardipine

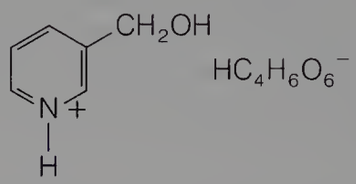
Nimodipine. 3,5-Pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-2-methoxyethyl, 1-methylethyl ester (Nimotop). Nimodipine is another dihydropyridine calcium channel blocker but differs in that it more effectively dilates the cerebral blood vessels than the other dihydropyridine derivatives. The drug is indicated for treatment of subarachnoid hemorrhage-associated neurological deficits.



Nimodipine

MISCELLANEOUS VASODILATORS

Nicotinyl Alcohol Tartrate. β-Pyridylcarbinol bitartrate; 3-pyridinemethanol tartrate (the alcohol corresponding to nicotinic acid) (Roniacol).



Nicotinyl Alcohol Tartrate

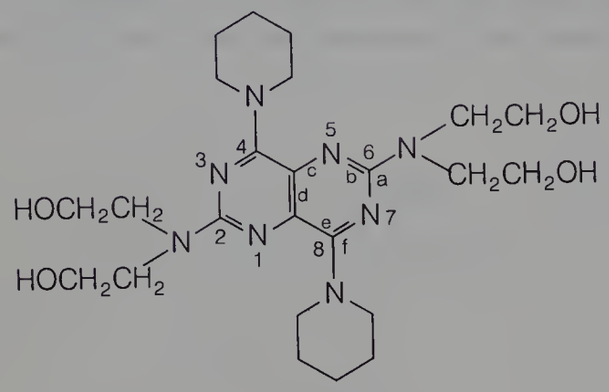
The free amine alcohol is a liquid with a boiling point of 145°C. It forms salts with acids. The bitartrate is crystalline and is soluble in water, alcohol, and ether. An aqueous solution has a sour taste, partly because of the bitartrate form of the salt.

In 1950, it was introduced as a vasodilator, following the lead that nicotinic acid dilated peripheral blood vessels. The drug, in fact, is converted by the body into nicotinic acid. The drug also has a direct relaxing effect on peripheral blood vessels, producing a flushing effect. It is given orally in tablets or as an elixir. Medicinal use includes the treatment of vascular spasm, Raynaud's disease, Buerger's disease, ulcerated varicose veins, chilblains (frostbite), migraine, Meniere's syndrome, and most conditions requiring a vasodilatory effect. The usual dose is 150 to 300 mg.

Dipyridamole. 2, 2′,2″,2‴-[(4,8-Di-1-piperidino-pyrimido[5,4-*d*]pyrimidine-2,6-diyl)-dinitrilo]tetrakisethanol (Persantine) is used for coronary and myocardial insufficiency. It is a bitter, yellow, crystalline powder, soluble in dilute acids, methanol, or chloroform.

Dipyridamole is a long-acting vasodilator. Its vasodilating action is selective on the coronary system; it is indicated for long-term therapy of chronic angina pectoris. The drug also inhibits adenosine deaminase in erythrocytes and interferes with the uptake of the vasodilator adenosine by erythrocytes. These actions potentiate the effect of prostacyclin acting as

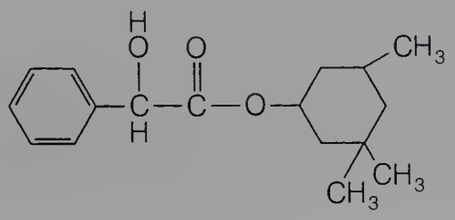
an inhibitor to platelet aggregation (see Platelet Aggregation and Inhibitors).



Dipyridamole

The recommended oral dose is 50 mg two or three times daily before meals. Optimum response may not be apparent until the third or fourth week of therapy. Dipyridamole is available in 25, 50, and 75 mg sugar-coated tablets.

Cyclandelate. 3,5,5-Trimethylcyclohexyl mandelate (Cyclospasmol). This compound was introduced in 1956 for use especially in peripheral vascular disease in which there is vasospasm. It is a white to off-white, crystalline powder, practically insoluble in water and readily soluble in alcohol and other organic solvents. Its actions are similar to those of papaverine.



Cyclandelate

Cyclandelate produces peripheral vasodilation by acting directly on vascular smooth muscle. When cyclandelate is effective, the improvement in peripheral circulation usually occurs gradually, and treatment must often be continued over long periods. At the maintenance dose of 100 mg four times daily, there is little incidence of serious toxicity. At higher doses, as high as 400 mg four times daily, which may be needed initially, there is a greater frequency of unpleasant side effects, such as headache, dizziness, and flushing. It must be used with caution in patients with glaucoma. The oral dosage forms are 200 mg capsules and 100 mg tablets.

ANTIARRHYTHMIC DRUGS

Cardiac arrhythmias are caused by a disturbance in the conduction of the impulse through the myocardial tissue, by disorders of impulse formation, or by a combination of these factors. The antiarrhythmic agents used most commonly affect impulse conduction by altering conduction velocity and the duration of the refractory period of heart muscle tissue. They also depress spontaneous diastolic depolarization, causing a reduction of automaticity by ectopic foci.

There are many pharmacological agents available for the treatment of cardiac arrhythmias. Agents such as oxygen, potassium, and sodium bicarbonate relieve the underlying cause of some arrhythmias. Other agents, such as digitalis, propranolol, phenylephrine, edrophonium, and neostigmine, act on the cardiovascular system by affecting heart muscle or on the autonomic nerves to the heart. Finally, there are drugs that alter the electrophysiological mechanisms causing arrhythmias. The latter group of drugs is discussed in this chapter.

Within the last four decades, research on normal cardiac tissues and, in the clinical setting, on patients with disturbances of rhythm and conduction has brought to light information on the genesis of cardiac arrhythmias and the mode of action of antiarrhythmic agents. In addition, laboratory tests have been developed to measure blood levels of antiarrhythmic drugs, such as phenytoin, disopyramide, lidocaine, procainamide, and quinidine, to help evaluate the pharmacokinetics of these agents. As a result, it is possible to maintain steady-state plasma levels of these drugs that allow the clinician to use these and other agents more effectively and with greater safety. No other clinical intervention has been more effective at reducing mortality and morbidity in coronary care units.

CARDIAC ELECTROPHYSIOLOGY

The heart depends on the synchronous integration of electrical impulse transmission and myocardial tissue response to carry out its function as a pump. When the impulse is released from the SA node, excitation of the heart tissue takes place in an orderly manner by a spread of the impulse throughout the specialized automatic fibers in the atria, the AV node, and the Purkinje fiber network in the ventricles. This spreading of impulses produces a characteristic electrocardiographic pattern that can be equated to predictable myocardial cell membrane potentials and Na^+ and K^+ fluxes in and out of the cell.

A single fiber in the ventricle of an intact heart during the diastolic phase (see phase 4, Fig. 19-4) has a membrane potential (resting potential) of -90 mV. This potential is created by differential concentrations of K^+ and Na^+ in the intracellular and extracellular fluid. An active transport system (pump) on the membrane is responsible for concentrating the K^+ inside the cell and maintaining higher concentrations of Na^+ in the extracellular fluid. Diastolic depolarization is caused by a decreased K^+ ionic current into the extracellular tissue and a slow inward leakage of Na^+ until the threshold potential (-60 to -55 mV) is reached. At this time there is a sudden increase in the inward sodium current, and a self-propagated wave occurs to complete the

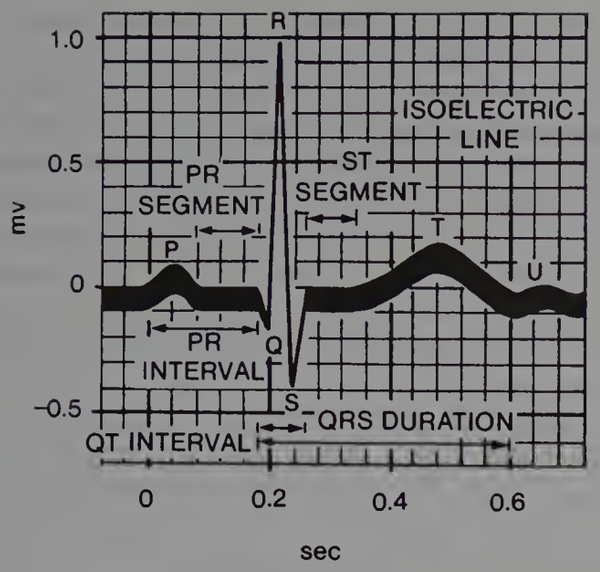


FIG. 19-10. *Normal electrocardiogram. (From Ganong, W. F.: Review of Medical Physiology, 9th ed., San Francisco, Lange Medical Publications, 1985.)*

membrane depolarization process. Pacemaker cells possess this property, which is termed *automaticity*. This maximal rate of depolarization (MRD) is represented by phase 0 or the spike action potential (Fig. 19-4).

The form, duration, resting potential level, and amplitude of the action potential are characteristic for different types of myocardial cells. The rate of rise of the response (phase 0) is related to the level of the membrane potential at the time of stimulation and has been termed *membrane responsiveness*. Less negative potentials produce smaller slopes of phase 0 and are characterized by slower conduction times. The phase 0 spike of the SA node corresponds to the inscription of the P wave on the electrocardiogram (Fig. 19-10). Repolarization is divided into three phases. The greatest amount of repolarization is represented by phase 3, in which there is a passive flux of K^+ ions out of the cell. Phase 1 repolarization is caused by an influx of chloride ions. During phase 2, a small inward movement of Ca^{2+} ions occurs through a slow channel mechanism that is believed to be important in the process of coupling excitation with contraction. The process of repolarization determines the duration of the action potential and is represented by the *QT* interval. The action potential duration is directly related to the refractory period of cardiac muscle.

MECHANISMS OF ARRHYTHMIAS

The current understanding of the electrophysiological mechanisms responsible for the origin and perpetuation of cardiac arrhythmias is that they are due to altered impulse formation, that is, change in automaticity; altered conduction; or both, acting simultaneously from different locations of the heart.

The generation of cardiac impulses in the normal heart usually is confined to specialized tissues that spontaneously depolarize and initiate the action potential. These cells are located in the right atrium and are referred to as the *SA node* or the *pacemaker cells*. Although the spontaneous electrical depolarization of the SA pacemaker cells is independent of the nervous system, these cells are innervated by both sympathetic and parasympathetic fibers, which may cause an increase or decrease of the heart rate, respectively. Other special cells in the normal heart, which possess the property of automaticity, may influence cardiac rhythm when the normal pacemaker is suppressed or when pathological changes occur in the myocardium to make these cells the dominant source of cardiac rhythm (i.e., ectopic pacemakers). Automaticity of subsidiary pacemakers may develop when myocardial cell damage occurs because of infarction or from digitalis toxicity, excessive vagal tone, excessive catecholamine release from sympathomimetic nerve fibers to the heart, or even high catecholamine plasma levels. The development of automaticity in specialized cells, such as that found in special atrial cells, certain AV node cells, bundle of His, and Purkinje fibers, may lead to cardiac arrhythmias. Because production of ectopic impulses is often due to a defect in the spontaneous phase 4 diastolic depolarization (i.e., "T wave"), drugs that are able to suppress this portion of the cardiac stimulation cycle are effective agents for these types of arrhythmia.

Arrhythmias also are caused by disorders in the conduction of impulses and changes in the refractory period of the myocardial tissue. Pharmacological intervention is based on these two properties. The Purkinje fibers branch into a network of interlacing fibers, particularly at their most distant positions. This creates several pathways in which a unidirectional block in a localized area may establish circular (circus) micro- or macrocellular impulse movements that reenter the myocardial fibers and create an arrhythmia (Fig. 19-11). Unidirectional block results from localized myocardial disease (*infarcts*) or from a change in dependence of the tissue to Na^+ fluxes that causes a longer conduction time and allows the tissue to repolarize to propagate the retrograde impulse.

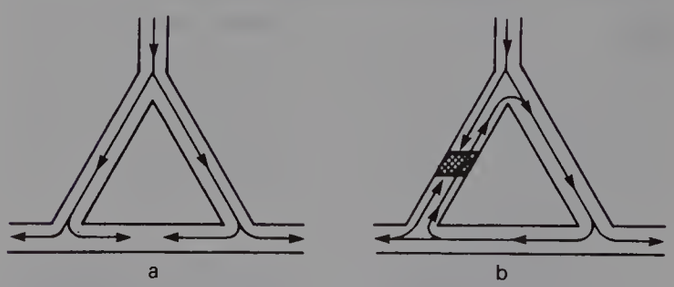


FIG. 19-11. *Reentry mechanism of Purkinje fibers.(a) Normal conduction of impulses through triangular arrangement of cardiac fibers. (b) Unidirectional block on left arm of triangular section allows impulse to reenter the regional conducting system and recycle.*

CLASSES OF ANTIARRHYTHMIC DRUGS

Antiarrhythmic drugs can be placed into four separate classifications, based on their mechanism of action or pattern of electrophysiological effects produced on heart tissue. Table 19-4 summarizes the four-part classification of antiarrhythmic drugs as first proposed by Vaughan Williams in 1970[19] and expanded in 1984.[20] It should be noted that drugs within the same category are placed there because they demonstrate similar clinical actions. However, if patients do not respond to a drug in this class, it should not rule out use of other drugs in the same class.[21] Despite the well-intentioned use of these agents, most antiarrhytmic drugs have the potential to aggravate the arrhythmia they treat (*proarrhythmia*). Proarrhythmia develops from an increase in the density of single ectopic beats and is more likely to occur in patients who have a dysfunction in the left ventricle or sustained ventricular tachycardia. Class I antiarrhythmic agents (see below) are especially proarrhythmic in myocardial infarction patients.

Class I. Membrane-Depressant Drugs

Class I antiarrhythmic agents are drugs that have membrane-stabilizing properties (i.e., they shift membranes to more negative potentials). Drugs in this class act on the fast Na^+ channels and interfere with the process by which the depolarizing charge is transferred across the membrane. It is assumed that these drugs bind to the Na^+ channel and block its function, preventing Na^+ conductance as long as the drug is bound. The prototypical drugs in this class are quinidine and procainamide. During the 1970s, several drugs were studied for their antiarrhythmic effects. Most of them were local anesthetics that affected Na^+ membrane channels and were grouped in a single class (class I). Studies on the antiarrhythmic properties of these chemicals have shown that there are sufficient differences to place them into separate subgroups.[21]

TABLE 19-4

CLASSES OF ANTIARRHYTHMIC DRUGS

Class	*Drugs*	*Mechanism of Action*
IA	Quinidine, procainamide, disopyramide	Lengthens refractory period
IB	Lidocaine, phenytoin, tocainide, mexiletine	Shortens duration of action potential
IC	Encainide, flecainide, lorcainide, moricizine, propafenone	Slows conduction
II	β-Adrenergic blockers (e.g., propranolol)	Slows AV conduction time, suppresses automaticity
III	Amiodarone, bretylium, sotalol	Prolongs refractoriness
IV	Calcium channel blockers (e.g., verapamil, diltiazem)	Blocks slow inward Ca^{2+} channel

Class I antiarrhythmic drugs can be subdivided on the basis of the relative ease with which they dissociate from the Na^+ ion channel. Drugs in class IC, such as encainide and lorcainide, are the most potent sodium channel-blocking agents of the class I antiarrhythmic drugs. They slowly dissociate from the Na^+ channel, causing a slowing down of the conduction time of the impulse through the heart. Class IB drugs, which include lidocaine, tocainide, and mexiletine, have the property of rapidly dissociating from the Na^+ channels and, thus, have the lowest potency as sodium channel blockers. They produce little, if any, change in action potential duration. Quinidine, procainamide, and disopyramide are drugs that have an intermediate rate of dissociation from Na^+ channels. These are categorized as class IA antiarrhythmic agents, and they lengthen the refractory period of cardiac tissue to cause cessation of arrhythmias.[22]

Studies have shown that Na^+ channels on the membranes of Purkinje fiber cells normally exist in at least three states: R = rested, closed near the resting potential but able to be opened by stimulation and depolarization; A = activated, allowing Na^+ ions to pass selectively through the membrane; and I = inactivated and unable to be opened (i.e., inactive).[23] The affinity of the antiarrhythmic drug for the receptor on the ion channel varies with the state of the channel or with the membrane potential. Because of this, R, A, and I ion channels can have different kinetics of interaction with antiarrhythmic drugs. A review of the recent literature shows that the antiarrhythmic drugs have a low affinity for R channels but a relatively high affinity for the A or I channels or both. Regardless of which channel state is blocked by class I antiarrhythmic drugs, the unblocking rate directly determines the amount of depression present at normal heart rates.

Class II. β-Adrenergic Blocking Agents

β-Adrenergic blocking drugs have the property of causing membrane-stabilizing or depressant effects on myocardial tissue. However, their antiarrhythmic properties are considered to be due principally to inhibition of adrenergic stimulation to the heart. The principal electrophysiological property of these β-blocking agents is reduction of the phase 4 slope of potential sinus or ectopic pacemaker cells such that the heart rate decreases and ectopic tachycardias are either slowed down or converted to sinus rhythm.

Class III. Repolarization Prolongators

Drugs in this class (e.g., amiodarone, bretylium, sotalol) cause several different electrophysiological changes on myocardial tissue but share one common effect, that of prolonging the action potential which increases the effective refractory period of the membrane action potential without

altering the phase of depolarization or the resting membrane potential. Drugs in this class produce their effects by more than one mechanism. Sotalol is a K^+ channel blocker and has some β-adrenergic blocking properties.[24] Amiodarone and bretylium, drugs that also prolong the action potential by means that are unclear, also have Na^+ channel-blocking properties.

Class IV. Calcium Channel Blockers

Although not all Ca^{2+} channel blockers possess antiarrhythmic activity, some members of this class of antiarrhythmic drugs (verapamil, diltiazem) block the slow inward current of Ca^{2+} ions during phase 2 of the membrane action potential in cardiac cells. For example, the prototypical drug in this group, verapamil, selectively blocks entry of Ca^{2+} into the myocardial cell. It acts on the slow response fibers found in the sinus node and the AV node, slowing conduction velocity and increasing refractoriness in the AV node.

*p*H AND ACTIVITY

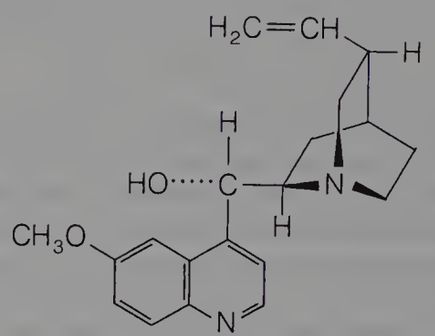
The action of class I local anesthetic-type antiarrhythmic drugs is *p*H-dependent and may vary with each drug.[25] Antiarrhythmic drugs are weak bases, with most having pK_a values ranging from 7.5 to 9.5. At physiological *p*H of 7.40 these bases exist in an equilibrium mixture consisting of both the free base and the cationic form. Ionizable drugs, such as lidocaine (pK_a 7.86), have stronger electrophysiological effects in ischemic than in normal myocardial cells. This potentiation has been attributed in part to the increase in H^+ concentration within the ischemic areas of the heart. Acidosis increases the proportion of Na^+ ion channels occupied by the protonated form of the antiarrhythmic agent. Nevertheless, the effect of *p*H on the antiarrhythmic activity of drugs can be complex, as both the free base and cationic species have been proposed as the active form of some drugs. The uncharged form of the Na^+ channel blocker can penetrate directly from the lipid phase of the surrounding cell membrane to block the channel.

Small changes in *p*H can alter these drugs' effectiveness by changing the charged/uncharged molecular ratio in the myocardial cells. Acidosis external to the myocardial cell promotes the cationic form. Because this species does not partition in the membrane as readily, onset of these drugs' action would be delayed. Furthermore, concentration of these drugs in the membrane would be reduced. Therefore, drugs that act on the channel only in the inactivated (closed) state would have a reduced effect from acidotic conditions. Acidosis may also cause prolongation of the effect of these drugs. External acidosis facilitates protonation of receptor-bound drugs. Because only neutral drugs can dissociate from closed channels, recovery is prolonged by acidosis.

Alkalosis tends to cause hyperpolarization of the cell membrane and, thereby, reduces the effect of antiarrhythmic drugs. Because of this, alkalosis promotes the formation of more of the free-base antiarrhythmic agent, increasing the rate of recovery from the block. Alkalosis-inducing salts, such as sodium lactate, have been used to counteract toxicity caused by the antiarrhythmic quinidine.

PRODUCTS

Quinidine Sulfate, USP. Quinidine sulfate is the sulfate of an alkaloid obtained from various species of *Cinchona* and their hybrids. It is a dextrorotary diastereoisomer of quinine. The salt crystallizes from water as the dihydrate in the form of fine, needle-like, white crystals. Quinidine sulfate contains a hydroxymethyl group that serves as a link between a quinoline ring and a quinuclidine moiety. The structure contains two basic nitrogens, of which the quinuclidine nitrogen is the stronger base (pK_a 10). Quinidine sulfate is bitter and light-sensitive. Aqueous solutions are nearly neutral or slightly alkaline. It is soluble to the extent of 1% in water and more highly soluble in alcohol or chloroform.



Quinidine Sulfate

Quinidine sulfate is the prototype of antiarrhythmic drugs and a class IA antiarrhythmic agent according to the Vaughan Williams classification. It reduces Na^+ current by binding the open ion channels (i.e., state *A*). The decrease of Na^+ entry into the myocardial cell causes depression of phase 4 diastolic depolarization and shifts the intracellular threshold potential toward zero. These combined actions diminish the spontaneous frequency of pacemaker tissues, depress the automaticity of ectopic foci, and, to a lesser extent, reduce impulse formation in the SA node. This last action results in bradycardia. During the spike action potential, quinidine sulfate decreases transmembrane permeability to passive influx of Na^+, causing a slowing down of the process of phase 0 depolarization, which decreases conduction velocity. This is shown as a prolongation of the QRS complex of electrocardiograms. Quinidine sulfate also causes a prolongation of action potential duration, which results in a proportionate increase in the QT interval. It is used to treat supraventricular and ventricular ectopic arrhythmias, such as atrial and ventricular premature beats, atrial and ventricular tachycardia, atrial flutter, and atrial fibrillation.

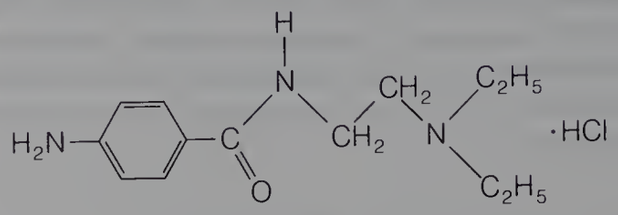
Quinidine sulfate is used most frequently as an oral preparation and is occasionally given intramuscularly. Quinidine sulfate that has been absorbed from the gastrointestinal tract or from the site of intramuscular injection is bound 80% to serum albumin.[18] The drug is taken up quickly from the bloodstream by body tissues; consequently, a substantial concentration gradient is established within a few minutes. Onset of action begins within 30 minutes, the peak effect being attained in 1 to 3 hr. Quinidine is metabolized primarily in the liver by hydroxylation, and a small amount is excreted by the liver.[25] Because of serious side effects and the advent of more effective oral antiarrhythmic agents, quinidine is now used less, except in selected patients for long-term oral antiarrhythmic therapy.

Quinidine Gluconate, USP. Quinidinium gluconate (Duraquin, Quinaglute). This occurs as an odorless, very bitter, white powder. In contrast with the sulfate salt, it is freely soluble in water. This is important because there are emergencies when the condition of the patient and the need for a rapid response may make the oral route of administration inappropriate. The high water solubility of the gluconate salt along with a low irritant potential makes it of value when an injectable form is needed in these emergencies. Quinidine gluconate forms a stable aqueous solution. When used for injection, it usually contains 80 mg/mL, equivalent to 50 mg of quinidine or 60 mg of quinidine sulfate.

Quinidine Polygalacturonate (Cardioquin). This is formed by reacting quinidine and polygalacturonic acid in a hydroalcoholic medium. It contains the equivalent of approximately 60% quinidine. This salt is only slightly ionized and slightly soluble in water, but studies have shown that although equivalent doses of quinidine sulfate give higher peak blood levels earlier, a more uniform and sustained blood level is achieved with the polygalacturonate salt.

In many patients, the local irritant action of quinidine sulfate in the gastrointestinal tract causes pain, nausea, vomiting, and especially diarrhea, often precluding oral use in adequate doses. In studies with the polygalacturonate salt, no evidence of gastrointestinal distress was encountered. It is available as 275 mg tablets. Each tablet is the equivalent of 200 mg of quinidine sulfate or 166 mg of free alkaloid.

Procainamide Hydrochloride, USP. *p*-Amino-*N*-[2-(diethylamino)ethyl]benzamide monohydrochloride; procainamidium chloride (Pronestyl). Procainamide hydrocholoride has emerged as a major antiarrhythmic drug in the treatment of cardiac arrhythmias. It was developed in the course of research for compounds structurally similar to procaine, which had limited effect as an antiarrhythmic agent because of its central nervous system side effects and short-lived action resulting from the rapid hydrolysis of its ester linkage by plasma esterases. Procainamide hydrocholoride is also more stable in water than procaine because of its amide structure. Aqueous solutions of procainamide hydrochloride have a *p*H of about 5.5. A kinetic study of the acid-catalyzed hydrolysis of procainamide hydrocholoride has shown it to be unusually stable to hydrolysis in the *p*H range 2 to 7, even at elevated temperatures.[26]

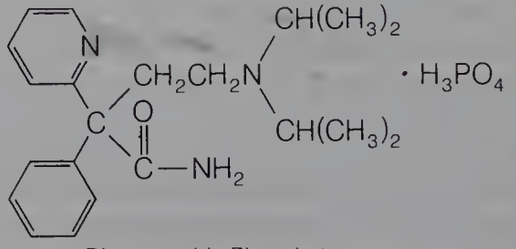


Procainamide Hydrochloride

Metabolism of procainamide hydrocholoride occurs through the action of *N*-acetyltransferase. The product of enzymatic metabolism of procainamide hydrocholoride is *N*-acetylprocainamide (NAPA), which possesses only 25% of the activity of the parent compound.[25] A study of the disposition of procainamide hydrocholoride showed 50% of the drug to be excreted unchanged in the urine, with 7% to 24% recovered as NAPA.[27,28] Unlike quinidine, procainamide hydrocholoride is bound only minimally to plasma proteins. Between 75% and 95% of the drug is absorbed from the gastrointestinal tract. Plasma levels appear 20 to 30 minutes after administration and peak in about 1 hr.[29]

Procainamide hydrocholoride appears to have all of the electrophysiological effects of quinidine: it diminishes automaticity, decreases conduction velocity, and increases action potential duration and, thereby, the refractory period of myocardial tissue. Clinicians have favored the use of procainamide hydrocholoride for ventricular tachycardias and quinidine for atrial arrhythmias, even though the two drugs are effective in either type of disorder.

Disopyramide Phosphate, USP. α-[2(Diisopropylamino)ethyl]-α-phenyl-2-pyridineacetamide phosphate (Norpace) is an oral and intravenous class IA antiarrhythmic agent. It is quite similar to quinidine and procainamide in its electrophysiological properties in that it decreases phase 4 diastolic depolarization, decreases conduction velocity, and has vagolytic properties.[30] It is used clinically in the treatment of refractory, life-threatening ventricular tachyarrhythmias. Oral administration of the drug produces peak plasma levels within 2 hr. The drug is bound approximately 50% to plasma protein and has a half-life of 6.7 hr in humans. More than 50% is excreted unchanged in the urine. Therefore, patients with renal insufficiency should be monitored carefully for evidence of overdose. Disopyramide phosphate commonly exhibits side effects of dry mouth, constipation, urinary retention, and other cholinergic-blocking actions because of its structural similarity to anticholinergic drugs.



Disopyramide Phosphate

Lidocaine Hydrochloride, USP. 2-(Diethylamino)-2′,6′-acetoxylidide monohydrochloride (Xylocaine). This drug, which was conceived as a derivative of gramine (3-dimethylaminomethylindole) and introduced as a local anesthetic, is now being used intravenously as a standard parenteral agent for suppression of arrhythmias associated with acute myocardial infarction and cardiac surgery. It is the drug of choice for the parenteral treatment of premature ventricular contractions.

Lidocaine hydrochloride is a class IB antiarrhythmic agent and has a different effect on the electrophysiological properties of myocardial cells from that of procainamide and quinidine. It binds with equal affinity to the active (*A*) and inactive (*I*) Na^+ ion channels. It depresses diastolic depolarization and automaticity in the Purkinje fiber network and increases the functional refractory period relative to action potential duration, as do procainamide and quinidine. However, it differs from the latter two drugs in that it does not decrease, but may even enhance, conduction velocity, and it increases membrane responsiveness to stimulation. There are fewer data available on the subcellular mechanisms responsible for the antiarrhythmic actions of lidocaine than on the more established drug quinidine. It has been proposed that lidocaine has little effect on membrane cation exchange of the atria. Sodium ion entrance into ventricular cells during excitation is not influenced by lidocaine because it does not alter conduction velocity in this area. Lidocaine hydrochloride does depress Na^+ influx during diastole, as do all other antiarrhythmic drugs, to diminish automaticity in myocardial tissue. It also alters membrane responsiveness in Purkinje fibers, allowing an increase of conduction velocity and ample membrane potential at the time of excitation.[31]

Lidocaine hydrochloride administration is limited to the parenteral route and is usually given intravenously, though adequate plasma levels are achieved after intramuscular injections. Lidocaine hydrochloride is not bound to any extent to plasma proteins and is concentrated in the tissues. It is metabolized rapidly by the liver (Fig. 19-12). The first step is deethylation, with the formation of monoethylglycinexylidide, followed by hydrolysis of the amide.[32] Metabolism is rapid, the half-life of a single injection ranging from 15 to 30 minutes. Lidocaine hydrochloride is a popular drug because of its rapid action and its relative freedom from toxic effects on the heart, especially in the absence of hepatic disease. Monoethylglycinexylidide, the initial metabolite of lidocaine, is an effective antiarrhythmic agent; however, its rapid hydrolysis by microsomal amidases prevents its use in humans.

Precautions must be taken so that lidocaine hydrochloride solutions containing epinephrine salts are not used as cardiac depressants. Such solutions are intended only for local anesthesia and are not used intravenously. The aqueous solutions without epinephrine may be autoclaved several times, if necessary.

Phenytoin Sodium, USP. 5,5-Diphenyl-2,4-imidazolidinedione; 5,5-diphenylhydantoin; diphenyl-hydantoin sodium (Dilantin). This drug has been used for decades in the control of grand mal types of epileptic seizure. It is structurally analogous to the barbiturates but does not possess their extensive sedative properties. The compound is available as the sodium salt. Solutions for parenteral administration contain 40% propylene glycol and 10% alcohol to dissolve the sodium salt.

Phenytoin sodium's cardiovascular effects were uncovered during observation of toxic manifestations of the drug in patients being treated for seizure disorders. Phenytoin sodium was found to cause bradycardia, prolong the PR interval, and produce T-wave abnormalities on electrocardiograms. It is a class IB antiarrhythmic agent. Today, phenytoin sodium's greatest clinical use as an antiarrhythmic drug is in the treatment of digitalis-induced arrhythmias.[33] Its action is similar to that of lidocaine. It causes a depression of ventricular automaticity produced by digitalis, without adverse intraventricular conduction. Because it also reverses the prolongation of AV conduction by digitalis, phenytoin sodium is useful in supraventricular tachycardias caused by digitalis intoxication.

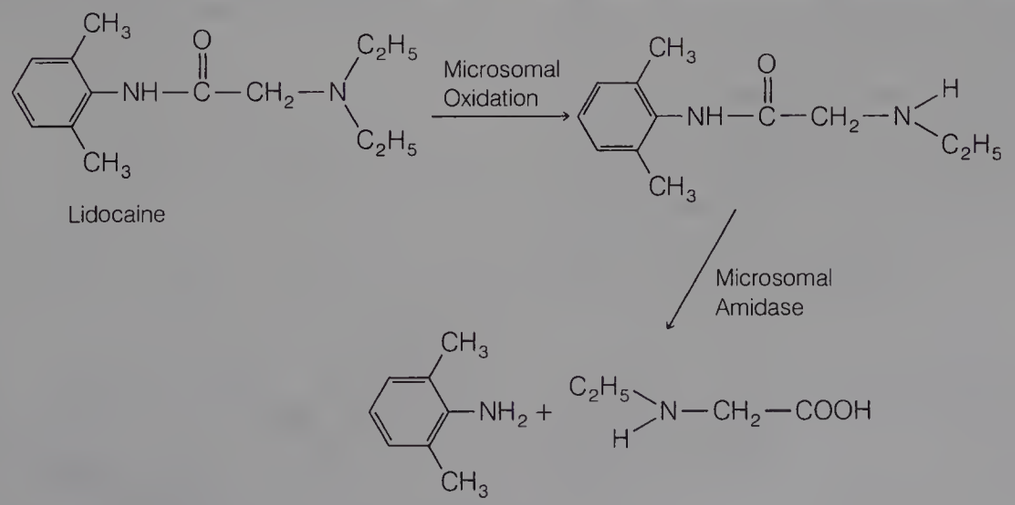
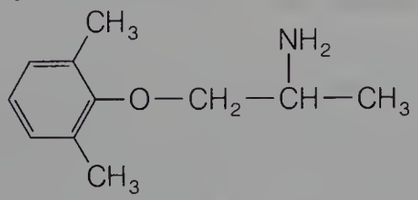


FIG. 19-12. *Metabolism of lidocaine.*

Phenytoin sodium is located in high amounts in the body tissues, especially fat and liver, leading to large gradients between the drug in tissues and the plasma concentrations. It is metabolized in the liver.

Mexiletine Hydrochloride. 1-Methyl-2-(2,6-xylyloxy)ethylamine hydrochloride (Mexitil). Mexiletine hydrochloride (pK_a 8.4) is a class IB antiarrhythmic agent that is effective when given either intravenously or orally. It resembles lidocaine in possessing a xylyl moiety but otherwise is different chemically. Mexiletine hydrochloride is an ether and is not subject to the hydrolysis common to the amides lidocaine and tocainide. Its mean half-life on oral administration is approximately 10 hr.



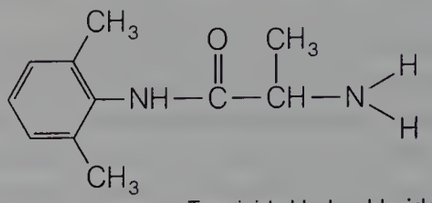
Mexiletine Hydrochloride

Although not subject to hydrolysis, mexiletine hydrochloride is metabolized by oxidative and reductive processes in the liver. Its metabolites, *p*-hydroxymexiletine and hydroxymethylmexiletine, are not pharmacologically active as antiarrhythmic agents.[34]

Mexiletine hydrochloride, similar to class I antiarrhythmic agents, blocks the fast Na^+ channel in cardiac cells. It is especially effective on the Purkinje fibers in the heart. The drug increases the threshold of excitability of myocardial cells by reducing the rate of rise and amplitude of the action potential and decreases automaticity.

Mexiletine hydrochloride is used for long-term oral prophylaxis of ventricular tachycardia. The drug is given in 200- to 400-mg doses every 8 hr.

Tocainide Hydrochloride. 2-Amino-2′,6′-propionoxylidide hydrochloride (Tonocard). Tocainide hydrochloride (pK_a 7.7) is an analogue of lidocaine. It is orally active and has electrophysiological properties, similar to lidocaine.[35] Total body clearance of tocainide hydrochloride is only 166 mL/minute, suggesting that hepatic clearance is not large. Because of low hepatic clearance, the hepatic extraction ratio must be small; therefore, tocainide hydrochloride is unlikely to be subject to a substantial first-pass effect. The drug differs from lidocaine in that it lacks two ethyl groups, which provides tocainide hydrochloride some protection from first-pass hepatic elimination after oral ingestion. Tocainide hydrochloride is hydrolyzed in a manner similar to lidocaine. None of its metabolites are active.



Tocainide Hydrochloride

Tocainide hydrochloride is classed as a IB antiarrhythmic agent and used orally to prevent or treat ventricular ectopy and tachycardia. The drug is given in 400- to 600-mg doses every 8 hr.

Encainide Hydrochloride. 4-Methoxy-*N*-[2-[2-(1-methyl-2-piperidinyl)ethyl]phenyl] benzamide, monohydrochloride (Enkaid). Encainide hydrochloride is categorized as a class IC antiarrhythmic agent. The main effect of this compound on the heart, along with the other class IC drugs, is to depress the upstroke velocity of phase 0 of the action potential and to lengthen the time-dependent refractoriness.

Encainide hydrochloride is a benzanilide derivative that has local anesthetic properties in addition to its class IC antiarrhythmic action. The drug is metabolized extensively, producing products that also have antiarrhythmic properties.[36] The metabolite, 3-methoxy-*O*-demethylencainide (MODE), is about equipotent to encainide hydrochloride. *O*-Demethylencainide (ODE) is considerably more potent than the parent drug. The half-life of encainide hydrochloride is 2 to 4 hr. The active metabolites have longer half-lives, estimated as being up to 12 hr, and may play an important part in the use of this drug in long-term therapy. Encainide hydrochloride also undergoes *N*-demethylation to form *N*-demethylencainide (NDE). The probable metabolic pathway for encainide hydrochloride is shown in Fig. 19-13.

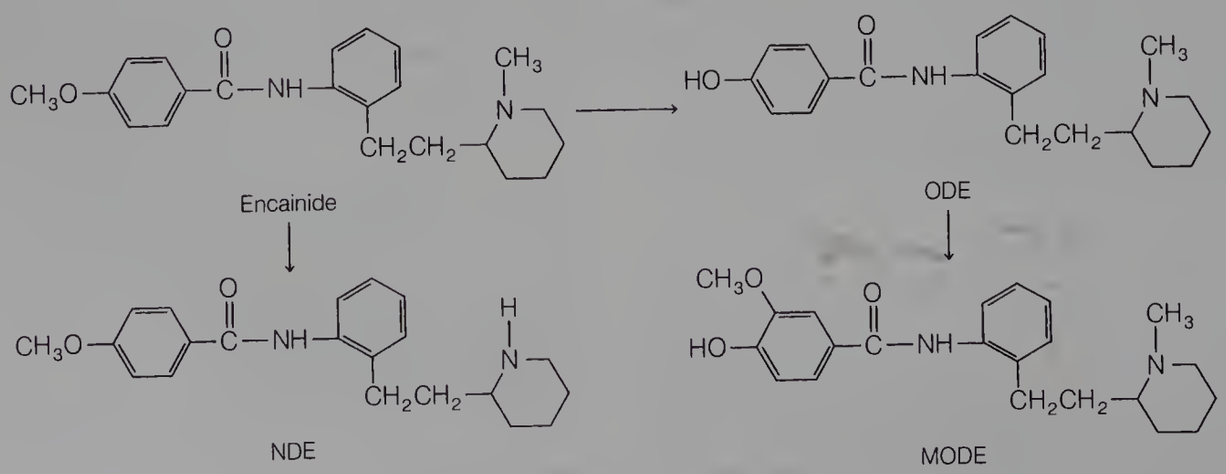
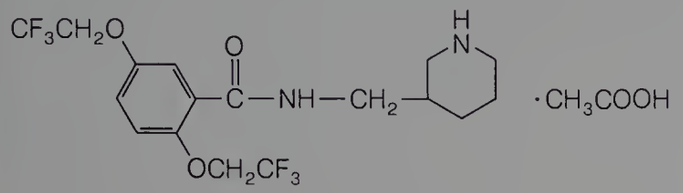


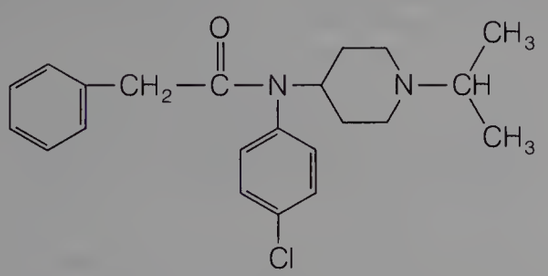
FIG. 19-13. *Metabolism of encainide.*

Flecainide Acetate. *N*-(2-Piperidinylmethyl)-2,5-bis (2,2,2-trifluoroethoxy)benzamide monoacetate (Tambocor). Flecainide acetate, like encainide, is a class IC antiarrhythmic drug with local anesthetic activity; it is a chemical derivative of benzamide. The drug undergoes biotransformation, forming a *meta-O*-dealkylated compound, the antiarrhythmic properties of which are one-half as potent as those of the parent drug, and a *meta-O*-dealkylated lactam of flecainide with little pharmacological activity.[37] Flecainide acetate is given orally to suppress chronic ventricular ectopy and ventricular tachycardia. It has some limitations because of central nervous system side effects.

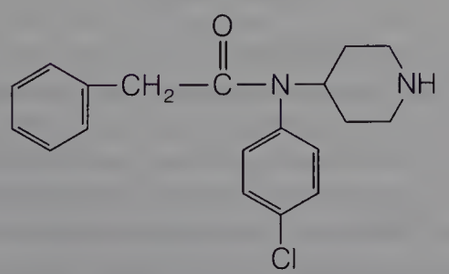


Flecainide Acetate

Lorcainide Hydrochloride. *N*-(4-Chlorophenyl)-*N*-[1-(1-methylethyl)-4-piperidinyl]benzeneacetamide monohydrochloride. Lorcainide hydrochloride is a local anesthetic-type antiarrhythmic agent. It is categorized as a class IC type drug because it effectively causes a slowing down of conduction in the His–Purkinje fiber network and ventricles of the heart. It is used to suppress chronic ventricular ectopy. Lorcainide hydrochloride undergoes metabolic *N*-dealkylation, forming norlorcainide. Metabolism is the product of the first-pass clearance after oral administration. The basis for this observation is that norlorcainide is not produced in significant amounts in the body following intravenous administration. Norlorcainide is an important metabolite of the parent drug, as it is cleared slowly from the body and has a half-life that is approximately three times longer. Accumulation of norlorcainide is of considerable clinical importance because the metabolite is equipotent to the original drug.

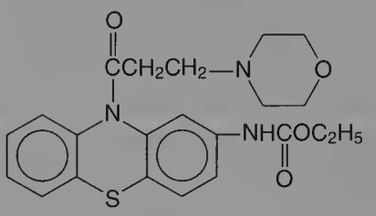


Lorcainide Hydrochloride



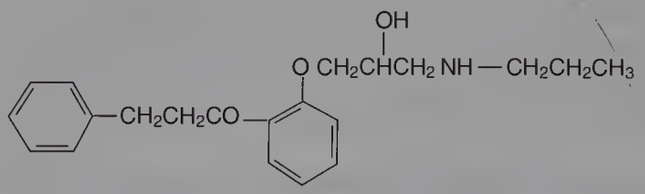
Norlorcainide

Moricizine. Ethyl 10-(3-morpholinopropionyl)phenothiazine-2-carbamate (Ethmozine). Moricizine is a phenothiazine derivative used for the treatment of malignant ventricular arrhythmias. It is categorized as a class IC antiarrhythmic agent, blocking the Na^+ channel with a 1:1 stochiometry. The drug has a higher affinity for the inactivated state than the activated or resting states. It appears to bind to a site on the external side of the Na^+ channel membrane.[38] It has been used to suppress life-threatening ventricular arrhythmias.



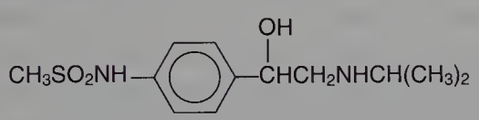
Moricizine

Propafenone. 2-[2′-Hydroxy-3-(propylamino)propoxy]-3-phenylpropiophenone (Rythmol). Propafenone, a class IC antiarrhythmic drug, contains a chiral center and is marketed as the racemic mixture. Therapy with the racemic mixture of propafenone produces effects that can be attributed to both (*S*)- and (*R*)-enantiomers. While both (*R*)- and (*S*)-enantiomers exert similar Na^+ channel-blocking effects, the (*S*)-enantiomer also produces a β-adrenergic blockade. As a result, the (*S*)-enantiomer is reported to be 40-fold more potent than the (*R*)-enantiomer as an antiarrhythmic agent.[39] The enantiomers also display stereoselective disposition characteristics. The (*R*)-enantiomer is cleared more quickly. Hepatic metabolism is polymorphic and determined genetically. Ten percent of Caucasians have a reduced capacity to hydroxylate the drug to form 5-hydroxypropafenone. This polymorphic metabolism accounts for the interindividual variability in the relationships between dose and concentration and, thus, variability in the pharmacodynamic effects of the drug. The 5-hydroxy metabolites of both enantiomers are as potent as the parent compound in blocking Na^+ channels. Propafenone also depresses the slow inward current of Ca^{2+} ions. This drug has been used for acute termination or long-term suppression of ventricular arrhythmias. It is bound in excess of 95% to α1-acid glycoprotein in the plasma. It is absorbed effectively, but bioavailability is estimated at less than 20% due to first-pass metabolism. Less than 1% is eliminated as unchanged drug. Therapy with propafenone may produce effects that can be attributed to both (*S*)- and (*R*)-enantiomers. Thus, the effects may be modulated because of an enantiomer–enantiomer interaction when patients are treated with the racemate.[40]



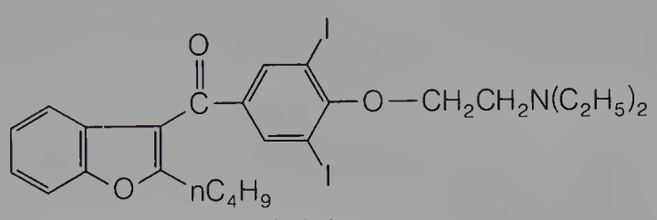
Propafenone

Sotalol. 4 '[1-Hydroxy-2-(isopropylamino)ethyl]methylsulfonanilide (Betapace). Sotalol is a relatively new antiarrhythmic drug and is characterized most often as a class III agent. It contains a chiral center and is marketed as the racemic mixture. Because of its enantiomers, its mechanism of action spans two of the antiarrhythmic drug classes. The *l*(−)-enantiomer has both β-blocking (class II) and potassium channel-blocking (class III) activity. The d(+)-enantiomer has class III properties similar to those of the (−)-isomer, but its affinity for the β-adrenergic receptor is 30 to 60 times lower. The sotalol enantiomers produce different effects on the heart. Class III action of d-sotalol in the sinus node are associated with slowing of sinus heart rate, whereas β-adrenergic blockade contributes to the decrease in heart rate observed with *l*- or *d,l*-sotalol. Sotalol is not metabolized nor is it bound significantly to proteins. Elimination occurs by renal excretion, with more than 80% of the drug being eliminated unchanged. Sotalol is characteristic of class III antiarrhythmic drugs in that it prolongs the duration of the action potential and, thus, increases the effective refractory period of myocardial tissue. It is distinguished from the other class III drugs (amiodarone and bretylium) because of its β-adrenergic receptor-blocking action.



Sotalol

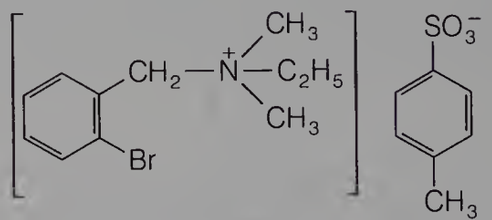
Amiodarone. 2-Butyl-3-benzofuranyl 4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl ketone (Cordarone). Amiodarone was introduced as an antianginal agent. It has a very pronounced class III action and is especially effective in maintaining sinus rhythm in patients who have been treated by direct current shock for atrial fibrillation.[41] Similar to class III antiarrhythmic drugs, amiodarone lengthens the effective refractory period by prolonging the action potential duration in all myocardial tissues. Amiodarone is eliminated very slowly from the body, with a half-life of about 25 to 30 days after oral doses.[42] Although the drug has a broad spectrum of antiarrhythmic activity, its main limitation is a slow onset of action. Initiation of drug action may not be for several days, and the peak effect may not be obtained for several weeks.



Amiodarone

Amiodarone has an adverse effect involving many different organ systems. It also inhibits metabolism of drugs cleared by oxidative microsomal enzymes. It contains iodine in its molecular structure and, as a result, has an effect on thyroid hormones. Hypothyroidism occurs in up to 11% of patients receiving amiodarone.[43] The principal effect is the inhibition of peripheral conversion of T_4 to T_3. Serum reverse T_3 (rT_3) is increased as a function of the dose as well as the length of amiodarone therapy. As a result, rT_3 levels have been used as a guide for judging adequacy of amiodarone therapy and predicting toxicity.[44]

Bretylium Tosylate. (*o*-Bromobenzyl)ethyl dimethylammonium *p*-toluenesulfonate (Bretylol) is an extremely bitter, white, crystalline powder. The chemical is freely soluble in water and alcohol. Bretylium tosylate is an adrenergic neuronal blocking agent that accumulates selectively into the neurons and displaces norepinephrine. Because of this property, bretylium was used initially, under the trade name of Darenthin, as an antihypertensive agent. It caused postural decrease in arterial pressure.[44] This use was discontinued because of the rapid development of tolerance, erratic oral absorption of the quaternary ammonium compound, and persistent pain in the parotid gland on prolonged therapy. Currently, bretylium is reserved for use in ventricular arrhythmias that are resistant to other therapy. Bretylium does not suppress phase 4 depolarization, a common action of other antiarrhythmic agents. It prolongs the effective refractory period relative to the action potential duration but does not affect conduction time and is categorized as a class III antiarrhythmic agent. Because bretylium does not have properties similar to those of the other antiarrhythmic agents, it has been suggested that its action is due to its adrenergic neuronal blocking properties; however, the antiarrhythmic properties of the drug are not affected by administration of reserpine. Bretylium is also a local anesthetic, but it has not been possible to demonstrate such an effect on atria of experimental animals, except at very high concentrations.[45] Therefore, the precise mechanism of the antiarrhythmic action of bretylium remains to be resolved.



Bretylium Tosylate

Verapamil and Diltiazem. Both of these drugs have the property of blocking the slow inward Ca^{2+} currents (voltage-sensitive channel) in cardiac fibers. This causes a slowing down of AV conduction and the sinus rate. These drugs are used in controlling atrial and paroxysmal tachycardias and are categorized as class IV antiarrhythmic agents according to the Vaughan Williams classification.[20] (A more detailed description of calcium channel blockers is given in a preceding section.)

ANTIHYPERTENSIVE AGENTS

Hypertension is a consequence of many diseases. Hemodynamically, blood pressure is a function of the amount of blood pumped by the heart and the ease with which the blood flows through the peripheral vasculature (i.e., resistance to blood flow by peripheral blood vessels). Diseases of components of the central and peripheral nervous systems that regulate blood pressure and abnormalities of the hormonal system and of the kidney and peripheral vascular network that affect blood volume can create a hypertensive state in humans. Hypertension is generally defined as mild when the diastolic pressure is between 90 and 104 mm Hg, moderate if the pressure is 105 to 114 mm Hg, and severe when above 115 mm Hg. It is estimated that about 15% of the adult population in the United States (about 40 million) are hypertensive.

Primary (essential) hypertension is the most common form of hypertension. Although advances have been made in the identification and control of primary hypertension, the etiology of this form of hypertension has not yet been resolved. *Renal hypertension* can be created by experimentally causing renal artery stenosis in animals. Renal artery stenosis also may occur in pathological conditions of the kidney, such as nephritis, renal artery thrombosis, renal artery infarctions, or other conditions that restrict blood flow through the renal artery. Hypertension also may originate from pathological states in the central nervous system, such as malignancies. Tumors in the adrenal medulla that cause release of large amounts of catecholamines create a hypertensive condition known as *pheochromocytoma*. Excessive secretion of aldosterone by the adrenal cortex, often because of adenomas, also produces hypertensive disorders.

Arterial blood pressure is regulated by several physiological factors, such as heart rate, stroke volume, peripheral vascular network resistance, blood vessel elasticity, blood volume, and viscosity of blood. Endogenous chemicals also play an important part in the regulation of arterial blood pressure. The peripheral vascular system is influenced greatly by the sympathetic–parasympathetic balance of the autonomic nervous system, the control of which originates in the central nervous system. Enhanced adrenergic activity is recognized as a principal contributor to primary (essential) hypertension.

Therapy using antihypertensive agents evolved rapidly between 1950 and 1960. During that time, a number of discoveries of drugs for the treatment and control of hypertensive disease were made. Despite the many years of experience, treatment remains empiric because the etiology of the principal form of hypertension, primary hypertension, is unknown. The first drugs used to produce symptomatic relief of hypertension were α-adrenergic blocking agents. These drugs had limitations because their duration of action was far too short and side effects precluded long-term therapy. Contemporary therapy of primary hypertension uses one of several drug classes as the first course. These drugs may be diuretics to reduce blood volume, inhibitors of the renin–angiotensin system (angiotensin-converting enzyme [ACE] inhibitors), and agents that reduce peripheral vascular resistance—for example, calcium channel blockers, vasodilators, and sympathetic nervous system depressants. The antihypertensive drug classes discussed in this section include ACE inhibitors, sympathetic nervous system depressants, and vasodilators acting on smooth muscle. Calcium channel blockers and other vasodilators have been included in earlier sections of this chapter. Diuretics are discussed in Chapter 18.

THE RENIN–ANGIOTENSIN SYSTEM AND HYPERTENSION

Renin–Angiotensin System Inhibitors

The renin–angiotensin system is a hormonal system that plays a central role in the control of sodium excretion and body fluid volume. It interacts closely with the sympathetic nervous system and aldosterone secretion in the regulation of blood pressure. Figure 19-14 shows the relationship of the component parts of the renin–angiotensin system and their main physiological effects.

The relationship between the renin–angiotensin system and blood pressure in humans has been known since before the beginning of the 20th century. Tigerstedt and Bergman[46] demonstrated in 1898 that kidney extract produced a potent vasopressor response when injected in a host. The substance was named "renin." Many years later, it was noted that this substance required a cofactor to produce vasoconstriction.[47] Eventually, in 1939, this hypertensive substance was isolated, identified as a decapeptide, and later called "angiotensin." It was observed that this cofactor existed as an inactive precursor, angiotensinogen. Even later, it was discovered that angiotensin existed in two forms, the biologically inactive decapeptide angiotensin I and the active octapeptide angiotensin II.[48]

The precursor of angiotensin, angiotensinogen, is a glycoprotein of molecular weight 58,000 to 61,000, synthesized primarily in the liver the brought into the circulatory system. Renin, an aspartyl protease (MW 35,000 to 40,000), whose primary source is the kidney, cleaves the Leu-Val bond from the aspartic acid end of the angiotensinogen polypeptide molecule to release the decapeptide angiotensin I (Fig. 19-15). The biochemical conversion continues with the cleavage of a dipeptide (His-Leu) from the carboxyl terminal of angiotensin I by ACE to form the octapeptide angiotensin II, a potent vasoconstrictor. Angiotensin III is formed by removal of the N-terminal aspartate residue of angiotensin II, a reaction catalyzed by glutamyl aminopeptidase. In contrast to angiotensin II, angiotensin III has a less potent but significant regulatory effect on sodium excretion by the renal tubules.

The regulatory action of the renin–angiotensin system in controlling sodium and potassium balance and arterial blood

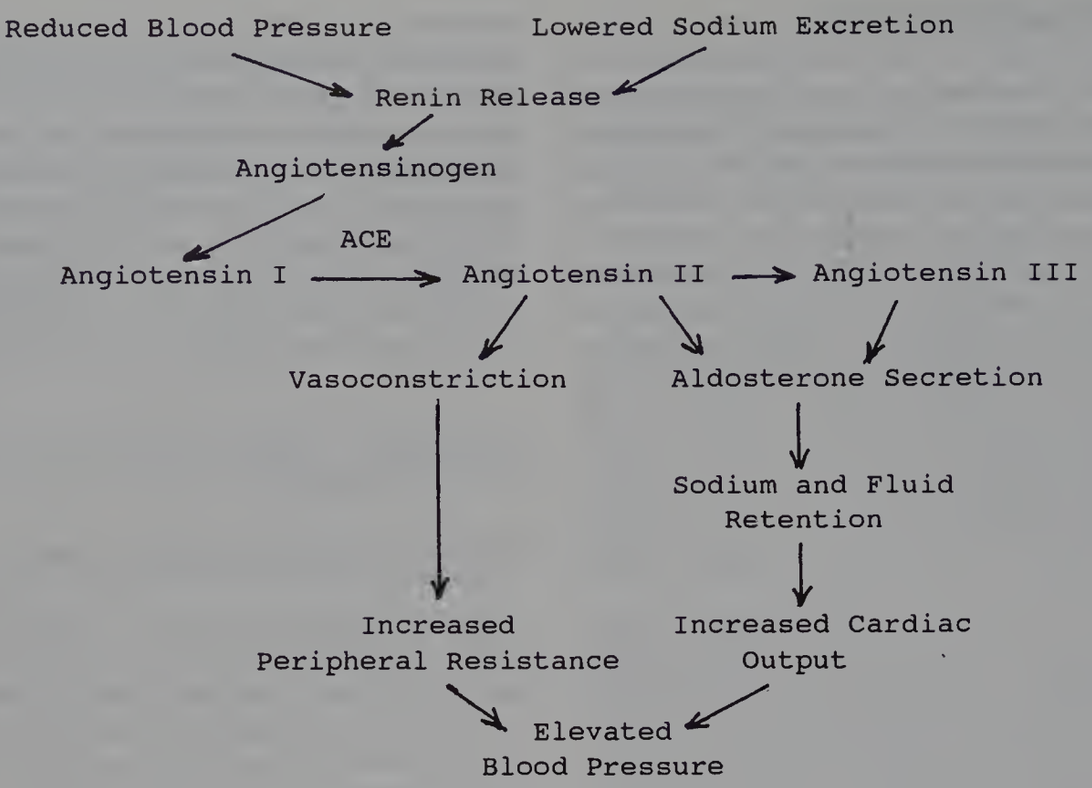


FIG. 19-14. *The renin-angiotensin system of blood pressure control.*

pressure is modified by vasodilators called "kinins." Kinins are formed by proteolytic enzymes that circulate in the plasma. Kallikrein is activated in plasma by noxious influences to act on a kinin, callidin, which is converted to bradykinin by tissue enzymes. Bradykinin enhances release of the prostaglandins PGE_2 and prostacyclin within certain tissues to produce a vasodilatory effect (Fig. 19-16). Bradykinin is converted to inactive products by ACE and other carboxypeptidases. Although ACE causes activation of angiotensin and inactivation of bradykinin, actions that appear to be opposite, the balance of the system seems to favor vasoconstriction.

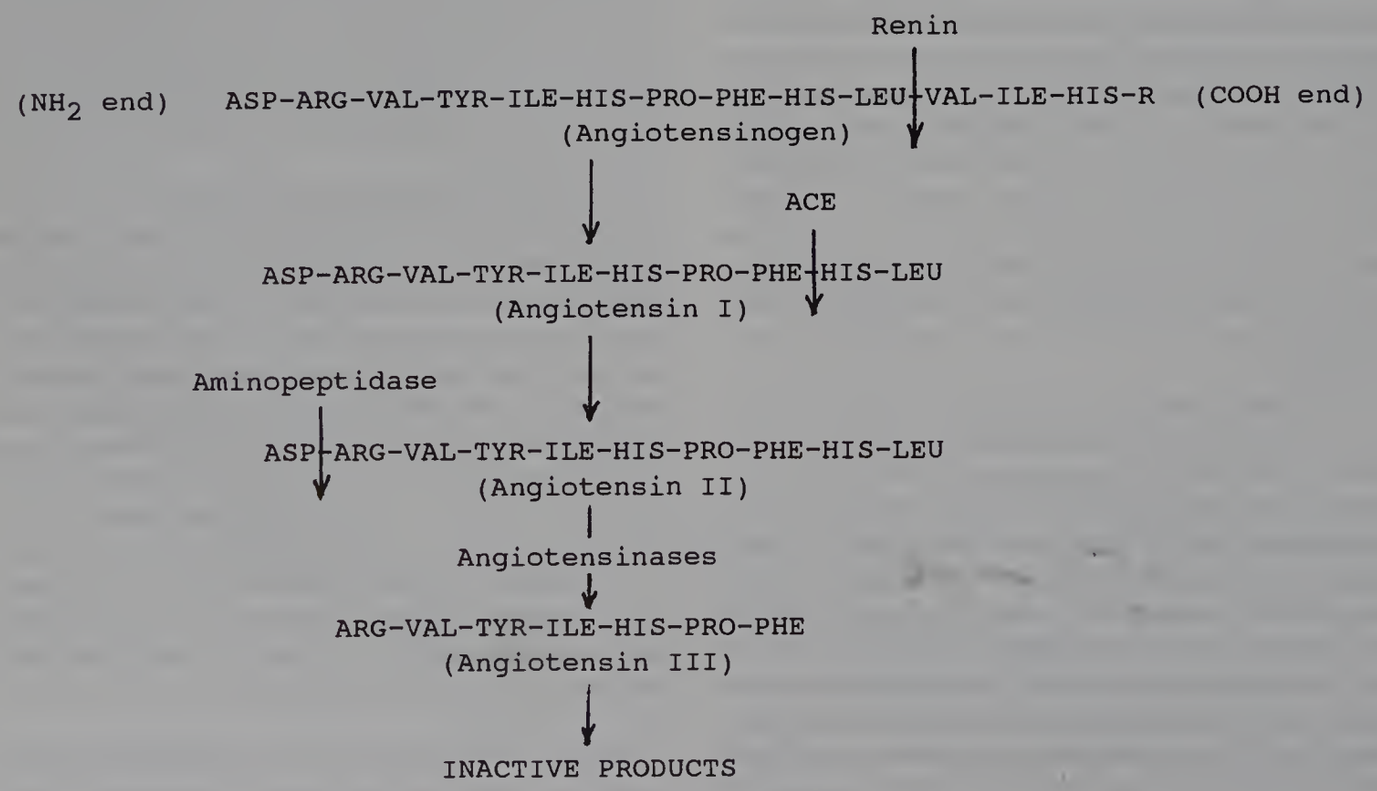


FIG. 19-15. *Biochemistry of the renin–angiotensin system. Formation of angiotensins from angiotensinogen.*

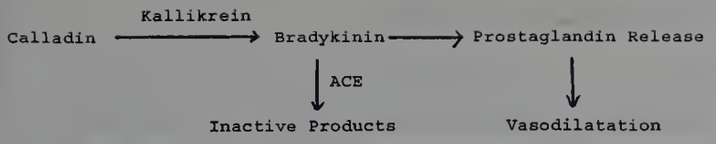


FIG. 19-16. *Bradykinin formation and action.*

ACE is a membrane-bound enzyme anchored to the cell membrane through a single transmembrane domain located near the carboxy-terminal extremity. The enzyme is a zinc-containing glycoprotein with a molecular weight of about 130,000. It is a nonspecific peptidyldipeptide hydrolase, widely distributed in mammalian tissues, that cleaves dipeptides from the carboxy-terminus of a number of endogenous peptides. The minimum structural requirement for binding and cleavage of a substrate by ACE is that it be a tripeptide with a free carboxylate group. A general exception is that peptides with a penultimate prolyl residue are not cleaved by this enzyme. This accounts for the biological stability of angiotensin II.[49] The important binding points at the active site of ACE are a cationic site to attract a carboxylate ion and a zinc ion that can polarize a carbonyl group of an amide function to make it more susceptible to hydrolysis. In the active site, there is a nucleophilic attack of the amide carbonyl by the γ-carbonyl group of a glutamic acid residue to cause hydrolysis of the peptide. Figure 19-17 shows a hypothetical model of the hydrolysis of angiotensin I by the active site of ACE. ACE exists in more than one form. Somatic ACE that regulates blood pressure found in most tissues differs from the isoenzyme ACE found in the testis. Somatic ACE, in contrast to testicular ACE, contains two binding domains. The principal active site for hydrolysis is the domain located in the C-terminal half of somatic ACE.[50]

Captopril. 1-[(2*S*)-3-mercapto-2-methyl-1-oxopropionyl]proline (Capoten). Captopril blocks the conversion of angiotensin I to angiotensin II by inhibiting the converting

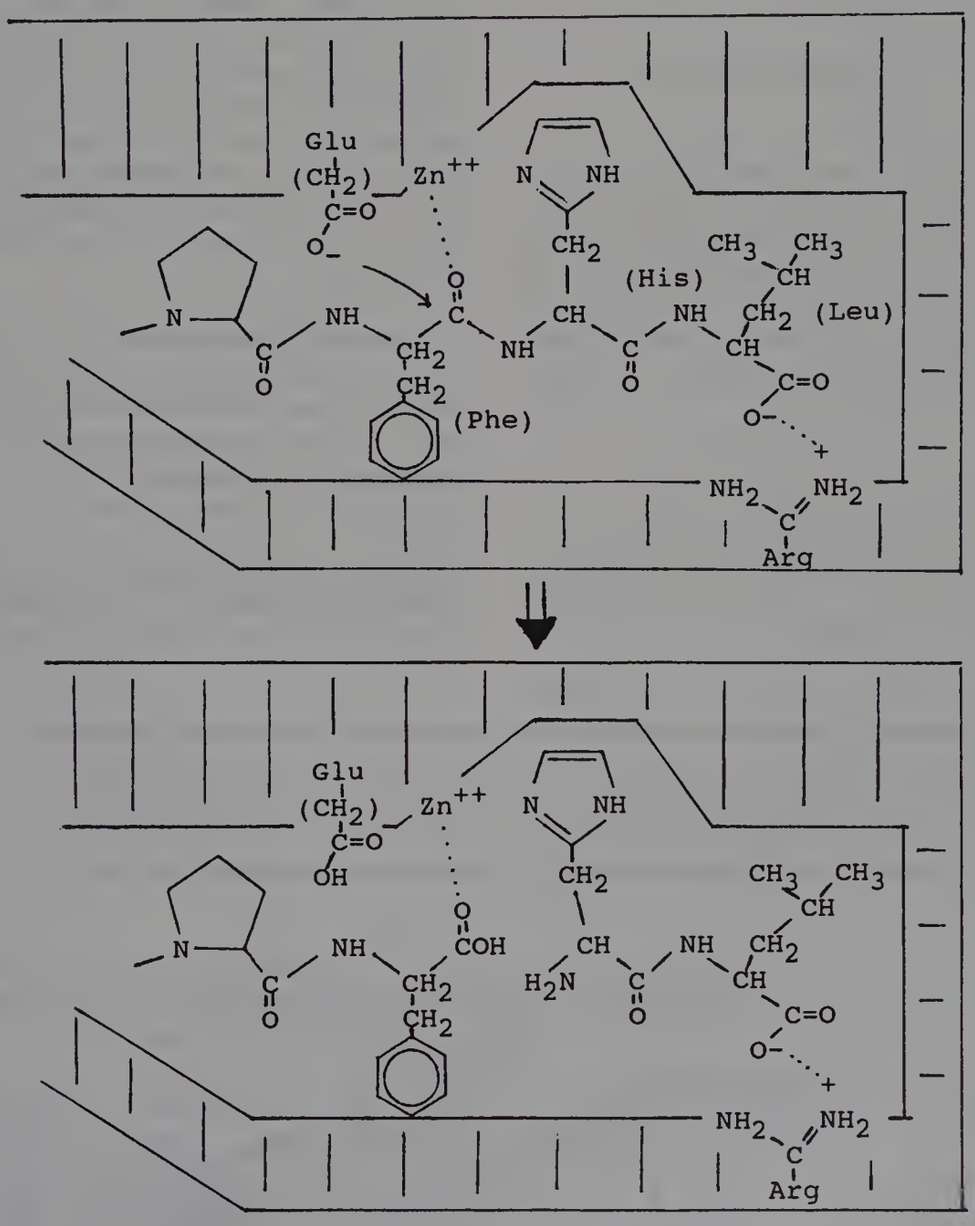
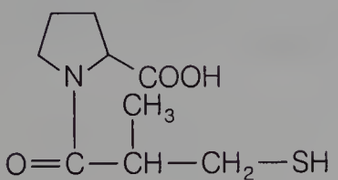


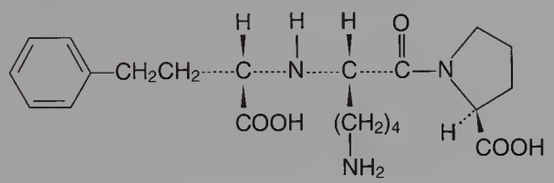
FIG. 19-17. *A model showing cleavage of the histidine-phenylalanine residue of angiotensin I by ACE to form the octapeptide antiotensin II and the dipeptide reside of histidine and leucine.*

enzyme. The rational development of Captopril as an inhibitor of ACE was based on the hypothesis that ACE and carboxypeptidase A functioned by similar mechanisms. It was noted that *d*-2-benzylsuccinic acid[49] was a potent inhibitor of carboxypeptidase A but not ACE. Using this small molecule as a prototype, captopril was designed with a carboxyl group on a proline and the introduction of a thiol group to enhance the binding to the zinc ion of ACE. The important binding points at the active site of ACE are thought to be an arginine residue, which provides a cationic site that attracts a carboxylate ion, and a zinc ion, which can polarize a carbonyl group of an amide function to make it more susceptible to hydrolysis. Hydrophobic pockets lie between these groups in the active site, as does a functional group that forms a hydrogen bond with an amide carbonyl. Figure 19-18 shows the hypothetical binding of captopril in the active site of ACE.



Captopril

Lisinopril. 1-[N^2-[*S*-1-Carboxy-3-phenylpropyl]-L-lysyl] L-proline (Prinivil, Zestil). Lisinopril is a lysine derivative of enalaprilat, the active metabolite of enalapril. Like all ACE inhibitors, it is an active site-directed inhibitor of the enzyme, using the zinc ion in an effective binding interaction at a stoichiometric ratio of 1 : 1. The pharmacological effects of lisinopril are similar to those of captopril and enalapril.



Lisinopril

ACE Inhibitor Prodrugs

Many new ACE inhibitors have become available for the treatment of hypertension following the clinical effectiveness of enalapril. Enalapril is a non–thiol-containing ACE inhibitor devoid of the side effects of skin rashes and loss of the sense of taste characteristic of the thiol-containing compound captopril. With the exception of the phosphorus-containing fosinopril, these antihypertensive agents have a 2-(*S*)-aminophenylbutyric acid ethyl ester moiety differing only in the substituents on the amino group. They have the common property of acting as prodrugs, being converted to the active enzyme inhibitor following absorption and metabolism by liver and intestinal enzymes. These drugs (Fig. 19-19) are used, as is the prototypical drug captopril, in the treatment of mild to moderate hypertension either alone or in conjunction with diuretics or calcium channel blockers. Table 19-5 compares some of their properties.

Enalapril Maleate. 1-[*N*[(*S*)-1-Carboxy-3-phenylpropyl]-L-alanyl]-L-proline 1′-ethyl ester, maleate (Vasotec). Enalapril maleate is a long-acting ACE inhibitor. It requires activation by hydrolysis of its ethyl ester to form the diacid enalaprilat. Enalapril is devoid of the side effects of rashes and loss of taste seen with captopril. These side effects are similar to those of the mercapto-containing drug penicillamine. The absence of the thiol group in enalapril maleate may give it its freedom from these side effects. The half-life is 11 hr.

Benazepril Hydrochloride. (3*S*)-3-[[(1*S*)-1-Carbethoxy-3-phenylpropyl] amino]-2,3,4,5-tetrahydro-2-oxo-1*H*-1-benzazepine-1-acetic acid hydrochloride (Lotensin). Benazepril hydrochloride is metabolized rapidly to the active diacid benazaprilat. As with the ACE prodrugs, no mutagenicity has been found, even though these drugs cross the placenta.

Quinapril Hydrochloride. (*S*)-[(*S*)-*N*-[(*S*)21-Carboxy-3-phenylpropyl]alanyl]-1, 2, 3, 4-tetrahydro-3-isoquinolinecarboxylic acid 1-ethyl ester hydrochloride (Acuretic). Quin-

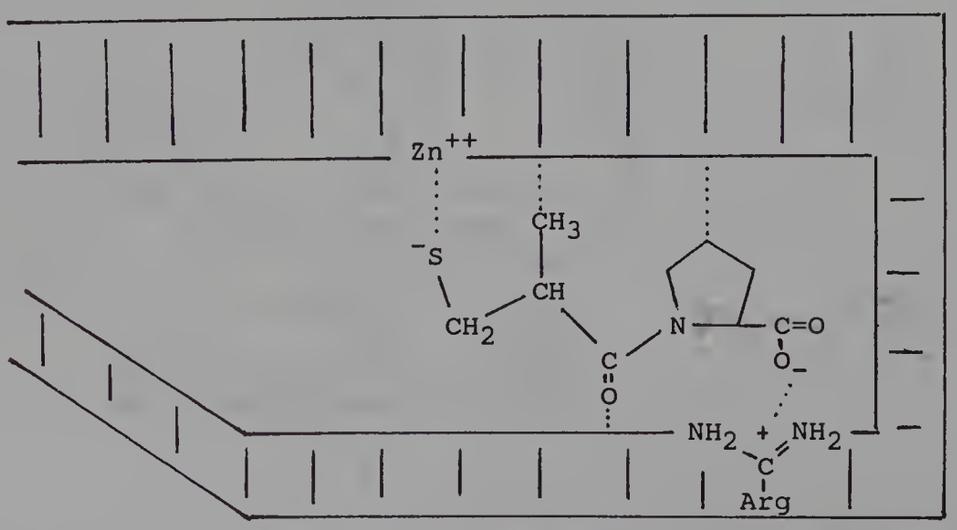
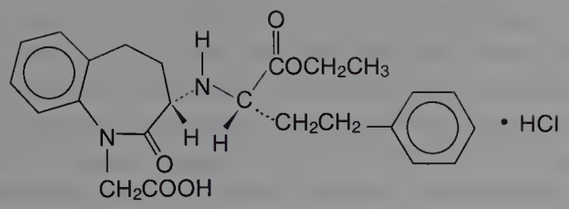
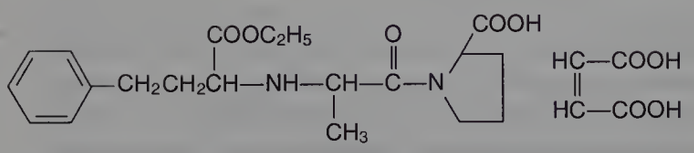


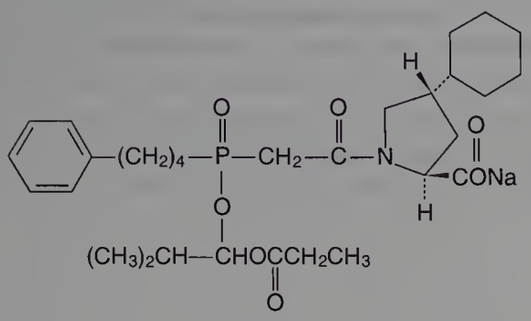
FIG. 19-18. Accommodation of captopril to the active site of ACE.



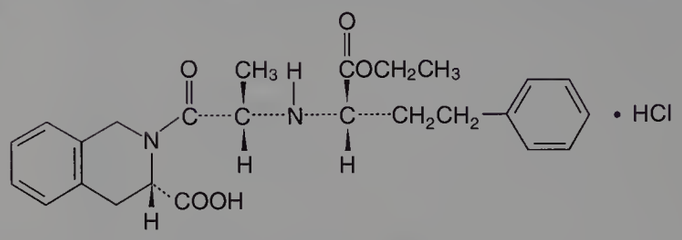
Benzapril Hydrochloride



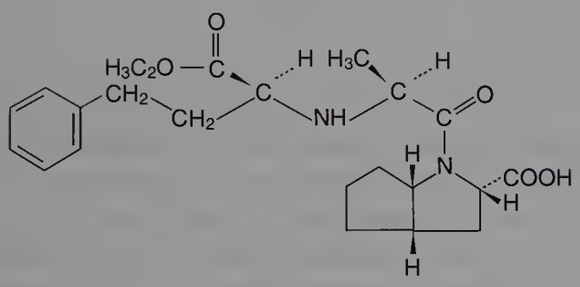
Enalapril Maleate



Fosinopril Sodium



Quinapril Hydochloride



Ramipril

FIG. 19-19. *Angiotensin-converting enzyme–inhibitor prodrugs.*

april hydrochloride forms the diacid quinaprilate in the body. It is more potent than captopril and equipotent to the active form of enalapril.

Ramipril. (2*S*, 3a*S*, 6a*S*)-1-[(S)-*N*-[(*S*-1-Carboxy 3-phenylpropyl]alanyl] octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid, 1-ethyl ester (Altace). Ramipril is hydrolyzed to ramiprilat, its active diacid form, faster than enalapril is hydrolyzed to its active diacid form. Peak serum concentrations from a single oral dose are achieved between 1.5 and 3.0 hr. The ramiprilate formed completely suppresses ACE activity for up to 12 hr, with 80% inhibition of the enzyme still observed after 24 hr.

Fosinopril Sodium. (4*S*)-Cyclohexyl-1-[[(*RS*)-1-hydroxy-2-methyl-propoxy] (4-phenylbutyl)-phosphinyl] acetyl]-L-proline, sodium salt (Monopril). Fosinopril is a phosphorus-containing ACE inhibitor. It is inactive but serves as a prodrug, being completely hydrolyzed by intestinal and liver enzymes to the active diacid fosinoprilat.

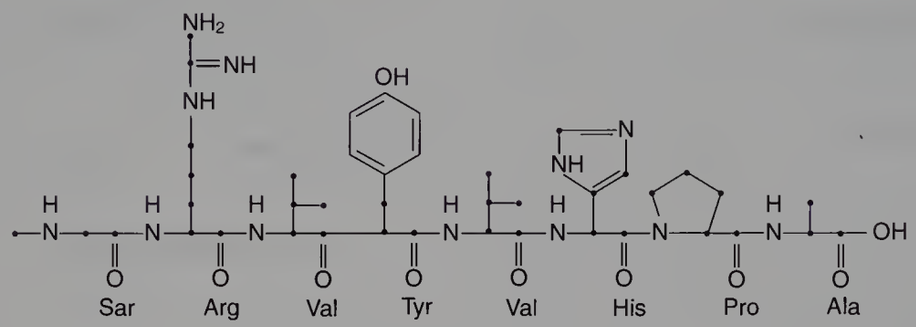
TABLE 19-5

ANGIOTENSIN-CONVERTING ENZYME–INHIBITOR PRODRUGS

Prodrug	*Metabolite*	*Metabolite Protein Binding (%)*	*Metabolite Plasma $t_{1/2}$ (Hours)*	*Mode of Excretion*
Benazepril	Benazeprilat	95	10–11	Renal
Enalapril	Enalaprilat	50–60	11.0	Renal
Fosinopril	Fosinoprilat	97	11.5	Renal/fecal
Quinapril	Quinaprilat	97	3.0	Renal/fecal
Ramipril	Ramiprilat	56	13–17	Renal/fecal

Angiotensin Antagonists

Inhibition of angiotensin II to produce a vasodilatory effect can be accomplished by administration of the competitive antagonist saralasin, an octapeptide that differs from angiotensin by two amino acids. Saralasin is not a pure competitive antagonist but a weak partial agonist. It is most effective when administered to patients who have a renin-dependent form of hypertension, lowering their blood pressure. In patients whose renin–angiotensin system effect on blood pressure is low, it tends to increase aldosterone secretion, causing sodium retention, and can cause blood pressure to rise. The drug is limited because it must be administered as an intravenous injection and has a short duration of action.



Saralasin

Adrenergic System Inhibitors

Drugs that reduce blood pressure by depressing the activity of the sympathetic nervous system have been used as effective agents in the treatment of hypertension. This can be accomplished in several ways: (1) depleting the stores of neurotransmitter, (2) reducing the number of impulses traveling in sympathetic nerves, (3) antagonizing the actions of the neurotransmitter on the effector cells, and (4) inhibiting neurotransmitter release.

Agents Depleting Neurotransmitter Stores. Folk remedies prepared from the species of *Rauwolfia*, a plant genus belonging to the Apocynaceae family, have been reported as early as 1563. The root of the species *R. serpentina* has been used for centuries as an antidote to stings and bites of insects, to reduce fever, as a stimulant to uterine contractions, for insomnia, and particularly for the treatment of insanity. Its use in hypertension was recorded in the Indian literature in 1918, but it was not until 1949 that hypotensive properties of *Rauwolfia* species appeared in the Western literature.[51] *Rauwolfia* preparations were introduced in psychiatry for the treatment of schizophrenia in the early 1950s, following confirmation of the folk remedy reports on their use in mentally deranged patients. By the end of the 1960s, however, the drug had been replaced by more efficacious neurotropic agents. Reserpine and its preparations remain useful in the control of mild essential hypertension.

The effects of reserpine do not correlate well with tissue levels of the drug. The pharmacological effects of reserpine were still present in animals when it could no longer be detected in the brain.[52] Reserpine depletes catecholamines and serotonin from central and peripheral neurons by interfering with the uptake of these amines from the cytosol into the vesicles and granules.[53,54] As a consequence, norepinephrine cannot be stored intraneuronally in adrenergic neurons and much of the norepinephrine in the cytosol is metabolized by monoamine oxidase (Fig. 19-20). The binding of reserpine to the storage vesicle membrane is firm, and as a result, the storage granule is destroyed, reducing the ability of the nerve to concentrate and store norepinephrine. Since reserpine acts on both central and peripheral adrenergic neurons, its antihypertensive effects may be the result of neurotransmitter depletion from both of these sites.

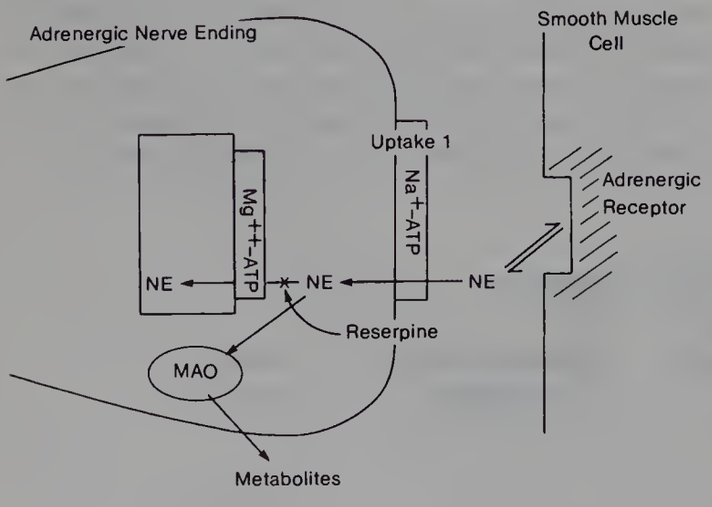
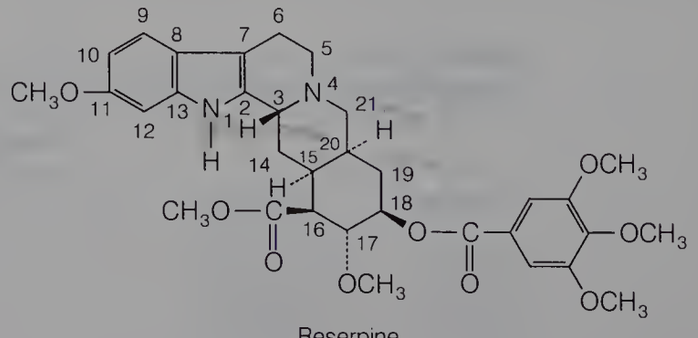
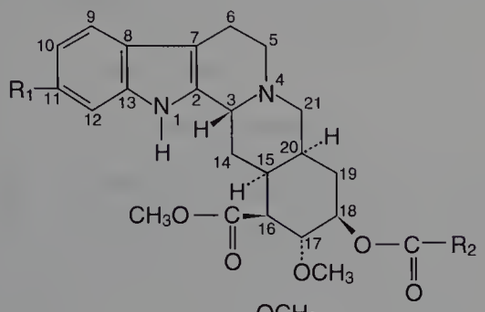


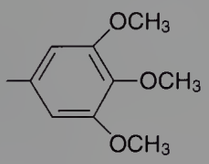
FIG. 19-20. Action of reserpine at adrenergic nerve ending.

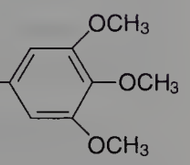


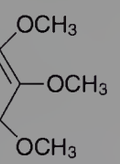
Reserpine

Chemical investigations of the active components of *R. serpentina* roots have yielded several alkaloids (e.g., ajmaline, ajmalicine, ajmalinine, serpentine, serpentinine, and others). Reserpine, which is the major active constituent of *Rauwolfia*,[55] was isolated in 1952 and is a much weaker base than the alkaloids just mentioned. Reserpinoid alkaloids are yohimbine-like bases that have an additional functional group on C-18. Only three naturally occurring alkaloids possess reserpine-like activity strong enough for use in treating hypertension: reserpine, deserpidine, and rescinnamine.

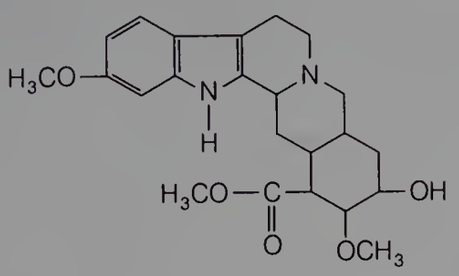


Reserpine $R_1 = OCH_3$; $R_2 =$ 

Rescinnamine $R_1 = OCH_3$; $R_2 = -CH=CH-$ 

Deserpidine $R_1 = H$; $R_2 =$ 

Reserpine is absorbed rapidly after oral administration. Fat tissue accumulates reserpine slowly, with a maximal level being reached between 4 and 6 hr. After 24 hr there are small amounts of reserpine in the liver and fat but none in the brain or other tissues. Reserpine is metabolized by the liver and intestine to methyl reserpate and 3,4,5-trimethoxybenzoic acid.



Methyl Reserpate

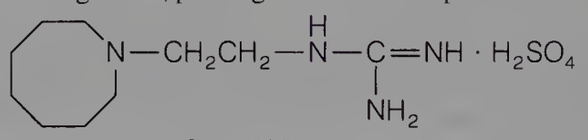
Powdered Rauwolfia Serpentina, USP (Raudixin, Rauserpal, Rauval) is the powdered whole root of *R. serpentina* (Benth). It is a light tan to light brown powder, sparingly soluble in alcohol and only slightly soluble in water. It contains the total alkaloids, of which reserpine accounts for about 50% of the total activity. Orally, 200 to 300 mg is roughly equivalent to 500 μg of reserpine. It is used in the treatment of mild or moderate hypertension or in combination with other hypotensive agents in severe hypertension.

Reserpine, USP (Serpasil, Reserpoid, Rau-Sed, Sandril). This is a white to light yellow, crystalline alkaloid, practically insoluble in water, obtained from various species of *Rauwolfia*. In common with other compounds with an indole nucleus, it is susceptible to decomposition by light and oxidation, especially when in solution. In the dry state, discoloration occurs rapidly when exposed to light, but the loss in potency is usually small. In solution, reserpine may break down when exposed to light, especially in clear glass containers, with no appreciable color change; thus, color change cannot be used as an index of the amount of decomposition.

Reserpine is effective orally and parenterally for the treatment of hypertension. After a single intravenous dose, the onset of antihypertensive action usually begins in about 1 hr. After intramuscular injection, the maximum effect occurs within approximately 4 hr and lasts about 10 hr. When given orally, the maximum effect occurs within about 2 weeks and may persist up to 4 weeks after the final dose. When used in conjunction with other hypotensive drugs in the treatment of severe hypertension, the daily dose varies from 100 to 250 μg.

Guanethidine and Related Compounds. Guanethidine has been classified traditionally as an adrenergic blocking agent because it is able to prevent the release of norepinephrine from postganglionic neurons in response to adrenergic stimulation. Guanethidine and other compounds discussed in this section have other actions on catecholamine metabolism and can cause significant depletion of these amines in adrenergic neurons. They do not interfere with release of epinephrine from the adrenal medulla.

Guanethidine Monosulfate, USP. [2-(Hexahydro-1(2*H*)-azocinyl)ethyl]guanidine sulfate (Ismelin sulfate) is a white, crystalline material that is very soluble in water. It was one of a series of guanidine compounds prepared in the search for potent antitrypanosomal agents. There is an absence of central nervous system effects, such as depression, because the drug is highly polar and does not easily cross the blood–brain barrier. Guanethidine monosulfate produces a gradual, prolonged fall in blood pressure. Usually



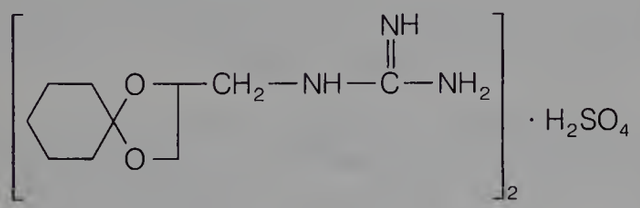
Guanethidine monosulfate

2 to 7 days of therapy are required before the peak effect is reached, and usually, this peak effect is maintained for 3 or 4 days; then, if the drug is discontinued, the blood pressure returns to pretreatment levels over a period of 1 to 3 weeks. Because of this slow onset and prolonged duration of action, only a single daily dose is needed.

Guanethidine monosulfate is metabolized by microsomal enzymes to 2-(6-carboxyhexylamino)ethylguanidine and

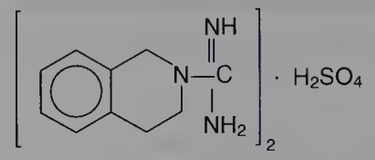
guanethidine *N*-oxide (Fig. 19-21). Both metabolites have very weak antihypertensive properties. Guanethidine monosulfate is taken up by the amine pump located on the neuronal membrane and retained in the nerve, displacing norepinephrine from its storage sites in the neuronal granules. The displaced norepinephrine is metabolized to homovanillic acid by mitochondrial monoamine oxidase, depleting the nerve ending of the neurotransmitter. The usefulness of guanethidine monosulfate also resides in the fact that once it is taken up by the nerve, it produces a sympathetic blockade by inhibiting release of nonepinephrine that would occur on neuronal membrane response to stimulation[28] by the nerve action potential. Guanethidine monosulfate stored in the granules is released by the nerve action potential but has a very low intrinsic activity for the adrenergic receptors on the postjunctional membrane. Moderate doses for a prolonged period or large doses may produce undesirable side effects by causing neuromuscular blockade and adrenergic nerve conduction blockade.

Guanadrel Sulfate. (1,4-Dioxaspiro[4.5]dec-2-ylmethyl)guanadine sulfate (Hylorel). Guanadrel sulfate is similar to guanethidine monosulfate in the manner in which it reduces elevated blood pressure. It acts as a postganglionic adrenergic blocking agent by displacing norepinephrine in adrenergic neuron storage granules, thereby preventing release of the endogenous neurotransmitter on nerve stimulation. Guanadrel sulfate has a much shorter half-life (10 hr) than guanethidine monosulfate, the half-life of which is measured in days. In the stepped-care approach to hypertension, guanadrel sulfate is usually a step 2 agent.



Guanadrel Sulfate

Debrisoquin Sulfate. 3,4-Dihydro-2(1*H*)-isoquinoline carboxamidine sulfate (Declinax). Debrisoquin sulfate has antihypertensive properties similar to those of guanethidine monosulfate but is of shorter duration because of rapid hepatic metabolism. It is converted rapidly to the 4-hydroxydebrisoquin metabolite by the cytochrome oxidases in the liver.[55] It also differs from guanethidine monosulfate in that it has relatively little ability to block neuronal uptake of norepinephrine. Debrisoquin sulfate is marketed in Canada but not in the United States.



Debrisoquin Sulfate

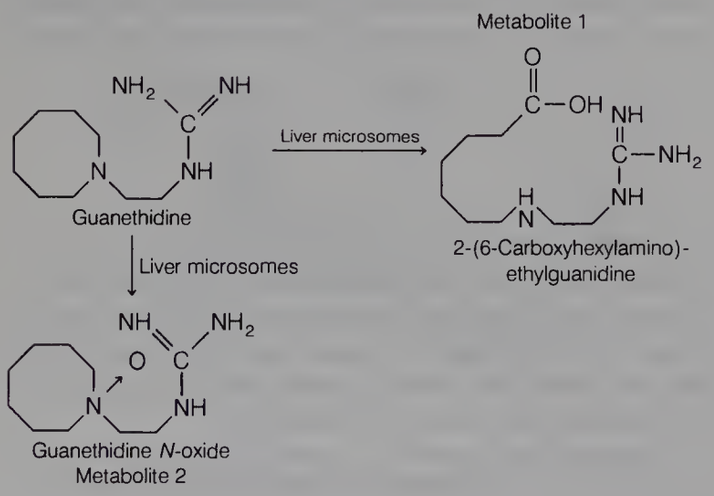
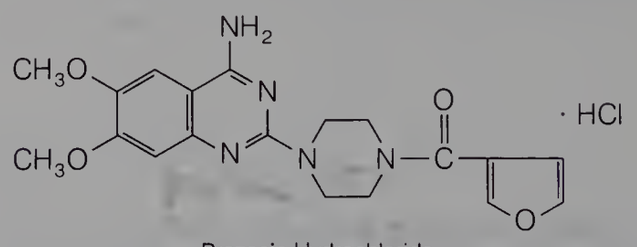


FIG. 19-21. *Metabolism of guanethidine monosulfate.*

Selective α-Adrenergic Antagonists. The principal clinical use of α-adrenergic antagonists is in the treatment of catecholamine-dependent hypertension. Classic drugs such as phentolamine and phenoxybenzamine are nonspecific blocking agents of both α_1- and α_2-receptors on the presynaptic membrane of the adrenergic neuron. Specific antagonists of α_1-receptors are effective antihypertensive agents by blocking the vasocontricting effect on smooth muscle and not interfering with the activation of α_2-receptors on the adrenergic neuron, which when activated inhibit further release of norepinephrine.

Prazosin Hydrochloride. 1-(4-Amino-6,7-dimethoxy-2-quinazolinyl)4-(2-furoyl)-piperazine monohydrochloride (Minipress). The antihypertensive effects of this drug are due to its peripheral vasodilation as a result of its blockade of α_1-adrenergic receptors. In ligand-binding studies, prazosin hydrochloride has 5000-fold greater affinity for α_1 than for some α_2-adrenergic receptors.[57]

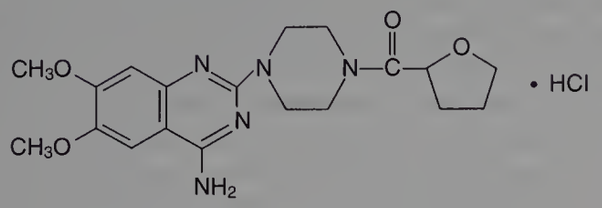
Prazosin hydrochloride is readily absorbed, and plasma concentrations reach a peak about 3 hr after administration. Plasma half-life is between 2 and 3 hr. Prazosin hydrochloride is highly bound to plasma protein; however, it does not cause adverse reactions with drugs that might be displaced from their protein-binding sites (e.g., cardiac glycosides). It may cause severe orthostatic hypertension because of its α-adrenergic blocking action, which prevents the reflex venous constriction that is activated when an individual sits up from a prone position.



Prazosin Hydrochloride

Terazosin Hydrochloride. 1-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-4-(tetrahydro-2-furoyl)piperazine monohydrochloride (Hytrin). Terazosin hydrochloride is a structural congener of prazosin hydrochloride. It possesses similar selective properties of specifically inhibiting α_1-adrenergic re-

ceptors. The drug is slightly less potent than prazosin hydrochloride. Terazosin hydrochloride has a half-life of approximately 12 hr, which is much longer than that of prazosin. This lends itself to a once-daily dose to control hypertension in many patients.



Terazosin Hydrochloride

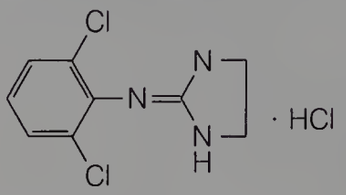
Centrally Acting Adrenergic Drugs. The use of agents that directly affect the peripheral component of the sympathetic nervous system represents an important approach to the treatment of hypertension. A second approach to modifying sympathetic influence on the cardiovascular system is through inhibition or reduction of central nervous system control of blood pressure. Several widely used medications act by stimulating α_2-receptors, which in the central nervous system reduces sympathetic outflow to the cardiovascular system and produces a hypotensive effect.

Methyldopate Hydrochloride, USP. L-3-(3,4-Dihydroxyphenyl)-2-methylalanine ethyl ester hydrochloride (Aldomet ester hydrochloride). Alpha methyldopa lowers blood pressure by inhibiting the outflow of sympathetic vasoconstrictor impulses from the brain. Early studies had suggested that the hypotensive action of α-methyldopa was due to the peripheral properties of the drug as a decarboxylase inhibitor or as a false transmitter.

The current hypothesis concerning the hypotensive activity of methyldopa involves the central nervous system as the site of action.[58] Methyldopa, upon conversion to α-methylnorepinephrine, acts on α_2-adrenergic receptors to inhibit the release of norepinephrine, resulting in a decrease of sympathetic outflow from the central nervous system and an activation of parasympathetic outflow.

Methyldopa is used as a step 2 agent and is recommended for patients with high blood pressure who are not responsive to diuretic therapy alone. Methyldopa, suitable for oral use, is a zwitterion and is not soluble enough for parenteral use. The problem was solved by making the ester, leaving the amine free to form the water-soluble hydrochloride salt. It is supplied as a stable, buffered solution, protected with antioxidants and chelating agents.

Clonidine Hydrochloride. 2-[(2,6-Dichlorophenyl)imino]imidazolidine monohydrochloride (Catapres) was the first antihypertensive known to act on the central nervous system. It was synthesized in 1962 as a derivative of the known α-sympathomimetic drugs naphazoline and tolazoline, potential nasal vasoconstrictors, but instead it has proved to be effective in the treatment of mild to severe hypertension.

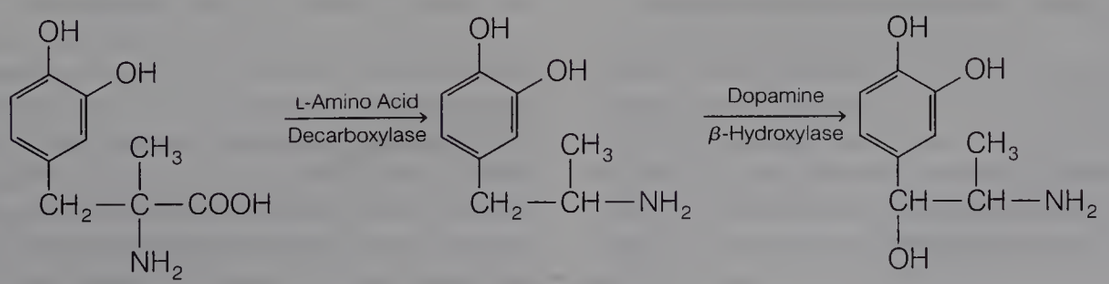


Clonidine Hydrochloride

Clonidine hydrochloride acts by both peripheral and central mechanisms in the body to affect blood pressure. It stimulates the peripheral α-adrenergic receptors to produce vasoconstriction, resulting in a brief period of hypertension. Clonidine hydrochloride acts centrally to inhibit the sympathetic tone and cause hypotension that is of much longer duration than the initial hypertensive effect. Administration of clonidine hydrochloride thus produces a biphasic change in blood pressure, beginning with a brief hypertensive effect and followed by a hypotensive effect that persists for about 4 hr. This biphasic response is altered by dose only: larger doses produce a greater hypertensive effect and delay the onset of the hypotensive properties of the drug. Clonidine hydrochloride acts on α_2-adrenoreceptors located in the hindbrain to produce its hypotensive action. Clonidine hydrochloride also acts centrally to cause bradycardia and to reduce plasma levels of renin. Sensitization of baroreceptor pathways in the central nervous system appears to be responsible for the bradycardia transmitted by way of the vagus nerve. However, the central mechanism that results in plasma renin decrease is not known. The hypotensive properties of clonidine in animals can be blocked by applying α-adrenergic blocking agents directly to the brain.[59]

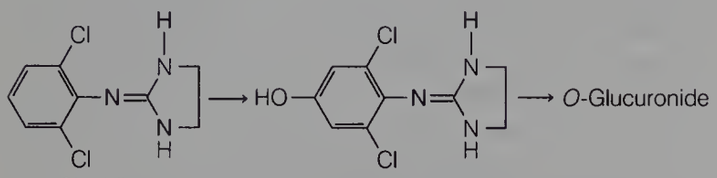
Clonidine hydrochloride has advantages over antihypertensive drugs such as guanethidine monosulfate and prazosin hydrochloride in that it seldom produces orthostatic hypotensive side effects. However, it does have some sedative properties that are undesirable; it also may cause constipation and dryness of the mouth.

Clonidine hydrochloride is distributed throughout the body, with the highest concentrations found in the organs



Methyldopa | α-Methyldopamine | α-Methylnorepinephrine

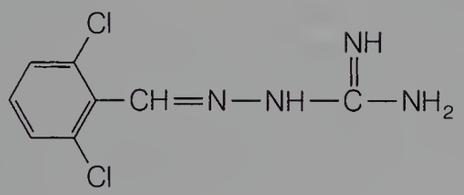
of elimination: kidney, gut, and liver. Brain concentrations are low but higher than plasma concentrations. The high concentration in the gut is due to an enterohepatic cycle wherein clonidine hydrochloride is secreted into the bile in rather high concentrations. The half-life in humans is about 20 hr. Clonidine hydrochloride is metabolized by the body to form two major metabolites, *p*-hydroxyclonidine and its glucuronide. *p*-Hydroxyclonidine does not cross the blood–brain barrier and has no hypotensive effect in humans.



Clonidine Hydrochloride — *p*-Hydroxyclonidine

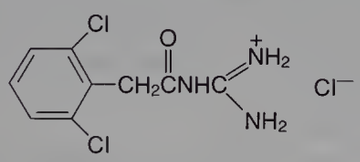
Metabolism of Clonidine

Guanabenz Acetate. [(2,6-Dichlorobenzylidene)amino]-guanidine monoacetate (Wytensin). Guanabenz acetate is a central α_2-adrenergic agonist that reduces the release of norepinephrine from the neuron when stimulated. The effect of the drug results in a decreased sympathetic tone in the heart, kidneys, and peripheral blood vessels. The drug does not produce orthostatic hypotension.



Guanabenz Acetate

Guanfacine Hydrochloride. *N*-(Aminoiminomethyl)-2,6-dichlorobenzeneacetamide (Tenex). Guanfacine hydrochloride is structurally related to clonidine hydrochloride and guanabenz acetate, sharing many of their pharmacological properties. The drug has a longer duration of action than either clonidine hydrochloride or guanabenz acetate. It lasts up to 24 hr. It also requires much longer (8 to 12 hr) for a peak effect to occur after the drug is administered.



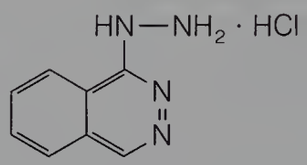
Guanfacine Hydrochloride

Vasodilatory Drugs Acting on Smooth Muscle

Reduction of arterial smooth muscle tone may occur by many mechanisms, such as reduction in sympathetic tone, stimulation of β-adrenergic receptors, or even direct action on the vasculature without interference from the autonomic innervation. Drugs acting on the arteriolar smooth muscle also have the property of increasing sympathetic reflex activity, causing an increase in heart rate and cardiac output, and stimulating renin release, which increases sodium retention and plasma volume. As a result, it is common to coadminister saluretics and β-adrenergic blocking drugs with these agents.

Antihypertensive agents that produce vasodilation of smooth muscle can be divided into two categories: direct-acting and indirect-acting vasodilators. Indirect-acting vasodilators may be distinguished from direct-acting in that they produce their effect by interfering with the vasoconstrictor stimuli and that their primary site of action is not necessarily the vascular smooth muscle itself. Indirect-acting vasodilators are characterized by the following: sympatholytic drugs, such as reserpine; α-adrenergic antagonists, such as prazosin hydrochloride; ACE inhibitors; and angiotensin II receptor antagonists, such as saralysin. Direct-acting vasodilators are characterized by hydralazine hydrochloride, sodium nitroprusside, potassium channel openers, and calcium channel-blocking agents.[59]

Hydralazine Hydrochloride, USP. 1-Hydrazinophthalazine monohydrochloride (Apresoline hydrochloride) originated from the work of a chemist[60] attempting to produce some unusual chemical compounds and from the observation[61] that this compound had antihypertensive properties. It occurs as yellow crystals and is soluble in water to the extent of about 3%. A 2% aqueous solution has a *p*H of 3.5 to 4.5.



Hydralazine Hydrochloride

Hydralazine hydrochloride is useful in the treatment of moderate to severe hypertension. It is often used in conjunction with less potent antihypertensive agents because when used alone in adequate doses there is a frequent occurrence of side effects. In combinations, it can be used in lower and safer doses. Its action appears to be centered on the smooth muscle of the vascular walls, with a decrease in peripheral resistance to blood flow. This results in an increased blood flow through the peripheral blood vessels. Also of importance is its unique property of increasing renal blood flow, an important consideration in patients with renal insufficiency.

Hydralazine hydrochloride acts on vascular smooth muscle to cause relaxation. Its mechanism of action is unclear. It interferes with Ca^{2+} entry and Ca^{2+} release from intracellular stores and has been reported to cause activation of guanylate cyclase, resulting in increased levels of cGMP. All of these biochemical events can cause vasodilation.

Absorption of hydralazine hydrochloride taken orally is rapid and nearly complete. The maximal hypotensive effect is demonstrable within 1 hr. The drug is excreted rapidly by the kidneys, and within 24 hr 75% of the total amount administered appears in the urine as metabolites or unchanged drug. Hydralazine hydrochloride undergoes benzylic oxidation, glucuronide formation, and *N*-acetylation by the microsomal enzymes in the tissues (Fig. 19-22). Acetyla-

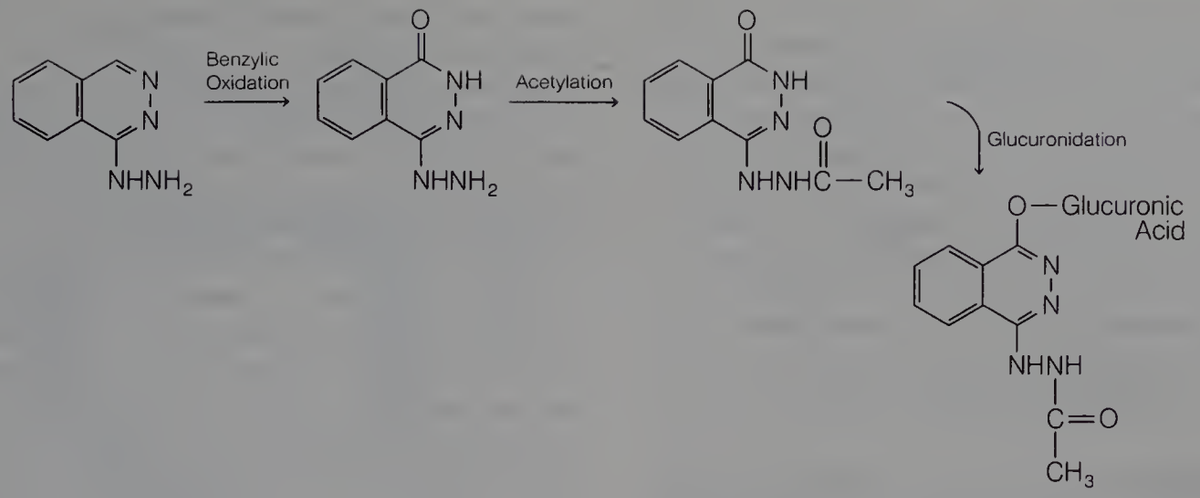


FIG. 19-22. *Metabolism of hydralazine hydrochloride.*

tion appears to be a major determinant of the rate of hepatic removal of the drug from the blood and, therefore, of systemic availability.[62] Rapid acetylation results in a highly hepatic extraction ratio from blood and a greater first-pass elimination.

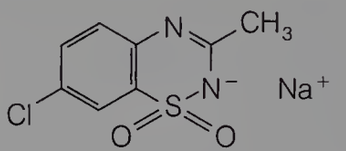
Hydralazine hydrochloride is more effective clinically when coadministered with drugs that antagonize adrenergic transmission (e.g., β-adrenergic antagonists, reserpine, guanethidine monosulfate, methyldopa, and clonidine hydrochloride). When given with diuretics, it is useful in the treatment of congestive heart failure.

Sodium Nitroprusside, USP. Sodium nitroferricyanide; disodium pentacyanonitrosylferrate(2) $Na_2[Fe(CN)_5NO]$ (Nipride) is one of the most potent blood pressure-lowering drugs. Its use is limited to hypertensive emergencies because of its short duration of action. The effectiveness of sodium nitroprusside as an antihypertensive has been known since 1928, but it was not until 1955 that its efficacy as a drug was established.[63] The drug differs from other vasodilators in that vasodilation occurs in both venous and arterial vascular beds. Sodium nitroprusside is a reddish-brown water-soluble powder that is decomposed by light when in solution. The hypotensive effect of the chemical is due to the formation of NO in situ (see "Nitrovasodilators"), elevating cellular levels of cGMP. Sodium nitroprusside is metabolized by the liver, yielding thiocyanate. Because thiocyanate is excreted by the kidney, patients with impaired renal function may suffer thiocyanate toxicity.

Potassium Channel Agonists

The two agents that can be classified in this category are diazoxide and minoxidil. These drugs are also called "potassium channel openers." These agents activate ATP-sensitive potassium channels, which leads to a decrease of intracellular Ca^{2+} and reduces the excitability of smooth muscle. The primary action of these drugs is to open potassium channels in the plasma membrane of vascular smooth muscle. An efflux of potassium from the cell follows, resulting in hyperpolarization of the membrane that produces an inhibitory influence on membrane excitation and subsequent vasodilation.

Diazoxide, USP. (Hyperstat IV). This drug is used as the sodium salt of 7-chloro-3-methyl-2*H*-1,2,4-benzothiadiazine 1,1-dioxide.



Sodium Diazoxide

Diazoxide lowers peripheral vascular resistance, increases cardiac output, and does not compromise renal blood flow.

This is a des-sulfamoyl analogue of the benzothiazine diuretics and has a close structural similarity to chlorothiazide. It was developed intentionally to increase the antihypertensive action of the thiazides and to minimize the diuretic effect.

It is used by intravenous injection as a rapidly acting antihypertensive agent for the emergency reduction of blood pressure in hospitalized patients with accelerated or malignant hypertension. Over 90% is bound to serum protein, and caution should be exercised when it is used in conjunction with other protein-bound drugs, which may be displaced by diazoxide. The injection is given rapidly by the intravenous route to ensure maximal effect. The initial dose is usually 1 mg/kg body weight, with a second dose given if the first injection does not elicit a satisfactory lowering of blood pressure within 30 minutes. Further doses may be given at 4- to 24-hr intervals if needed. Oral antihypertensive therapy is begun as soon as possible.

The injection has a *p*H of about 11.5, which is necessary to convert the drug to its soluble sodium salt. There is no significant chemical decomposition after storage at room temperature for 2 years. When the solution is exposed to light, it darkens.

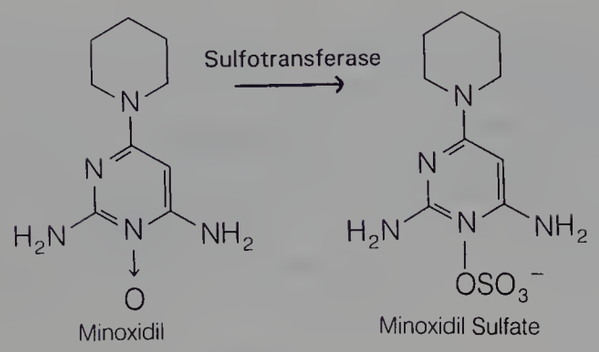


FIG. 19-23. *Activation of minoxidil.*

Minoxidil, USP. 2,4-Diamino-6-piperidinopyrimidine-3-oxide (Loniten) was developed as a result of isosteric replacement of a triaminotriazine moiety by triaminopyrimidine. The triaminotriazines were initially observed to be potent vasodilators in cats and dogs following their formation of *N*-oxides in these animals. The triazines were inactive in humans because of their inability to form *N*-oxide metabolites; this led to the discovery of minoxidil. Minoxidil is the only direct-acting vasodilator that requires metabolic activation to produce its antihypertensive effect (Fig. 19-23). It is converted to minoxidil sulfate in the liver by a sulfotransferase enzyme.[64]

The antihypertensive properties of minoxidil are similar to those of hydralazine hydrochloride in that minoxidil is able to decrease arteriolar vascular resistance. Minoxidil exerts its vasodilatory action by a direct effect on arteriolar smooth muscle and appears to have no effect on the central nervous system or on the adrenergic nervous system in animals.[65] The serum half-life is 4.5 hr, and the antihypertensive effect may last up to 24 hr.

Minoxidil is used for severe hypertension that is difficult to control with other antihypertensive agents. The drug has some of the characteristic side effects of direct vasodilatory drugs. It causes sodium and water retention and may require coadministration of a diuretic. Minoxidil also causes reflex tachycardia, which can be controlled by use of a β-adrenergic blocking agent.

Minoxidil topical solution is used to treat alopecia androgenitica (male pattern baldness). Although the mechanism is not clearly understood, topical minoxidil is believed to increase cutaneous blood flow, which may stimulate hair growth. The stimulation of hair growth is attributed to vasodilation in the vicinity of application of the drug, resulting in better nourishment of the local hair follicles.

ANTIHYPERLIPIDEMIC AGENTS

The major cause of death in the Western world today is attributed to vascular disease, of which the most prevalent form is atherosclerotic heart disease. Although many causative factors of this disease are recognized (e.g., smoking, stress, diet), atherosclerotic disease can be treated through medication or surgery.

Hyperlipidemia is the most prevalent indicator for susceptibility to atherosclerotic heart disease; it is a term used to describe elevated plasma levels of lipids that are usually in the form of lipoproteins. Hyperlipidemia may be caused by an underlying disease involving the liver, kidney, pancreas, or thyroid; or it may not be attributable to any recognizable disease. Within recent years lipids have been indicated in the development of atherosclerosis in humans. *Atherosclerosis* may be defined as degenerative changes in the intima of medium and large arteries. The degeneration includes the accumulation of lipids, complex carbohydrates, blood, and blood products and is accompanied by the formation of fibrous tissue and calcium deposition on the intima of the blood vessels. These deposits, or *plaques*, decrease the lumen of the artery, reduce its elasticity, and may create foci for thrombi and subsequent occlusion of the blood vessel.

LIPOPROTEIN CLASSES

Lipoproteins are macromolecules consisting of lipid substances (cholesterol, triglycerides) noncovalently bound with protein and carbohydrate. These combinations solubilize the lipids and prevent them from forming insoluble aggregates in the plasma. They have a spherical shape and consist of a nonpolar core surrounded by a monolayer of phospholipids whose polar groups are oriented toward the lipid phase of the plasma. Included in the phospholipid monolayer is a small number of cholesterol molecules and proteins termed "apolipoproteins." The apolipoproteins appear to be able to solubilize lipids for transport in an aqueous surrounding such as plasma (Fig. 19-24).

The various lipoproteins found in plasma can be separated by ultracentrifugal techniques into chylomicrons, very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL). These correlate with the electrophoretic separations of the lipoproteins as follows: chylomicrons, pre-β-lipoprotein (VLDL), broad β-lipoprotein (IDL), β-lipoprotein (LDL), and α-lipoprotein (HDL).

Chylomicrons contain 90% triglycerides by weight and originate from exogenous fat from the diet. They are the least dense of the lipoproteins and migrate the least under the influence of an electric current. Chylomicrons are normally absent in plasma after 12 to 24 hr of fasting. The VLDL is composed of about 60% triglycerides, 12% cholesterol, and 18% phospholipids. It originates in the liver from free fatty acids. Although VLDL can be isolated from plasma, it is catabolized rapidly into IDL, which is degraded further into LDL. Normally, IDL also is catabolized rapidly to LDL, but it is not usually isolated from plasma. The LDL consists of 50% cholesterol and 10% triglycerides. This is the major cholesterol-carrying protein. In normal persons, this lipopro-

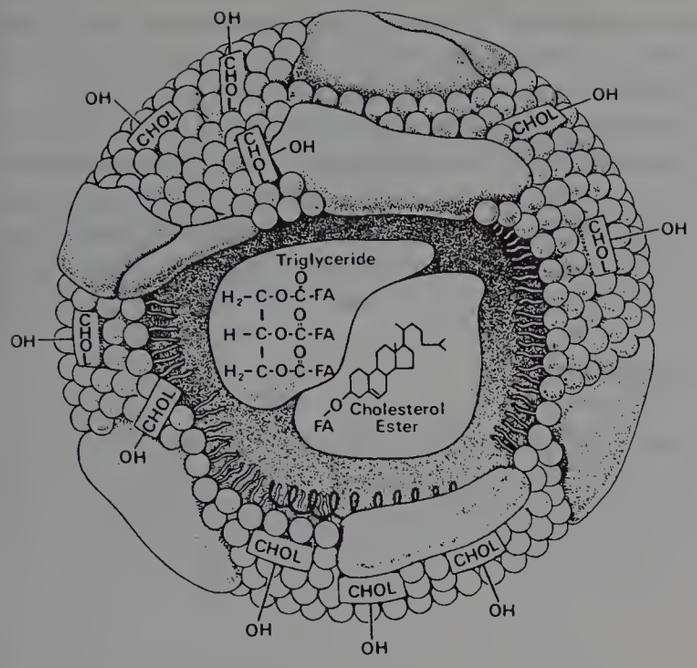


FIG. 19-24. *Hypothetical model of lipoprotein particle.*

tein accounts for about 65% of the plasma cholesterol and is of major concern in hyperlipidemic disease states. The LDL is formed from the intravascular catabolism of VLDL. The HDL is composed of 25% cholesterol and 50% protein and accounts for about 17% of the total cholesterol in plasma.

LIPOPROTEIN METABOLISM

The rate at which cholesterol and triglycerides enter the circulation from the liver and small intestine depends on the supply of the lipid and proteins necessary to form the lipoprotein complexes. Although the protein component must be synthesized, the lipids can be obtained either from a de novo biosynthesis in the tissues or from the diet. Reduction of plasma lipids by diet can delay the development of atherosclerosis. Furthermore, the use of drugs that decrease assimilation of lipids into the body plus diet has been demonstrated to decrease mortality from cardiovascular disease.[66]

Lipid transport mechanisms that shuttle cholesterol and triglycerides among the liver, intestine, and other tissues exist. Normally, plasma lipids, including lipoprotein cholesterol, are cycled into and out of plasma and do not cause extensive accumulation of deposits in the walls of arteries. Genetic factors and changes in hormone levels affect lipid transport by altering enzyme concentrations and apoprotein content, as well as the number and activity of lipoprotein receptors. This complex relationship makes the treatment of all hyperlipoproteinemias by a singular approach difficult, if not impractical.

Lipids are transported by both *exogenous* and *endogenous* pathways. In the exogenous pathway, dietary fat (triglycerides and cholesterol) is incorporated into large lipoprotein particles (chylomicrons), which enter the lymphatic system and are then passed into the plasma. The chylomicrons are acted upon by lipoprotein lipase in the adipose tissue capillaries, forming triglycerides and monoglycerides. The free fatty acids cross the endothelial membrane of the capillary and are incorporated into triglycerides in the tissue for storage as fat or are used for energy by oxidative metabolism. The chylomicron remnant in the capillary reaches the liver and is cleared from the circulation by binding to a receptor that recognizes the apoprotein E and B-48 protein components of the chylomicron remnant.

In the endogenous pathway of lipid transport, lipids are secreted from the liver. These are triglycerides and cholesterol combined with apoprotein B-100 and apoprotein E to form VLDL. The VLDL is acted upon by lipoprotein lipase in the capillaries of adipose tissue to generate free fatty acids and an IDL. Some IDL may bind to LDL receptors in the liver and is cleared from plasma by endocytosis. Approximately one-half of the circulating IDL is converted to LDL in the plasma by additional loss of triglycerides. This LDL has a half-life in plasma of about 1.5 days and represents 60% to 70% of the cholesterol in plasma. These LDL particles bind to LDL receptors in extrahepatic tissues and are removed from the plasma. Levels of LDL receptors vary according to the need by extrahepatic tissues to bind LDL for the purpose of utilizing cholesterol. The extrahepatic tissue subsequently releases HDL. Free plasma cholesterol can be adsorbed onto HDL and the cholesterol esters formed by the enzyme lecithin-cholesterol acyltransferase (LCAT). These esters are transferred from HDL to VLDL or LDL in plasma to complete the cycle. The pathways for plasma lipoprotein metabolism by the exogenous and endogenous routes are shown in Fig. 19-25.

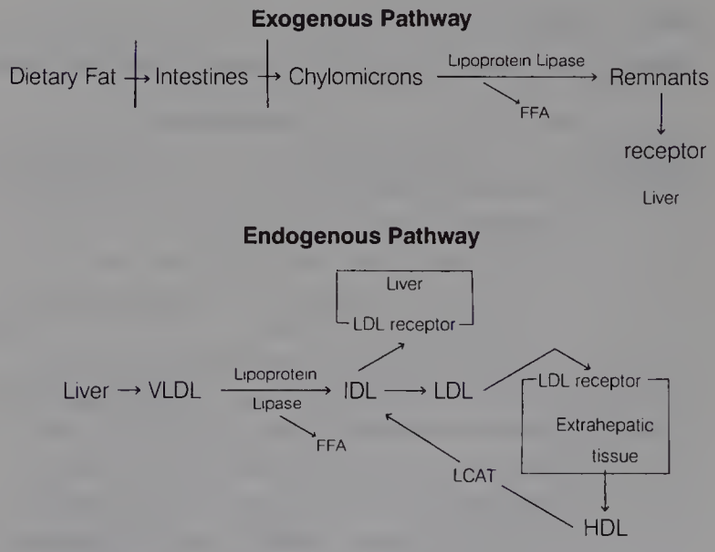


FIG. 19-25. *Exogenous and endogenous pathways of lipoprotein metabolism.*

HYPERLIPOPROTEINEMIAS

It is now recognized that lipid disorders are related to problems of lipoprotein metabolism[67] that create conditions of hyperlipoproteinemia. The hyperlipoproteinemias have been classified into six types, each of which is treated differently (Table 19-6).

The abnormal lipoprotein pattern characteristic of type I is caused by a decrease in the activity of lipoprotein lipase, an enzyme that normally hydrolyzes the triglycerides present in chylomicrons and clears the plasma of this lipoprotein fraction. Because the triglycerides found in chylomicrons come primarily from exogenous sources, this type of hyperlipoproteinemia may be treated by decreasing the intake of dietary fat. There are no drugs at present that can be used effectively to counteract type I hyperlipidemia.

Type II hyperlipoproteinemia has been divided into types IIa and IIb. Type IIa is characterized by elevated levels of LDL (β-lipoproteins) and normal levels of triglycerides. This subtype disorder is very common and may be caused by disturbed catabolism of LDL. Type IIb differs from type IIa in that this hyperlipidemia has elevated VLDL levels in addition to LDL. Type II hyperlipoproteinemia is often clearly familial and frequently inherited as an autosomal dominant abnormality with complete penetrance and expression in infancy. Patients have been treated by use of dietary restrictions on cholesterol and saturated fats. This type of hyperlipoproteinemia responds to some form of chemotherapy. The combined therapy may bring LDL levels back to normal.

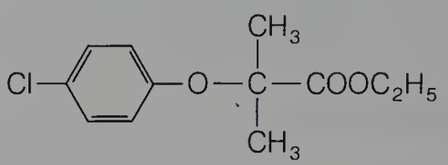
Type III is a rare disorder characterized by a broad band of β-lipoprotein. Similar to type II, it is also familial. Patients respond favorably to diet and drug therapy.

In type IV, hyperlipoproteinemia levels of VLDL are elevated. Because this type of lipoprotein is rich in triglycerides, plasma triglycerides are elevated. The metabolic defect that causes type IV is still unknown; however, this form of hyperlipidemia responds to diet and drug therapy.

Type V hyperlipoproteinemia has high levels of chylomicrons and VLDL, resulting in high levels of plasma triglycerides. The biochemical defect of type V hyperlipoproteinemia is not understood. Clearance of dietary fat is impaired, and a reduction of dietary fat is indicated along with drug therapy.

PRODUCTS

Clofibrate, USP. Ethyl 2-(*p*-chlorophenoxy)-2-methylpropionate (Atromid-S). Clofibrate is a stable, colorless to pale yellow liquid with a faint odor and a characteristic taste. It is soluble in organic solvents but insoluble in water.



Clofibrate

Clofibrate is prepared by a Williamson synthesis, condensing *p*-chlorophenol with ethyl α-bromoisobutyrate, or by the interaction of a mixture of acetone, *p*-chlorophenol, and chloroform in the presence of excess potassium hydroxide. The acid obtained by either of these methods is esterified to give clofibrate. Both acid and ester are active; however, the latter is preferred for medicinal use. Clofibrate is hydrolyzed rapidly to 2-*p*-chlorophenoxy-2-methylpropionic acid by esterases in vivo and circulates in blood bound to serum albumin. The acid has been investigated as a hypolipidemic agent. It is absorbed more slowly and to a smaller extent than is the ester. The aluminum salt of the acid gave even

TABLE 19-6

CHARACTERIZATION OF HYPERLIPOPROTEINEMIA TYPES

Hyperlipoproteinemia	Abnormality		Appearance of Plasma*	Triglycerides	Cholesterol (Total)
	Electrophoresis	Ultracentrifuge			
I	Massive chylomicronemia		Clear, on top creamy layer of chylomicronemia	Massively elevated	Slightly to moderately elevated
IIa	β-Lipoproteins elevated	LDL increased	Clear	Normal	Heavily elevated
IIb	Pre-β-lipoproteins elevated	LDL + VLDL increased	Slightly turbid	Slightly elevated	Heavily elevated
III	Broad β-lipoprotein band	VLDL/LDL of abnormal composition	Slightly turbid to turbid	Elevated	Elevated
IV	Pre-β-lipoproteins elevated	VLDL increased	Turbid	Moderately to heavily elevated	Normal to elevated
V	Pre-β-lipoproteins elevated, chylomicronemia present	VLDL increased, chylomicronemia present	Turbid, on top chylomicronemia	Massively elevated	Slightly elevated

* After having been kept standing at 4°C for 25 hours.
Adapted from Witte, E. C.: Prog. Med. Chem. 11:199, 1975.

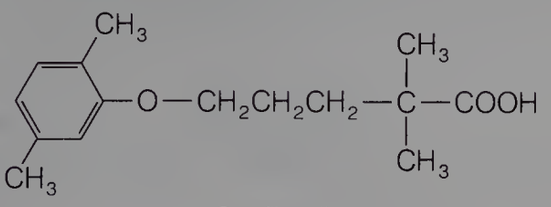
lower blood levels than *p*-chlorophenoxy-2-methylpropionic acid.[68]

Clofibrate is the drug of choice in the treatment of type III hyperlipoproteinemias and may also be useful, to a lesser extent, in types IIb and IV hyperlipoproteinemias. The drug is not effective in types I and IIa.

Clofibrate can lower plasma concentrations of both triglycerides and cholesterol, but it has a more consistent clinical effect on triglycerides. It also affects lipoprotein plasma levels by enhancing removal of triglycerides from the circulation and causes reduction of VLDL by stimulating lipoprotein lipase to increase the catabolism of this lipoprotein to LDL.[69] Clofibrate lowers triglyceride levels in the serum, much more so than those of cholesterol, and decreases free fatty acids and phospholipids. The lowering of cholesterol may be the result of more than one mechanism. Clofibrate inhibits the incorporation of acetate into the synthesis of cholesterol, between the acetate and mevalonate step, by inhibiting *sn*-glyceryl-3-phosphate acyltransferase. Clofibrate also regulates cholesterol synthesis in the liver by inhibiting microsomal reduction of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA), catalyzed by HMG-CoA reductase. Clofibrate may lower plasma lipids by means other than impairment of cholesterol biosynthesis, such as increasing excretion through the biliary tract.

Clofibrate is tolerated well by most patients, the most common side effects being nausea and, to a smaller extent, other gastrointestinal distress. The dosage of anticoagulants, if used in conjunction with this drug, should be reduced by one-third to one-half, depending on the individual response, such that the prothrombin time may be kept within the desired limits.

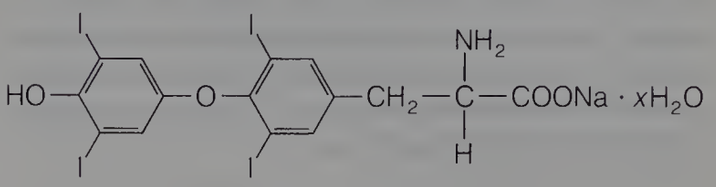
Gemfibrozil. 5-(2,5-Dimethylphenoxy)-2,2-dimethylpentanoic acid (Lopid). This is a congener of clofibrate that was used first in the treatment of hyperlipoproteinemia in the mid-1970s. Its mechanism of action and use are similar to those of clofibrate. Gemfibrozil reduces plasma levels of VLDL triglycerides and stimulates clearance of VLDL from plasma. The drug has little effect on cholesterol plasma levels but does cause an increase of HDL.



Gemfibrozil

Gemfibrozil is absorbed quickly from the gut and excreted unchanged in the urine. The drug has a plasma half-life of 1.5 hr, but the observed reduction of plasma VLDL concentration takes between 2 and 5 days to become evident. The peak effect of its hypolipidemic action may take up to 4 weeks to become manifest.

Dextrothyroxine Sodium, USP. *O*-(4-Hydroxy-3,5-diiodophenyl)-3,5-diiodo-D-tyrosine monosodium salt hydrate; sodium D-3,3′,5,5′-tetraiodothyronine (Choloxin). This compound occurs as a light yellow to buff powder. It is stable in dry air but discolors on exposure to light; hence, it should be stored in light-resistant containers. It is very slightly soluble in water, slightly soluble in alcohol, and insoluble in acetone, chloroform, and ether.



Dextrothyroxine Sodium

The hormones secreted by the thyroid gland have marked hypocholesterolemic activity along with their other well-known actions. With the finding that not all active thyroid principles possessed the same degree of physiological actions, a search was made for congeners that would cause a decrease in serum cholesterol without other effects such as angina pectoris, palpitation, and congestive failure. D-Thyroxine has resulted from this search. However, at the dosage required, the L-thyroxine contamination must be minimal; otherwise, it will exert its characteristic actions. One route to optically pure (at least 99% pure) D-thyroxine is the use of an L-amino acid oxidase from snake venom, which acts only on the L-isomer and makes separation possible.

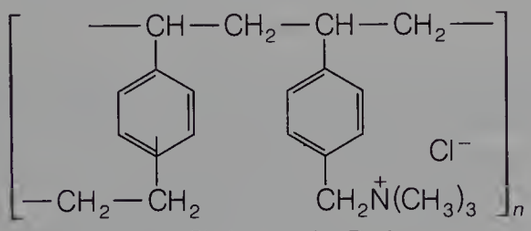
The mechanism of action of D-thyroxine appears to be stimulation of oxidative catabolism of cholesterol in the liver through stimulation of 7α-cholesterol hydroxylase, the rate-limiting enzyme in the conversion of cholesterol to bile acids. The bile acids are conjugated with glycine or taurine and excreted by the biliary route into the feces. Although cholesterol biosynthesis is not inhibited by the drug, thyroxine increases the number of LDL receptors, enhancing removal of LDL from plasma.

Use of thyroxine in the treatment of hyperlipidemias is not without adverse effects. The drug increases the frequency and severity of anginal attacks and may cause cardiac arrhythmias.

D-Thyroxine potentiates the action of anticoagulants, such as warfarin or dicumarol; thus, dosage of the anticoagulants should be reduced by one-third if used concurrently and then further modified, if necessary, to maintain the prothrombin time within the desired limits. Also, it may increase the dosage requirements of insulin or of oral hypoglycemic agents if used concurrently with them.

Cholestyramine Resin, USP. (Cuemid, Questran) is the chloride form of a strongly basic anion-exchange resin. It is a styrene copolymer with divinylbenzene with quaternary ammonium functional groups. After oral ingestion, cholestyramine resin remains in the gastrointestinal tract, where it readily exchanges chloride ions for bile acids in the small intestine, to be excreted as bile salts in the feces. Cholestyramine resin is also useful in lowering plasma lipids. The reduction of the amounts of reabsorbed bile acids results in

increased catabolism of cholesterol in bile acids in the liver. The decreased concentration of bile acids returning to the liver lowers the feedback inhibition by bile acids of 7α-hydroxylase, the rate-limiting enzyme in the conversion of cholesterol to bile acids, increasing the breakdown of hepatic cholesterol. Although the biosynthesis of cholesterol is increased, it appears that the rate of catabolism is greater, resulting in a net decrease in plasma cholesterol levels by affecting LDL clearance. The increase of LDL receptors in the liver that occurs when its content of cholesterol is lowered augments this biochemical event.

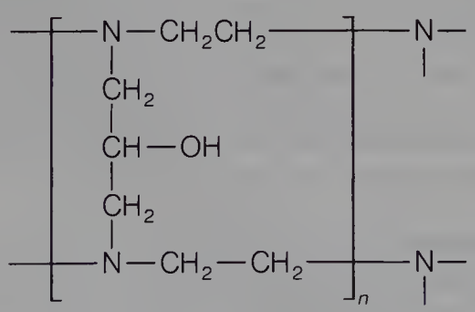


Cholestyramine Resin

Cholestyramine resin does not bind with drugs that are neutral or with amine salts; however, it is possible that acidic drugs (in the anion form) could be bound. For example, in animal tests, absorption of aspirin given concurrently with the resin was depressed only moderately during the first 30 minutes.

Cholestyramine resin is the drug of choice for type IIa hyperlipoproteinemia. When used in conjunction with a controlled diet, it reduces β-lipoproteins. The drug is an insoluble polymer and, thus, probably one of the safest because it is not absorbed from the gastrointestinal tract to cause systemic toxic effects.

Colestipol Hydrochloride (Colestid) is a high-molecular-weight, insoluble, granular copolymer of tetraethylenepentamine and epichlorohydrin. It functions as an anion-exchange, resin-sequestering agent in a manner similar to that of cholestyramine resin. Colestipol hydrochloride reduces cholesterol levels without affecting triglycerides and seems to be especially effective in the treatment of type II hyperlipoproteinemias.



Colestipol Hydrochloride

Niacin, USP. Nicotinic acid, 3-pyridinecarboxylic acid, is effective in the treatment of all types of hyperlipoproteinemia, with the exception of type I, at doses above those given as a vitamin supplement. The drug reduces VLDL synthesis and, subsequently, its plasma products, IDL and LDL. Plasma triglyceride levels are reduced because of the decreased VLDL production. Cholesterol levels are lowered, in turn, because of the decreased rate of LDL formation from VLDL. Although niacin is the drug of choice for type II hyperlipoproteinemias, its use is limited because of the vasodilating side effects. Flushing occurs in practically all patients but generally subsides when the drug is discontinued.

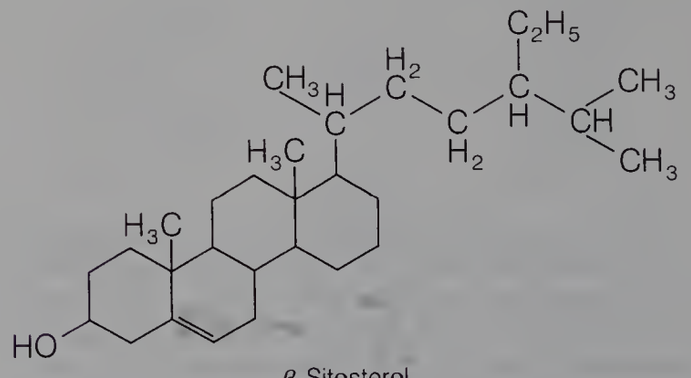
The basis of the hypolipidemic effects of niacin may be due to its ability to inhibit lipolysis (i.e., prevent the release of free fatty acids [FFAs] and glycerol from fatty tissues]. As a consequence, there is a reduced reserve of FFA in the liver, and diminution of lipoprotein biosynthesis occurs, which results in a reduction of the production of VLDL. The decreased formation of lipoproteins leads to a pool of unused cholesterol normally incorporated in VLDL. This excess cholesterol is then excreted through the biliary tract.

Niacin (nicotinic acid) may be administered as aluminum nicotinate (Nicalex). This is a complex of aluminum hydroxy nicotinate and niacin. The aluminum salt is hydrolyzed to aluminum hydroxide and niacin in the stomach. There seems to be no advantage of the aluminum salt over the free acid. The frequency of hepatic reaction appears more prevalent than with niacin.

Nicotinic acid has been esterified with the purpose of prolonging its hypolipidemic effect. Pentaerythritol tetranicotinate has been more effective experimentally than niacin in reducing cholesterol in rabbits. Sorbitol and *myo*-inositol hexanicotinate polyesters have been employed in the treatment of patients with atherosclerosis obliterans.

The usual maintenance dose of niacin is 3 to 6 g/day given in three divided doses. The drug usually is given at mealtimes to reduce gastric irritation that often accompanies large doses.

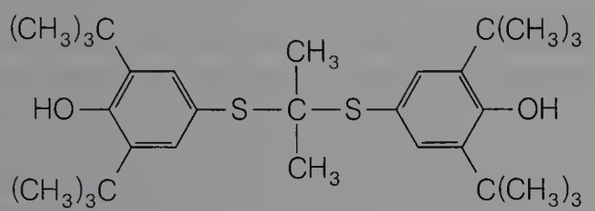
β-Sitosterol is a plant sterol, the structure of which is identical with that of cholesterol, except for the substituted ethyl group on C-24 of its side chain. Although the mechanism of its hypolipidemic effect is not clearly understood, it is suspected that the drug inhibits the absorption of dietary cholesterol from the gastrointestinal tract. Sitosterols are absorbed poorly from the mucosal lining and appear to compete with cholesterol for absorption sites in the intestine.



β-Sitosterol

Probucol, USP. 4,4′[(1-Methylethylidene) bis(thio)][2,6-bis(1,1-dimethylethyl)]phenol, DH-581 (Lorelco) is a chemical agent that was developed for the plastics and rubber

industry in the 1960s. The probucol molecule has two tertiary butylphenol groups linked by a dithiopropylidene bridge, giving it a high lipophilic character with strong antioxidant properties. In humans, it causes reduction of both liver and serum cholesterol levels, but it does not alter plasma triglycerides. It reduces LDL and, to a lesser extent, HDL levels by a unique mechanism that is still not clearly identified. The reduction of HDL may be due to the ability of probucol to inhibit the synthesis of apoprotein A-1, a major protein component of HDL.[70] It is effective at reducing levels of LDL and is used in those hyperlipoproteinemias characterized by elevated LDL levels.



Probucol

HMG-CoA REDUCTASE INHIBITORS

Drugs in this class of hypolipidemic agents inhibit the enzyme HMG-CoA reductase, responsible for the conversion of HMG-CoA to mevalonate in the synthetic pathway for the synthesis of cholesterol (Fig. 19-26). HMG-CoA reductase is the rate-limiting catalyst for the irreversible conversion of 3-hydroxy-3-methylglutaryl-CoA to mevalonic acid in the synthesis of cholesterol. The activity of HMG-CoA reductase is also under feedback regulation. When cholesterol is available in sufficient amounts for body needs, the enzyme activity of HMG-CoA reductase is suppressed.

Elevated plasma cholesterol levels have been correlated with an increase in cardiovascular disease. Of the plasma lipoproteins, the LDL fraction contains the most cholesterol. The source of cholesterol in humans is either the diet or de novo synthesis with HMG-CoA as the rate-limiting step. Ingested cholesterol as the free alcohol or ester is taken up after intestinal absorption and transported to the liver and other body organs through the exogenous pathway (Fig. 19-25). The LDL delivers cholesterol to peripheral cells. This process occurs after binding of LDL to specific LDL receptors located on the surface of cell membranes. After binding and endocytosis of the receptor and LDL, lysosomal degradation of this complex in the cell makes cholesterol available for use in cellular membrane synthesis. It is generally accepted that the lowering of total plasma cholesterol is accomplished most effectively by reducing LDL levels. Therefore, the population of LDL receptors is an important component of clearing the plasma of cholesterol. HMG-CoA reductase inhibitors contribute to this by directly blocking the active site of the enzyme. This action has a twofold effect on cholesterol plasma levels: causing a decrease in de novo cholesterol synthesis and causing an increase in hepatic LDL receptors. These HMG-CoA reductase inhibitors are effective hypocholesteremic agents in patients with familial hypercholesteremia.

Three drugs, lovastatin, simvastatin, and pravastatin, compose the list of approved HMG-CoA reductase inhibitors for the treatment of hyperlipidemia in patients. The three drugs have similar structures to the substrate, HMG-CoA of the enzyme HMG-CoA reductase. Lovastatin and simvastatin are lactones and prodrugs, being activated by hydrolysis in the liver to their respective β-hydroxy acids. Pravastatin, in contrast, is administered as the sodium salt of the β-hydroxy acid.

Lovastatin

2-Methylbutanoic acid 1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2*H*-pyran-2-yl) ethyl]-1-naphthalenyl ester; mevinolin; MK-803 (Mevacor). Lovastatin (formerly mevinolin) is a potent inhibitor of HMG-CoA. The drug was obtained originally from the fermentation products of the fungi *Aspergillus terreus* and *Monascus ruber*. Lovastatin was one of two original HMG-CoA reductase inhibitors. The other drug, mevastatin (formerly called compactin), was isolated from cultures of *Penicillium cillium citrum*. Mevastatin was withdrawn from clinical trials because it altered intestinal morphology in dogs. This effect was not observed with lovastatin.

Simvastatin

2,2-Dimethyl butanoic acid, 1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2-pyran-2-yl)-ethyl]-1-naphthalenyl ester (Zocor). Simvastatin is an ana-

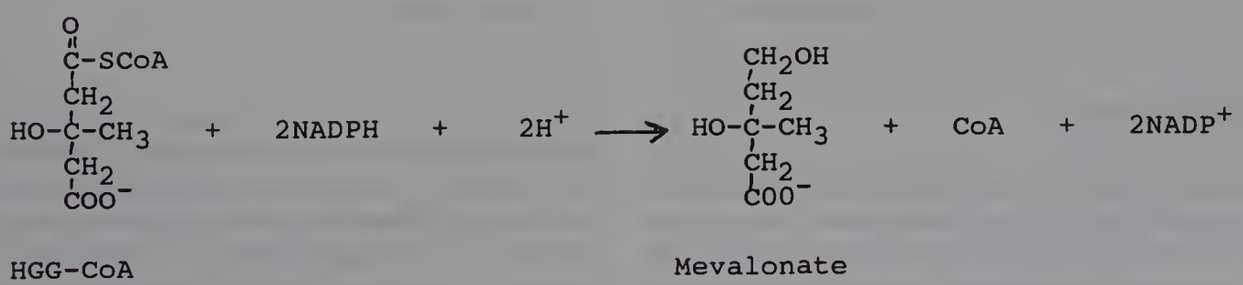


FIG. 19-26. *The HMG-CoA reductase reaction.*

logue of lovastatin. These two drugs have many similar properties. Both drugs, in the prodrug form, reach the liver unchanged after oral administration, where they undergo extensive metabolism to a number of open ring hydroxy acids, including the active β-hydroxy acids. They are also highly bound to plasma proteins. These actions make the bioavailability of simvastatin rather poor but better than that of lovastatin, which has been estimated at 5%.

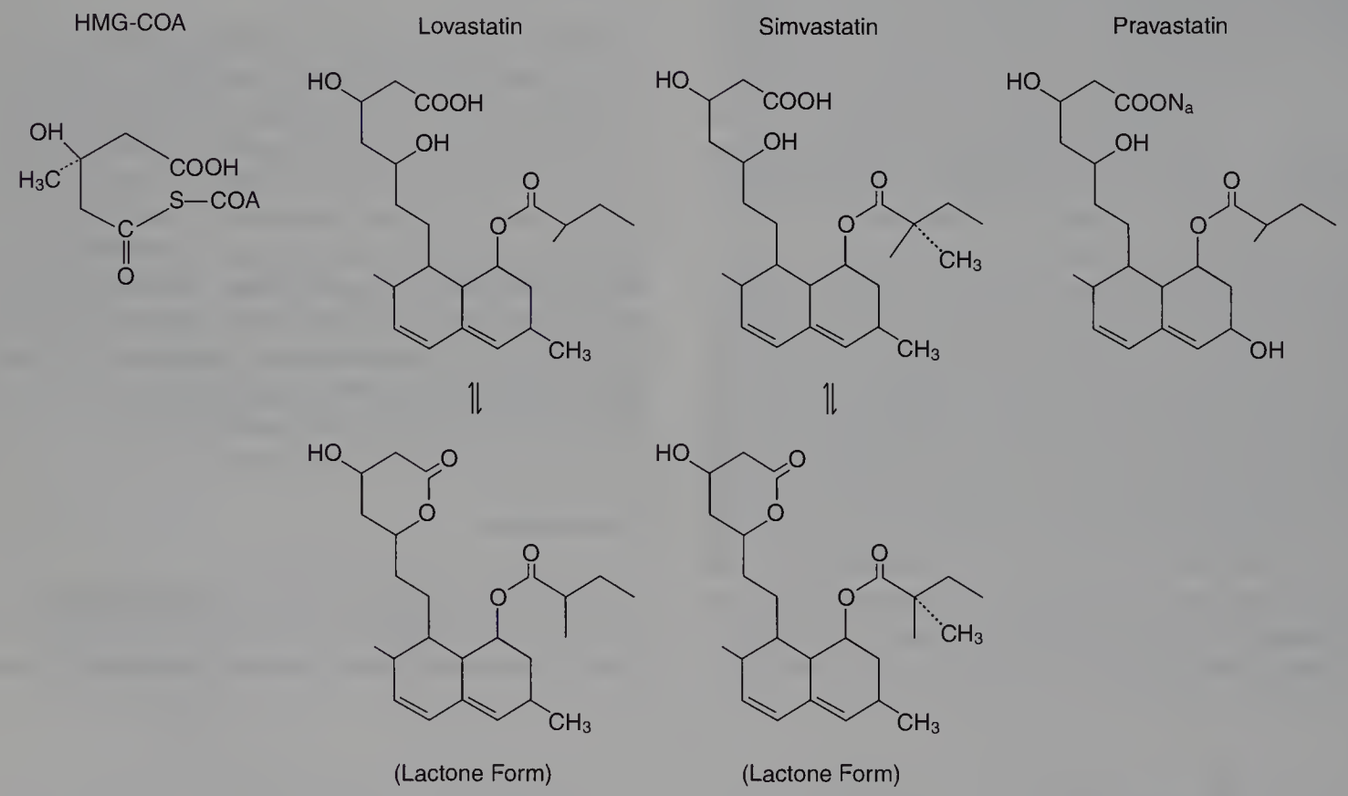
Pravastatin

Sodium 1,2,6,7,8,8a-hexahydro-β,δ, 6,8-trihydroxy-2-methyl-1-8-(2-methyl-1-oxobutoxy)-naphthaleneheptanoate (Pravachol). Pravastin is the most rapid-acting of the three HMG-CoA reductase inhibitor drugs, reaching a peak concentration in about 1 hr. The more hydrophylic nature of the sodium salt of the β-hydroxy acid, in contrast to the lactone forms of the other two agents, may explain this property. Absorption of pravastatin following oral administration can be inhibited by resins such as cholestyramine because of the presence of the carboxylic acid function on the drug. The lactone forms of lovastatin and simvastatin are less affected by cholestyramine.

MECHANISM OF BLOOD COAGULATION

The fluid nature of blood can be attributed to the flat cells (endothelial) that maintain a nonthrombogenic environment in the blood vessels. This is a result of at least four phenomena: (1) the maintenance of a transmural negative electric charge that prevents adhesion between platelets; (2) the release of a plasmalogen activator, which activates the fibrinolytic pathway; (3) the release of thrombomodulin, a cofactor that activates protein C, a coagulation factor inhibitor; and (4) the release of prostacyclin (PGI_2), a potent inhibitor of platelet aggregation.

The process of blood coagulation (Fig. 19-27) involves a



ANTICOAGULANTS

A theory of blood clotting introduced in 1905 was based on the existence of four factors: thromboplastin (thrombokinase), prothrombin, fibrinogen, and ionized calcium. The clotting sequence proposed was that when tissue damage occurred, thromboplastin entered the blood from the platelets and reacted with prothrombin in the presence of calcium to form thrombin. Thrombin then reacted with fibrinogen to form insoluble fibrin, which enmeshed red blood cells to create a clot. The concept remained unchallenged for almost 50 years, but it has now been modified to accommodate the discovery of numerous additional factors that enter into the clotting mechanism (Table 19-7).

series of steps that occur in a cascade fashion and terminate in the formation of a fibrin clot. Blood coagulation occurs by activation of either an intrinsic pathway, a relatively slow process of clot formation, or an extrinsic pathway, which has a much faster rate of fibrin formation. Both pathways merge into a common pathway for the conversion of pro-

TABLE 19-7

THE ROMAN NUMERICAL NOMENCLATURE OF BLOOD-CLOTTING FACTORS AND SOME COMMON SYNONYMS

Factor	*Synonyms*
I	Fibrinogen
II	Prothrombin
III	Thromboplastin, tissue factor
IV	Calcium
V	Proaccelerin, accelerator globulin, labile factor
VI	(This number is not now used)
VII	Proconvertin, stable factor, autoprothrombin I, SPCA
VIII	Antihemophilic factor, antihemophilic globulin, platelet cofactor I, antihemophilic factor A
IX	Plasma thromboplastin component (PTC), Christmas factor, platelet cofactor II, autoprothrombin II, antihemophilic factor B
X	Stuart-Power factor, Stuart factor, autoprothrombin III
XI	Plasma thromboplastin antecedent (PTA), antihemophilic factor C
XII	Hageman factor
XIII	Fibrin stabilizing factor, fibrinase, Laki-Lorand factor

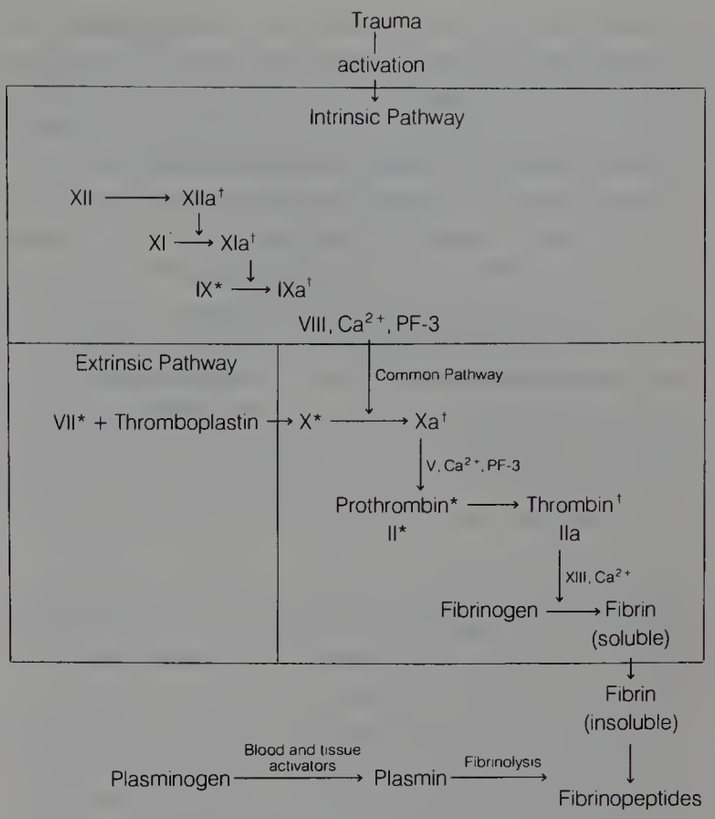


FIG. 19-27. *Scheme of blood coagulation and fibrinolysis. The asterisk denotes a vitamin K-dependent factor. The dagger denotes inhibition by heparin and antithrombin III.*

thrombin to thrombin and subsequent transformation of fibrinogen to the insoluble strands of fibrin. Lysis of intravascular clots occurs through a plasminogen–plasmin system, which consists of plasminogen, plasmin, urokinase, kallikrein, plasminogen activators, and some undefined inhibitors.

The *intrinsic* pathway refers to the system for coagulation that occurs from the interaction of factors circulating in the blood. It is activated when blood comes into contact with a damaged vessel wall or a foreign substance. Each of the plasma coagulation factors (Table 19-7), with the exception of factor III (tissue thromboplastin), circulates as an inactive proenzyme. Except for fibrinogen, which precipitates as fibrin, these factors are usually activated by enzymatic removal of a small peptide in the cascade of reactions that make up the clotting sequence (Fig. 19-27). The *extrinsic* clotting system refers to the mechanism by which thrombin is generated in plasma after the addition of tissue extracts. When various tissues, such as brain or lung (containing thromboplastin), are added to blood, a complex between thromboplastin and factor VII in the presence of calcium ions activates factor X, bypassing the time-consuming steps of the intrinsic pathway that form factor X. The intrinsic and extrinsic pathways interact in vivo. Small amounts of thrombin formed early after stimulation of the extrinsic pathway accelerate clotting by the intrinsic pathway by activating factor VIII. Thrombin also speeds up the clotting rate by activation of factor V, located in the common pathway. Thrombin then converts the soluble protein fibrinogen into a soluble fibrin gel by acting on Gly-Arg bonds to remove small fibrinopeptides from the N-terminal, enabling the remaining fibrinogen molecule to polymerize. It also activates factor XIII, which stabilizes the fibrin gel in the presence of calcium by cross-linking between the chains of the fibrin monomer through intermolecular γ-glutamyl ζ-lysine bridges to form an insoluble mass.

ANTICOAGULANT MECHANISMS

In the milieu of biochemicals being formed to facilitate the clotting of blood, the coagulation cascade in vivo is controlled by a balance of inhibitors in the plasma to prevent all of the blood in the body from solidifying. Thrombin plays a pivotal role in blood coagulation. It cleaves fibrinogen, a reaction that initiates formation of the fibrin gel, which constitutes the framework of the blood clot. As mentioned above, it activates the cofactors factor V and factor VIII, to accelerate the coagulation process. Intact endothelial cells express a receptor, thrombomodulin, for thrombin. Thrombin, when bound to thrombomodulin, does not have coagulant activity and, thus, prevents clot formation beyond damaged areas and onto intact endothelium. In this bound state, however, it does activate protein C, which then inactivates two cofactors and impedes blood clotting. Thrombin also activates factor XIII, leading to cross-linking of the fibrin gel. The activity of thrombin is regulated by its inactivation by plasma protein inhibitors: α_1-proteinase inhibitor, α_2-macroglobulin, antithrombin (antithrombin III), and heparin cofactor II. These belong to a family of proteins called "serpins," an acronym for serine protease inhibitors.

Antithrombin III, an α_2-globin, neutralizes thrombin and the serine proteases in the coagulation cascade—Xa, IXa, XIa, and XIIa. Although antithrombin III is a slow-acting inhibitor, it becomes a very rapid-acting inhibitor of thrombin in the presence of heparin. Heparin is a naturally occurring anticoagulant that requires antithrombin III (see above) for its biological property of preventing blood clot formation. It binds at the lysine site of the antithrombin III molecule, causing a change in the conformation of antithrombin III and increasing its anticoagulant properties. Heparin can then dissociate from antithrombin III to bind to another antithrombin III molecule. An additional system which controls unwanted coagulation involves protein C, a vitamin K-dependent zymogen in the plasma. Protein C is converted to a serine protease when thrombin and factor Xa, formed in the blood in the coagulation cascade, interact with thrombomodulin. The now activated protein C inhibits factors V and VIII and, in so doing, blocks further production of thrombin. Protein C also enhances fibrinolysis by causing release of the tissue plasminogen activator.

The biosynthesis of prothrombin (factor II) depends on an adequate supply of vitamin K. A deficiency of vitamin K results in the formation of a defective prothrombin molecule. The defective prothrombin is antigenically similar to normal prothrombin but has reduced calcium-binding ability and no biological activity. In the presence of calcium ions, normal prothrombin adheres to the surface of phospholipid vesicles and greatly increases the activity of the clotting mechanism. The defect in the abnormal prothrombin is in the NH_2-terminal portion, in which the second carboxyl residue has not been added to the γ-carbon atom of some glutamic acid residues on the prothrombin molecule to form γ-carboxyglutamic acid.[71] Administration of vitamin K antagonists results in the decreased synthesis of a biologically active prothrombin molecule and increases the clotting time of blood in humans.[72]

Vitamin K is critical to the formation of clotting factors VII, IX, and X. These factors are glycoproteins that have γ-carboxyglutamic acid residues at the N-terminal end of the peptide chain. The enzyme involved in forming an active prothrombin is a vitamin K-dependent carboxylase located in the microsomal fraction of liver cells. It has been suggested that vitamin K drives the carboxylase reaction by abstracting a proton from the relatively unreactive methylene carbon of the glutamyl residue, forming a 2,3-epoxide. Oral anticoagulants interfere with the γ-carboxylation of glutamic acid residues by preventing the reduction of vitamin K to its hydroquinone form (Fig. 19-28).

Hemophilia A, a blood disease characterized by a deficiency of coagulation factor VIII, is the most common inher-

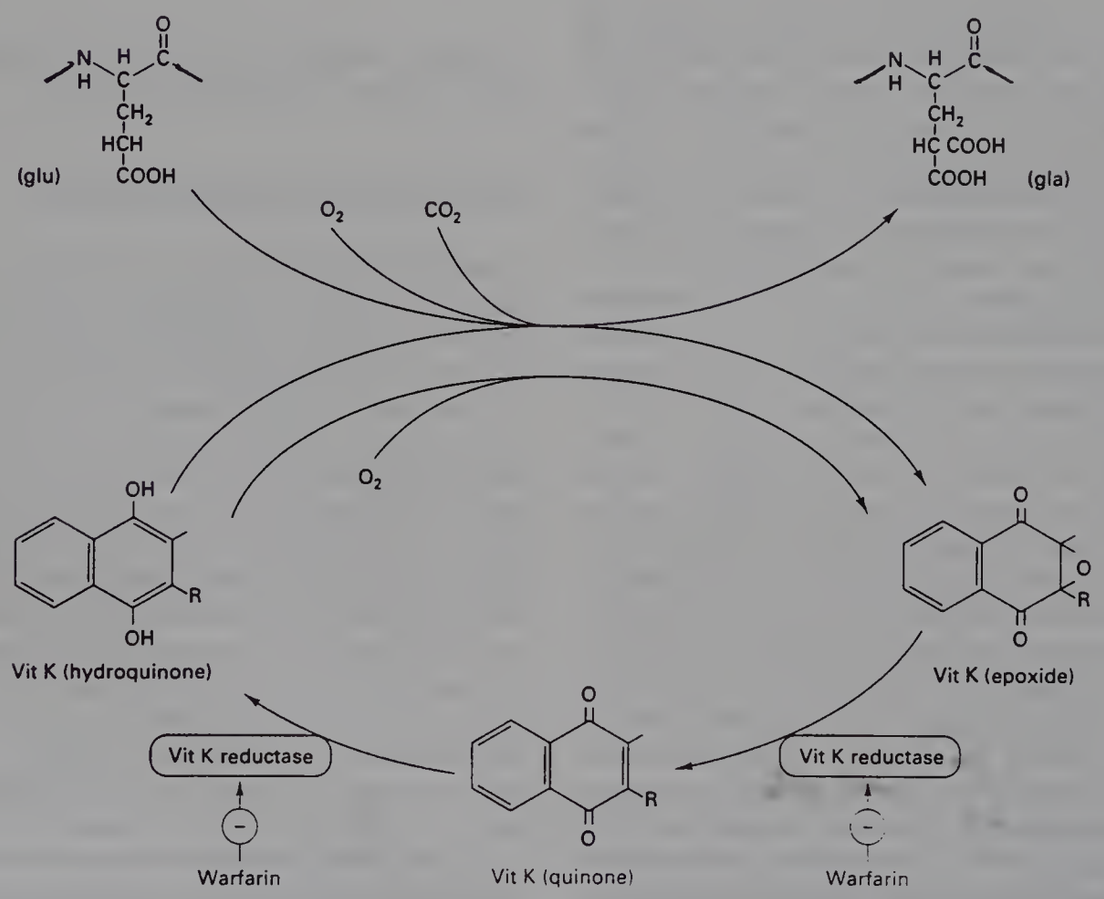


FIG. 19-28. *Mechanism of action of vitamin K and sites of action of warfarin.*

ited blood coagulation disorder. Treatment of this disease over the past 25 years has depended on the concentration of the antihemophilic factor (factor VIII) by cryoprecipitation and immunoaffinity chromatography separation technology. The impact of this therapy has been diminished by the presence of viruses that cause the acquired immunodeficiency syndrome (AIDS) and other less tragic viral diseases in humans. Recombinant antihemophilic factor preparations have been produced since 1989, using mammalian cells genetically altered to secrete human factor VIII. Kogenate and Helixate are recombinant preparations, obtained from genetically altering baby hamster kidney cells, that contain high concentrations of factor VIII. Recombinant factor VIIa, an active factor in the extrinsic pathway, now in phase III clinical trials (Novo Seven), has been used to treat patients with hemophilia A factor VII deficiency. Hemophilia B, another genetic blood disorder, which constitutes about 20% of hemophilia cases, is caused by a deficiency of factor IX and has been treated from cryoprecipitated fractions obtained from plasma. Monoclonal antibody technology has produced an essentially pure, carrier-free preparation of native factor IX (Mononine). Recombinant technology has solved the problem of limited supply and viral contamination of these critical blood factors.

PLATELET AGGREGATION AND INHIBITORS

Blood platelets play a pivotal role in hemostasis and thrombus formation. Actually, they have two roles in the cessation of bleeding: a hemostatic function, in which platelets, through their mass, cause a physical occlusion of openings in blood vessels, and a thromboplastic function, in which the chemical constituents of the platelets take part in the blood coagulation mechanism. The circulatory system is self-sealing because of the clotting properties of blood. However, the pathological formation of clots within the circulatory system creates a potentially serious clinical situation that must be dealt with through the use of anticoagulants.

Platelets do not adhere to intact endothelial cells. They do become affixed to subendothelial tissues, which have been exposed by injury, to cause hemostasis. Platelets bind to collagen in the vessel wall and trigger other platelets to adhere to them. This adhesiveness is accompanied by a change in shape of the platelets and may be caused by mobilization of calcium bound to the platelet membrane. The growth of the platelet mass depends on the adenosine diphosphate (ADP) released by the first few adhering cells and enhances the aggregation process. A secondary phase (phase II) immediately follows, with additional platelet aggregation. In this secondary phase, the platelets undergo a secretory process during which enzymes, such as cathepsin and acid hydrolases along with fibrinogen, are released from α-granules in the platelets, and ADP, ATP, serotonin, and calcium are released from dense bodies in the platelets. The dense bodies

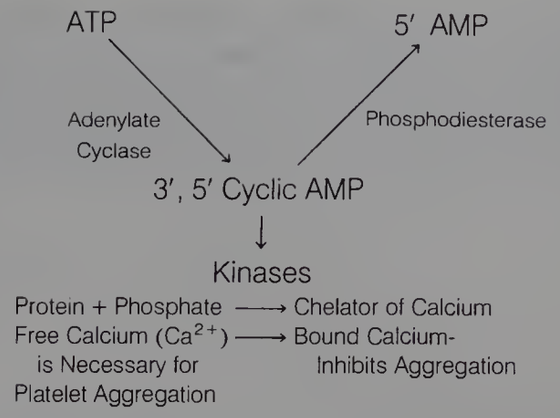


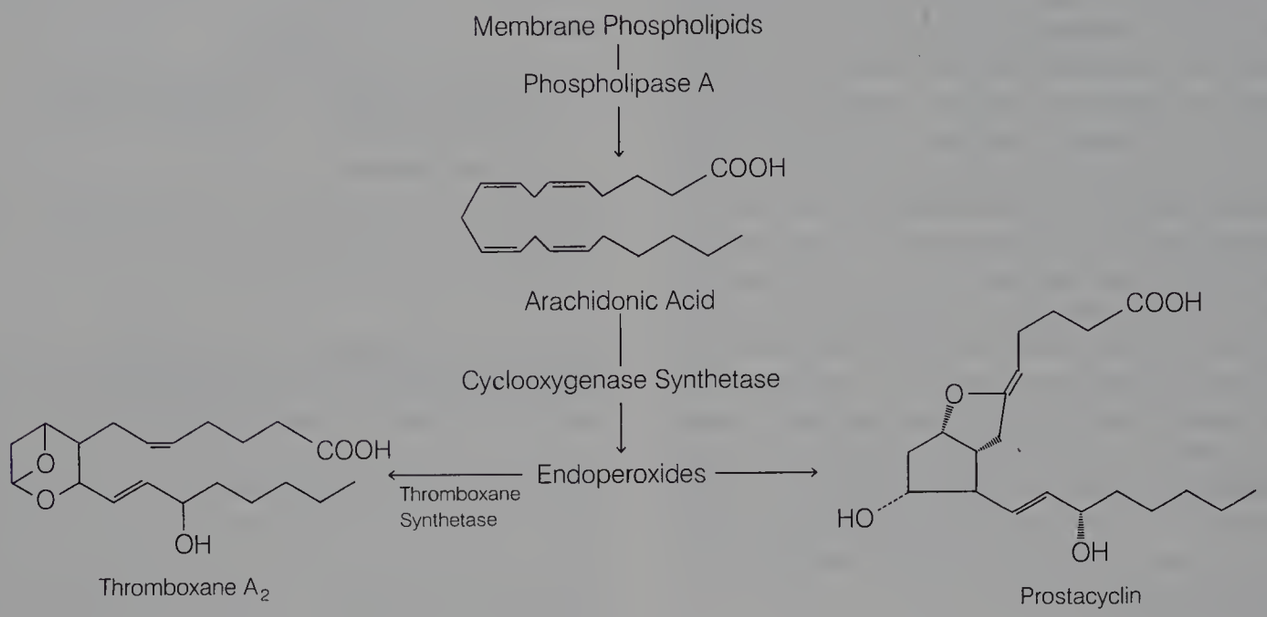
FIG. 19-29. *Role of adenosine 3′,5′-cyclic monophosphate (cAMP) in inhibition of platelet aggregation.*

are likened to the storage granules associated with adrenergic neurons. Increased levels of cAMP inhibit platelet aggregation. Cyclic AMP activates specific dependent kinases, which form protein–phosphate complexes that chelate calcium ions. The reduced levels of calcium inhibit aggregation (Fig. 19-29). Inhibitors of platelet aggregation can increase cAMP levels by either stimulating adenylate cyclase or inhibiting phosphodiesterase.[72] Substances such as glucagon, adenosine, and isoproterenol increase cAMP levels and inhibit platelet aggregation. Drugs such as theophylline, aminophylline, dipyramidole, papaverine, and adenosine inhibit phosphodiesterase and aggregation of platelets. Epinephrine, collagen, and serotonin inhibit adenylate cyclase and stimulate platelet aggregation.[73] The role of platelets in arterial thrombosis is similar to that in hemostasis. The factors contributing to venous thrombosis are circulatory stasis, excessive generation of thrombin formation of fibrin, and, to a lesser extent than in the artery, platelet aggregation.

Aspirin, sulfinpyrazone, and indomethacin have an inhibitory effect on platelet aggregation. They inhibit cyclooxygenase, the enzyme that controls the formation of prostaglandin endoperoxides and increases the tendency for platelets to aggregate. Aspirin also inhibits the platelet-release reaction. Dipyridamole inhibits adenosine deaminase and adenosine uptake by platelets. As a result, the increased plasma concentrations of adenosine inhibit ADP-induced aggregation of platelets.

Among the many pharmacological actions of prostaglandins is the ability of some to stimulate or inhibit the aggregation of platelets and alter the clotting time of blood. Prostaglandins are synthesized from 20-carbon polyunsaturated fatty acids containing from three to five double bonds. These fatty acids are present in the phospholipids of cell membranes of all mammalian tissues. The main precursor of prostaglandins is arachidonic acid. Arachidonic acid is released from membrane phospholipids by the enzyme phospholipase A_2. Once released, arachidonic acid is metabolized by cyclooxygenase synthetase to form unstable cyclic endoperoxides, PGG_2 and PGH_2, which subsequently are trans-

formed into prostacyclin and thromboxane A_2 (TXA_2). The conversion to TXA_2 occurs with the aid of the enzyme thromboxane synthetase. The formation of prostacyclin can occur nonenzymatically. Blood platelets convert arachidonic acid to TXA_2, whereas prostacyclin is formed mainly by the vascular endothelium. Both prostacyclin and TXA_2 are unstable at physiological *p*H and temperatures. Their half-lives are 2 to 3 minutes.



Prostacyclin inhibits platelet aggregation by stimulating adenylate cyclase to increase cAMP levels in the platelets. Prostacyclin is also a vasodilator and, as a result, has potent hypotensive properties when given intravenously or by intra-arterial administration. TXA_2 induces platelet aggregation. Together with prostacyclin, TXA_2 plays a role in the maintenance of vascular homeostasis. In addition to being a platelet aggregator, TXA_2 is a potent vasoconstrictor.

Retardation of clotting is important in blood transfusions, to avoid thrombosis after surgery or from other causes, to prevent recurrent thrombosis in phlebitis and pulmonary embolism, and to lessen the propagation of clots in the coronary arteries. This retardation may be accomplished by agents that inactivate thrombin (heparin) or those substances that prevent the formation of prothrombin in the liver, the coumarin derivatives and the phenylindanedione derivatives.

Although heparin (see Chap. 25) is a useful anticoagulant, it has limited applications. Many of the anticoagulants in use today were developed following the discovery of dicumarol, an anticoagulant present in spoiled sweet clover. These compounds are orally effective, but there is a lag period of 18 to 36 hr before they significantly increase the clotting time. Heparin, in contrast, produces an immediate anticoagulant effect after intravenous injection. A major disadvantage of heparin is that the only effective therapeutic route is parenteral.

Dicumarol and related compounds are not vitamin K antagonists in the classic sense. They appear to act by interfering with the function of vitamin K in the liver cells, which are the sites of synthesis of the clotting factors, including prothrombin. This lengthens the clotting time by decreasing the amount of biologically active prothrombin in the blood.

The discovery of dicumarol and related compounds as potent reversible* competitors of vitamin K coagulant-promoting properties led to the development of antivitamin K compounds, such as phenindione, which was designed in part according to metabolite–antimetabolite concepts. The active compounds of the phenylindanedione series are characterized by a phenyl, a substituted phenyl, or a diphenylacetyl group in the 2-position. Another requirement for activity is a keto group in the 1- and 3-positions, one of which may form the enol tautomer. A second substituent, other than hydrogen, at the 2-position prevents this keto–enol tautomerism, and the resulting compounds are ineffective as anticoagulants.

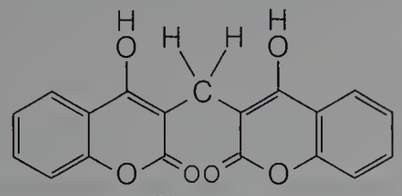
PRODUCTS

Protamine Sulfate, USP. has an anticoagulant effect, but it counteracts the action of heparin if used in the proper amount and is used as an antidote for the latter in cases of overdosage. It is administered intravenously in a dose depending on the circumstances.

Dicumarol, USP. 3,3′-Methylenebis[4-hydroxycoumarin] is a white or creamy white crystalline powder with a faint, pleasant odor and a slightly bitter taste. It is practically insoluble in water or alcohol, slightly soluble in chloroform, and dissolved readily by solutions of fixed alkalies. The ef-

* At high levels dicumarol is not reversed by vitamin K.

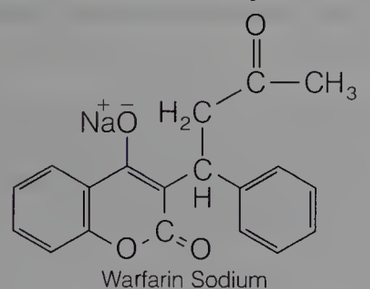
fects after administration require 12 to 72 hr to develop and persist for 24 to 96 hr after discontinuance.



Dicumarol

Dicumarol is used alone or as an adjunct to heparin in the prophylaxis and treatment of intravascular clotting. It is employed in postoperative thrombophlebitis, pulmonary embolus, acute embolic and thrombotic occlusion of peripheral arteries, and recurrent idiopathic thrombophlebitis. It has no effect on an already formed embolus but may prevent further intravascular clotting. Because the outcome of acute coronary thrombosis is largely dependent on extension of the clot and formation of mural thrombi in the heart chambers, with subsequent embolization, dicumarol has been used in this condition. It has also been administered to arrest impending gangrene after frostbite. The dose, after determination of the prothrombin clotting time, is 25 to 200 mg, depending on the size and the condition of the patient, the drug being given orally in the form of capsules or tablets. On the second day and thereafter, it may be given in amounts sufficient to maintain the prothrombin clotting time at about 30 seconds. If hemorrhages should occur, 50 to 100 mg of menadione sodium bisulfite is injected, supplemented by a blood transfusion.

Warfarin Sodium, USP. 3-(α-Acetonylbenzyl)-4-hydroxycoumarin sodium salt (Coumadin, Panwarfin) is a white, odorless, crystalline powder, having a slightly bitter taste; it is slightly soluble in chloroform and soluble in alcohol or water. A 1% solution has a *p*H of 7.2 to 8.5.

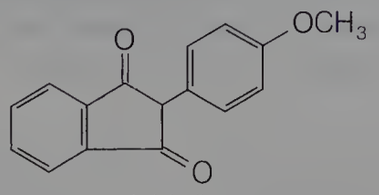


Warfarin Sodium

By virtue of its great potency, warfarin sodium at first was considered unsafe for use in humans and was utilized very effectively as a rodenticide, especially against rats. However, when used in the proper dosage level, it can be used in humans, especially by the intravenous route.

Warfarin Potassium, USP. 3-(α-Acetonylbenzyl)-4-hydroxycoumarin potassium salt (Athrombin-K). Warfarin potassium is readily absorbed after oral administration, with a therapeutic hypoprothrombinemia being produced in 12 to 24 hr after administration of 40 to 60 mg. This salt is therapeutically interchangeable with warfarin sodium.

Anisindione, USP. 2-(*p*-Methoxyphenyl)-1,3-indandione; 2-(*p*-anisyl)-1,3-indandione (Miradon) is a *p*-methoxy congener of phenindione. It is a white, crystalline powder, slightly soluble in water, tasteless, and absorbed well after oral administration.

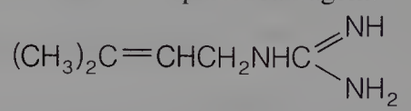


Anisindione

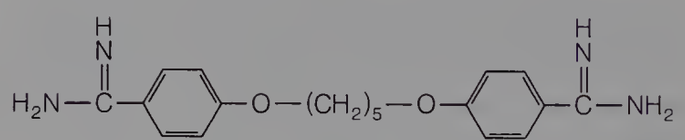
In instances when the urine may be alkaline, an orange color may be detected. This is due to metabolic products of anisindione and is not hematuria.

SYNTHETIC HYPOGLYCEMIC AGENTS

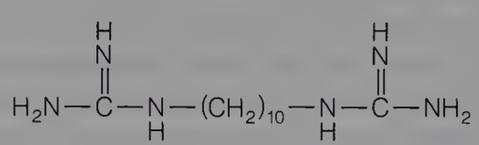
The discovery that certain organic compounds will lower the blood sugar level is not recent. In 1918, guanidine was shown to lower the blood sugar level. The discovery that certain trypanosomes need much glucose and will die in its absence was followed by the discovery that galegine lowered the blood sugar level and was weakly trypanocidal. This led to the development of several very active trypanocidal agents, such as the bisamidines, diisothioureas, bisguanidines, and others. Synthalin (trypanocidal at 1:250 million) and pentamidine are outstanding examples of very active trypanocidal agents. Synthalin lowers the blood sugar level in normal, depancreatized, and completely alloxanized animals. This may be due to a reduction in the oxidative activity of mitochondria, resulting from inhibition of the mechanisms that simultaneously promote phosphorylation of ADP and stimulate oxidation by nicotinamide adenine dinucleotide (NAD) in the citric acid cycle. Hydroxystilbamidine isethionate USP is used as an antiprotozoan agent.



Galegine



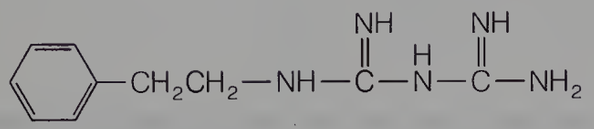
Pentamidine



Synthalin

In 1942, *p*-aminobenzenesulfonamidoisopropylthiadiazole (an antibacterial sulfonamide) was found to produce hypoglycemia. These results stimulated research for the development of synthetic hypoglycemic agents, several of which are in use today.

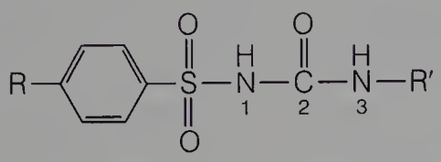
Sulfonylureas became widely available in 1955 for the treatment of non–ketosis-prone mild diabetes and are still the drugs of choice. A second class of compounds, the biguanides, in the form of a single drug, phenformin, has been used since 1957. However, it has been withdrawn from the United States market because of its toxic effect. Phenformin causes lactic acidosis, from which fatalities have been reported.



Phenformin

SULFONYLUREAS

The sulfonylureas may be represented by the following general structure:



These are urea derivatives with an arylsulfonyl group in the 1-position and an aliphatic group at the 3-position. The aliphatic group, R′, confers lipophilic properties to the molecule. Maximal activity results when R′ consists of three to six carbon atoms, as in chlorpropamide, tolbutamide, and acetohexamide. Aryl groups at R′ generally give toxic compounds. The R group on the aromatic ring primarily influences the duration of action of the compound. Tolbutamide disappears quite rapidly from the bloodstream through being metabolized to the inactive carboxy compound, which is excreted rapidly. However, chlorpropamide is metabolized more slowly and persists in the blood for a much longer time.

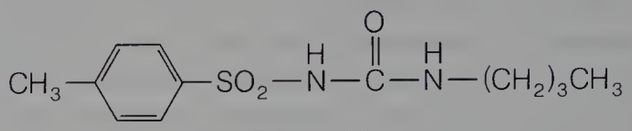
The mechanism of action of the sulfonylureas is to stimulate the release of insulin from the functioning β-cells of the intact pancreas. In the absence of the pancreas, they have no significant effect on blood glucose. The sulfonylureas may have other actions, such as inhibition of secretion of glucagon and action at postreceptor intracellular sites to increase insulin activity.

For a time, tolbutamide, chlorpropamide, and acetohexamide were the only oral hypoglycemic agents. Subsequently, a second generation of these drugs became available. Although they did not present a new method of lowering blood glucose levels, they were more potent than the existing drugs. Glipizide and glyburide are the second-generation oral hypoglycemic agents.

Whether they are first- or second-generation oral hypoglycemic drugs, this group of agents remains a valuable adjunct to therapy in the diabetic patient whose disease had its onset in adulthood. Accordingly, the group of sulfonylureas is not indicated in the juvenile-onset diabetic.

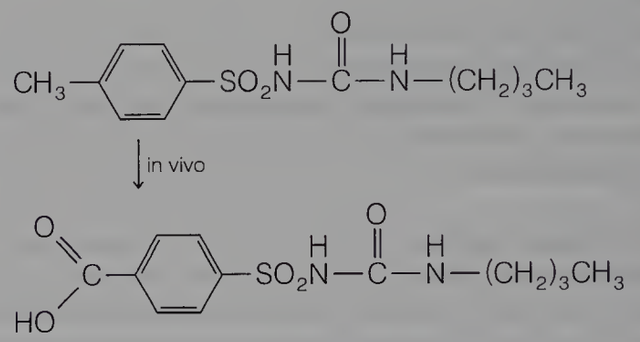
PRODUCTS

Tolbutamide, USP. 1-Butyl-3-(*p*-tolylsulfonyl)urea (Orinase) occurs as a white, crystalline powder that is insoluble in water and soluble in alcohol or aqueous alkali. It is stable in air.



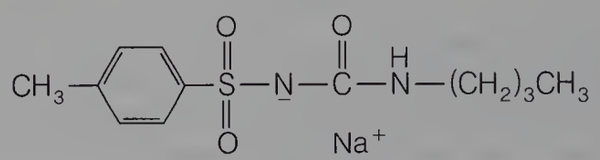
Tolbutamide

Tolbutamide is absorbed rapidly in responsive diabetic patients. The blood sugar level reaches a minimum after 5 to 8 hr. It is oxidized rapidly in vivo to 1-butyl-3-(*p*-carboxyphenyl)sulfonylurea, which is inactive. The metabolite is freely soluble at urinary *p*H; however, if the urine is strongly acidified, as in the use of sulfosalicylic acid as a protein precipitant, a white precipitate of the free acid may be formed.



Tolbutamide should be used only when the diabetic patient is an adult or shows maturity-onset diabetes, and the patient should adhere to dietary restrictions.

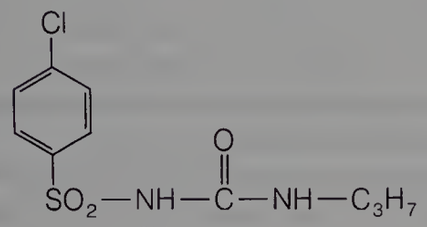
Tolbutamide Sodium, USP. 1-Butyl-3-(*p*-tolylsulfonyl)-urea monosodium salt (Orinase Diagnostic). Tolbutamide sodium is a white, crystalline powder, freely soluble in water, soluble in alcohol and chloroform, and very slightly soluble in ether.



Tolbutamide Sodium

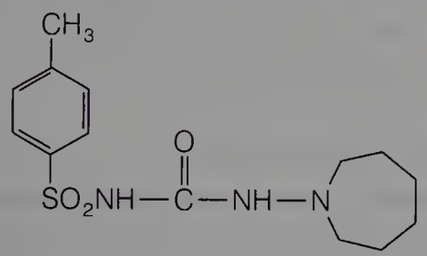
This water-soluble salt of tolbutamide is used intravenously for the diagnosis of mild diabetes mellitus and of functioning pancreatic islet cell adenomas. The sterile dry powder is dissolved in sterile water for injection to make a clear solution, which then should be administered within 1 hr. The main route of breakdown is to butylamine and sodium *p*-toluenesulfonamide.

Chlorpropamide, USP. 1-[(*p*-Chlorophenyl)-sulfonyl]-3-propylurea (Diabinese). Chlorpropamide is a white, crystalline powder, practically insoluble in water, soluble in alcohol, and sparingly soluble in chloroform. It will form water-soluble salts in basic solutions. This drug is more resistant to conversion to inactive metabolites than is tolbutamide and, as a result, has a much longer duration of action. One study showed that about half of the drug is excreted as metabolites, the principal one being hydroxylated in the 2-position of the propyl side chain.[75] After control of the blood sugar level, the maintenance dose is usually on a once-a-day schedule.



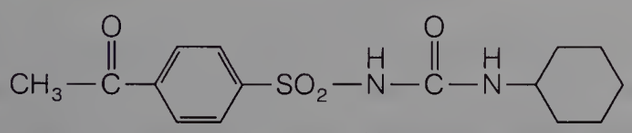
Chlorpropamide

Tolazamide, USP. 1-(Hexahydro-1*H*-azepin-1-yl)-3-(*p*-tolylsulfonyl)urea (Tolinase). This agent is an analogue of tolbutamide and is reported to be effective, in general, under the same circumstances for which tolbutamide is useful. However, tolazamide appears to be more potent than tolbutamide and is nearly equal in potency to chlorpropamide. In studies with radioactive tolazamide, investigators found that 85% of an oral dose appeared in the urine as metabolites that were more soluble than tolazamide itself.



Tolazamide

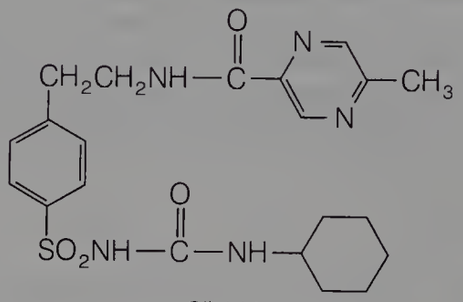
Acetohexamide, USP. 1-[(*p*-Acetylphenyl)sulfonyl]-3-cyclohexylurea (Dymelor). Acetohexamide is related chemically and pharmacologically to tolbutamide and chlorpropamide. Like the other sulfonylureas, acetohexamide lowers the blood sugar, primarily by stimulating the release of endogenous insulin.



Acetohexamide

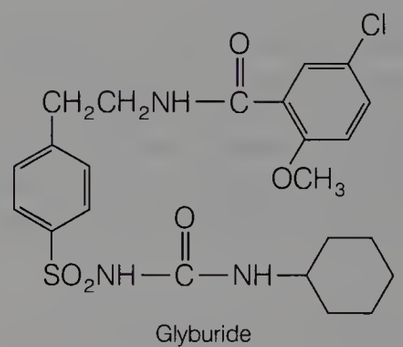
Acetohexamide is metabolized in the liver to a reduced form, the α-hydroxyethyl derivative. This metabolite, the main one in humans, possesses hypoglycemic activity. Acetohexamide is intermediate between tolbutamide and chlorpropamide in potency and duration of effect on blood sugar levels.

Glipizide. 1-Cyclohexyl-3-[[*p*-[2-(methylpyrazinecarboxamido)ethyl]phenyl]sulfonyl]urea (Glucotrol). Structurally, glipizide is a cyclohexylsulfonylurea analogue similar to acetohexamide and glyburide. The drug is absorbed rapidly on oral administration. Its serum half-life is 2 to 4 hr, and it has a hypoglycemic effect that ranges from 12 to 24 hr.



Glipizide

Glyburide. 1-[[*p*-[2-(5-Chloro-*o*-anisamido)ethyl]-phenyl]sulfonyl]-3-cyclohexylurea (DiaBeta, Euglucon, Micronase). Similar to glipizide, this is a second-generation oral hypoglycemic agent. The drug has a half-life elimination of 10 hr, but its hypoglycemic effect remains for up to 24 hr.



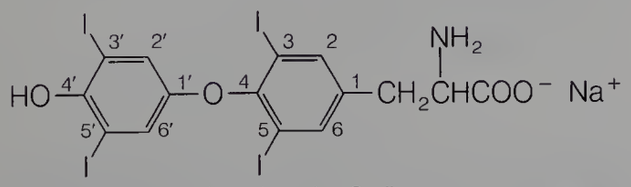
Glyburide

THYROID HORMONES

Desiccated, defatted thyroid substance has been used for many years as replacement therapy in thyroid gland deficiencies. The efficacy of the whole gland is now known to depend on its thyroglobulin content. This is an iodine-containing globulin. Thyroxine was obtained as a crystalline derivative by Kendall[76] of the Mayo Clinic in 1915. It showed much the same action as the whole thyroid substance. Later, thyroxine was synthesized by Harington and Barger in England.[77] Later studies showed that an even more potent iodine-containing hormone existed, which is now known as triiodothyronine. There is now evidence that thyroxine may be the storage form of the hormone, whereas triiodothyronine is the circulating form. Another point of view is that, in the blood, thyroxine is bound more firmly to the globulin fraction than is triiodothyronine, which can then enter the tissue cells.

PRODUCTS

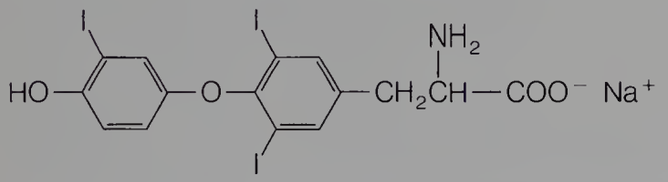
Levothyroxine Sodium, USP. *O*-[4-Hydroxy-3,5-diiodo-(phenyl)-3,5-diiodo-2-tyrosine monosodium salt, hydrate (Synthroid, Letter, Levoroxine, Levoid). This compound is the sodium salt of the *levo*-isomer of thyroxine, which is an active physiological principle obtained from the thyroid gland of domesticated animals used for food by humans. It is also prepared synthetically. The salt is a light yellow, tasteless, odorless powder. It is hygroscopic but stable in dry air at room temperature. It is soluble in alkali hydroxides, 1:275 in alcohol and 1:500 in water, to give a *p*H of about 8.9.



Levothyroxine Sodium

Levothyroxine sodium is used in replacement therapy of decreased thyroid function (hypothyroidism). In general, 100 μg of levothyroxine sodium is clinically equivalent to 30 to 60 mg of Thyroid USP.

Liothyronine Sodium, USP. *O*-(4-Hydroxy-3-iodophenyl)-3,5-diiodo-L-thyroxine monosodium salt (Cytomel) is the sodium salt of L-3,3′,5-triiodothyronine. It occurs as a light tan, odorless, crystalline powder, which is slightly soluble in water or alcohol and has a specific rotation of + 18° to +22° in a mixture of diluted HCl and alcohol.



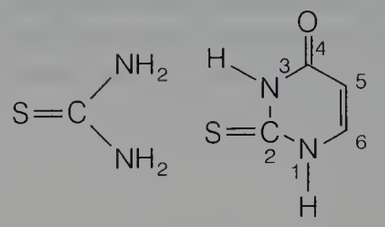
Liothyronine Sodium

Liothyronine sodium occurs in vivo together with levothyroxine sodium; it has the same qualitative activities as thyroxine but is more active. It is absorbed readily from the gastrointestinal tract, is cleared rapidly from the bloodstream, and is bound more loosely to plasma proteins than is thyroxine, probably because of the less acidic phenolic hydroxyl group.

Its uses are the same as those of levothyroxine sodium, including treatment of metabolic insufficiency, male infertility, and certain gynecological disorders.

ANTITHYROID DRUGS

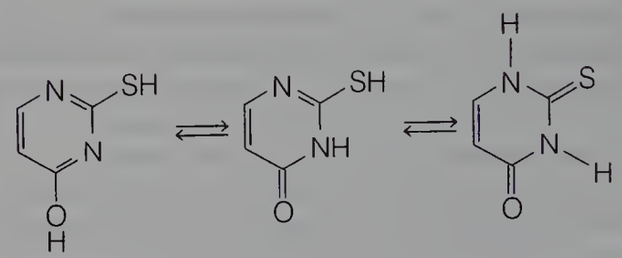
When *hyperthyroidism* (excessive production of thyroid hormones) exists, the condition usually requires surgery, but before surgery the patient must be prepared by preliminary abolition of the hyperthyroidism through the use of antithyroid drugs. Thiourea and related compounds show an antithyroid activity, but they are too toxic for clinical use. The more useful drugs are 2-thiouracil derivatives and a closely related 2-thioimidazole derivative. All of these appear to have a similar mechanism of action (i.e., prevention of the iodination of the precursors of thyroxine and triiodothyronine). The main difference in the compounds lies in their relative toxicities.



Thiourea 2-Thiouracil

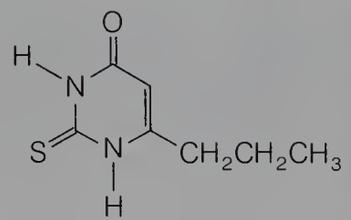
These compounds are absorbed well after oral administration and excreted in the urine.

The 2-thiouracils, 4-keto-2-thiopyrimidines, are undoubtedly tautomeric compounds and can be represented as follows:



Some 300 related structures have been evaluated for antithyroid activity, but, of these, only the 6-alkyl-2-thiouracils and closely related structures possess useful clinical activity. The most serious side effect of thiouracil therapy is agranulocytosis.

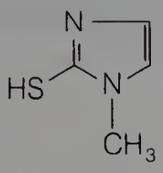
Propylthiouracil, USP. 6-Propyl-2-thiouracil (Propacil). Propylthiouracil is a stable, white, crystalline powder with a bitter taste. It is slightly soluble in water but readily soluble in alkaline solutions (salt formation).



Propylthiouracil

This drug is useful in the treatment of hyperthyroidism. There is a delay in appearance of its effects because propylthiouracil does not interfere with the activity of thyroid hormones already formed and stored in the thyroid gland. This lag period may vary from several days to weeks, depending on the condition of the patient. The need for three equally spaced doses during a 24-hr period often is stressed, but there is now evidence that a single daily dose is as effective as multiple daily doses in the treatment of most hyperthyroid patients.[78]

Methimazole, USP. 1-Methylimidazole-2-thiol (Tapazole) occurs as a white to off-white, crystalline powder with a characteristic odor and is freely soluble in water. A 2% aqueous solution has a *p*H of 6.7 to 6.9. It should be packaged in well-closed, light-resistant containers.



Methimazole

Methimazole is indicated in the treatment of hyperthyroidism. It is more potent than propylthiouracil. The side effects are similar to those of propylthiouracil. As with other antithyroid drugs, patients using this drug should be under medical supervision. Also, similar to the other antithyroid drugs, methimazole is most effective if the total daily dose is subdivided and given at 8-hr intervals.

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CHAPTER **20**

Local Anesthetic Agents

Gareth Thomas
David E. Thurston

Local anesthetics are blocking drugs that, when administered locally in the correct concentration, "block" the nerves that carry the pain sensation and automatic impulses in local areas of the body. They do not block coarse touch or movement, and the action is reversible. Their method of administration is governed by such properties as toxicity, stability, duration of action, water solubility, membrane permeability, and point of application, while their modes of action depend on their lipid solubility, pK_a, vasodilator, and protein binding characteristics.

Although given locally, the drug may exert a systemic effect due to transport in the blood from the site of administration to other areas such as the kidney, liver, etc. These systemic effects, which are dependent on the concentration of the local anesthetic in the blood, are usually sedation and light-headedness, but restlessness, nausea, and anxiety may also occur. High plasma concentrations can result in convulsions, chiropidy, and coma with respiratory and cardiac depression.

Local anesthetics are used in dentistry, ophthalmology, and minor surgical operations, including endoscopy. They are also used topically for the temporary relief of pain from insect bites, burns, and other types of surface wounds. Local anesthetics are particularly effective when used on mucus membranes such as the mouth, vagina, or rectum.

HISTORICAL DEVELOPMENT

The start of the search for ways to relieve pain is lost in the past. People have used religious exorcism, hypnotism, acupuncture, hypothermia, nerve compression, and drugs. Each of these methods has had its periods of popularity, and most are still used in one form or another. The modern development of the use of drugs to induce local anesthesia probably started in the mid-19th century. However, the earliest recorded use of hypothermia as a local anesthetic is believed to be by Larrey, Napoleon's chief army surgeon during the retreat from Moscow. He recorded that amputations carried out on patients in sub-zero temperatures had a higher survival rate than those carried out in warmer conditions. Later in the century, Thompson reported that ether acted as a refrigerant when poured on to the skin. These observations lay dormant until 1848, when Arnott reported that he had used a pig's bladder filled with crushed ice to alleviate the pain caused when incisions were made in the skin. This was followed by Snow's unsuccessful attempts to find a viable way of using refrigeration as a local anesthetic. Success was achieved eventually by his protege Richardson, who replaced the cologne in the then recently introduced Eau-de-Cologne spray with ether. This achieved temperatures that allowed minor surgery to be carried out. Richardson and other workers improved the efficiency of the procedure by using a petroleum distillate, then ethyl bromide, and ultimately ethyl chloride. The success of the Richardson spray inspired Koller to search for a local anesthetic that could be safely applied to the eye.

Koller qualified in medicine in 1882 and went on to specialize in ophthalmology in Vienna. His experiences as an eye surgeon made him increasingly aware of the need for a local anesthetic that could be used in the eye. In 1884, while he was collaborating with Freud to study the effect of cocaine on fatigue, a colleague remarked that the drug numbed his tongue. Koller and Gaertner investigated this claim and found that a dilute aqueous solution of cocaine hydrochloride caused local anesthesia of the cornea. Brettauer presented Koller's results on his behalf at an ophthalmology meeting in Heidelberg in September 1884 since Koller could not afford the train fare from Vienna to Heidelberg. Koller's paper resulted in the immediate widespread use of cocaine in Europe and the United States. Koller also recommended the use of cocaine as a local anesthetic in ear, nose, and throat operations. However, at the time, little was known about its addictive properties.

In 1885, degradation studies by Camels and Gossin suggested that there were some structural similarities between

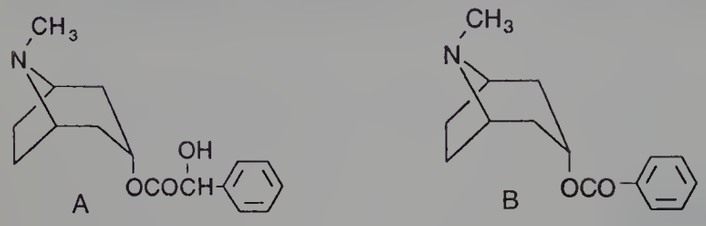


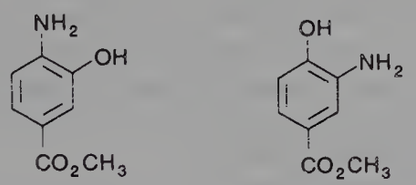
FIG. 20-1. **A** and **B:** Homatropine and benzoyltropine.

cocaine and atropine. This led Filehne at the University of Breslau (now Wroclaw) in Poland to determine whether atropine had any local anesthetic activity in the eye. Atropine had been isolated from the roots of belladonna in 1831 by Mein, a German apothecary. Filehne found that atropine had little local anesthetic activity and was toxic, causing eye irritation at the doses required for any activity. Earlier, Lossen showed that atropine could be split into tropic acid and a nitrogenous base called "tropine." Later in 1880, Ladenburg synthesized a series of physiologically active compounds, which he called "tropeines," by esterifying tropine with a variety of aromatic acids. Filehne investigated these semisynthetic analogs of atropine for local anesthetic activity and found that homatropine (Fig. 20-1) was less irritating to the eyes and a better local anesthetic than atropine, whereas benzoyltropine was a strong local anesthetic but caused too much irritation to be of any clinical use. However, the identification of a benzoyl group in the most active atropine analogs and in cocaine led Filehne to test the activity of the benzoyl derivatives of quinine, cinchonine, hydrocotarnine, and morphine. His results, which were published in 1887, showed that these benzoate esters acted as local anesthetics, but many had unwanted side effects.

By 1888, the toxic and addictive effects of cocaine were beginning to concern the medical world, and many workers were seeking a safe substitute. In 1892, Einhorn,[1] a professor of chemistry at the University of Munich, suggested a structure for cocaine based on the structure of tropine proposed by Merling[2] at the University of Berlin (Fig. 20-2A,B). Merling decided, on the basis of these incorrect structures, to synthesize a benzoyl analog containing only a piperidine ring. He produced a compound whose structure was similar to that of the weakly active methyltriacetone alkamine analog of atropine (Fig. 20-2C). It was marketed under the name *alpha-Eucaine* (Fig. 20-2D) but was not popular as it caused a burning sensation when applied to the eyes. It was rapidly replaced by *beta-Eucaine* (Fig. 20-2E), but this also caused eye irritation.

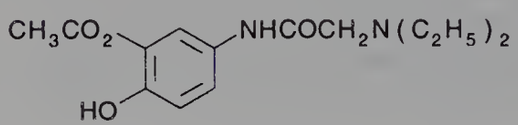
Einhorn, on realizing Merling's success with the benzoate derivatives of piperidine, attempted to synthesize active benzoate compounds based on the simpler hexane ring. His syntheses, which were based on the reduction of aromatic benzoate esters, failed, so he decided to have a number of unrelated aromatic benzoate esters tested for local anesthetic activity. Some were found to be active, but, more importantly, several of the phenols formed by the hydrolysis of the esters were also found to be active, and in 1896 Einhorn introduced *Orthoform* (orthocaine) into clinical use. Problems with its production and its side effects led him to introduce *Orthoform New* in 1897. Einhorn's work was important in that it gave the first indication that a benzoate ester was not essential for local anesthesia.

Although the *Orthoforms* were relatively successful as topical anesthetics, their poor water solubility made them unsuitable for other medicinal uses. Consequently, Einhorn attempted to improve their water solubility by introducing amine-containing aliphatic side chains. He reasoned that the formation of their amine hydrochlorides would improve water solubility without making the preparation too acidic.



Orthoform

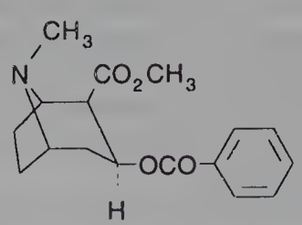
Orthoform New



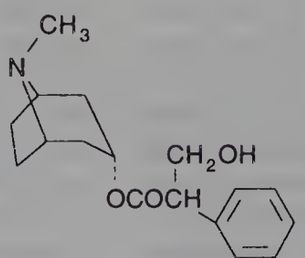
Nirvanin

One of Einhorn's compounds, Nirvanin, was introduced in 1898. Its activity was low, and it had to be used in high doses, which caused toxic effects.

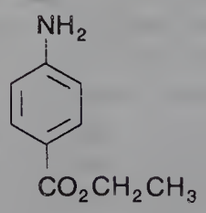
In 1898, Willstatter determined the correct structures of both atropine and cocaine. He followed this by synthesizing cocaine in 1901.



Cocaine



Atropine



Benzocaine

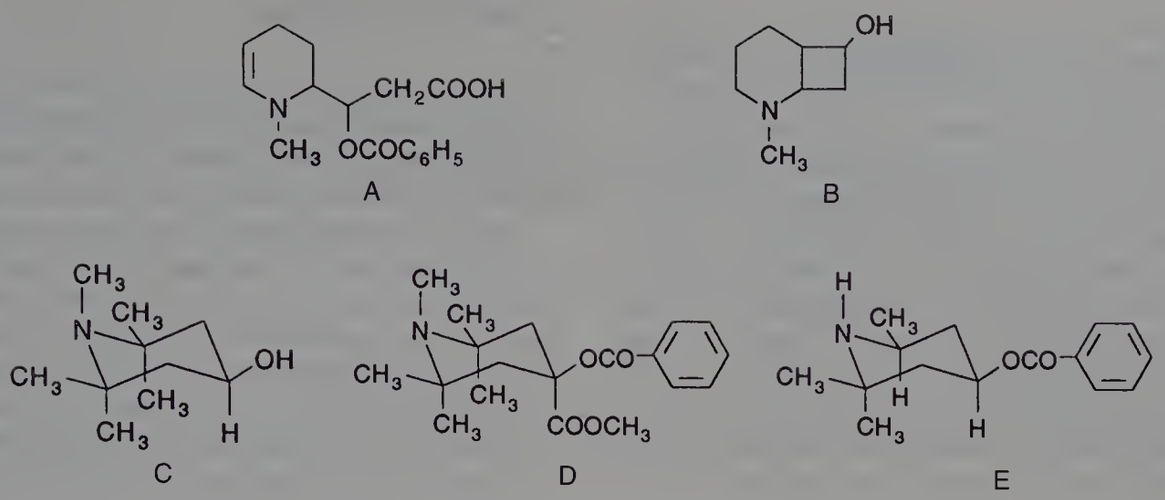
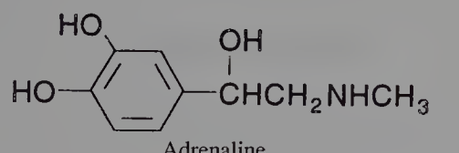


FIG. 20-2. *The incorrect structures proposed for* **(A)** *cocaine and* **(B)** *tropine by Albert Einhorn and Georg Merling. The structures of* **(C)** *methyltriacetone alkamine,* **(D)** *α-Eucaine and* **(E)** *β-Eucaine.*

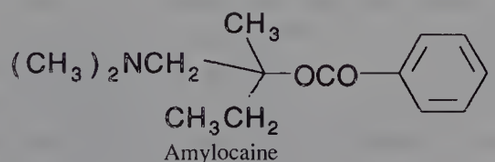
Einhorn's clinical success with the poorly water-soluble *Orthoforms* resulted in the introduction of ethyl 4-aminobenzoate into clinical use in 1902 under the name *Anaesthesine*. It was later given the approved name of *Benzocaine*. Ritsert had already noticed, in 1890, that this water-insoluble compound had numbed his tongue, and so to improve its water solubility it had been formulated as the hydrochloride. However, as aromatic amines are weak bases, the resulting solution had proved too acidic for clinical use, and he had discounted its use.

In 1902, Fourneau in France designed a drug whose structure incorporated functional groups similar to those found in the structure of the cocaine molecule. However, he did not include the piperidine ring, which he considered to be responsible for the toxicity of cocaine. His compound, which he marketed under the name of *Stovaine*, was the first nonirritant local anesthetic that could be given by injection and used as a safe substitute for cocaine. Stovaine was later given the approved name of *Amylocaine.*



Adrenaline

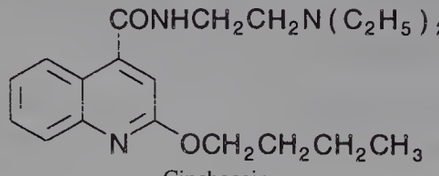
Procaine



Amylocaine

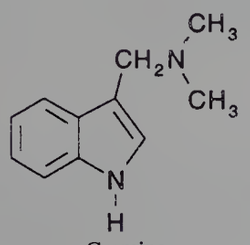
Earlier work in 1885 by Corning in the United States had shown that the anesthetic effect of cocaine could be enhanced by the use of tourniquets to prevent the drug being carried away from the site of action. This increased the effectiveness of the drug and allowed lower doses to be used. However, applications of the tourniquet technique were limited. In 1900, the publication of the observation that adrenal extracts caused blood vessels to contract resulted in Braun demonstrating that mixtures of cocaine and adrenal extracts were more effective than cocaine alone. The isolation of adrenaline, the active component of adrenal extracts, and its subsequent structural determination led Braun in 1904 to design a drug based on the structure of both adrenaline and Einhorn's local anesthetics. It was marketed as *Novocaine* and was later given the approved name of *Procaine*. *Procaine* dominated the local anesthetic market for half a century and is still in use today.

In the next 30 or so years after the synthesis of procaine, large numbers of compounds were tested for local anesthetic activity, but none of importance emerged. However, Miescher working for Ciba in Switzerland found that some of the acetanilide analogs he had synthesized as potential antipyretic agents also exhibited local anesthetic activity. In 1931, his synthetic work led to the production of cinchocaine (*Nupercaine*), a long-acting local anesthetic particularly useful in spinal anesthesia.

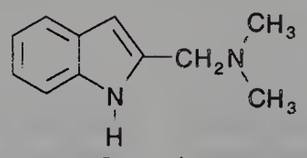


Cinchocaine

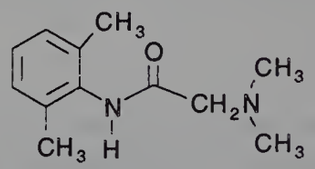
At about the same time that cinchocaine was developed, an investigation of the chemical structure of the alkaloid gramine at Stockholm University resulted in Erdtman synthesizing its isomer, *Iosgramine*. As luck would have it, Erdtman tasted isogramine and found that his tongue went numb. Realizing its potential, he tested its open chain precursor and found that it also exhibited local anesthetic activity. For the next 7 years, Erdtman and his student Lofregen synthesized and tested compounds with similar structural characteristics. Their search was rewarded 57 compounds later, by the discovery of lignocaine (*Lidocaine, Xylocaine*). This drug was marketed in 1948 by Astra in Sweden, and, because of its rapidity of action and nonirritant and relatively safe nature, it has become the leading local anesthetic.



Gramine
3-(dimethylaminomethyl)indole

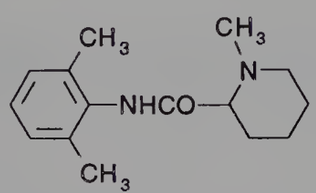


Isogramine
2-(dimethylaminomethyl)indole

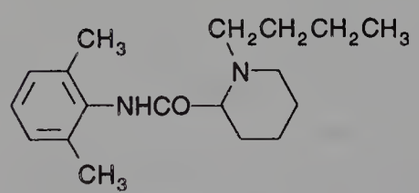


Lignocaine

In 1957, scientists at AB Bofors replaced the acyclic tertiary amino side chain of lignocaine with a cyclic tertiary amine for no reason other than that novel compounds were thereby produced. This irrational approach led to two useful local anesthetics, mepivacaine (*Carbocaine*) and bupivacaine (*Marcain*). Bupivacaine was long acting, producing nerve blocks for up to 8 hr.



Mepivacaine



Bupivacaine

A large number of active compounds have now been synthesized, but lignocaine, procaine, and many of the pre-1957 compounds are still in current use. However, in 1974 Hughes isolated and in 1975[3] determined the structures of the natural pain control agents, methionine-enkephalin (met-enkephalin) and leucine-enkephalin (leu-enkephalin). The isolation of these and other peptides with similar activity has opened up a new structural route to the synthesis of local anesthetic agents. This has yet to be fully exploited.

H-Try-Gly-Gly-Phe-Met (OH)
Met-enkephalin

H-Try-Gly-Gly-Phe-Leu (OH)
Leu-enkephalin

NERVOUS SYSTEM

The nervous system has two main properties: it responds to various stimulations and is able to transmit the stimulus to the brain and other parts of the nervous system. Both stimulation and transmission may be blocked by local anesthetic agents.[4]

The basic building blocks of the nervous system are the *nerve cells* or *neurons*. Associated with the neurons are the *glial cells*. In humans, the complete system contains more than 10 billion neurons and ~10 to 15 times that number of glial cells. Extending from the brain, the *command center* of the nervous system, is the cluster of neurons and glial cells that form the spinal cord. The brain together with the spinal cord form the central nervous system (*CNS*). Extending outward from the CNS is the peripheral nervous system (*PNS*). This system is divided into three parts: the first extends to the skin and muscles, the second links the brain to the ear, nose, throat, and eyes, and the third controls the unconscious bodily functions such as breathing, digestion, and heartbeat. This last is referred to as the autonomic or involuntary nervous system.[5]

Neurons receive, conduct, and transmit electrical signals in the form of ionic currents. A typical neuron usually consists of a central cell body from which radiate out a number of thin branch-like protruberances (Fig. 20-3). These branches are of two types, a single branch known as the axon, which acts as a conductor of signals from the cell body, and a number of other separate branches known as the dendrites, which act as antennae receiving signals from the axon of other neurons. Both the axons and dendrites of neurons can exhibit an astonishing variety of branching, but axon branching is usually simpler.

The terminal branches of the axon end in *synaptic knobs*,

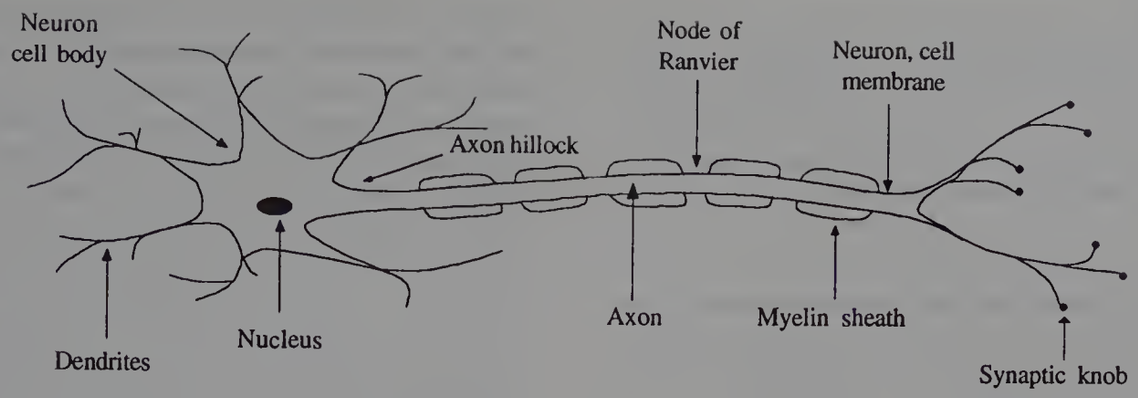


FIG. 20-3. *A schematic diagram of a neuron. Representation of the variety of branching found in dendrites.*

which are also known as *terminal buttons* or *axon telondria*. The junction between two neurons where a nerve impulse is transmitted from one neuron to the other consists of the synaptic knob of the transmitting neuron separated by a gap of ~ 30 to 50 nm from either the dendrite, axon hillock, or cell body of the other neuron. This gap is known as the synaptic cleft, and the whole area where transmission and reception of the impulse from one neuron to the other occurs, a *synapse*.

The axon arises from a thickened area of the cell body called the *axon hillock*. Its membrane is mainly composed of lipids and proteins and is known as the *axolemma*. Many of the axons of the CNS and PNS are partly covered from near the axon hillock to the synaptic knob by a sheath of *myelin* (myelinated axons), but some axons do not have this type of covering (unmyelinated axons). The myelin sheath of PNS myelinated axons is not continuous but is broken at ~ 1 mm intervals to expose the axolemma to the extracellular fluid. These exposed areas, which are ~ 1 μm in length, are known as the *nodes of Ranvier*. The distance between the nodes is often referred to as the *internodal distance*.

A segment of the PNS myelin sheath consists of a single glial cell known as a Schwann cell, tightly wrapped around the axon so as to form several tightly bound layers of the same cell (Fig. 20-4A). In unmyelinated axons the Schwann cells simply surround the axon and are not tightly wrapped around it (Fig. 20-4B). The CNS myelinated sheath is a much more complicated structure. In all cases, the main function of myelin is to act as an insulating material, electrically insulating the axon from the extracellular fluid.

A nerve consists of chains of neurons (nerve fiber) enclosed in different layers of protective tissues (Fig. 20-4C). For example, in a spinal nerve the individual nerve fibers whether myelinated or unmyelinated are wrapped in a layer of protective connective tissue known as the *endoneurium*. These endoneurium coated fibers are grouped in bundles known as *fascicles*. Each fascicle is coated with a layer of connective tissue called the "perineurim." The complete nerve consists of a number of fascicles embedded in tissue through which run various blood vessels, the whole structure being covered by a layer of connective tissue known as the epineurium.

Neurons are excited by electrical, chemical, and mechanical stimuli. They convey the information provided by this stimulation in the form of electrical signals. However, the precise nature of the information carried will depend on the type of neuron; for example, a *motor neuron* will convey electrical signals that cause a particular muscle to contract. In all cases, the signals take the form of changes in the electrical potential across the neuron membranes. In myelinated neurons, a change in the membrane potential in one node of Ranvier will stimulate further changes in an adjacent node and so on; that is, a change in electrical potential at A stimulates a change in electrical potential at B, which in turn initiates a change in the electrical potential at C and so on (Fig. 20-5). This process, whereby the change in potential

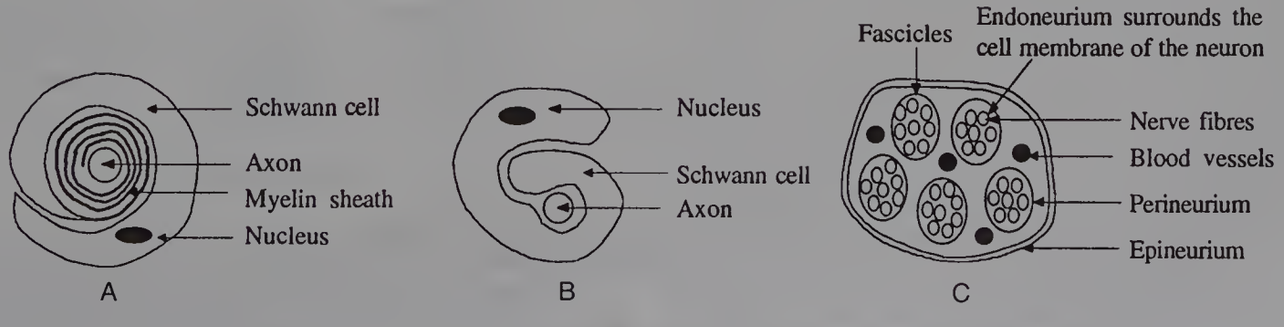


FIG. 20-4. *Representations of* **(A)** *a myelinated,* **(B)** *an unmyelinated axon, and* **(C)** *a cross section of a nerve.*

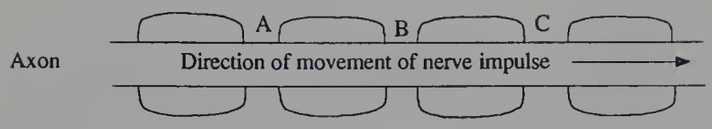


FIG. 20-5. *A representation of the transmission of a nerve impulse along a neuron fiber by saltatory conduction.*

jumps from one node to another, is known as *saltatory conduction*. It results in the movement of an electrical impulse, which is referred to as either the *nerve impulse* or *action potential,* along the axon. In unmyelinated nerves, the change in potential of one section of the membrane stimulates a change in potential of an immediately adjacent section of the membrane. These nerve impulses are transmitted or conducted along an axon sheathed with myelin at speeds up to 120 m s^{-1} or more but only up to 10 m s^{-1} in unmyelinated axons. In large neurons, the strength of the nerve impulse is maintained by an automatic amplification system, but many smaller neurons have no such systems.

In most cells, the electrical potential difference between the inner and outer surfaces of the cell membrane is due to the movement of ions across that membrane.[5] In all axons, the interior face of the membrane is the negative side of the potential difference. For a cell at rest that is not undergoing outside stimulation, this electrical potential is known as the *resting potential* and can vary from -20 to -200 mV. The resting potential of neurons is about -70 mV. The *action potential* of the axon is the series of potential changes that occurs when the axon is stimulated. Microelectrodes implanted in the axon (*intracellular recording*) show that there is an initial depolarization of the membrane by ~20 mV. This is followed by the rapid rise of the membrane's potential to a maximum value of about $+35$ mV. The membrane is then said to be *depolarized*. This is immediately followed by the potential dropping back toward the resting potential (*repolarization*). The repolarization overshoots the resting potential (*hyperpolarization*) before slowly recovering to the resting potential (Fig. 20-6). The rapid rise and fall of the potential is termed the *spike potential*, and the point at which it starts the *firing level* or *threshold* of the axon. No action potential is produced if the stimulus is below the threshold potential. However, once the threshold level is reached, the action potential will occur regardless of the strength of the stimulus. Furthermore, the amplitude of this action potential is independent of the intensity of the stimulant. The action potential is said to obey the "*all or nothing*" *law*.

The peripheral nerves of mammals consist of bundles of neurons held together in a fibrous envelope called the *epineurium*. The changes in potential for these systems is the sum of the action potentials of all the axons in the system if extracellular recording is attempted. Each axon in the system will have a different threshold potential, and so the number of axons firing will initially increase with increase in the intensity of the stimulus. Eventually, all the axons in the nerve will fire, and at this point further increases in the intensity of the stimulus will cause no further increase in the size of the action potential. However, in bundles of mixed nerves, there will be multiple peaks in the action potential profile because the differing types of nerve fiber will have different conduction speeds.

The electrical potential across the lipid membrane of an axon is mainly due to the transport of small inorganic ions such as Ca^{2+}, Na^{+}, K^{+}, and Cl^{-} across the membrane. These ions move, by a process known as passive transport, through water-filled channels (ion channels) formed by the integral proteins of the membrane. The ions move from high concentration to low concentration (*downhill*) at rates of the order of $\geq 10^6$ ions per second, which is a hundred times faster than transport by any known carrier protein. Active transport of the ions is usually in the opposite direction to passive transport. For example, sodium ions are transported into an axon by passive transport but out by active transport. Similarly, potassium ions may be transported out of an axon by passive transport but in by active transport. A small movement of ions across a membrane can lead to the generation of electric fields that are enormous by macroscopic standards. For example, the transfer of one ion pair per million, the cation leaving and the anion entering the neuron across a membrane, results in an electrical potential of ~150,000 V cm^{-1}.

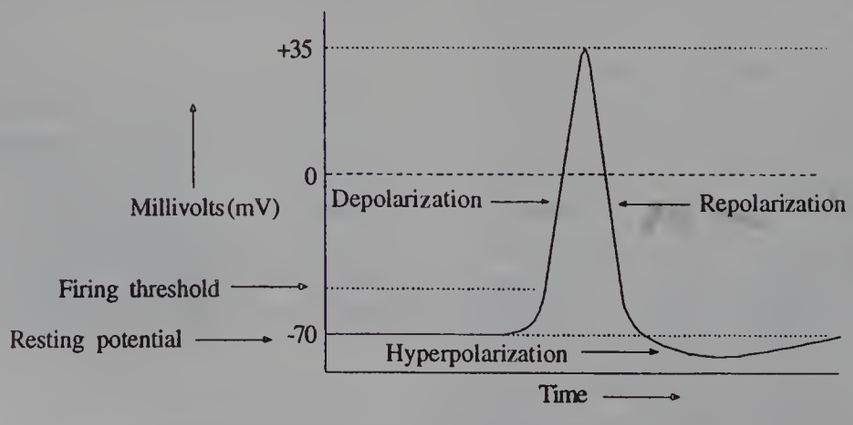


FIG. 20-6. *The changes in electrical potential observed during a nerve impulse.*

The highest density of Na^+ ion channels occurs at the nodes of Ranvier.[6] The myelinated internodal sections of the neuron contain far fewer Na^+ channels. In addition, these internodal regions are electrically insulated and so do not contribute to the action potential. Consequently, the potential produced at the nodes of Ranvier must be of sufficient strength to produce an effect up to 1 mm away.

Ion channels are formed by groups of integral proteins that run from one side of the membrane to the other. The channels are selective, allowing the passage of certain ions but preventing the passage of others. This suggests that parts of the channel must act as a selective filter. Furthermore, some of the channels are not permanently open; changes in the conformation of the proteins that form the channel effectively open and close the channel like a gate. These *gates* usually open briefly in response to various membrane changes such as a change in voltage across the membrane (*voltage-gated channels*) or the binding of a ligand to a receptor (*ligand-gated channels*). Over 50 types of gated channels have been discovered.[7]

The axolemma is more permeable to K^+ ions than to Na^+ ions. These ions diffuse out of the neuron through the so-called *potassium leak channels*, whose opening does not appear to require a specific membrane change. The movement of K^+ ions is concentration driven; the K^+ ions move from inside the neuron, where the concentration is high, to the extracellular fluid, where the concentration of K^+ ions is lower (Fig. 20-7a). This tendency of K^+ ions to leak out of the cell (*driven by the concentration gradient*) is balanced by the electrical forces of anions permanently held in the cell that attract potassium ions into the cell via potassium channels and also active transport mechanisms such as the sodium pump. These movements of K^+ ions result in a potential difference across the membrane, which is a major contributor to the *equilibrium potential* which exists between the opposite faces of a biological membrane in a normal cell at rest with a switched on sodium pump. Its value for a particular ion when the system is in equilibrium and the cell is at rest may be calculated using the Nernst equation:

$$V = V_i - V_o = \frac{RT}{zF} \ln \frac{C_o}{C_i}$$

where V is the equilibrium potential, V_i the internal potential, V_o the external potential, C_i the internal concentration of the ion (mol dm^{-3}), C_o the external concentration of the ion (mol dm^{-3}), R the ideal gas constant and T the temperature (°K). F is Faraday's constant (96,487 coulombs), and z is the charge on the ion.

The axolemma of a neuron at rest with a switched on sodium pump does not allow the free movement of Na^+ ions even though it contains channels that are specific for them. However, if the sodium pump is switched off the concentration of sodium starts to build up in the cell, as the membrane is not completely impermeable to sodium ions. The sodium ions leak into the cell down the concentration gradient through the so called *sodium ion channels* attracted by the permanent anions in the cell. This inward movement of sodium ions neutralizes the negative charges of some of these anions and so reduces (*depolarizes*) the membrane potential, which allows a greater concentration of potassium ions to leave the cell (Fig. 20-7B).

Calcium ions will also move into a cell, attracted by the anions permanently present inside, and will also leak out of cells via *calcium channels*. Similarly, under the appropriate conditions, chloride ions move in and out of cells. The movements of both these ions also contributes to the membrane potential of the cell, which, for a cell at rest, can vary from -20 mV to -200 mV. The more permeable a membrane is to a particular ion, the more closely the membrane potential approaches the equilibrium potential for that ion.

The initial depolarization of the neuron (Fig. 20-7) was shown by Hodgkin and Huxley in 1953 to be due to an increase in the movement of sodium ions into the neuron, which is followed almost immediately by an increase in the movement of potassium ions out. Consequently, the gated channels of neurons are believed to be responsible for the transmittance of the action potentials that carry information to and from the body of the nerve cell. It is thought that the action potential is triggered by a momentary shift of the membrane potential of a small section of the membrane to a less negative value (*depolarization of the membrane*). This causes the gated sodium ion channels in this section of the membrane to open, which allows sodium ions to enter the cell. This process depolarizes the membrane still further until

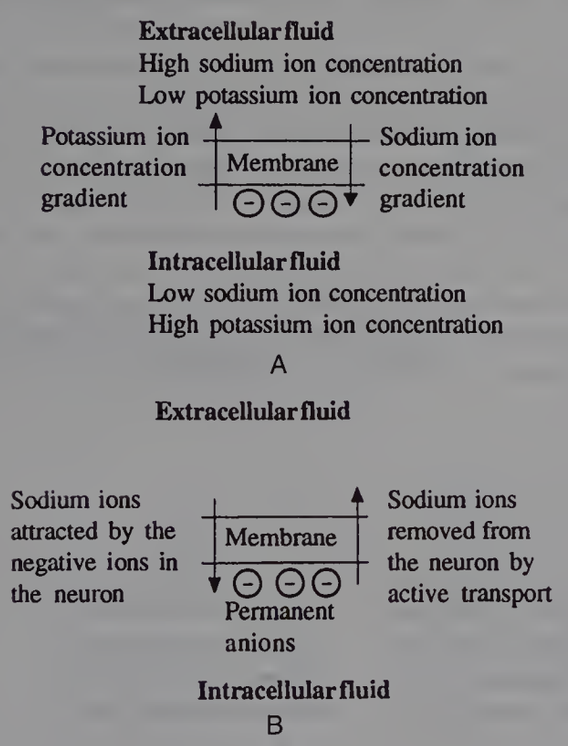


FIG. 20-7. **(A)** *The movement of Na^+ and K^+ ions across a membrane due to differences in concentration.* **(B)** *The attraction of positive ions into a cell by electrostatic attraction and their removal by active transport.*

the action potential reaches a critical value (the *firing threshold*), when it triggers the opening of large numbers of adjacent sodium channels so that Na^+ ions flood into the axon. This process continues until the membrane potential of this section of membrane reaches about +60 mV, which is the equilibrium potential for sodium ions when the cell is at rest (calculated using the Nerst equation). At this point, all the sodium ion channels of the membrane should be permanently open. However, this situation is not reached because each channel has an automatic closing mechanism which operates even though the cell membrane is still depolarized (Fig. 20-8). Once closed, the ion channel cannot open again until the membrane potential in its vicinity returns to its original negative value which is brought about by the leakage of potassium ions out of the neuron through potassium channels. Hodgkin and Huxley showed that a membrane becomes more permeable to potassium ions a fraction of a millisecond after the sodium channels have started to open. As a result, potassium ions flow out of the neuron which reduces the electrical potential of the membrane and so, at the peak of the action potential, the membrane potential has a value of about +40 mV relative to the exterior of the axon. The movement of the potassium ions out of the axon, coupled with the automatic closing of the sodium channel gates and the action of the sodium pump transporting Na^+ ions out of the neuron, result in a net flow of positive ions out of the neuron. This repolarizes the membrane and causes its membrane potential to drop below its resting potential to almost reach that of the potassium ion equilibrium value. As the sodium channels close and K^+ ions flow back into the axon, the membrane potential returns to its resting value. The entire process of depolarization and repolarization is normally accomplished within one millisecond. However, the action of the sodium pump is very slow compared to that of the Na^+ and K^+ ion channels.

An action potential originating from the cell body will trigger the depolarization of an adjacent section of the axon membrane, which in turn will produce an action potential in this area through the same process. These new areas will in turn affect further adjacent sections of the membrane and so on. In this way, the initial electrical impulse will move along the neuron. The impulse will flow in one direction since the channels will automatically close and will not open until the next stimulus reaches them down the neuron. Furthermore, as each action potential requires the same membrane trigger potential of about −20mV, the amplitude of the impulse remains the same as it proceeds along the axon. Moreover, since only a small change in the Na^+ and K^+ ion concentrations is required to form the action potential, the axon is able to transmit a nerve impulse every few milliseconds.

Neurons contain many thousands of sodium ion channels. Experimental work by Catterall in 1984 using radioactive neurotoxins and antibodies showed that their distribution in the plasma membranes of electrically excitable cells is nonuniform. Sodium channels appear to occupy at least 15% of the surface area of the nodes of Ranvier, but very few have been detected in the internodal regions of myelinated axons. Experimental work by Moczydlowski and co-workers in 1986[8] indicated that the nature of sodium channels varied with the type of membrane. The results obtained demonstrated the existence of three different Na-channel subtypes or "isochannels" and the fact that each of these individual isochannels are the predominant Na-channel types in mammalian brain, skeletal muscle, and cardiac muscle, respectively. Further work by Schneider and Dubois in 1986 attributed the action of benzocaine on the voltage clamped frog nodes of Ranvier to two different types of channel with different rates of inactivation and affinities for the drug.[9] Since then, numerous different types of ion channels have been identified, and it is likely that these different type respond differently to local anesthetic agents.

Patch clamp recording has shown that a sodium channel either opens completely to allow the passage of a sodium ion or remains shut. Sodium channels appear to open on a random basis but when open, a channel always has the same conductance. In other words, the rate of ion transfer from one side of the membrane to the other is always the same. Since a membrane is ~5 nm thick, the voltage gradient across a membrane is of the order of 150,000 Vcm^{-1}. Consequently, the proteins forming the channels are subjected to very strong electrical fields. Changes in this electrical field are believed to be responsible for the changes in the conformations of the proteins that form the gated channels. That is, changes in the electrical field of the membrane cause

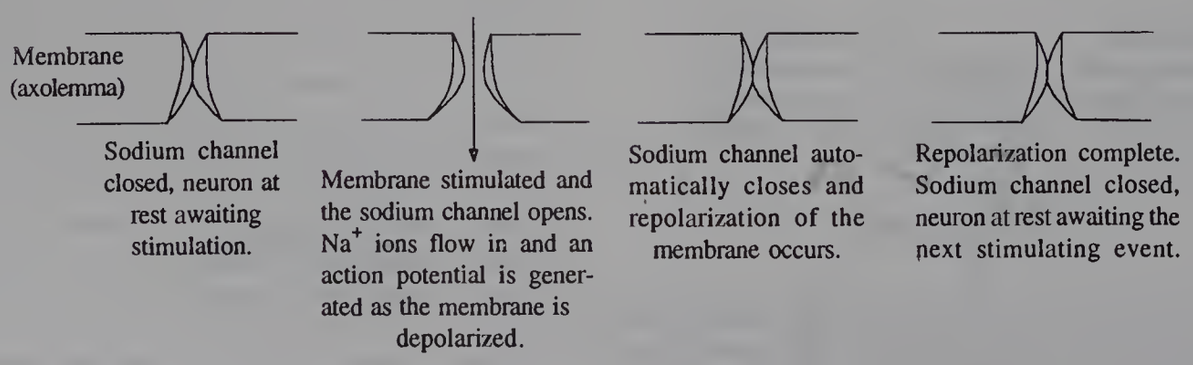


FIG. 20-8. *The cycle of conformational changes that occur in a sodium channel.*

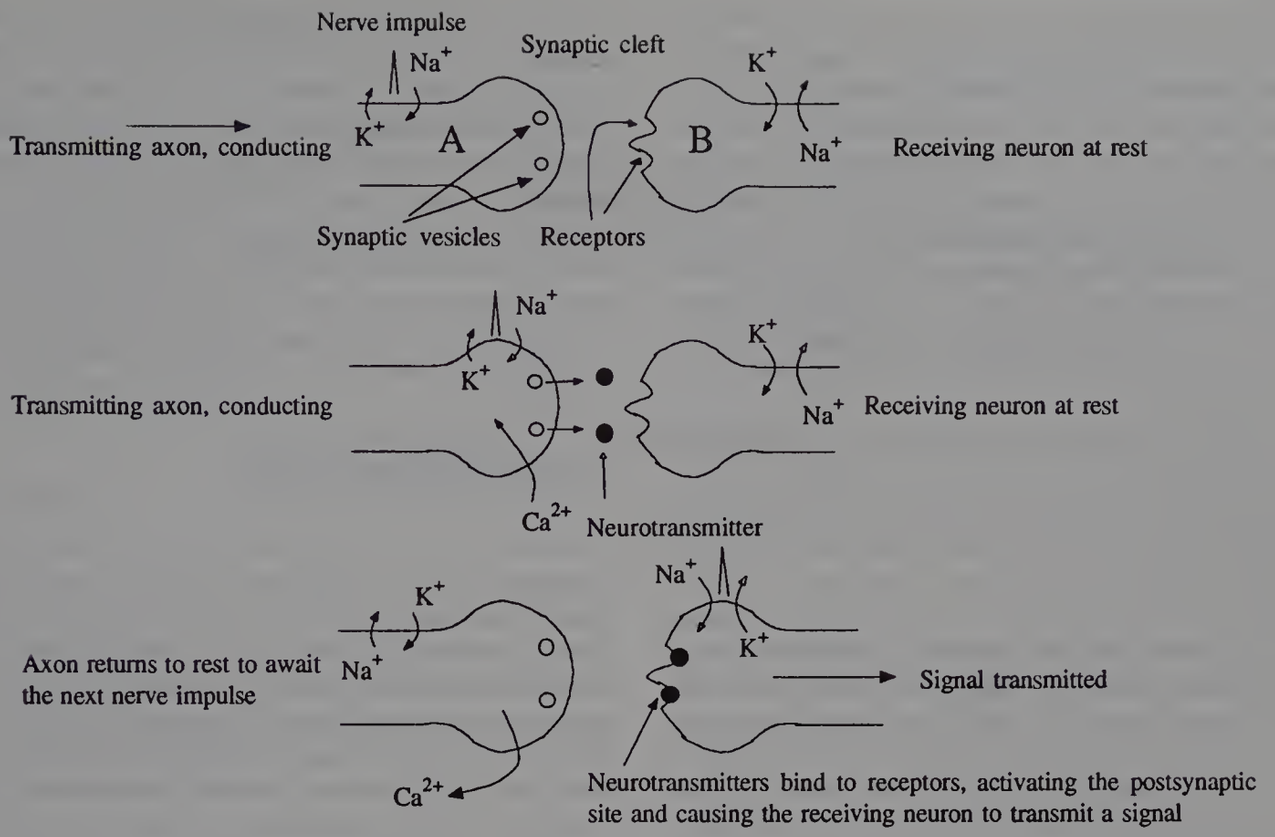


FIG. 20-9. *A representation of the action of a neurotransmitter.*

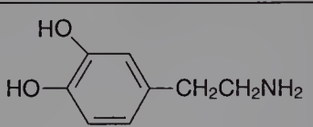
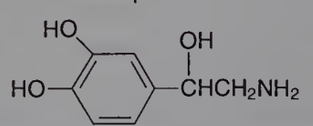
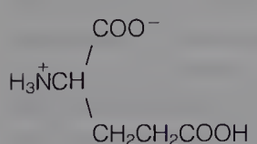
the opening and closing of the so called gates in the ion channels.

Neurons communicate with each other, and with muscle cells and glands at sites known as synapses. A nerve impulse arriving at the synapse (*presynaptic site or cell*) of a neuron (A) triggers the release, by exocytosis, of a chemical known as a *neurotransmitter,* which is stored in the synaptic vesicles (Fig. 20-9). The neurotransmitter diffuses across the synaptic cleft to a receptor (*postsynaptic cell or site*) on either a dendrite of a neuron (B) or another suitable cell. This causes a change in the electrical potential of the postsynaptic site which, in the case of the neuron, is then transmitted as an electrical impulse to the body of the neuron. Synapses usually permit the conduction of impulses in one direction only.

The chemical structures of neurotransmitters are very varied and include acetylcholine, simple amino acids, small peptides, β-phenylethanolamines, and catecholamines (Table 20-1). Their release is triggered by a change in the membrane potential at the presynaptic site. For example, the process that stimulates the contraction of a muscle cell is

TABLE 20-1

EXAMPLES OF NEUROTRANSMITTERS

Amino Acids	*Small Peptides*	*Miscellaneous Amines and Their Derivatives*
$H_3\overset{+}{N}CH_2COO^-$	H-Try-Gly-Gly-Phe-Met(OH)	
Glycine	Met-enkephalin	Dopamine
$H_3\overset{+}{N}CH_2CH_2CH_2COO^-$	H-Try-Gly-Gly-Phe-Leu(OH)	
γ-Aminobutanoic acid (GABA)	Leu-enkephalin	Noradrenaline
		$(CH_3)_3\overset{+}{N}CH_2CH_2OCOCH_3$
Glutamicacid		Acetylcholine

initiated by the opening of a calcium ion channel caused by the arrival of an action potential at the presynaptic site. Since the concentration of calcium ions is far higher in the extracellular fluid, calcium ions flow into the neuron. This increase in intracellular calcium ion concentration triggers the release of acetylcholine into the synaptic cleft by exocytosis of the synaptic vesicles. The mechanism of this Ca^{2+}-stimulated release of acetylcholine is not known. However, the quantity of neurotransmitter released is proportional to the concentration of Ca^{2+} flowing into the neuron. The released acetylcholine is rapidly metabolized by the action of the enzyme acetylcholinesterase.

The arrival of the neurotransmitter at the postsynaptic receptor activates the relevant *transmitter-gated ion channel*, which changes the membrane's permeability to ions, which produces a change in the membrane potential.[5] However, transmitter-gated ion channels are not sensitive to changes in the membrane potential and so cannot transmit an action potential. Consequently, the opening of transmitter-gated channels must stimulate the opening of voltage-gated channels, which can transmit an action potential through the neuron in the manner previously described. If the neurotransmitter is not removed from the site of its action by blood circulation, it will continue to act until it is metabolized. This metabolism is usually rapid. However, some toxins prevent the metabolism of the neurotransmitter and, in effect, leave the action potential system switched on with potentially fatal results. Many drugs act by interfering with either the synthesis and/or action and/or metabolism of the neurotransmitter. For example, the body's own local anesthetic agents, the enkephalins, inhibit the release of substance P, a neurotransmitter known to transmit pain signals across the synaptic cleft. It is believed that when a pain-transmitting neuron is activated, adjacent neurons release enkephalins, which inhibit the action of substance P. Other drugs act by replacing the neurotransmitter.

Some neurotransmitters such as glycine and GABA act as inhibitors by opening Cl^- ion channels and allowing Cl^- ions to flow into the neuron causing hyperpolarization. This makes the internal face of the membrane relatively more electronegative, and so the neuron will require a more intense depolarization if it is to transmit a signal. Ethanol is believed to act by inducing GABA receptors to open their Cl^- channels in the brain. This inhibits the excitability of the affected neurons and so reduces their ability to transmit nerve impulses. However, the ability of a neurotransmitter to either excite or inhibit a neuron appears to depend on the nature of its receptor rather than its structure.

Transmitter-gated channels are ion selective, and their receptor sites are highly selective toward a particular neurotransmitter.[5] The first to be characterized was the acetylcholine receptor. This is a glycoprotein that consists of five integral proteins. There are ~20,000 mm^2 of these receptors in the synapse sites of muscle cells. When two acetylcholine molecules bind to the receptor, they cause a change in conformation of the proteins, which opens the channel. The channel has a cluster of negatively charged amino acid residues at its entrance, which is thought to prevent the passage of negative ions. Its diameter is ~0.65 nm, and so it will allow the passage of positive ions such as sodium, potassium, and calcium. In muscle cells, sodium ions are the main contributors to the change in membrane potential (~30,000 ions/channel/msec). Calcium ions make a small contribution because their extracellular concentration is much lower than that of sodium ions, while in the case of potassium ions the leakage out almost balances the voltage gradient-driven inward movement of potassium ions.

MECHANISM OF ACTION

Experimental work has shown that the main site of local anesthetic action is on the cell membrane. They do not appear to have any appreciable effect on the intracellular fluid (*axoplasm*). Various theories have been put forward to explain the mechanism of the action.[10] Many of these postulate a prevention of conduction and formation of an action potential by either fully or partially blocking the sodium ion channels. Blocking is believed to be achieved either by the drug molecule causing a physical block in the channel, like a cork in a bottle, or by the drug molecule distorting the channel. If sufficient sodium channels are blocked, there would be no significant changes in membrane potential and so the conduction of an action potential along the neuron would be prevented. Blocking of conduction would automatically prevent the release of neurotransmitter at the presynaptic site. It is interesting to note that increasing the calcium ion concentration of the extracellular fluid may either enhance or reduce the activity of a local anesthetic by affecting the opening of sodium channels.

Shanes[11] suggested in 1958 that local anesthetics acted by increasing the surface pressure of the cell membrane, which would result in the closure of ion channels. In 1968, Metcalfe and Burgen[12] proposed that a block was caused, increasing the degree of disorder of the membrane, which would distort the ion channel. However, based on the work of Ritchie[13] in 1975, Hille[14] in 1980, Strichartz[15] in 1980, and Strichartz and Ritchie[13] in 1985, it is now believed that the main mechanism of local anesthetic action is associated with the blocking of sodium channels (Fig. 20-10). Strichartz[15] also showed in 1981 that the receptor for the blocking action appears to be about halfway down the sodium channel. Work by Schneider and Dubois in 1986 indicated that benzocaine blocks two different types of sodium channel. The work suggested that these channels have different affinities for the drug and so differing rates of inactivation. Other investigations in 1986 by Moczydlowski et al.[8] of the blocks imposed by local anesthetic agents indicated that there are at least two sites of action of local anesthetic agents and not one in the interior entrance of the channel, as previously proposed by Hille. Their work also supported the idea of a wide internal entryway into the channel but a constricted

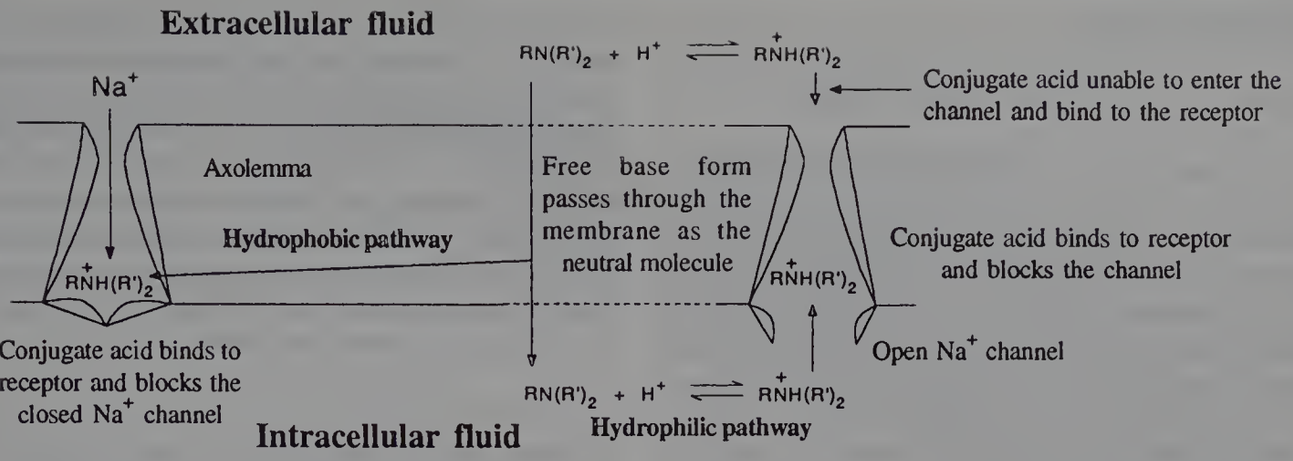


FIG. 20-10. *A representation of the mechanism of action of local anesthetics in blocking closed and open sodium channels.*

external entry. This internal entry was large enough to allow the passage of organic molecules, but the external entry was small enough to prevent the ingress of organic molecules with a single methyl group. It was, however, large enough to permit the entry of divalent cations such as Ca^{2+} and Co^{2+}. The observations made by Moczydlowski et al. were supported by the work of MacIver and Roth[16] in 1987 on a single isolated neuron (crayfish stretch receptor), which also suggested the existence of receptor sites that can discriminate between the structures of different anesthetics. These deductions were supported by those of Elliot et al.[17] in 1987, who concluded, from their investigation of the inhibition of sodium current in giant squid axon by benzocaine, that there were at least two sites for the action of the drug.

It is an important feature of the local anesthetics in clinical use that their structures include tertiary amine groups that coexist in equilibrium with the conjugate acid at physiological pH:

$$\underset{\text{Local anesthetic (neutral molecule)}}{RN(R')_2} + H^+ \rightleftharpoons \underset{\text{Conjugate acid}}{RNH(R')_2}$$

Experimental studies carried out by Narahashi and Frazier[18] in 1971 and Strichartz and Ritchie[13] in 1985 have indicated that the site of action of local anesthetics is only accessible from the interior of the neuron. Consequently, as neutral molecules cross membranes more easily than charged molecules the drug has to cross the membrane in its uncharged form before it can enter and block the ion channel. Once inside the neuron, experimental evidence suggests that the action of the drug is mainly due to its charged form and its binding to the receptor is voltage dependent (Fig. 20-9)[18,19]

Analysis of the work of Strichartz, Hille, and Ritchie has shown that the block caused by many local anesthetic agents is dependent on the number of channels open; the greater the number open, the greater the block. This suggests that the activity of the local anesthetic agent is dependent on it entering the channel from inside the neuron (''the hydrophilic pathway''). However, blocks can arise even if the channel is not open. This is explained by the local anesthetic agent entering the channel directly from the membrane (''the hydrophobic pathway''). The relative effects of these two pathways appears to depend on the lipid solubility of the drug, but both contribute to the blocking effect.

Local anesthetic agents are removed from the site of application by the blood flowing through the tissues and membranes in the area of application. The drugs are metabolized by a variety of routes; ester type agents are partly eliminated by hydrolysis catalyzed by plasma esterases, while amide-based local anesthetics are extensively metabolized usually through N-dealkylation and hydrolysis. Consequently, the use of amide local anesthetic agents in patients with severe liver damage should be avoided, as any toxic effects of the agent will be increased.

The delivery of local anesthetic agents to the liver for metabolism appears to be related to their degree of binding to plasma proteins. Experimental work by Tucker et al. in 1970 showed that amide-based local anesthetic agents bind more readily to plasma and tissue proteins than do ester-based agents. The binding of amide-based agents often involves the anesthetic binding to α_1-acid glycoprotein. This binding is usually significant, ranging from 55% to 95% of the drug. However, many factors will influence the concentration of plasma proteins; for example, cancer, smoking, and trauma will decrease the concentration of plasma proteins, while oral contraceptives will increase their concentration. Plasma protein concentration may also be altered in many diseases. Obviously, these changes will influence the quantity and rate of delivery of the local anesthetic to the liver, with subsequent changes in the systemic toxicity of the drug.

The elimination of local anesthetics and their metabolites from the liver is slow since it depends on hepatic blood flow. If this flow is reduced, it can result in an increase in concentration of agents and their metabolites in the body when large doses are administered over long periods of time. This build-up may result in an increase in the systemic toxicity of the local anesthetic agent.[20]

ADMINISTRATION

TOPICAL OR SURFACE ANESTHESIA

Direct application to the skin or a mucous membrane blocks the sensory nerve endings. The local anesthetic may be applied in the form of a liquid, spray, cream, ointment, or gel. It appears that the form used is often selected on a subjective basis. For example, in the use of local anesthetic agents as premedication in gastrointestinal endoscopy, sprays were preferred by the patients even though the degree of anesthesia was the same for sprays and gargles.[10,21]

Anesthesia of the mucous membranes of the ear, nose, and throat is usually brought about by using aqueous solutions of the salts of tetracaine, lignocaine, or cocaine. The vasoconstrictor effect of cocaine reduces bleeding in surgical procedures. However, all local anesthetics are rapidly absorbed through mucous membranes, and so their use may be accompanied by an increased risk of toxic systemic reactions. As a result, dosage must be carefully controlled.

INFILTRATION ANESTHESIA

A set dose of the local anesthetic in a suitable solvent system is injected directly into the area of the body that is to be anesthetized. These areas range from the skin to deeper tissues. The most frequently used local anesthetics for infiltration are lignocaine, bupivacaine, and procaine.

The technique produces a good degree of anesthesia in a localized area without disrupting general bodily functions. However, the use of this technique may require large concentrations of anesthetic to bring about the desired degree of anesthesia with an attendant increase in the risk of toxic systemic reactions.

FIELD BLOCK ANESTHESIA

A solution of the local anesthetic is injected subcutaneously at a point adjacent to the area that is to be anesthetized so that it blocks the nerve transmissions to that region. Field block anesthesia is brought about by the same drugs used for infiltration anesthesia. However, the technique produces a larger region of anesthesia with a lower dose of the local anesthetic than is required by the infiltration technique.

REGIONAL NERVE BLOCK ANESTHESIA

This is usually brought about either by injection of the anesthetic in a suitable solvent system into the nerve, or infiltration of the anesthetic into the tissue surrounding a nerve or plexus supplying the region to be anesthetized. For example, spinal anesthesia may be brought about by injection into the cerebrospinal fluid in the subarachnoid space. Dental anesthesia is brought about by flooding the area around the nerve by injecting the anesthetic into the adjacent tissue. The local anesthetic agent used for a nerve block depends on which nerve is to be blocked, the length of time the anesthesia is required, and the medical condition and physique of the patient. Duration of action is usually prolonged by the use of vasodilators rather than by increasing the dose. This approach reduces the chances of the drug spreading to regions that do not require anesthesia.

INTRAVENOUS REGIONAL ANESTHESIA

This is used to anesthetize a large region such as a limb. The anesthetic is injected into a suitable vein in a limb that has had its blood flow restricted by a tourniquet. The efficiency and safety of the technique are dependent on preventing arterial flow for the duration of the anesthesia. Lignocaine is frequently used to produce intravenous regional anesthesia, but bupivacaine is not approved for this purpose because of its long duration of action.

SPINAL ANESTHESIA

This is carried out by injecting the anesthetic agent into the subarachnoid space in the spinal cord. The anesthetic acts mainly on the nerve fibers and blocks the pain regions of the body served by the sections of the spinal cord affected.

EPIDURAL ANESTHESIA

The drug is injected into the epidural space between the vertebrae and spinal cord. This numbs the nerves leading to the uterus and the pelvic area, and leads to pain relief during labor. Epidural anesthesia may sometimes cause headaches.

FACTORS INFLUENCING THE EFFECTIVENESS OF THE ANESTHETIC ACTION

SUSCEPTIBILITY OF THE NEURON TO ANESTHESIA

Pain information is carried by the largely unmyelinated C-fibers, whereas sharp pain is transmitted by myelinated Aδ-fibers. The sensitivity of nerve fibers to local anesthetic appears to vary according to the length, anatomical type, and degree of conductance of the nerve fiber. In general, the

order of onset of local anesthesia with increasing concentration of agent is often as follows:

small nonmyelinated fibers > small myelinated fibers > large fibers

However, this order is not strictly followed in practice. Some myelinated fibers are blocked with lower concentrations of local anesthetics than some nonmyelinated fibers, while large fibers are often blocked before smaller fibers. Furthermore, in experimental work, it is the outer fibers in the nerve that are affected first regardless of their nature.

Experimental work by Franz and Perry[22] in 1974, supported by the work of Chiu and Ritchie[23] in 1984, suggests that the differential blocking of nerve fibers depends on the length of the axon that has to be exposed to the local anesthetic to bring about anesthesia. Shorter nerve fibers have shorter internodal distances and in the early stages of anesthesia are more fully exposed to the local anesthetic, with the result that they are more readily blocked than longer fibers. In most patients, the sensation of pain is the first to be lost, followed by temperature and touch.

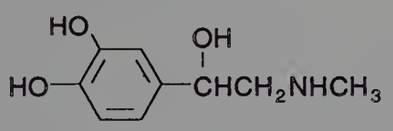
*p*H OF THE EXTRACELLULAR AND INTRACELLULAR FLUIDS

Local anesthetics are normally weak bases (pK_a c.a. 8.5), which are only slightly soluble in water. Consequently, they are usually marketed as aqueous solutions of their more soluble salts. These solutions are often quite acidic, which makes them less prone to bacterial and fungal contamination. However, an aqueous solution of the salt of a local anesthetic will normally contain between 2% and 15% of the free base in equilibrium with the salt.

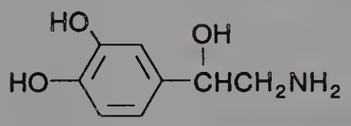
Although the drug is mainly transferred through the cell membrane in its free base form, administration of the drug in alkaline solution does not enhance its activity. This is because the structure of the drug is controlled by the *p*H of the extracellular fluid and not the *p*H of the dosage form. Once inside the neuron, equilibrium is reestablished. Both the free base and its protonated form are known to be active, but it is not known whether they bind to the same receptor site. However, it does appear that the protonated base plays the major part in anesthetic activity.[12,24,25]

VASOCONSTRICTORS

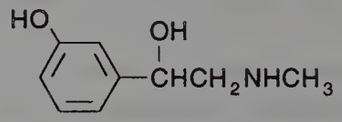
The anesthetic action of local anesthetics is proportional to the time that the agent is in contact with nerve tissue. As early as 1903, Braun discovered that the addition of adrenaline to solutions of local anesthetics increased and prolonged their action. It is now accepted that the addition of vasoconstrictors, such as adrenaline, to local anesthetic solutions prolongs and intensifies their action. The agent is confined to its site of action by reducing the rate at which the blood carries it away. Vasoconstrictors, such as adrenaline, also reduce the rate of absorption of a drug by allowing the metabolic rate of the local anesthetic to keep pace with the rate at which it is absorbed into the blood stream. This also reduces systemic toxicity. However, prolonged use of a vasoconstrictor on major arteries may cause irreversible tissue damage and can lead to gangrene.



Adrenaline (Epinephrine)



Noradrenaline (Norepinephrine)



Phenylephrine

Felypressin

The main vasoconstrictors in current use with local anesthetics are adrenaline (epinephrine), noradrenaline (norepinephrine), and felypressin. Solutions of local anesthetics often contain adrenaline, or a synthetic analogue such as phenylephrine. The effect of vasoconstrictors depends on the local anesthetic agent used; for example, adrenaline significantly prolongs the action of lignocaine but has less of an effect with prilocaine. The concentrations of vasoconstrictors are kept as low as possible in order to reduce the possibility of unwanted side effects such as chest pains, palpitations, and increased heart rate. In this context, local anesthetic preparations containing vasoconstrictors should never be used on digits. Additionally, preparations containing adrenaline should not normally be used on patients suffering from diseases, including poorly controlled diabetes, cardiovascular disease, and thyrotoxicosis. It is interesting to note that cocaine is a vasoconstrictor and so probably owes some of its effectiveness to this property.

NEURON STIMULATION

The effectiveness of the blocking action of a given concentration of a local anesthetic agent depends on the frequency and extent to which a neuron has been recently stimulated. The greater the frequency of this stimulation, the more effective the local anesthetic agent is in blocking a response.[14,26]

RATE OF ONSET AND DURATION OF ANESTHESIA

The time for the onset of action appears to be related to the type of tissue being anesthetized, the method of administration, and the percentage of the local anesthetic agent in its unprotonated form at physiological *p*H. Since the degree of protonation is indicated by the pK_a value of the drug, local anesthetic agents with a low pK_a value and a high lipid solubility usually have a more rapid onset of action than those with higher pK_a values and lower lipid solubility. For example, lignocaine, which is ~35% unprotonated at *p*H 7.4, usually has a more rapid onset of action than bupivacaine, which is ~8% unprotonated at this *p*H. The time taken for the drug to diffuse from its site of application to its site of action will also affect the rate of onset of anesthesia.[10]

It has been reported[27,28] that the time taken for the onset of anesthesia can be reduced by the use of the hydrogen carbonate form of the drug. This does not increase the toxicity of the local anesthetic agent but has been reported to reduce the pain associated with injection and improve the effectiveness of the block in some cases.

The duration of action appears to be related to the lipid solubility of the local anesthetic agent and its ability to bind to protein. As a general rule, the more lipid soluble the drug, the longer the duration of its action. However, it is difficult to classify local anesthetics in terms of the duration of anesthesia, because, although the period of action depends on the dose, the relationship between dose and duration of anesthesia is not clear. In most cases, increasing the dose increases the duration of the anesthesia, but the relationship is not linear. For example, doubling a dose will not necessarily double the time of action.

The dose used clinically is usually determined by factors such as systemic toxicity, potency, and the time for which the anesthesia is required. When long periods of anesthesia are required, it is better to repeat applications rather than use large doses. This keeps dose levels to a minimum, which reduces the level of any possible systemic toxicity.

SECONDARY PHARMACOLOGICAL ACTION

Local anesthetics do not rely on blood circulation to reach their site of action, as they are usually administered at or close to their site of action. Systemic side effects arise because the local anesthetic agent is carried away in the blood before it can be fully metabolized. Consequently, the chemical and pharmacological properties of local anesthetics are of major importance not only in determining the effectiveness of the drug but also its systemic side effects.

Local anesthetic agents can affect the function of any organs in which electrical impulse transmission occurs. The nature and extent of these unwanted side effects will depend on the drug used, the concentration of the drug in circulation, the site of application, and the technique used. The secondary effects of local anesthetic agents in these situations are discussed in this section.

CARDIOVASCULAR SYSTEM

Local anesthetic agents usually affect the cardiovascular system by decreasing the force of contraction, electrical excitability, and conduction of the myocardium. However, a high systemic concentration of local anesthetic is usually necessary before any of these effects are observed. Occasionally, low concentrations administered by infiltration will cause cardiovascular collapse and death. The reason for cardiovascular collapse is not known;[29] however, it appears that local anesthetic agents may act as antiarrhythmic agents by blocking the Na^+, K^+, and Ca^{2+} channels responsible for the excitation of heart muscle. For example, many workers believe that lignocaine may reduce the possibility of Na^+ channels opening during depolarization. However, recovery from this type of block is usually rapid.

CNS

All amide-based local anesthetics can stimulate the CNS, causing symptoms ranging from restlessness to clonic convulsions. Stimulation may be followed by depression of the CNS and death, usually from respiratory failure. These unwanted side effects appear to be related to the potency of the anesthetic. It is therefore possible to predict these side effects from a knowledge of the drug and its concentration in the bloodstream. Unfortunately, convulsions can occur with little or no warning, but these can be prevented or stopped by the use of sedatives, such as diazepam or barbiturates, although near-anesthetic doses of the latter are required.

Other types of local anesthetic can stimulate the CNS system but often lead to drowsiness. However, individual compounds may cause other unwanted side effects. For example, at blood concentrations of 5 $\mu g\ cm^{-3}$, lignocaine may produce muscle twitching, dysphoria, and euphoria. Both lignocaine and procaine can produce symptoms of sedation followed by unconsciousness. Cocaine, in common with some other local anesthetic agents, has an effect on mood and behavior.

BLOOD

Amethocaine (tetracaine), benzocaine, lignocaine, and prilocaine have been reported to induce methemoglobinemia. This is a condition in which the level of methemoglobin in erythrocytes is greater than the normal 1% to 2%. Methemoglobin is hemoglobin that contains iron III instead of iron II and so is incapable of transporting oxygen. Concentrations of ~15% result in the appearance of cyanosis in which the lips take on a purple-blue coloration. High concentrations are rare, but concentrations above 70% are associated with a high mortality rate. It has been suggested that the condition may be due to the presence of either an aromatic amine in the local anesthetic or its metabolism to an aniline type structure.[30–32]

WOUND HEALING

Local anesthetics may interfere with wound healing. This is particularly important in surgery carried out on the hands and feet.

HYPERSENSITIVITY

Hypersensitivity to local anesthetics appears to be related to both chemical structure and the method of administration. Allergic reactions occur most frequently with ester-based local anesthetic agents (benzoic acid derivatives). Adverse effects include allergic dermatitis, asthmatic attack, or, in extreme cases, death due to anaphylactic shock. Individuals suffering a hypersensitive reaction from one local anesthetic agent are often sensitive to compounds with a similar structure. For example, patients sensitive to procaine are often also sensitive to amethocaine.

Amide-based local anesthetic agents do not usually produce hypersensitivity reactions. However, they may be responsible for other unwanted effects and have been implicated in malignant hyperpyrexia. Families with a history of this disease should only be treated with ester-based local anesthetics.

Patch testing frequenlty provides adequate warning of hypersensitivity. However, when Ruzicka et al. (1987) conducted allergy tests on 104 patients who were known to have had an allergic reaction to benzocaine and procaine, the results showed that prick testing did not indicate an allergic reaction and intracutaneous testing rarely gave a hypersensitive reaction. They concluded that contact allergic patients could be injected with local anesthetic agents without a significant risk of a reaction.

Hypotension caused by local anesthetic agents is often unrelated to the type of drug used and can be prevented by premedication with a suitable sedative.[33]

STRUCTURE-ACTION

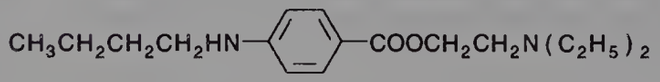
A large number of compounds will produce a conduction block. However, *most* of the local anesthetic agents in general use may conveniently be classified into four basic types, namely, those that act by hypothermia, alkaloids, derivatives of benzoic acid and aniline, and miscellaneous compounds. The benzoic acid and aniline group contains most of the local anesthetic agents currently in clinical use.

It is not possible to relate the chemical structures of local anesthetics to their activity because little is known about the structures of the receptors. However, it is possible to pick out similar structural features among some of the active compounds in common use.

AGENTS WITH HYPOTHERMIC ACTION

Local anesthetics that act by reducing the temperature of the area being anesthetized are largely of historical interest. Most of the chemical agents used produced an intense cold through rapid evaporation and hence an anesthetic action. One of the most effective was ethyl chloride, which is still in use today as a topical local anesthetic. However, this agent should not be used on mucous membranes or broken skin, and prolonged use may cause chemical frostbite.

Procaine

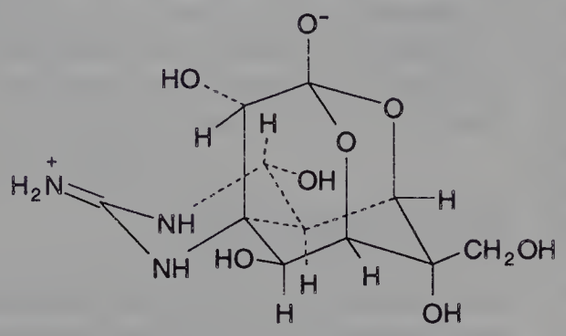


Amethocaine

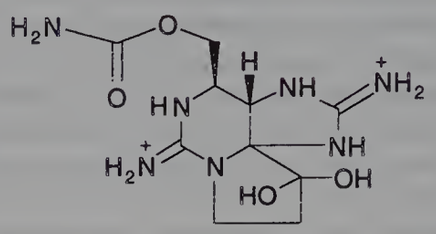
ALKALOIDS[34]

These compounds are obtained from plants and trees. The only one in general clinical use is cocaine, which, because of its addictive properties, is largely restricted to use with

the ear, nose, and throat. However, cocaine is of considerable interest because of the active agents that were developed from its structure.



Tetrodotoxin



Saxitoxin

Tetrodotoxin and saxitoxin are naturally occurring local anesthetic agents but are too toxic to be of clinical use. Tetrodotoxin is found in the tissue and organs of fish of the order of *Tetraodontiformis*, and saxitoxin is isolated from some marine dinoflagellates. These compounds, which are highly toxic to humans, are structurally different but appear to have the same mechanism of action. They are thought to block the external opening of the Na^+ channel. Interest in these compounds centers on the fact that they could lead to the development of new local anesthetic agents, on their use as tools in neurochemical research work, and in investigating the molecular nature of action potentials and sodium channels.[10]

BENZOIC ACID AND ANILINE DERIVATIVES WITH LOCAL ANESTHETIC ACTIVITY

Most of the local anesthetic agents in current medical use are of this type. The benzoic acid derivatives are esters developed from cocaine, whereas the aniline derivatives are amides developed from isogramine. Both types of derivative have chemical structures that normally have the following general format:

Both the ester and N-substituted amide functional groups are bioisosteres (Fig. 20-11), which explains the occurrence of these groups in similar positions in the structures of local anesthetics.

The lipophilic center is usually either a carbocyclic or heterocyclic ring system, whereas the hydrophilic center is normally a secondary or tertiary amine, which may or may not be cyclic. Tertiary amines are more useful since they are less irritating to tissue. The hydrophilic center may be attached to the ester or amide by either a short hydrocarbon chain, or oxygen, nitrogen, or sulphur atoms. However, most of the local anesthetic agents in common use utilize a short hydrocarbon chain. The lipophilic center is believed to be largely responsible for the lipid solubility of the local anesthetic agent. Lipid solubility plays an important part in the action of local anesthetics since it is well established that their action depends on their ability to penetrate the cell membrane of the axon. The hydrophilic center provides the local anesthetic agent with some of its water solubility. This is an essential factor in transporting the drug to the membrane and, once inside the cell, to the receptor. The hydrophilic center is also believed to be involved in the binding of the drug to the receptor.

The best local anesthetic action is obtained when the lipophilic and hydrophilic centers are in balance. If the hydrophilic center is the dominant structure, the anesthetic action of the drug is weak since its membrane penetration is poor. Similarly, if the lipophilic center is the dominant structure, local anesthetic action will again be poor. In this case, the agent can penetrate the lipid membrane of the axon, but its solubility in both the extracellular and intracellular fluids is poor.

The pK_a values of local anesthetic agents have been used as a measure of the degree of its ionization and hence as a measure of the lipophilic/hydrophilic ratio. In general, clinically useful local anesthetics have pK_a values in the range 7.5 to 9.5. This implies that compounds with pK_a values below 8.0 are not sufficiently ionized at physiological *p*H to be effective in bringing about anesthesia even though they can penetrate the axon. In contrast, drugs with pK_a values above 9.5 are almost fully ionized at physiological

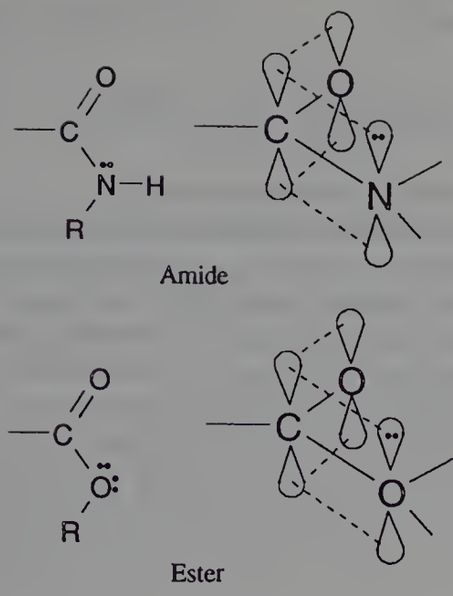


FIG. 20-11. Ester and amide functional groups are bioisosteres. Bioisosteres are structures that have similar sizes, shapes,and electronic structures.

*p*H. Consequently, these drugs are less effective because they have difficulty in penetrating the cell membrane.

The partition coefficients of structurally similar local anesthetics have been used as a measure of their relative activity. In vivo experiments have shown that, with a series of related structures, an increase in activity corresponds to an increase in partition coefficient until a maximum activity is reached. After this point is reached, the activity decreases even though the partition coefficient increases. Unfortunately, the increase in activity is often accompanied by an increase in toxicity.

A study[35] of the homologous series formed by substituting the aryl ring of local anesthetics by alkyl, alkyloxy, and alkylamino groups showed that the partition coefficients of the members of a series increased with an increase in the number of methylene groups in the substituent of the series. It was observed that, in general, the maximum activity in a series was achieved for the C_4 to C_6 homologues. Similarly, substitution of the hydrophilic center showed that the partition coefficient increased with the number of carbon atoms, which was also accompanied by an increase in activity. The use of piperidino and pyrrolidino groups as hydrophilic groups gave products with a similar degree of activity to that obtained with a diethylamino group. However, the morpholino group gave products with a lower activity.[36]

Local anesthetics are believed to bind to plasma and tissue proteins by van der Waals' forces, dipole-dipole attractions, and electrostatic forces (Fig. 20-12). Local anesthetic activity of benzoic acid–based drugs improves if the aryl lipophilic center has electron donor substituents, but decreases with electron acceptor substituents. Therefore, it is likely that electron donor substituents increase the binding of the local anesthetic agent to the receptor, but the electron acceptors substituents reduce this binding. Buchi and Perlia suggested that this latter was a consequence of an electron acceptor withdrawing electrons from the carbon of the carbonyl group, which decreases the polarization of this group. This reduces the strength of the carbonyl group's dipole, which consequently weakens its dipole-dipole attraction with the receptor.

Compounds whose structures contain amide functional groups tend to bind more strongly. For example. Tucker and co-workers, in 1970, reported that 95% of bupivacaine bound to plasma and tissue proteins compared to 55% for prilocaine. Tucker and Mather[20] in 1975 also showed that the more potent and longer acting agents are more extensively bound to plasma proteins. However, this is not the only factor affecting potency. For example, amide bonds are more resistant to hydrolysis, which will also affect the duration of action.

MISCELLANEOUS AGENTS

A wide variety of compounds other than those discussed in the preceding sections exhibit some local anesthetic activity.

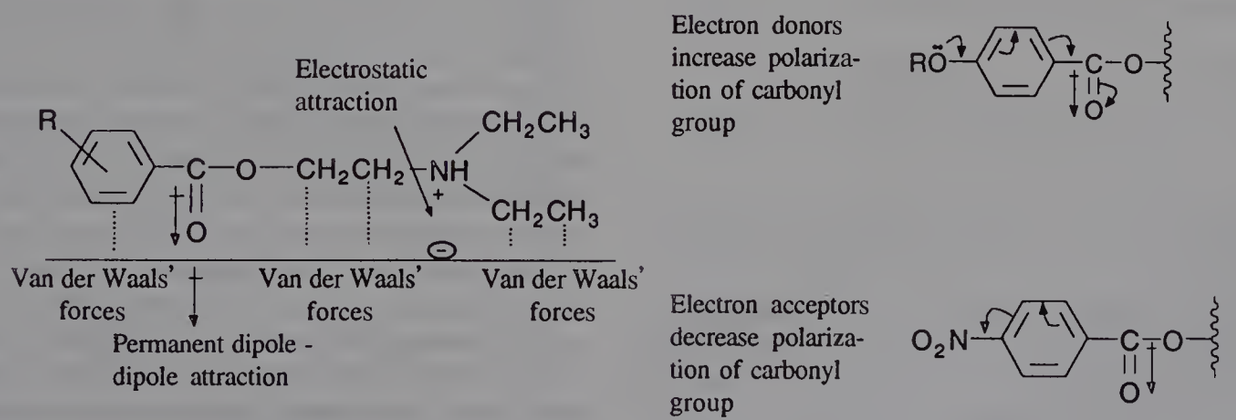


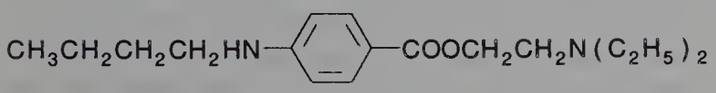
FIG. 20-12. A schematic representation of the binding of ester-type local anesthetic agent to a receptor site (Buchi and Perlia, 1960).

These include benzyl alcohol, phenol, and some antihistamines.

LEXICON OF LOCAL ANESTHETICS

A large number of synthetic local anesthetics has been produced. This discussion is mainly limited to those mentioned in the USP and BP. Many agents are used in the form of their salts, as these are more soluble in water and so easier to formulate. Many clinical formulations also contain a vasoconstrictor, which confines the action to the desired site and hence reduces systemic toxicity.

Amethocaine (Tetrocaine).

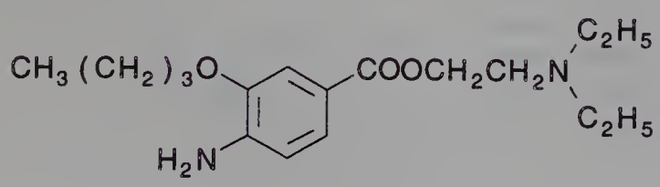


2-Dimethylaminoethyl 4-butylaminobenzoate (Amethocaine).

Amethocaine is a white or yellow waxy solid. It is supplied as its mono hydrochloride (Tetracaine hydrochloride), which is a white, slightly hydroscopic, slightly bitter, crystalline solid. Other crystalline forms are known. The hydrochloride is freely soluble in water (pK_a 8.5) and ethanol, sparingly soluble in chloroform, and insoluble in diethyl ether and benzene. It has a high lipid solubility. Aqueous solutions may be sterilized by boiling for a short period of time. Amethocaine is unstable with alkalies.

The anesthetic strength of amethocaine is about a quarter of that of cocaine. The onset of action is slow but is of long duration. It is hydrolyzed by plasma esterases to 4-aminobenzoic acid and other metabolites. The high toxicity of amethocaine has resulted in it being used mainly for topical applications such as ENT surgery, bronchoscopy, and local treatment of hemorrhoids. Amethocaine is also used for spinal anesthesia.

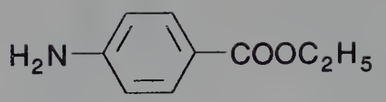
Benoxinate, USP.



2-Diethylaminoethyl 4-amino-3-butoxybenzoate (Benoxinate).

The drug is a liquid supplied as its white crystalline hydrochloride with a salty taste. Benoxinate hydrochloride is very soluble in water (*p*H 4.5 to 5.2), soluble in chloroform and ethanol, and almost insoluble in diethyl ether. The hydrochloride is stable to air, heat, and light. Benoxinate hydrochloride is used mainly in ophthalmology because there is no significant absorption into the eye.

Benzocaine.

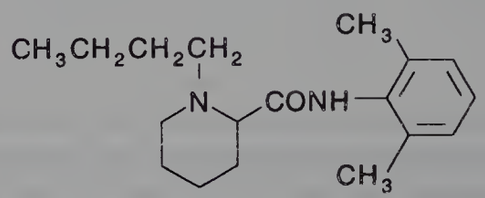


Ethyl 4-aminobenzoate (Benzocaine).

Benzocaine exists as odorless, white rhombohedric crystals. It is sparingly soluble in water (pK_a 2.5), very soluble in chloroform, ethanol, diethyl ether, and lipids. The drug is also soluble in many solutions of dilute aqueous acids as the appropriate salt. Benzocaine is sensitive to light and prolonged exposure to temperatures above 30°.

The drug has a low potency and a low systemic toxicity. It is used as a topical local anesthetic in combination with other agents, although these mixtures can give rise to allergic reactions. Benzocaine is also a possible sulphonamide antagonist.

Bupivacaine, USP.



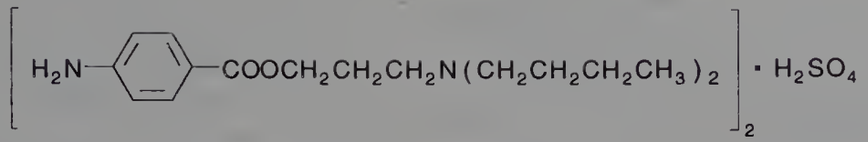
(RS)-1-Butyl-N-(2,6-dimethylphenyl)-2-piperidinecarboxamide (Bupivacaine)

Bupivacaine (Anekain, Marcaine) is sparingly soluble in water (*p*K_a 8.1), but its lipid solubility is good. Bupivacaine is used as its monohydrochloride (Marcaina, Carbostesin), which is a white, odorless, crystalline powder, soluble in water (1% *p*H 4.5 to 6.0) and ethanol but only slightly soluble in acetone, diethyl ether, and chloroform. Solutions may be sterilized by autoclaving but should be protected from light.

Bupivacaine hydrochloride has been resolved into its enantiomers {(+)-Bupivacaine hydrochloride; [α] + 12.5° and (−)-Bupivacaine hydrochloride; [α] −12.3°} by Tullar[37] in 1971, who showed that the longer acting (−) isomer had the S configuration.

The drug is used in solution with or without adrenaline. Its plasma protein binding is good (95%), and its anesthetic action is about a quarter of the strength of cocaine. Bupivacaine hydrochloride has a similar action to lignocaine but is longer acting and more toxic. Onset of action is slow, but the duration ranges from 4 to 10 hr depending on the type of nerve blocked. Bupivacaine hydrochloride is used mainly for infiltration and regional nerve blocks. It has a minimal motor block, which makes it particularly suitable for certain types of surgery. Bupivacaine hydrochloride is not recommended for intravenous regional anesthesia, as prolonged blocks have been reported. Strong solutions should not be used in obstetrical anesthesia. It is metabolized in the liver, and its metabolites are mainly excreted in the urine. Very little free drug is excreted.

Butacaine Sulphate, USP.

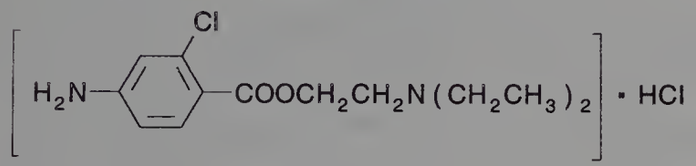


3-(Dibutylamino)-1-propanyl 4-aminobenzoate sulphate (Butacaine sulphate).

Butacaine is a liquid that is normally used as its white crystalline sulphate obtained by crystallization from propanol. Butacaine sulphate is very soluble in water and quite soluble in acetone and warm ethanol. It is slightly soluble in chloroform but almost insoluble in diethyl ether. Aqueous solutions can be sterilized by boiling, but the sulphate should be protected from light. The free base is liberated from aqueous solutions as an insoluble oily liquid when the solution is made alkaline. Butacaine sulphate has been used as a topical local anesthetic in dentistry, and also as a constituent of ear and nose drops.

Cinchocaine. See Dibucaine hydrochloride, USP.

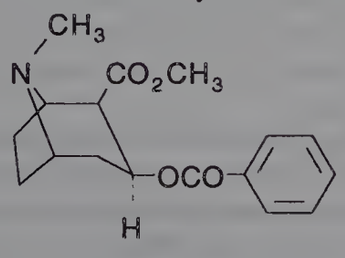
Chloroprocaine Hydrochloride, USP.



2-(Diethylamino)ethyl 4-amino-2-chlorobenzoate hydrochloride (Chloroprocaine hydrochloride)

Chloroprocaine hydrochloride (Nesacaine) is supplied as bitter tasting crystals. It slowly dissolves in water (~1 g in 22 cm^3) and is sparingly soluble in 95% ethanol (1 g in 100 cm^3). Aqueous solutions are slightly acidic and slowly turn yellow. Chloroprocaine has a similar action to procaine but has a more rapid onset of action and is at least twice as potent. It is used for infiltration and nerve block anesthesia. Adrenaline may be used with chloroprocaine to reduce toxicity. Chloroprocaine is not a good topical anesthetic and should not be used for spinal anesthesia. The systemic toxicity of chloroprocaine is lower than procaine because it is metabolized more rapidly in the plasma by plasma cholinesterase. The drug has a half-life of ~23 sec in adults and is excreted in the urine, mainly in the form of its metabolites. However, there is a possibility of neurological toxicity.

Cocaine. Cocaine is a naturally occurring compound first isolated from the leaves of *Erythroxylon coca* Lam. It has now been isolated from other species of *Erythroxylon* and *Erythroxylaceae* and has been synthesized in the laboratory.



(1R,2R3S,5S)-2-Methoxycarbonyltropan-3-yl benzoate (Cocaine).

Cocaine is a white crystalline powder that is slightly volatile, subliming above 90° to give a noncrystalline sublimate. It is slightly soluble in water (~1 g in 600 cm^3, pK_a 5.59 at 15°) to give a solution alkaline to litmus. Cocaine is very soluble in ethanol, chloroform, diethyl ether, and lipids. The drug should be protected from light.

Cocaine is optically active (four pairs of enantiomers are theoretically possible, but only three pairs have been synthesized[34]), the naturally occurring form having a specific rotation of $-35°$ at 18° in 50% aqueous ethanol.

Cocaine was the first drug to be used as a local anesthetic. It also acts as a vasoconstrictor, which probably accounts for some of its effectiveness. However, it is a CNS stimulant, giving rise to a feeling of euphoria and because of this is subject to abuse. High doses can cause convulsions, and it is too toxic for general use, although it is still used in ear, nose, and throat surgery and in ophthalmology, although the latter use may result in damage to the cornea.

Cocaine blocks the uptake of catecholamines at adrenergic nerve endings. Consequently, the use of the drug with adrenaline should be avoided. Cocaine does not penetrate intact skin, but it is rapidly absorbed through mucous membranes. This absorption is increased in the presence of inflammation, which may result in increased systemic toxic effects. Cocaine is largely metabolized by hydrolysis catalyzed by plasma esterases (Fig. 20-13), the drug having a half-life of ~1 to 1.5 hr depending on the method of administration. Small quantities are also excreted in the urine, and in some animals hepatic enzymes are involved in its metabolism.

Cocaine is stable; a sample kept under dry conditions in a well-closed container may show no decomposition after 5 years. Aqueous solutions are stable below *p*H 4, but hydrolysis is rapid above *p*H 5.5.

Cocaine is a controlled drug and subject to the relevant drug abuse regulations of the country in which it is being used.[21]

Cocaine Monohydrochloride, USP. Cocaine monohydrochloride is a hygroscopic, colorless, or white crystalline powder. It is very soluble in water and ethanol, and soluble in chloroform and glycerol. It is almost insoluble in ether and fixed oils. Cocaine monohydrochloride has a pK_a of 8.9, while a 2.5% w/v aqueous solution has a specific rotation of $-79°$ to $-81°$. Aqueous solutions of the monohydrochloride are unstable when heated but can be sterilized by autoclaving at 98° to 100° for not more than 30 min.[33] Aqueous solutions are also unstable to alkalies, and the drug is precipitated by alkaloidal precipitates such as sodium borate, silver nitrate, and mercury salts. Cocaine hydrochloride has the

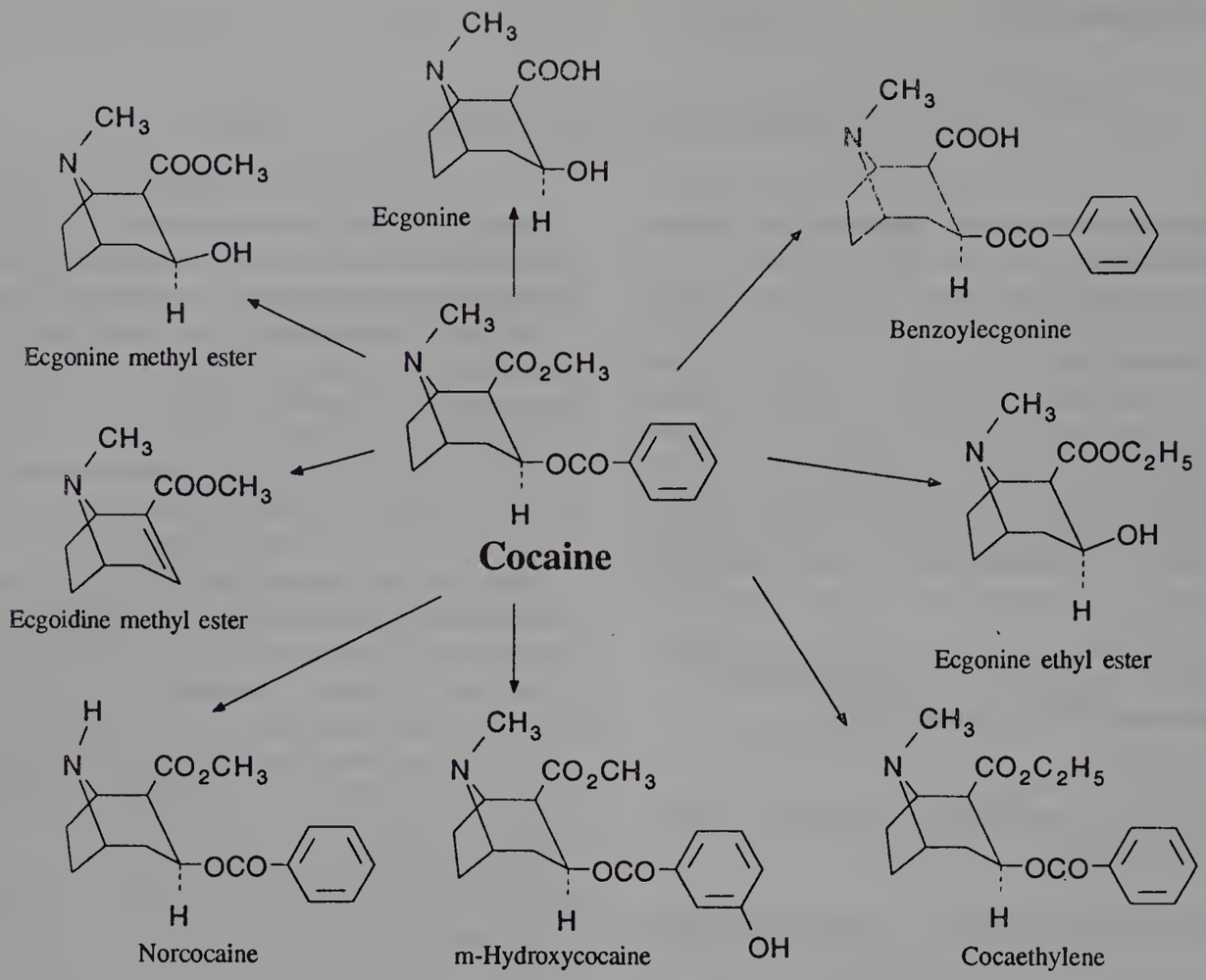
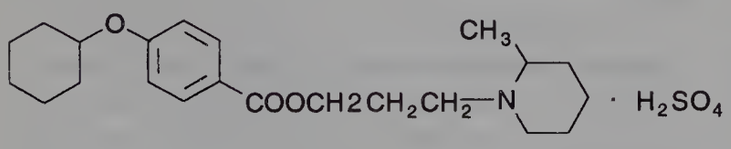


FIG. 20-13. *The metabolism of cocaine.*

same action as cocaine, and it is used for the administration of cocaine in aqueous solution. Its clinical uses are similar to those of the free base; however, it should never be administered by injection. The use of cocaine hydrochloride is subject to the same restrictions as cocaine as it is addictive and subject to abuse. Cocaine abuse can lead to renal failure, sexual dysfunction, and an increase in the risk of spontaneous abortion.

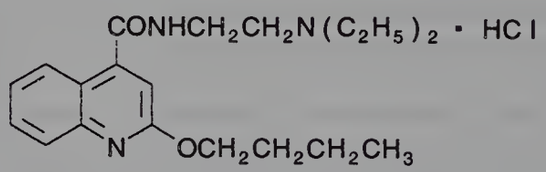
Cyclomethycaine sulphate, USP.



3-(2-Methyl-1-piperidinyl)propyl 4-cyclohexyloxybenzoate hydrogen sulphate (Cyclomethycaine sulphate)

Cyclomethycaine sulphate (Surfacaine) is a white crystalline powder that is sparingly soluble in water and ethanol. It is only slightly soluble in chloroform and mineral acids. It is used as a topical local anesthetic in the form of creams, ointment, gels, and suppositories.

Dibucaine Hydrochloride (Cinchocaine), USP.



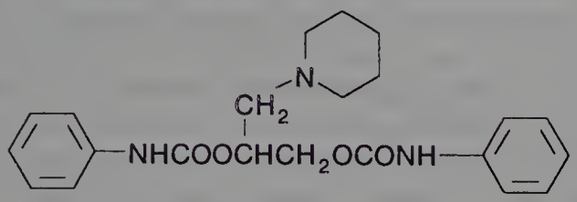
2-Butoxy-N-[2-(diethylamino) ethyl [-4-quinolinecarboxamide (Dibucaine hydrochloride)

Dibucaine hydrochloride (Nupercaine) is a colorless to off-white, hygroscopic, crystalline compound that has a faint characteristic odor and darkens on exposure to light. Dibucaine hydrochloride is very soluble in water (pK_a 8.15), ethanol, and acetone, soluble in chloroform and lipids, but insoluble in diethyl ether and other nonpolar solvents. Aqueous solutions of the drug may be sterilized by autoclaving, but the *p*H of the solution should not be above 6.2. Dibucaine hydrochloride should be protected from light and stored in air-tight containers.

Dibucaine hydrochloride has a strength of anesthetic action about a quarter that of cocaine. Onset of action is rapid and action is of long duration. The drug has an apparent half-life of 11 hr and is mainly metabolized in the liver. The amide

group of dibucaine hydrochloride is not hydrolyzed to any extent in serum, and the slow rate of metabolism is probably responsible for the high systemic toxicity of this drug.

Dibucaine hydrochloride is used as a topical local anesthetic. Its parenteral use is restricted to spinal anesthesia. The less-water-soluble free base is used as a topical anesthetic in the form of creams, ointments, and suppositories.



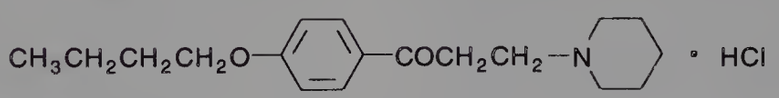
Diperodon Monohydrate, USP.

3-(1-Piperidinyl)-1,2-propandiol bis(phenylcarbamate) (Diperodon)

Diperodon monohydrate is a cream to white colored powder. It is almost insoluble in water but soluble in ethanol, diethyl ether, and chloroform. Diperodon is unstable to heat and is used as a topical local anesthetic.

Diperodon hydrochloride monochloride is a bitter tasting, white colored powder. It is slightly soluble in water, but this solubility may be increased by the addition of sodium chloride to the solution. Diperodon is slightly soluble in acetone and ethyl acetate but insoluble in diethylether. Traces of alkali will precipitate the free base, and so aqueous solutions should be used as soon as possible. However, aqueous solutions of the hydrochloride are stable to heat at *p*H 4.5 to 4.7 and so may be sterilized by autoclaving. The hydrochloride is used as a topical local anesthetic.

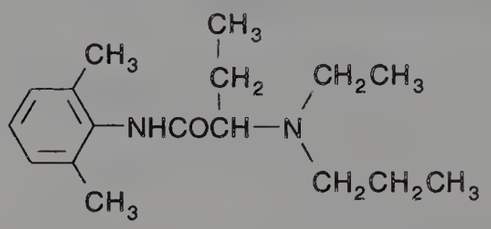
Dyclonine Monohydrochloride (Dyclocaine Hydrochloride), USP.



1-(Butoxyphenyl)-3-(1-piperidinyl)-1-propanone (Dyclonine monohydrochloride)

Dyclonine monohydrochloride (Dyclone, Tanaclone) is a white crystalline compound that may have a slight odor. It is soluble in water, chloroform, and ethyl alcohol. A 1% aqueous solution has a pH between 4 and 7. It is soluble in acetone but almost insoluble in hexane and diethyl ether. The drug is stable in acid solutions, but these should not be heated or sterilized by autoclaving. Dyclonine monohydrochloride should be stored in air-tight containers and protected from light. Dyclonine monohydrochloride is used for topical anesthesia of the skin and mucous membrane. Onset of action occurs in 5 to 10 min and usually results in an action that can last up to 1 hr. It is used in proprietary gargles, mouthwashes, lozenges, and sprays for treating sore throats and mouths. The drug should not be given by injection or used in the eyes as it is a tissue irritant.

Etidocaine, USP.

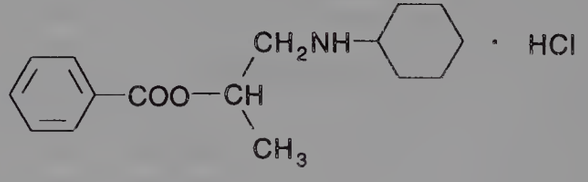


N-(2,6-Dimethylphenyl)-2-(ethylpropylamino) butanamide (Etidocaine).

This agent is soluble in water (pK_a 7) and has a high lipid solubility. It is usually used in the form of its hydrochloride.

Etidocaine monohydrochloride (Duranest) binds strongly to plasma protein (~94%) and has an action that is similar to that of bupivacaine and lignocaine. Onset of action is fast and action is of longer duration than that of lignocaine. The toxicity of etidocaine monohydrochloride is similar to that of bupivacaine except that it produces a stronger motor block. The primary site of the metabolism of etidocaine monohydrochloride is the liver, and its many metabolites are excreted in the urine.

The drug is usually used with adrenaline for infiltration, epidural, and peripheral nerve block anesthesia. It is not suitable for vaginal births because of the high degree of motor block and subsequent muscle relaxation caused by its use. Etidocaine monohydrochloride can cause cardiac arrest if plasma concentrations are too high.



Hexylcaine Hydrochloride, USP.

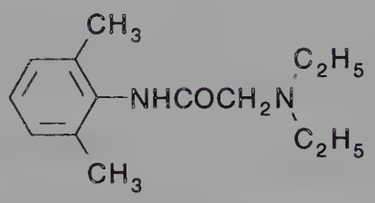
1-(Cyclohexylamino)-2-propanol benzoate hydrochloride (Hexylcaine hydrochloride)

Hexylcaine hydrochloride (Cyclaine) is formed as a crystalline solid from absolute ethanol. It is soluble in water, and a 1% aqueous solution (pH 4.4) may be sterilized by

autoclaving. The drug is also very soluble in ethanol and chloroform but insoluble in diethyl ether.

Hexylcaine hydrochloride has been used for topical anesthesia of mucous membranes. Both tissue irritation and necrosis have been reported after its topical use. It has also been used for spinal and nerve block anesthesia.

Lignocaine (Lidocaine), USP.



2(Diethylamino)-N-(2,6-dimethylphenyl)acetamide (Lignocaine)

Lignocaine (Lidocaine, Xylocaine) is obtained as white to slightly yellow crystals. It is almost insoluble in water but soluble in ethanol, benzene, diethyl ether, chloroform, and oils. The aqueous solubility is unusual in that it decreases with increase in temperature.[38] Aqueous solutions of lignocaine are resistant to hydrolysis under acid and basic conditions, but when hydrolysis occurs it yields 2,6-dimethylaminobenzene (2,6-xylidine) and diethylaminoethanoic acid.

The monohydrochloride monohydrate is very soluble in water (pK_a 7.9 at 25°) and ethanol and fairly soluble in chloroform. It is insoluble in diethyl ether. A 0.5% aqueous solution of lignocaine monohydrochloride has a *p*H of 4.0 to 5.5. Stability studies have shown that 2% aqueous solutions of lignocaine hydrochloride with a *p*H of 7.3 are relatively stable to heat. Aqueous solutions and gels may be sterilized by autoclaving. However, hydrolysis will occur more readily in acid solution. Anhydrous lignocaine monohydrochloride is hygroscopic.

Lignocaine is one of the most widely used local anesthetic agents, with a plasma protein binding of ~64% at therapeutic drug concentrations. The drug is able to penetrate the placenta, but fetal plasma binding is only about half that found in maternal plasma. Lignocaine is as powerful as cocaine and is usually administered in the form of its hydrochloride. Solutions of the drug are used for a variety of nerve blocks as well as for typical analgesia. For example, 15 to 50 cm^3 of a 1.5% solution is often used for epidural/caudal blocks, and 2% to 4% solution may be used for topical analgesia of the larynx. It may also be given intravenously and by infusion in accident cases. A single dose has a rapid onset of action, which lasts from 60 to 90 min. A toxic dose (~200 mg) results in stimulation followed by depression of the CNS and in some cases convulsions. Lignocaine depresses the cardiovascular system, and, because of this effect, it is also used intravenously for the management of cardiac arrhythmias. However, lignocaine should be used cautiously with patients with congestive heart failure, respiratory depression, and conduction disturbances.

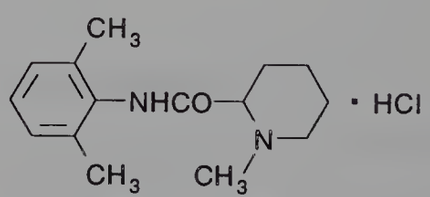
Common side effects are sleepiness and dizziness. Toxicity is reduced and activity prolonged when mixtures of lignocaine and adrenaline are administered. However, the drug may raise the *p*H of solutions above that required for the stability of additives such as adrenaline, noradrenaline, and isoprenaline, which results in their subsequent deterioration.

Lignocaine is mainly metabolized in the liver by a variety of pathways utilizing mixed function oxidases (Fig. 20-14). Both monoethylglycylxylidide and glycylxylidide exhibit both local anesthetic and toxic actions, especially as their half-lives are longer than that of lignocaine. About 75% of the glycylxylidide is metabolized to 4-hydroxy-2,6-dimethylaniline, which is excreted in the urine together with ~10% of the drug. The rate of clearance of lignocaine in the young, middle aged, and elderly is approximately the same.

Lignocaine is stable to light, the calculated shelf-life of a sample of the drug exposed to indirect window light being 8 years.[38] Liquid lignocaine preparations are chemically relatively stable provided certain additives are avoided. However, they may be physically unstable in that the drug is precipitated or the solution becomes cloudy. This is believed to be due to the presence of additives that either complex with the lignocaine or change the *p*H of the solution. Loss of drug potency may also occur because of the absorption of lignocaine onto the surface of the container, especially if it is plastic.

Lignocaine is used as a topical local anesthetic in creams, gels, ointments, aerosols, and solutions. The hydrochloride is used for infiltration, peripheral nerve block, and epidural anesthesia. It is also widely used in dentistry. Modification of the *p*H of lignocaine solutions by the use of sodium bicarbonate significantly reduces the discomfort produced when the local anesthetic is administered by infiltration. Intravenous administration has also been used to alleviate pain in chronic disorders.

Mepivacaine, USP.



N-(2,6-Dimethylphenyl)-1-methyl-2-piperidinecarboxamide monohydrochloride (Mepivacaine)

The hydrochloride is a white odorless crystalline compound. It is soluble in water and methanol but only slightly soluble in chloroform. It is almost insoluble in diethyl ether. A 2% aqueous solution has a *p*H of ~4.5. Mepivacaine has a pK_a of 7.69.

The free base may be obtained as crystals from diethyl ether. It is soluble in lipids. Mepivacaine was resolved into its isomers by Tullar,[37] who showed that the longer acting (+)-isomer had an S configuration.

Mepivacaine binds strongly to plasma proteins (78%) and has similar properties to lignocaine. It has about the same strength of anesthesia as cocaine. The onset of action of the

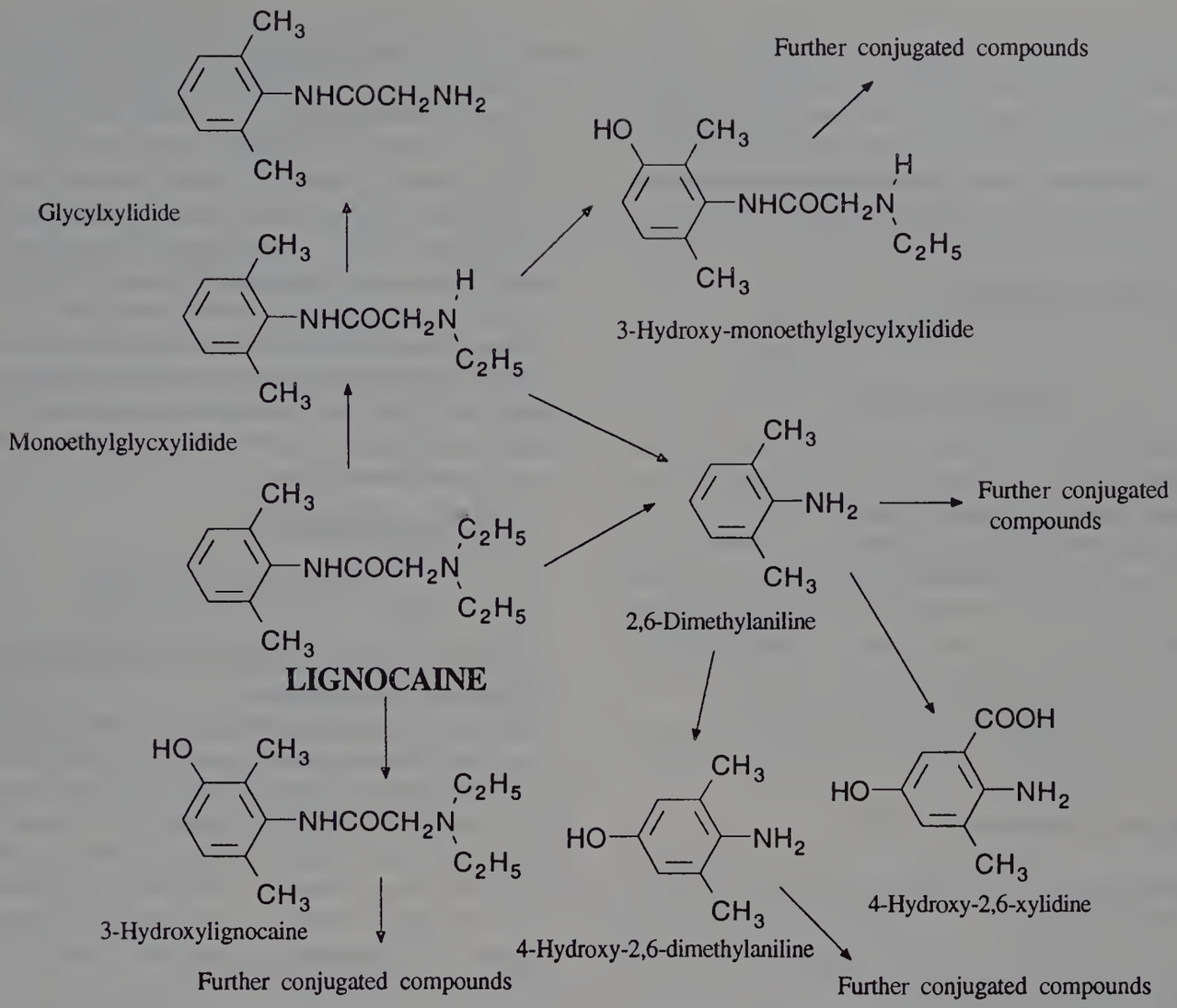


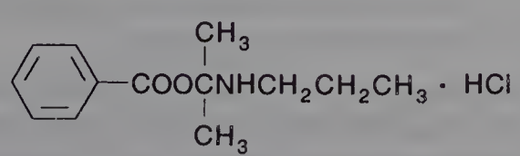
FIG. 20-14. *The metabolism of lignocaine.*

drug is slow, but the action lasts longer than lignocaine. Vasoconstrictors may be used to prolong action. However, mepivacaine is cumulative and so may be less safe to use than lignocaine. Mepivacaine is used in the form of its racemate, but earlier work by Aberg showed that the S (+) mepivacaine was less toxic than R (−) mepivacaine.

The drug is metabolized mainly in the liver, with <10% of the drug being excreted unchanged in the urine. Other metabolites are excreted via the kidney and the bile. Only small amounts of the latter metabolites are excreted in the feces.

Mepivacaine is used for infiltration, peripheral nerve block, and epidural anesthesia.

Meprylcaine HCl, USP.



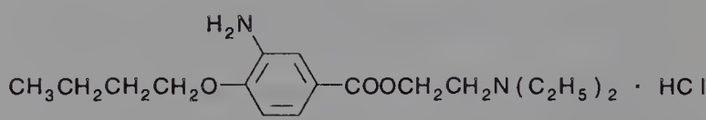
2-Methyl-2-propylamino-1-propyl benzoate monohydrochloride (Oricaine hydrochloride)

Meprylcaine hydrochloride is a white crystalline solid. It is very soluble in water, ethanol, and chloroform. Aqueous solutions are slightly acidic, a 2% solution having a pH of 5.7. The free base is almost insoluble in water but soluble in ethanol, diethyl ether, acetone, and lipids.

The drug is metabolized in the plasma by the plasma esterases.

Meprylcaine hydrochloride is used in dentistry for infiltration and nerve block anesthesia.

Oxybuprocaine Hydrochloride (Benoxinate Monohydrochloride), USP.



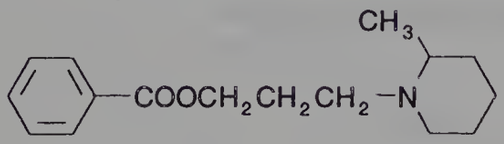
2-(Diethylamino)ethyl 4-amino-3-butoxybenzoate monohydrochloride (Oxybuprocaine hydrochloride)

Oxybuprocaine hydrochloride (Benoxinate, Dorsacaine, Conjuncain, Cebesine, Novesine, Benoxil, Lacrimin) is a white or slightly off-white crystalline solid. The hydrochloride is very soluble in water and chloroform, soluble in

ethanol, but insoluble in diethyl ether. Aqueous solutions are acidic with a *p*H of 4.5 to 5.2. The free base is a liquid.

Oxybuprocaine is used as a surface anesthetic in ophthalmology and the nose and throat. Moderate corneal swelling and fibrinous iritis have been noted in some patients.[39] However, oxybuprocaine is less irritant than amethocaine hydrochloride is when applied in similar concentrations to the conjunctiva of the eye.

Piperocaine Hydrochloride.

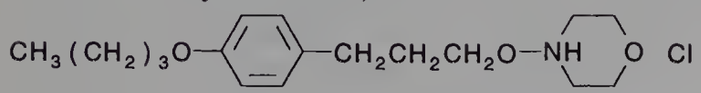


3-(2-Methylamino-1-piperidyl)propyl benzoate monohydrochloride (Piperocaine hydrochloride)

Piperocaine hydrochloride (Neothesin, Methycaine) is a bitter-tasting crystalline solid stable in air. It is very soluble in water (1 g in 1 cm^3), soluble in ethanol and chloroform, but insoluble in diethyl ether. Aqueous solutions may be sterilized by autoclaving. The free base is precipitated as an oil from aqueous solutions by alkalies and bases.

Piperocaine is used for surface, infiltration, and regional nerve block anesthesia.

Pramoxine Hydrochloride, USP.

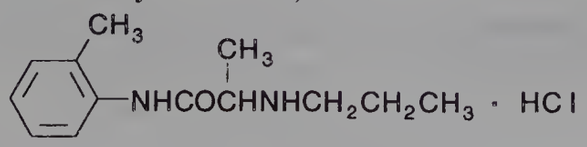


4-[3-(4-Butoxyphenoxy)propyl]morpholine monohydrochloride (Pramoxine hydrochloride)

Pramoxine hydrochloride (Proctofoam, Tronolane) is a white crystalline solid that may have a slight aromatic odor. It is soluble in water, with a 1% aqueous solution having a *p*H of ~4.5. Pramoxine hydrochloride is also soluble in chloroform but only slightly soluble in diethyl ether. The free base is a liquid.

Pramoxine hydrochloride is used as a topical anesthetic for the relief of insect bites, minor wounds, and hemorroids. Its use may initially be accompanied by a stinging and burning sensation. The drug should not be used for the eyes, nose, or throat.

Prilocaine Hydrochloride, USP.



N-(2-Methylphenyl)-2-(propylamino)-propanamide monohydrochloride (Prilocaine hydrochloride)

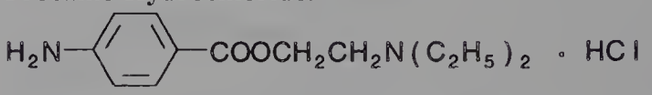
Prilocaine hydrochloride (Citanest, Xylonest) is a white, bitter, odorless, crystalline solid. It is soluble in water (pK_a 7.9) and ethanol and is reasonably soluble in lipids. The drug is almost insoluble in diethyl ether. The free base is obtained as needles.

Prilocaine hydrochloride has a plasma protein binding of 55% and an anesthetic strength similar to that of cocaine. Onset of action is slow, but duration is about the same as that of lignocaine. However, prilocaine hydrochloride is slightly less toxic than lignocaine, but large doses of ~800 mg or more can cause methemoglobinemia. The drug crosses the placenta and may produce methemoglobinemia in the fetus.

Prilocaine hydrochloride is mainly metabolized in the liver but also in the kidneys. One of the metabolites is 2-methylaniline, which is thought to be metabolized to the compounds that cause methemoglobinemia. 2-Methylaniline and other metabolites are excreted in the urine.

Prilocaine hydrochloride is used for infiltration, intravenous regional, and nerve block anesthesia. A mixture of prilocaine with lignocaine, which has a eutectic point below the melting point of either compound, is used for the preparation of topical dosage forms.

Procaine Hydrochloride.



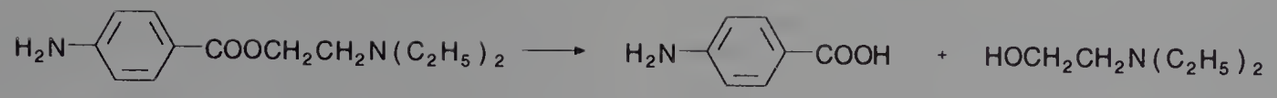
2-(Diethylamino)ethyl 4-aminobenzoate monohydrochloride (Procaine hydrochloride)

Procaine hydrochloride (Novocaine) is obtained as colorless or white odorless plates, monoclinic or triclinic crystals. It is very soluble in water (1 g in 1 cm^3, pK_a 8.9), soluble in ethanol, and slightly soluble in chloroform. It is almost insoluble in diethyl ether and has a low lipid solubility. A 2% aqueous solution has a *p*H of ~6.0. The stability of aqueous solutions of the drug depends on the *p*H of the solution; for example, at 20°, a solution with a *p*H of 3.6 has a 10% loss of potency after 2,300 days, but at a *p*H of 7.0 the same loss occurs after only 7 days at the same temperature. This drop in potency increases with increase in temperature. However, buffered solutions may be sterilized by carefully controlled autoclaving. Solutions for injection have *p*H values in the range 3.0 to 5.5.

The free base is obtained as either white odorless anhydrous plates or dihydrate needles. It is soluble in ethanol, diethyl ether, and chloroform. A number of the salts of procaine are also used as local anesthetics:

- Procaine nitrate: The nitrate is soluble in water (neutral solution) and ethanol. It is useful for formulating in solutions containing silver nitrate, as no precipitate is formed.
- Procaine butyrate (Probutylin): This agent occurs as hydroscopic crystals that are soluble in water, ethanol, and vegetable oils.
- Procaine borate (Borocaine): The borate is very soluble in water (1 g in 4 cm^3), soluble in ethanol, but insoluble in chloroform and diethyl ether. Aqueous solutions are stable enough to be sterilized by brief boiling.

The action of procaine is similar to that of lignocaine, but it has a slow onset of action and action is of shorter duration. It is a vasodilator, and this is probably partly responsible for the short duration of action. Its potency is about half that of cocaine. Vasoconstrictors such as adrenaline may be added in order to prolong its action.



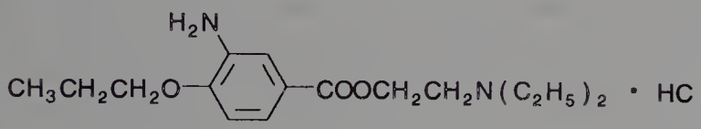
Procaine — Para-aminobenzoic acid — Diethylaminoethanol

Procaine is mainly hydrolyzed in the plasma by plasma cholinesterase to produce 4-aminobenzoic acid (this inhibits the action of sulphonamides) and diethylaminoethanol. Both these compounds are excreted in the urine, the para-aminobenzoic acid partly in the form of conjugates. However, ~70% of the diethylaminoethanol is metabolized by the liver.

Procaine prolongs the action of some drugs by forming salts that slowly decompose to release the drug. For example, intramuscular injection of procaine slows the absorption of penicillin G, thereby extending its antibiotic action. However, the injection of large doses of procaine can have unwanted toxic effects.

Procaine is usually given by injection because of its poor absorption through the mucous membrane. It is used mainly for infiltration, peripheral nerve block, and spinal anesthesia. Its use has been largely superseded by lignocaine and other local anesthetics. It is not used for topical application because of its poor mucous membrane absorption. Its use in malignant hyperpyrexia syndrome is controversial.

Proparacaine Hydrochloride, USP.

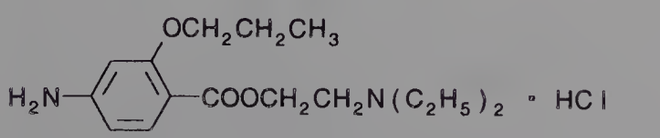


2-Diethylaminoethyl 3-amino-4-propoxybenzoate monohydrochloride (Proparacaine monohydrochloride)

Proparacaine monohydrochloride (Ak-Taine, Alcaine, Ophthaine, Ophthetic, Proxymetacaine). Proparacaine monohydrochloride is a white to light buff colored powder that crystallizes as prisms from a mixture of absolute ethanol and ethyl ethanoate. It is soluble in water (pK_a 3.22), warm ethanol, and methanol, but is insoluble in diethyl ether and benzene. Aqueous solutions are neutral to litmus, a 1% solution having a *p*H of ~6. Aqueous solutions should be protected from light and should not be used if they are discolored.

Proparacaine hydrochloride is a rapid-acting local anesthetic agent of similar potency to amethocaine. It is used as a topical surface local anesthetic agent in ophthalmology. It is too toxic to be used by injection.

Propoxycaine Hydrochloride, USP.

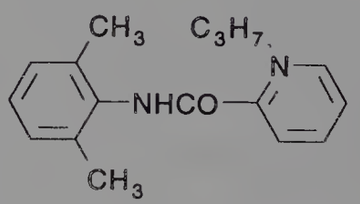


2-Diethylaminoethyl 4-amino-2-propoxybenzoate hydrochloride (Propoxycaine hydrochloride)

Propoxycaine hydrochloride (Ravocaine, Pravocaine hydrochloride, Blockaine hydrochloride) is a white odorless crystalline solid that discolors on prolonged exposure to air and light. The hydrocholoride is very soluble in water, soluble in ethanol and chloroform, but only slightly soluble in diethyl ether and acetone. A 2% solution has a pH of 5.4. Solutions cannot be sterilized by autoclaving.

Propoxycaine hydrochloride is a structural isomer of proparacaine being less toxic but slightly less potent than proparacaine. It has a rapid onset of action and a longer duration of action than procaine. The drug has been used for infiltration and nerve block anesthesia in dental work in combination with procaine and vasoconstrictors.

Ropivacaine.



(−)-1-Propyl-2′,6′-pipecoloxylidide (Ropivacaine).[40]

Ropivacaine is a crystalline solid. It has an identical pK_a value to bupivacaine (pK_a 8.1), similar protein binding strength, but a slightly lower solubility in lipids. It has been used in the form of its S-isomer monohydrochloride for infiltration and peripheral and central nerve blocks. It is a vasoconstrictor, and this probably explains why its action can be prolonged when used for infiltration and peripheral nerve block anesthesia. Ropivacaine has a similar action to bupivacaine but appears to be less toxic. In particular, it appears to be less arrhythmogenic than bupivacaine and causes a shorter and less-intense motor block.

Tetracaine. See Amethocaine.

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CHAPTER 21

Histamine and Antihistaminic Agents

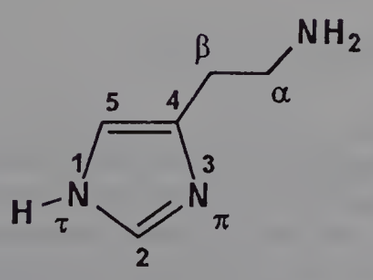
Thomas N. Riley
Jack DeRuiter

Histamine is a β-imidazolylethylamine derivative that is present in essentially all mammalian tissues. The major physiological actions of histamine are centered on the cardiovascular system, nonvascular smooth muscle, exocrine glands, and the adrenal medulla.[1] In a general sense, histamine plays an important role as a "chemical messenger" component of the various pathways that have evolved in multicellular organisms, allowing them to communicate efficiently and effectively. The involvement of histamine in the mediation of allergic and hypersensitivity reactions as well as in the regulation of gastric acid secretion has led to the development of important drug classes useful in the treatment of symptoms associated with allergic and gastric hypersecretory disorders.

HISTAMINE

NOMENCLATURE

Histamine, known trivially as 4(5-)(2-aminoethyl)-imidazole, consists of an imidazole heterocycle and ethylamine side chain. The methylene groups of the aminoethyl side chain are designated as α and β. The side chain is attached, via the β-CH_2 group, to the 4-position of an imidazole ring. The imidazole N at position 3 is designated as the pros (π) N, whereas the N at position 1 is termed the tele (τ) N. The side chain N is distinguished as Nα.



Histamine

IONIZATION AND TAUTOMERISM

Histamine is a basic organic compound (Nπ, $pK_{a1} = 5.80$; Nα, $pK_{a2} = 9.40$; Nτ, $pK_{a3} = 14.0$) capable of existing as a mixture of different ionic and uncharged tautomeric species (Fig. 21-1)[2,3] Histamine has been found to exist almost exclusively (96.6%) as the monocationic conjugate species (αNH_3^+) at physiologic *p*H (7.4). The ratio of the concentrations of the tautomers N^{τ}-H/N^{π}-H has been calculated to be 4.2, indicating that in aqueous solution 80% of the histamine monocation exists as N^{τ}-H and 20% as N^{π}-H.

Structure-activity relationship studies suggest that the α-NH_3^+ monocation is important for agonist activity at histamine receptors and that transient existence of the more lipophilic uncharged histamine species may contribute to translocation of cell membranes. Other studies support the proposal that the N^{τ}-H tautomer of the histamine monocation is the pharmacophoric species at the H_1-receptor, whereas a 1,3-tautomer system is important for selective H_2-receptor agonism.

STEREOCHEMISTRY

Histamine is an achiral molecule; however, histamine receptors are known to exert a high degree of stereoselectivity toward chiral ligands.[4] Molecular modeling and steric-activity relationship studies of the influence of conformational isomerism on the activity of histamine suggest the importance of trans-gauche rotameric structures (Fig. 21-2) in the receptor activities of this substance. Studies with conformationally restricted histamine analogs suggest that, while the trans rotamer of histamine possesses affinity for both H_1 and H_2 histamine receptors, the gauche conformer does not act at H_2-sites.

HISTAMINE LIFE CYCLE

Knowledge of the biodisposition of histamine is important to understanding the involvement of this substance in various

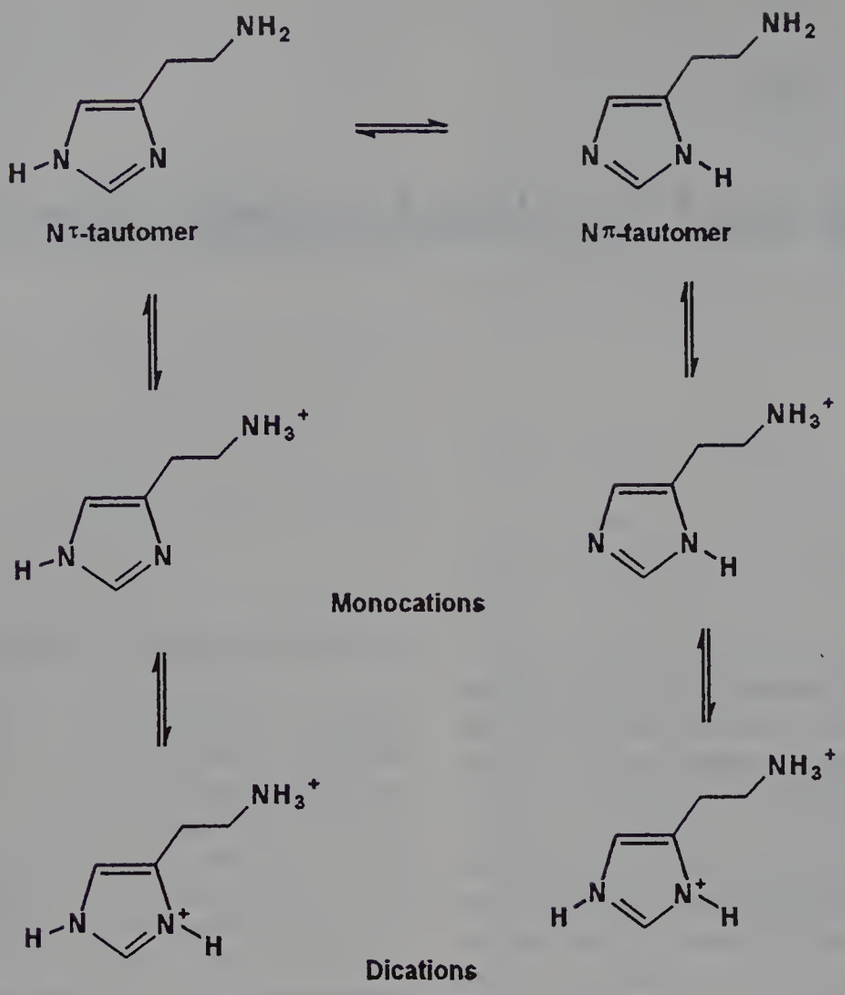


FIG. 21-1. *Histamine tautomers and cations.*

pathophysiologies as well as the actions of various ligands that either enhance or block the actions of histamine. Each of the steps in the "life cycle" of histamine represents a potential site for pharmacological intervention.

BIOSYNTHESIS AND DISTRIBUTION

Histamine is synthesized in cytoplasmic granules of its primary storage cells: mast cells and basophils.[5] Histamine is formed from the naturally occurring amino acid, *S*-histidine, via the catalysis of either the pyridoxal phosphate-dependent enzyme histidine decarboxylase (HDC, EC 4.1.1.22) or aromatic amino acid decarboxylase (Fig. 21-3). Substrate specificity is higher for HDC. Inhibitors of HDC include α-fluoromethyl histidine (FMH) and certain flavanoids, however, no HDCIs have proved useful clinically.

Histamine is found in almost all mammalian tissues in concentrations ranging from 1 to >100 μg/g. This substance is in particularly high concentration in skin, bronchial, and intestinal mucosa. It is found in higher concentrations in mammalian cerebrospinal fluid than in plasma and other body fluids.

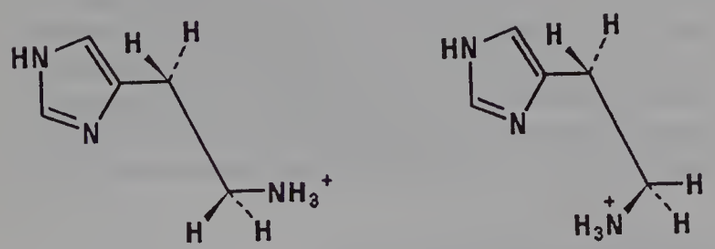


FIG. 21-2. *Histamine rotamers.*

STORAGE AND RELEASE

Most histamine is synthesized and stored in mast cells and basophil granulocytes.[6] Protein-complexed histamine is then stored in secretory granules and released by exocytosis in response to a wide variety of immune (antigen and antibody) and nonimmune (bacterial products, xenobiotics, physical effects, and cholinergic effects) stimuli. The release of hista-

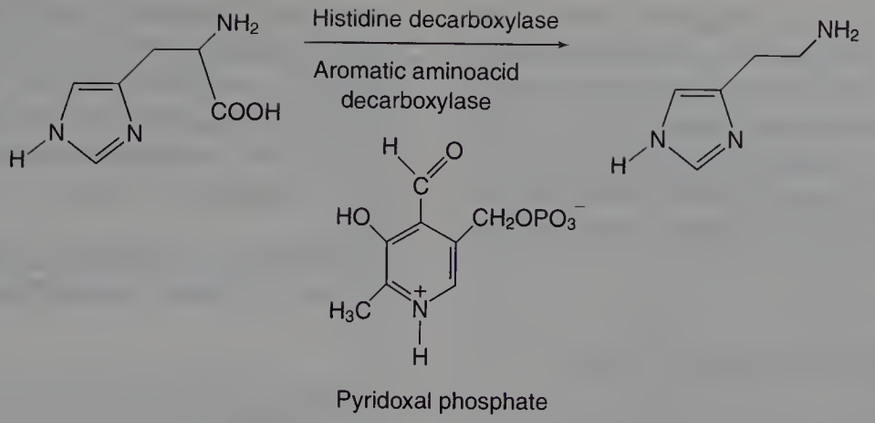


FIG. 21-3. *Biosynthesis of histamine.*

mine as one of the mediators of hypersensitivity reactions is initiated by the interaction of an antigen-IgE complex with the membrane of a histamine-storage cell. This interaction triggers activation of intracellular phosphokinase C (PKC) and accumulation of inositol phosphates, diacylglycerols, and Ca^{2+}. Exocytotic release of histamine follows the degranulation of histamine storage cells. Histamine is released from mast cells in the gastric mucosa by gastrin and acetylcholine. Neurochemical studies also suggest that histamine is stored in selected neuronal tracts in the CNS.

RECEPTORS

Once released, the physiological effects of histamine are mediated by specific cell-surface receptors.[7] Extensive pharmacological analysis suggests the existence of three different histamine-receptor subtypes: H_1, H_2, and H_3.

Histamine H_1-receptors mediate smooth muscle contraction, increased vascular permeability, pruritus, prostaglandin generation, decreased atrioventricular conduction time accompanied by tachycardia, and activation of vagal reflexes. Histamine H_1-receptors are $G^{q/11}$-protein–linked receptors, which, when activated, give rise intracellularly to the second messengers inositol triphosphate (IP_3) and diacylglcerol (DAG). The structure of the H_1-receptor has been determined and shown to display several important features that distinguish it from the H_2-receptor.[8] The H_1-receptor contains seven hydrophobic transmembrane domains (TMs) characteristic of most G-protein receptors. Analysis of the primary structure of this receptor indicates the presence of threonine and asparagine residues in TM5 proposed to serve as the histamine-imidazole binding site and an aspartate reside in TM3 thought to interact ionically with the histamine α-NH_3^+ monocation. A structural requirement for H_1-receptor agonism is the presence of an "aromatic" nitrogen with a nonbonded pair of elections oriented α to the point of attachment of the ethylamine side chain, i.e., Nπ in the case of histamine.

H_2-receptors are located on the cell membrane of acid-secreting cells (parietal) of the gastric mucosa and mediate the gastric acid secretory actions of histamine. The physiologic/pharmacologic effects of H_2-receptor ligands are mediated by a stimulatory G_s-protein coupled receptor, which, in turn, activates the adenylate cyclase/cyclic adenosine monophosphate (AMP) intracellular second messenger system. The H_2-receptor has been cloned and, similar to the H_1-site, found to consist of seven hydrophobic TMs.[9] Examination of the primary structure of the H_2-receptor has led to the proposal that an aspartate residue in TM3 is the primary biding site for the cationic nitrogen of histamine and that a threonine and an aspartate residue in TM5 may be important for hydrogen bonding with the nitrogen atoms of the imidazole ring of histamine. It has been further proposed that tautomerism of the imidazole ring of histamine or isosteric structural features of other H_2-agonists is an important component of the ability of agonist ligands to activate this receptor system (Fig. 21-4).[2]

The most recently described receptor for histamine, the H_3-receptor, is proposed to function as a neural autoreceptor

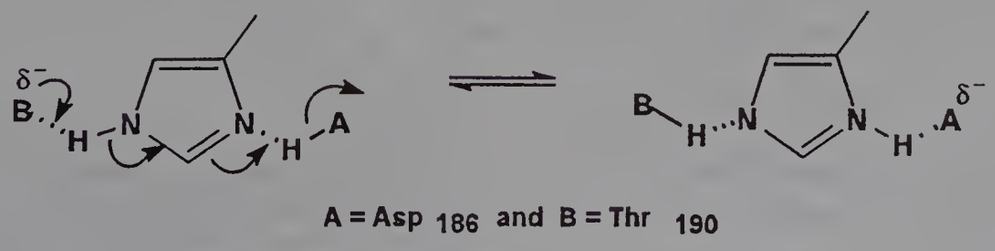


FIG. 21-4. *Functional requirements for H_2-receptor agonism.*

(presynaptic) serving to modulate histamine synthesis and release in the CNS.[10] Subsequent studies have also located H_3-sites in peripheral tissue, including the gastric mucosa, where this receptor may negatively control gastric acid secretion, and on the cardiac sympathetic terminals in the myocardium. Isolation and characterization of the H_3-receptor protein as well as identification of transmembrane signaling are just beginning.

TERMINATION OF HISTAMINE ACTION

Three principal ways exist for terminating the physiological effects of histamine.[3]

- *Cellular uptake:* Animal studies have documented the uptake of histamine by many cells. In particular, uptake is a temperature- and partially Na^+-dependent process in rabbit gastric glands and the histamine is metabolized once in the cell.
- *Desensitization of cells:* Some H_1-receptor-containing tissues exhibit a homogeneous loss of sensitivity to the actions of histamine perhaps as a result of receptor modification.
- *Metabolism:* The most common pathway for terminating histamine action involves enzymatic inactivation (Fig. 21-5).[11]

The enzyme histamine N-methyltransferase (HMT, EC 2.1.1.8) is widely and ubiquitously distributed among mammalian tissues and catalyzes the transfer of a methyl group from S-adenosyl-L-methionine (SAM) to the ring tele-nitrogen of histamine producing N^{τ}-methylhistamine and S-adenosyl-L-homocysteine. Histamine is also subject to oxidative

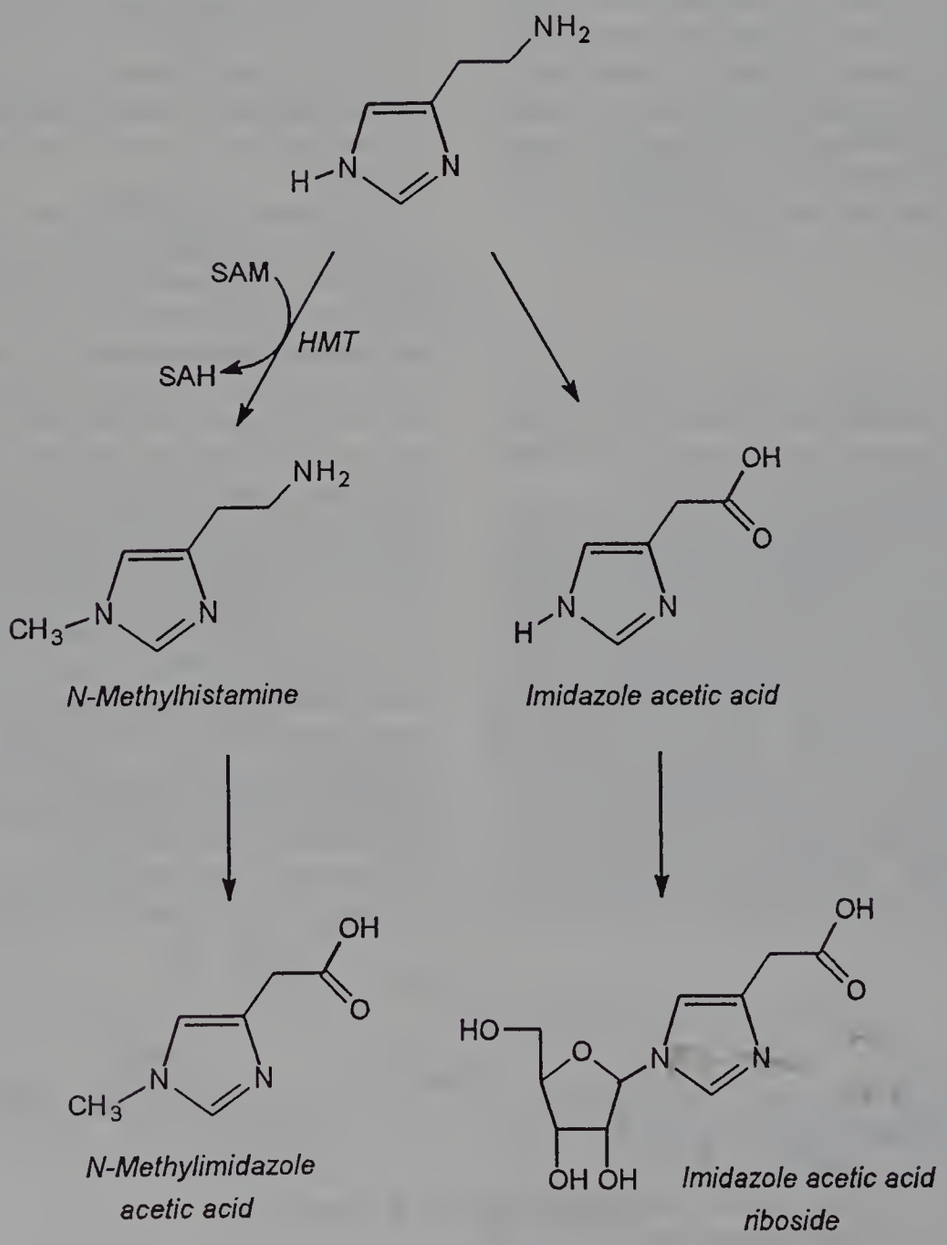


FIG. 21-5. *Metabolism of histamine.*

deamination by diamine oxidase (DAO, EC 1.4.3.6), yielding imidazole acetic acid, a physiologically inactive product, excreted in the urine. Similarly, N^{τ}-methylhistamine is converted by both DAO and monoamine oxidase (MAO) to N-methyl imidazole acetic acid.

FUNCTIONS OF ENDOGENOUS HISTAMINE AS RELATED TO PHARMACOLOGIC INTERVENTION

Histamine exhibits a wide variety of both physiologic and pathologic functions in different tissues and cells. The actions of histamine that are of interest from both a pharmacologic and therapeutic point of view include (a) its important but limited role as a chemical mediator of hypersensitivity reactions, (b) a major role in the regulation of gastric acid secretion, and (c) an emerging role as a neurotransmitter in the CNS.

HISTAMINE H_1-RECEPTOR ANTAGONISTS: ANTIHISTAMINIC AGENTS

The term ''antihistamine'' historically has referred to drugs that antagonize the actions of histamine at H_1-receptors rather than H_2-receptors. The development of antihistamine drugs began more than five decades ago with the discovery that piperoxan was able to protect animals from the bronchial spasm induced by histamine.[12] This finding was followed by the synthesis of a number of N-phenylethylenediamines with antihistaminic activities superior to piperoxan.[13] Further traditional structure-activity studies in this series based largely on the principles of isosterism and functional group modification led to the introduction in the 1940s to 1970s of a variety of H_1-antagonists containing the diarylalkylamine framework.[14,15] These H_1-antagonists, referred to now as the first generation or classical antihistamines, are related structurally and include a number of aminoalkyl ethers, ethylenediamines, piperazines, propylamines, phenothiazines, and dibenzocycloheptenes. In addition to H_1-receptor antagonism, these compounds display an array of other pharmacological activities that contribute toward therapeutic applications and adverse reactions. More recently, a number of second generation or ''nonsedating'' antihistamines have been developed and introduced.[16] The second generation agents bear some structural resemblance to the first generation agents, but have been modified to be more specific in action and limited in their distribution profiles.

MECHANISM OF ACTION

H_1-antagonists may be defined as those drugs that competitively inhibit the action of histamine on tissues containing H_1-receptors. Traditionally, H_1-antagonists have been evaluated in vitro in terms of their ability to inhibit histamine-induced spasms in an isolated strip of guinea pig ileum. Antihistamines may be evaluated in vivo in terms of their ability to protect animals against the lethal effects of histamine administered intravenously or by aerosol.

To distinguish competitive antagonism of histamine from other modes of action, the index pA is applied to in vitro assays. The index pA_2 is defined as the inverse of the logarithm of the molar concentration of the antagonist which reduces the response of a double dose of the agonist to that of a single one. The more potent H_1-antagonists exhibit a pA_2 value significantly higher than 6. Although there are many pitfalls[17] to be avoided in the interpretation of structure-activity relationship (SAR) studies using pA_2 values, the following example is illustrative in distinguishing competitive antagonism. It has been shown that pA_2 values for pyrilamine (mepyramine) antagonism ranged from 9.1 to 9.4 with human bronchii and guinea pig ileum.[15] By contrast, the pA_2 value in guinea pig atria (H_2 receptor) was 5.3. Thus, it may be concluded that pyrilamine is a weak, noncompetitive inhibitor of histamine at the atrial receptors and a competitive inhibitor at H_1-receptors. The structural features required for effective interaction with these receptors is discussed below. It should be noted that some H_1-antagonists also block histamine release. However the concentrations required to do so are considerably greater than those required to produce significant histamine receptor blockade. The H_1-antagonists do not block antibody production or antigen-antibody interactions.[18]

STRUCTURE-ACTIVITY RELATIONSHIPS

The H_1-antagonists are now commonly subdivided into two broad groups: the first generation or classical antihistamines and the second generation or ''nonsedating'' antihistamines—based primarily on their general pharmacological profiles.[16,23] The differences between these two series are discussed in more detail in the sections that follow. It is important to note, however, that most detailed structure-activity analyses for H_1-antagonists that have been published focus on the structural requirements for the first generation agents.[14,15] From these studies, the basic structural requirements for H_1-receptor antagonism have been identified as shown in Fig. 21-6. In this structure, Ar is aryl (including phenyl, substituted phenyl, and heteroaryl groups such as 2-pyridyl), Ar′ is a second aryl or arylmethyl group, X is a connecting atom of O, C, or N, $(CH_2)_n$ represents a carbon chain, and NRR′ represents a basic, terminal amine function.

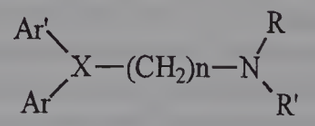


FIG. 21-6. *General antihistamine structure.*

The nature of the connecting atom, as well as the diaryl substitution pattern and amine moiety have been used to subclassify the first generation antihistamines as indicated in the sections that follow.

This diaryl substitution pattern is present in both the first and second generation antihistamines and is essential for significant H_1-receptor affinity. Furthermore, several SAR studies suggest that the two aryl moieties must be capable of adopting a noncoplanar conformation relative to each other for optimal interaction with the H_1-receptor.[19] The two aromatic systems may be linked as in the tricyclic antihistamines (phenothiazines, dibenzocycloheptanes and heptenes, etc.), but again they must be noncoplanar for effective receptor interaction. Most H_1-antagonists contain substituents in one of the aryl rings (usually benzene), and these influence antihistamine potency, as well as biodisposition, as is discussed for individual classes of compounds in the sections that follow.

In many of the first generation or classical antihistamines, the terminal nitrogen atom is a simple dimethylamino moiety. However, the amine may also be part of a heterocyclic structure, as illustrated by the piperazine, some propylamines (pyrrolidines and piperdines), some phenothiazines, the dibenzocycloheptenes, and the second generation antihistamines. In all cases, the amino moiety is basic, with pK_a's ranging from 8.5 to 10 and thus presumed to be protonated when bound on the receptor. The moiety is also important in the development of stable, solid dosage forms through salt formation.

The carbon chain of typical H_1-antagonists consists of two or three atoms. As a result, the distance between the central point of the diaryl ring system and the terminal nitrogen atom in the extended conformation of these compounds ranges from 5 to 6 angstroms. A similar distance between these key moieties is observed for those antihistamines with less conformational freedom. In some series, branching of the carbon chain results in a reduction of antihistaminic activity. However, there are exceptions, as evidenced by promethazine, which has a greater activity than its nonbranched counterpart. When the carbon adjacent to the terminal nitrogen atom is branched, the possibility of asymmetry exists. However, stereoselective H_1-receptor antagonism typically is not observed when chirality exists at this site.[20] Also, in those compounds that possess an asymmetrically substituted unsaturated carbon chain (pyrrobutamine and triprolidine), one geometric isomer typically displays higher receptor affinity than the other.

The X connecting moiety of typical H_1-antagonists may be a saturated carbon-oxygen moiety or simply a carbon or nitrogen atom. This group along with the carbon chain appear to serve primarily as a spacer group for the key pharmacophoric moieties. Many of the anthistamines containing a carbon atom in the connecting moiety are chiral and exhibit steroselective receptor binding. For example, in the pheniramine series and carbinoxamine, this atom is chiral and in vitro analyses indicate that those enantiomers with the S-configuration have higher H_1-receptor affinity.[21]

Generally, the first and second generation antihistamines are substantially more lipophilic than the endogenous agonist histamine (or the H_2-antagonists).[22] This lipophilicity difference results primarily from the presence of the two aryl rings and the substituted amino moieties, and thus may simply reflect the different structural requirements for antagonist versus agonist action at H_1-receptors.

The nature of this connecting moiety and the structural nature of the aryl moieties have been used to classify the antihistamines as indicated in the sections that follow. Furthermore, variations in the diaryl groups, X connecting moieties, and the nature of substitution in the alkyl side chain or terminal nitrogen among the various drugs account for differences observed in antagonist potency as well as pharmacologic, biodisposition, and adverse reaction profiles. The ability of these drugs to display an array of pharmacologic activities is due largely to the fact that they contain the basic pharmacophore required for binding to muscarinic as well as adrenergic, serotonergic receptors. The relationships of antihistamine structure to these overlapping actions (H_1-antagonist, anticholinergic, and local anesthetic) have been analyzed.

GENERAL PHARMACOLOGIC CONSIDERATIONS

The classical antihistamines have been used extensively for the symptomatic treatment (sneezing, rhinorrhea, and itching of eyes, nose, and throat) of allergic rhinitis (hay fever, pollinosis), chronic idiopathic urticaria, and a number of other symptoms of allergic diseases at the beginning of the season when pollen counts are low. Although the symptoms of the common cold might be modified by antihistamines, these agents do not prevent or cure colds, nor do they shorten the course of the disease.[23] The antihistamines also are of little or no value in diseases such as systemic anaphylaxis and bronchial asthma, in which autacoids other than histamine are important.[18]

A number of the antihistamines, particularly the phenothiazines and aminoalkyl ethers, have antiemetic actions and thus may be useful in the treatment of nausea, vomiting, and motion sickness.[18,23] Also, those agents that produce pronounced sedation have applications as nonprescription sleeping aids.[18,23] Several of the phenothiazines have limited utility in Parkinson-like syndromes as a result of their ability to block central muscarinic receptors.[18,23] And a number of antihistamines including promethazine, pyrilamine, tripelennamine, and diphenhydramine display local anesthetic activity that may be therapeutically useful.[24]

As the general pharmacologic profiles above suggest, the majority of antihistamines are capable of interaction with a variety of neurotransmitter receptors and other biomacromo-

lecular targets. This is most evident among the first generation agents many of which function as antagonists at muscarinic receptors and, to a lesser extent, adrenergic, serotonergic and dopamine receptors.[16,18,23] While some of these nontarget receptor interactions may be of some therapeutic value (as discussed above), more frequently they are manifested as adverse reactions that limit drug use. This is particularly true of the peripheral anticholingeric effects produced by these drugs, and interactions with a number of neurotransmitter systems in the CNS that result in sedation, fatigue, and dizziness.[16,18,23]

The primary objective of antihistamine research over the past 10 to 15 years has centered on development of new drugs with higher selectivity for H_1-receptors and lacking undersirable CNS actions. The pronounced sedative effects of some of the first generation agents were thought to result from the ability of these drugs to penetrate the blood-brain barrier, due to their lipophilic nature, and then block cerebral H_1-receptors and possibly other receptors.[16] Thus, research efforts were initiated to design novel antihistamines with reduced ability to penetrate the CNS and decreased affinity for central histamine receptors. These efforts led to the introduction of the second generation antihistamines, which are nonsedating and have little antagonist activity at other neurotransmitter receptors at therapeutic concentrations. The pharmacologic properties of these agents are discussed in more detail later in this chapter.

Surprisingly little information is available concerning the pharmacokinetic and biodisposition profiles of the first generation antihistamines.[22] Generally, the compounds are orally active and well absorbed, but oral bioavailability may be limited by first pass metabolism. The metabolites formed depend on drug structure to a large extent, but commonly involve the tertiary amino moiety. This functionality may be subject to successive oxidative N-dealkylation, deamination, and amino acid conjugation of the resultant acid. The amine group may also undergo N-oxidation, which may be reversible, or direct glucuronide conjugation. Those first generation agents with unsubstituted and activated aromatic rings (phenothiazines) may undergo aromatic hydrolyation to yield phenols, which may be eliminated as conjugates.[22] More detailed pharmacokinetic data are available for the second generation agents, and the following drug monographs.

The H_1-antagonists display a variety of significant drug interactions when co-administered with other therapeutic agents. For example, monoamine oxidase inhibitors prolong and intensify the anticholinergic actions of the antihistamines.[16,18,22,23] Also, the sedative effects of these agents may potentiate the depressant activity of barbiturates, alcohol, narcotic analgesics, and other depressants. In recent years, it has been discovered that several of the second generation antihistamines may produce life-threatening arrhythmias when coadministered with drugs that inhibit their metabolism.[16,18] These interactions are discussed in more detail in the sections that follow.

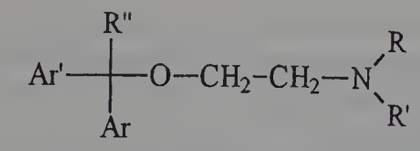


FIG. 21-7. *General structure of the aminoalkyl ethers.*

FIRST-GENERATION H_1-ANTAGONIST DRUG CLASSES

Aminoalkyl Ethers (Ethanolamines)

The aminoalkyl ether antihistamines are characterized by the presence of a CHO connecting moiety (X) and a two- or three-carbon atom chain as the linking moiety between the key diaryl and tertiary amino groups (Fig. 21-7). Most compounds in this series are simple N,N-dimethylethanolamine derivatives and are classified as such in a number of texts. Clemastine and diphenylpyraline differ from this basic structural pattern in that the basic nitrogen moiety and at least part of the carbon of a heterocyclic ring system, and that there are three carbon atoms between the oxygen and nitrogen atoms.

The simple diphenyl derivative diphenhydramine was the first clinically useful member of the ethanolamine series and serves as the prototype. Other therapeutically useful derivatives of diphenhydramine have been obtained by para substitution of methyl (methyldiphenhydramine), methoxy (medrylamine), chloro (chlorodiphenhydramine), or bromo (bromodiphenhydramine) of one of the phenyl rings. These derivatives are reported to have superior therapeutic profiles relative to diphenhydramine as a result of reduced side effects.[22]

Replacement of the one of the phenyl rings of the diphenhydramine with a 2-pyridyl group as in doxylamine and carbinoxamine results in an enhancement antihistaminic activity. These compounds display oral antihistaminic activities 40 and two times greater, respectively, than diphenhydramine in animals.[22]

As a result of an asymmetrically substituted benzylic carbon, most of the aminoalkyl ethers are optically active. Most studies indicate that the individual enantiomers differ significantly in antihistaminic activity, with activity residing predominantly in the S-enantiomer.[21]

The diaryl tertiary aminoalkyl ether structure that characterizes these compounds also serves as a pharmacophore for muscarinic receptors. As a result the drugs in this group possess significant anticholinergic activity, which may enhance the H_1-blocking action on exocrine secretions. Drowsiness is a side effect common to the tertiary aminoalkyl ethers, presumably as a result of the ability of these compounds to penetrate and BBB and occupy central H_1-receptors. Although this side effect is exploited in over-the-counter (OTC) sleeping aids, it may interfere with the patient's

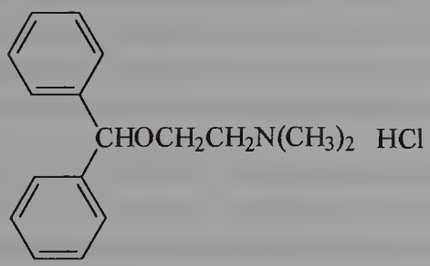
performance of tasks requiring mental alertness.[18,23] The frequency of gastrointestinal side effects in this series of antihistamines is relatively low compared to the ethylenediamine antihistamines.[18,23]

In spite of their extensive use, pharmacokinetic data on this series of compounds is relatively limited. Most members of this series appear to be extensively metabolized by pathways including N-oxidation, and successive oxidative N-dealkylation followed by amino acid conjugation of the resultant acid metabolites.[22]

The structures of the aminoalkyl ether derivatives, along with physico-chemical properties, basic therapeutic activity data and dosage form information are provided in the monographs that follow.

Diphenhydramine Hydrochloride, USP. 2-(Diphenylmethoxy)-N, N-dimethylethanamine hydrochloride (*Benadryl*). The oily, lipid-soluble free base is available as the bitter-tasting hydrochloride salt, which is a stable, white crystalline powder, soluble in water (1:1), alcohol (1:2), and chloroform (1:2). The salt has a pK_a value of 9, and a 1% aqueous solution has a *p*H of ~5.

In addition to antihistaminic action, diphenhydramine exhibits antidyskinetic, antiemetic, antitussive, and sedative properties. It is used in OTC sleep-aid products. In the usual dose range of 25 to 400 mg, diphenhydramine is not a highly active H_1-antagonist; it has anticholinergic and sedative properties. Conversion to a quaternary ammonium salt does not alter the an antihistaminic action greatly, but does increase the anticholinergic action.

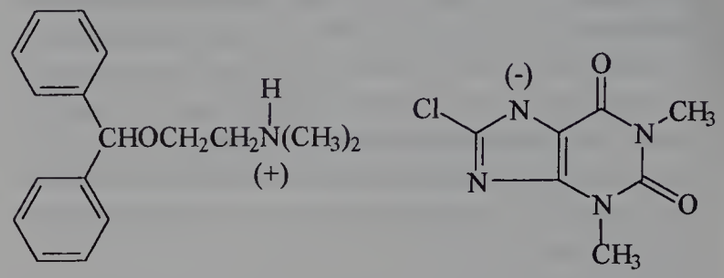


Diphenhydramine Hydrochloride

As an antihistaminic agent, diphenhydramine is recommended in various allergic conditions and, to a lesser extent, as an antispasmodic. It is administered either orally or parenterally in the treatment of urticaria, seasonal rhinitis (hay fever), and some dermatoses. The most common side effect is drowsiness, and the concurrent use of alcoholic beverages and other CNS depressants should be avoided. Usual adult dose: Oral, 25–50 mg; IM or IV, 10–50 mg.

Dosage forms: Capsules, elixir, syrup, tablets, injection.

Dimenhydrinate, USP. 8-Chlorotheophylline 2-(diphenylmethoxy)-N, N-dimethylethylamine compound (*Dramamine*). The 8-chlorotheophyllinate (theoclate) salt of diphenhydramine is a white crystalline, odorless powder that is slightly soluble in water and freely soluble in alcohol and chloroform.



Dimenhydrinate

Dimenhydrinate is recommended for the nausea of motion sickness and for hyperemesis gravidarum (nausea of pregnancy). For the prevention of motion sickness, the dose should be taken at least 30 min before beginning the trip. The cautions listed for diphenhydramine should be observed.

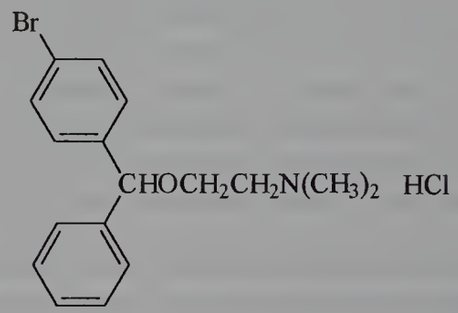
Usual adult dose: Oral, 50–100 mg/4 hr; IM or IV, 50 mg/4 hr; rectal, 100 mg qd or bid.

Dosage forms: Elixir, syrup, tablets, injection, suppositories.

Bromodiphenhydramine Hydrochloride, USP. 2-[(4-Bromophenyl)phenylmethoxy]-N, N-dimethylethanamine hydrochloride (*Ambodryl Hydrochloride*). The hydrochloride salt is a white to pale buff crystalline powder that is freely soluble in water and in alcohol. Relative to diphenhydramine, bromodiphenhydramine is more lipid-soluble and was found to be twice as effective in protecting guinea pigs against the lethal effects of histamine aerosols.

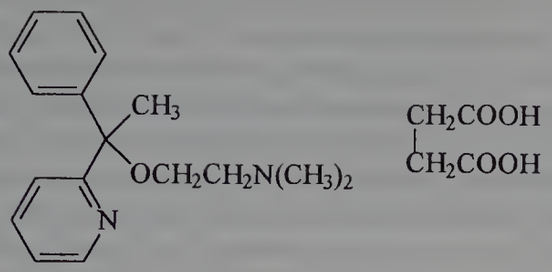
Usual adult dose: Oral, 25 mg/4–6 hr.

Dosage forms: Capsules and elixir.



Bromodiphenhydramine Hydrochloride

Doxylamine Succinate, USP. 2-[α-[2-(Dimethylamino)-ethoxy]-α-methyl -benzyl]pyridine bisuccinate (*Decapryn Succinate*). The acid succinate salt (bisuccinate) is a white to creamy-white powder that has a characteristic odor and is soluble in water (1:1), alcohol (1:2), and chloroform (1:2). A 1% solution has a *p*H of ~5.



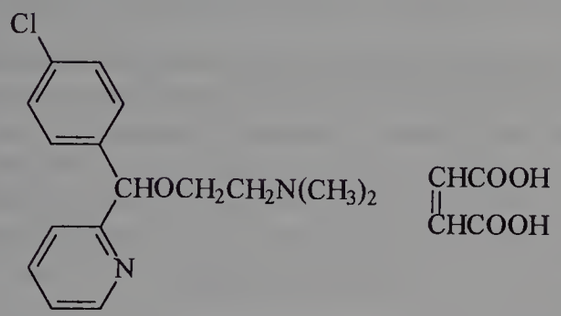
Doxylamine Succinate

Doxylamine succinate is comparable in potency to diphenhydramine. It is a good nighttime hypnotic when compared with secobarbital.[25] Concurrent use of alcohol and other CNS depressants should be avoided.

Usual adult dose: Oral, 12.5–25 mg/4–6 hr.

Dosage forms: Syrup and tablets.

Carbinoxamine Maleate, USP. (*d,l*)-2-[p-Chloro-α-[2-(dim ethylamino)ethoxy]benzyl]pyridine bimaleate (*Clistin*). The oily, lipid-soluble free base is available as the bitter bimaleate salt, a white crystalline powder that is very soluble in water and freely soluble in alcohol and in chloroform. The pH of a 1% solution is 4.6 to 5.1.



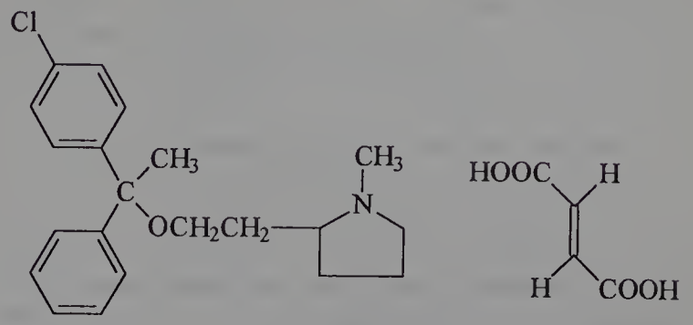
Carbinoxamine Maleate

Carbinoxamine is a potent antihistaminic and is available as the racemic mixture. Carbinoxamine differs structurally from chloropheniramine only in that an oxygen atom separates the asymmetric carbon atom from the aminoethyl side chain. The more active levo isomer of carbinoxamine has been shown to have the (S) absolute configuration[26] and to be superimposable upon the more active dextro isomer (S configuration[21]) of chlorpheniramine.

Usual adult dose: Oral, 4–8 mg/tid or qid.

Dosage forms: Elixir and tablets.

Clemastine Fumarate, USP. 2-[2-[1-(4-Chloro-phenyl)-1-phenylethoxy]ethyl]-1-methylp yrrolidine hydrogen fumarate (1:1) (*Tavist*). Dextrorotatory clemastine has two chiral centers, each of which is of the (R) absolute configuration. A comparison of the activities of the antipodes indicates that the asymmetric center close to the side chain nitrogen is of lesser importance to antihistaminic activity.[15]



Clemastine Fumarate

This member of the ethanolamine series is characterized by a long duration of action, with an activity that reaches a maximum in 5 to 7 hr and persists for 10 to 12 hr. It is well absorbed when administered orally and is excreted primarily in the urine. The side effects are those usually encountered with this series of antihistamines. Clemastine is closely related to chlorphenoxamine, which is used for its central cholinergic-blocking activity. Therefore, it is not surprising that clemastine has significant antimuscarinic activity.

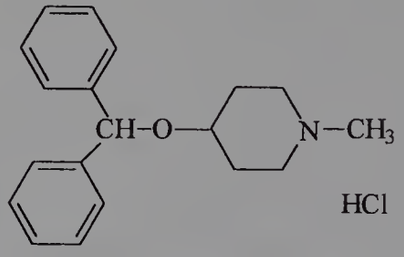
Usual adult dose: Oral, 1.34 mg bid or 2.68 mg qd to tid.

Dosage forms: Syrup and tablets.

Diphenylpyraline Hydrochloride, USP. 4-(Diphenylmethoxy)-1-methylpiperidine hydrochloride (*Hispril; Diafen*). The salt occurs as a white or slightly off-white crystalline powder that is soluble in water or alcohol. Diphenylpyraline is structurally related to diphenhydramine with the aminoalkyl side chain incorporated in a piperidine ring. It is a potent antihistaminic, and the usual dose is 2 mg three or four times daily. The hydrochloride is available as 5-mg sustained-release capsules.

Usual adult dose: Oral, 5 mg/12 hr.

Dosage forms: Extended release capsules.



Diphenylpyraline Hydrochloride

Ethylenediamines

The ethylenediamine antihistamines are characterized by the presence of a nitrogen connecting atom (X) and a two carbon atom chain as the linking moiety between the key diaryl and tertiary amino moieties (Fig. 21-8). All compounds in this series are simple diarylethylenediamines except for antazo-

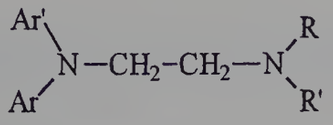


FIG. 21-8. General structure of the ethylenediamines.

line in which the terminal amine and a portion of the carbon chain are included as part of an imidazoline ring system. Because it differs significantly in its pharmacologic profile, antazoline is not always classified as an ethylenediamine derivative.

Phenbenzamine was the first clinically useful member of this class and served as the prototype for the development of more effective derivatives. Replacement of the phenyl moiety of phenbenzamine with a 2-pyridyl system yielded tripelennamine, a significantly more effective histamine receptor blocker.[22] Substitution of a para methoxy (pyrilamine or mepyramine), chloro (chloropyramine), or bromo (brom-tripelennamine) results in a further enhancement in activity.[22] Replacement of the benzyl group of tripelennamine with a 2-thienylmethyl group provided methapyrilene, and replacement of tripelennamine's 2-pyridyl group with a pyrimidinyl moiety (along with p-methoxy substitution) yielded thonzylamine, both of which function as potent H_1-receptor antagonists.[22]

In all of these compounds, the aliphatic or terminal amino group is significantly more basic than the nitrogen atom bonded to the diaryl moiety; the nonbonded electrons on the diaryl nitrogen are delocalized by the aromatic ring, and the resultant reduction in electron density on nitrogen decreases basicity. Thus, the aliphatic amino group in the ethylenediamines is sufficiently basic for the formation of pharmaceutically useful salts.

The ethylenediamines were among the first useful antihistamines.[22] They are highly effective H_1-antagonists, but they also display a relatively high frequency of CNS depressant and gastrointestinal side effects.[23] The anticholinergic and antiemetic actions of these compounds is relatively low compared to most other classical antihistamines. The piperazine and phenothiazine-type antihistamines also contain the ethylenediamine moiety, but these agents are discussed separately because they exhibit significantly different pharmacologic properties.

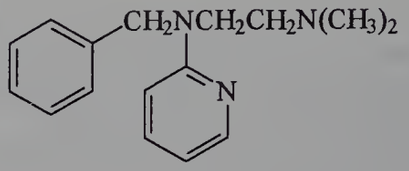
Relatively little information is available concerning the pharmacokinetics of this series of compounds. Tripelennamine is known to be metabolized in humans by N-glucuronidation, N-oxidation, and pyridyl oxidation followed by phenol glucuronidation. It is anticipated that other members of this series are similarly metabolized.[22]

The structures of the salt forms of the marketed ethylenediamine antihistamines, along with physicochemical properties, basic therapeutic activity profiles, and dosage form information follow.

Tripelennamine Citrate, USP. 2-[Benzyl[2-(dimethylamino)ethyl]amino]pyridine citrate (1:1); PBZ (*Pyrilbenzamine Citrate*). The oily free base is available as the less bitter monocitrate salt, which is a white crystalline powder freely soluble in water and in alcohol. A 1% solution has a *p*H of 4.25. For oral administration in liquid dose forms, the citrate salt is less bitter and, thus, more palatable than the hydrochloride. Because of the difference in molecular weights, the doses of the two salts must be equated: 30 mg of the citrate salt are equivalent to 20 mg of the hydrochloride salt.

Usual adult dose: Oral, 25–50 mg/4–6 hr.

Dosage forms: Elixir.



Tripelennamine
(Citrate or HCl)

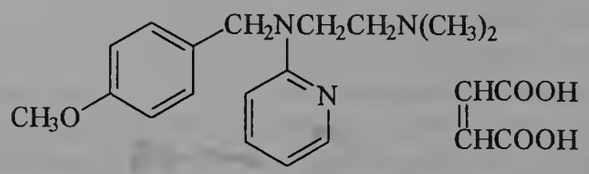
Tripelennamine Hydrochloride, USP. This compound is a white crystalline powder that darkens slowly on exposure to light. The salt is soluble in water (1:0.77) and in alcohol (1:6). It has a pK_a of ~9, and a 0.1% solution has a *p*H of ~5.5.

Tripelennamine, the first ethylenediamine developed in American laboratories, is well absorbed when given orally. On the basis of clinical experience, it appears to be as effective as diphenhydramine and may have the advantage of fewer and less severe side reactions. However, drowsiness may occur and may impair ability to perform tasks requiring alertness. The concurrent use of alcoholic beverages should be avoided.

Usual adult dose: Oral tablets, 25–50 mg/4–6 hr; extended release, 100 mg/8–12 hr.

Dosage forms: Tablets, extended-release tablets.

Pyrilamine Maleate, USP. 2-[[2-(Dimethylamino)ethyl](p-methoxybenzyl)amino]-pyridi ne maleate (1:1); mepyramine. The oily free base is available as the acid maleate salt, which is a white crystalline powder having a faint odor and a bitter, saline taste. The salt is soluble in water (1:0.4) and freely soluble in alcohol. A 10% solution has a pH of ~5. At a pH of 7.5 or above, the oily free base begins to precipitate.



Pyrilamine Maleate

Pyrilamine differs structurally from tripelennamine by having a methoxy group in the para position of the benzyl radical. It differs from its more toxic and less potent precur-

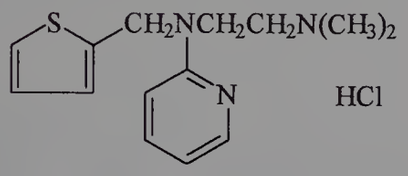
sor phenbenzamine (*Antergan*) by having a 2-pyridyl group on the nitrogen atom in place of a phenyl group.

Clinically, pyrilamine and tripelennamine are considered to be among the less potent antihistaminics. They are highly potent, however, in antagonizing histamine-induced contractions of guinea pig ileum.[14] Because of the pronounced local anesthetic action, the drug should not be chewed, but taken with food.

Usual adult dose: Oral, 25–50 mg/6–8 hr.

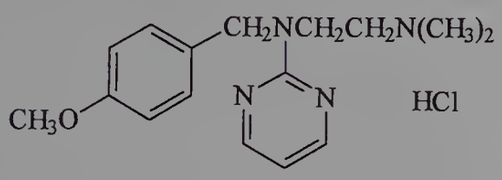
Dosage forms: Tablets.

Methapyrilene Hydrochloride. 2-[[2-(Dimethylamino) ethyl]-2-thenylamino]-pyri dine monohydrochloride (*Histadyl*). The oily free base is available as the bitter-tasting monohydrochloride salt, which is a white crystalline powder that is soluble in water (1:0.5), in alcohol (1:5), and in chloroform (1:3). Its solutions have a pH of ~5.5. It differs structurally from tripelennamine in having a 2-thenyl (thiophene-2-methylene) group in place of the benzyl group. The thiophene ring is considered isosteric with the benzene ring, and the isosteres exhibit similar activity. A study of the solid-state conformation of methapyrilene hydrochloride showed that the trans conformation is preferred for the two ethylenediamine nitrogen atoms. The Food and Drug Administration declared methapyrilene a potential carcinogen in 1979, and all products containing it have been recalled.



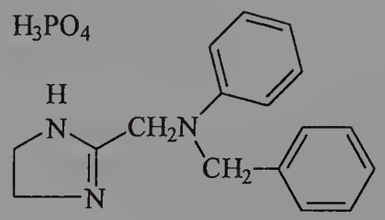
Methapyrilene Hydrochloride

Thonzylamine Hydrochloride. 2-[[2-(Dimeth-ylamino)ethyl](p-methoxybenzyl)amin o]pyrimidine hydrochloride. The hydrochloride is a white crystalline powder, soluble in water (1:1), alcohol (1:6), and chloroform (1:4). A 2% aqueous solution has a pH of 5.5. It is similar in activity to tripelennamine but is claimed to be less toxic. The usual dose is 50 mg up to four times daily. It is available in certain combination products.



Thonzylamine Hydrochloride

Antazoline Phosphate. 2-[(N-Benzylanilino)methyl]-2-imidazoline dihydrogen phosphate. The salt occurs as a bitter, white to off-white crystalline powder that is soluble in water. It has a pK_a of 10.0, and a 2% solution has a *p*H of ~4.5. Antazoline, similarly to the ethylenediamines, contains an N-benzylanilino group linked to a basic nitrogen through a two-carbon chain.



Antazoline Phosphate

Antazoline is less active than most of the other antihistaminic drugs, but it is characterized by the lack of local irritation. The more soluble phosphate salt is applied topically to the eye in a 0.5% solution. The less soluble hydrochloride is given orally. In addition to its use as an antihistamine, antazoline has over twice[27] the local anesthetic potency of procaine and also exhibits anticholinergic actions.

Piperazines (Cyclizines)

The piperazines or cyclizines can also be considered to be ethylenediamine derivatives or cyclic ethylenediamines (cyclizines); however, in this series the connecting moiety (X) is a CHN group and the carbon chain, terminal amine functionality as well as the nitrogen atom of the connecting group are all part of a piperazine moiety (Fig. 21-9). The both nitrogen atoms in these compounds are aliphatic and thus display comparable basicities. The primary structural differences within this series involves the nature of the para aromatic ring substituent (H or Cl) and, more importantly, the nature of the terminal piperazine nitrogen substituent.

The piperazines are moderately potent antihistaminics with a lower incidence of drowsiness.[18,22,23] However, warning of the possibility of some dulling of mental alertness is advised. The activity of the piperazine-type antihistaminics is characterized by a slow onset and long duration of action. These agents exhibit peripheral and central antimuscarinic activity, and this may be responsible for the antiemetic and antivertigo effects.[18,23] The agents diminish vestibular stimulation and may act on the medullary chemoreceptor trigger zone. Thus, as a group, these agents are probably more useful as antiemetics and antinauseants and in the treatment of motion sickness.

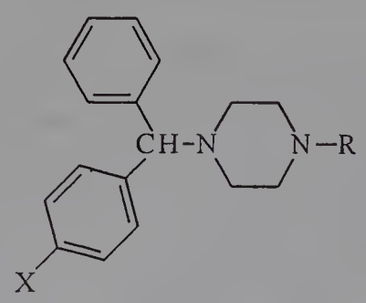


FIG. 21-9. *General structure of the piperazines.*

Some members of this series have exhibited a strong teratogenic potential, inducing a number of malformations in rats. Norchlorcyclizine, a metabolite of these piperazines, was proposed to be responsible for the teratogenic effects of the parent drugs.[28]

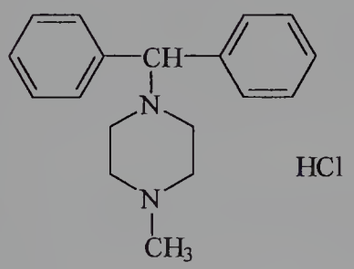
Metabolic studies in this series of compounds have focused primarily on cyclizine and chlorcyclizine, and these compounds undergo similar biotransformation. The primary pathways involve N-oxidation and N-demethylation, and both of these metabolites are devoid of antihistaminic activity.[22]

The structures of the marketed salt forms of the piperazine antihistamines, along with physicochemical properties, basic therapeutic activity profiles, and dosage form information, are provided below.

Cyclizine Hydrochloride, USP. 1-(Diphenylmethyl)-4-methylpiperazine monohydrochloride (*Marezine*). This drug occurs as a light-sensitive, white crystalline powder having a bitter taste. It is slightly soluble in water (1:115), alcohol (1:115), and chloroform (1:75). It is used primarily in the prophylaxis and treatment of motion sickness. The lactate salt (**Cyclizine Lactate Injection, USP**) is used for intramuscular injection because of the limited water solubility of the hydrochloride. The injection should be stored in a cold place, because a slight yellow tint may develop if stored at room temperature for several months. This does not indicate a loss in biologic potency.

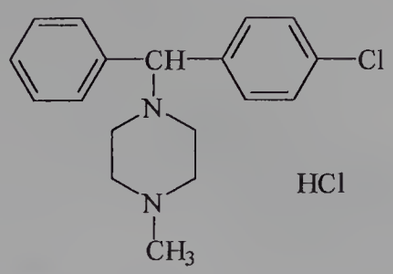
Usual adult dose: Oral, 50 mg/4–6 hr; IM, 50 mg/4–6 hr.

Dosage forms: Tablets (HCl) and injection (lactate).



Cyclizine Hydrochloride
Cyclizine Lactate

Chlorcyclizine Hydrochloride, USP. 1-(p-Chloro-α-phenylbenzyl)-4-methylpiperazine monohydrochloride. This salt, a light-sensitive, white crystalline powder, is soluble in water (1:2), in alcohol (1:11), and in chloroform (1:4). A 1% solution has a pH of 4.8 to 5.5.



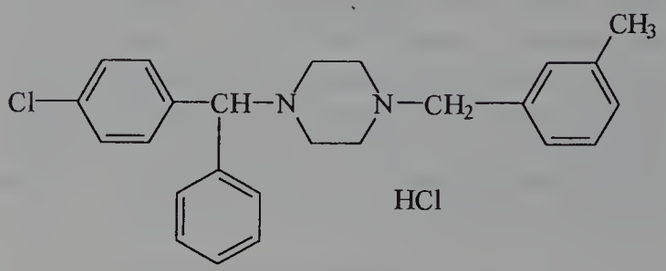
Chlorcyclizine Hydrochloride

Disubstitution or substitution of halogen in the 2- or 3-position of either of the benzhydryl rings results in a much less potent compound. Chlorcyclizine is indicated in the symptomatic relief of urticaria, hay fever, and certain other allergic conditions.

Meclizine Hydrochloride, USP. 1-(p-Chloro-α-phenylbenzyl)-4-(*m*-methylbenzyl) piperazine dihydrochloride monohydrate (*Bonine; Antivert*). Meclizine hydrochloride is a tasteless, white or slightly yellowish crystalline powder that is practically insoluble in water (1:1,000). It differs from chlorcyclizine by having an N-m-methylbenzyl group in place of the N-methyl group. Although it is a moderately potent antihistaminic, meclizine is used primarily as an antinauseant in the prevention and treatment of motion sickness and in the treatment of nausea and vomiting associated with vertigo and radiation sickness.

Usual adult dose: Oral, 25–50 mg.

Dosage forms: Tablets and chewable tablets.

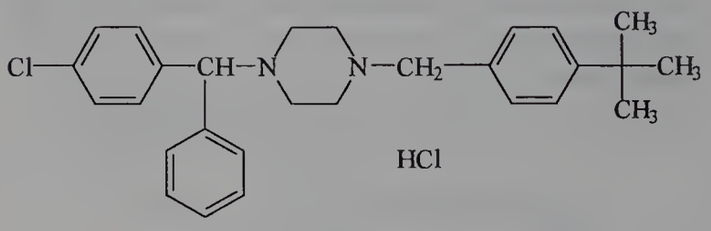


Meclizine Hydrochloride

Buclizine Hydrochloride, USP. 1-(p-*tert*Butylbenzyl)-4-(p-chloro-α-phenylbenzyl)pipe razine dihydrochloride (*Bucladin-S*). The salt occurs as a white to slightly yellow crystalline powder that is insoluble in water. The highly lipid-soluble buclizine has CNS depressant, antiemetic, and antihistaminic properties. The salt is available in 50-mg tablets for oral administration. The usual dose is 50 mg 30 min before travel and is repeated in 4 to 6 hr as needed.

Usual adult dose: Oral, 50 mg/4–6 hr.

Dosage forms: Tablets.



Buclizine Hydrochloride

Propylamines (Monoaminopropyl Derivatives)

The propylamine antihistamines are characterized structurally by an sp^3 or sp^2 carbon connecting atom with a carbon

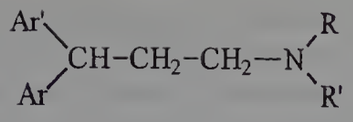


FIG. 21-10. *General structure of the propylamines.*

chain of two additional carbons linking the key tertiary amino and diaryl pharmacophore moieties (Fig. 21-10). Those propylamines with a saturated carbon connecting moiety are commonly referred to as the pheniramines. All of the pheniramines consist of a phenyl and 2-pyridyl aryl groups, and a terminal dimethylamino moiety. These compounds differ only in the phenyl substituent at the para-position; H (pheniramine), Cl (chlorpheniramine), and Br (brompheniramine). The halogenated pheniramines are significantly more potent (20–50 times) and have a longer duration of action.[22]

All pheniramines are chiral molecules, and the halogen-substituted derivatives have been resolved by crystallization of salts formed with d-tartaric acid. Antihistaminic activity resides almost exclusively in the *S*-stereoisomers.[21]

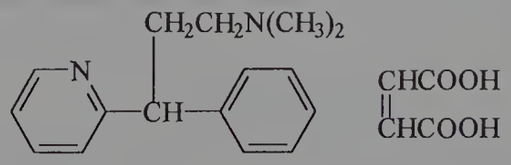
Those propylamines with an unsaturated connecting moiety include the open derivatives pyrrobutamine and triprolidine, and the cyclic analogs dimethindene and phenindamine. In the open-chain propylamines, it appears that a coplanar aromatic double-bond system is an important factor for antihistaminic activity. The pyrrolidino group of these compounds is the side chain tertiary amine that imparts greatest antihistaminic activity. The conformational rigidity of the unsaturated propylamines has provided a useful model to determine distances between the key diaryl and tertiary pharmacophoric moieties in H_1-receptor antagonists, a distance of 5 to 6 angstroms.

The antihistamines in this group are among the most active H_1-antagonists. The agents of this class also produce less sedation that the other classical antihistamines (yet a significant proportion of patients do experience this effect) and have little antiemetic action. They do, however, exhibit a significant degree of anticholinergic activity, albeit less than the aminoalkyl ethers and phenothiazines.[18,22,23]

In the propylamine series, the pharmacokinetics of chlorpheniramine have been studied most extensively in humans.[22] Oral bioavailability is relatively low (30–50%) and may be limited by first-pass metabolism. The primary metabolites for this compound and other members of this series are the mono- and di-N-dealkylation products. Complete oxidation of the terminal amino moiety followed by glycine conjugation has also been reported for brompheniramine. Chlorpheniramine plasma half-lives range from ~12 to 28 hr, depending on the route of administration (oral versus intravenous).[22]

The structures of the marketed salt forms of the propylamine antihistamines, along with physicochemical properties, basic therapeutic activity profiles, and dosage form information follow.

Pheniramine Maleate. 2-[α-[2-Dimethylaminoethyl]benzyl]pyridine bimaleate (*Trimeton; Inhiston*). This salt is a white crystalline powder, having a faint aminelike odor, that is soluble in water (1:5) and is very soluble in alcohol.



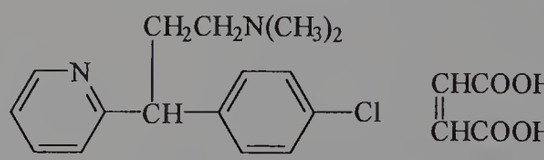
Pheniramine Maleate

This drug is the least potent member of the series and is marketed as the racemate. The usual adult dose is 20 to 40 mg three times daily. It is available in certain combination products.

Chlorpheniramine Maleate, USP. (±)2-[p-Chloro-α-[2-dimethylamino)ethyl]benzyl]pyridi ne bimaleate (*Chlor-Trimeton*). The bimaleate salt is a white crystalline powder that is soluble in water (1:3.4), in alcohol (1:10), and in chloroform (1:10). It has a pK_a of 9.2, and an aqueous solution has a pH of 4 to 5. Chlorination of pheniramine in the para position of the phenyl ring gave a 10-fold increase in potency with no appreciable change in toxicity. Most of the antihistaminic activity resides with the dextro enantiomorph (see dexchlorpheniramine below). The usual dose is 2 to 4 mg three or four times a day. It has a half-life of 12 to 15 hr.

Usual adult dose: Oral, 4 mg/4–6 hr; extended release, 8–12 mg/8–12 hr; IM, IV, or SC, 5–40 mg.

Dosage forms: Extended-release capsules, syrup, tablets, chewable tablets, extended-release tablets, injection.



Chlorpheniramine Maleate
Dextrochlorpheniramine Maleate

Dexchlorpheniramine Maleate, USP. *Polaramine.* Dexchlorpheniramine is the dextrorotatory enantiomer of chlorpheniramine. In vitro and in vivo studies of the enantiomorphs of chlorpheniramine showed that the antihistaminic activity exists predominantly in the *dextro*-isomer. As mentioned previously, the dextro-isomer has been shown[21] to have the (S)configuration, which is superimposable upon the (S)configuration of the more active levorotatory enantiomorph of carbinoxamine.

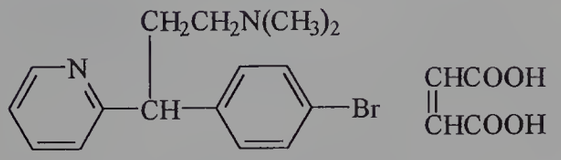
Usual adult dose: Oral, 2 mg/tid or qid.

Dosage forms: Syrup, tablets, extended release tablets.

Brompheniramine Maleate, USP. (±)2-[p-Bromo-α-[2-(dimethylamino)-ethyl]benzyl]pyrid ine bimaleate (*Dimetane®*). This drug differs from chlorpheniramine by the substitution of a bromine atom for the chlorine atom. Its actions and uses are similar to those of chlorpheniramine. It has a half-life of 25 hr, which is almost twice that of chlorpheniramine.

Usual adult dose: Oral, 4 mg/tid or qid; extended release, 8–12 mg/8–12 hr; IM, IV, or SC, 10 mg/8–12 hr.

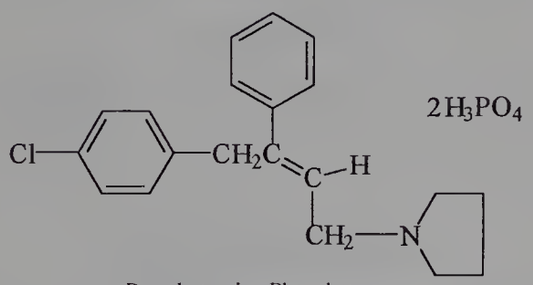
Dosage forms: Elixir, tablets, extended-release tablets, injection.



Brompheniramine Maleate
Dextrobrompheniramine Maleate

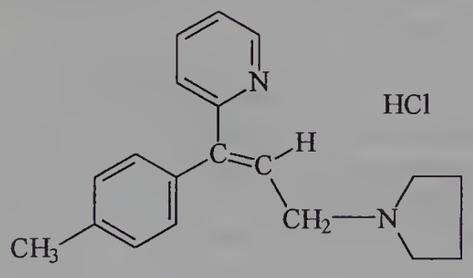
Dexbrompheniramine Maleate, USP. *Disomer®*. Like the chlorine congener, the antihistaminic activity exists predominantly in the dextroisomer and is of comparable potency.

Pyrrobutamine Phosphate. (*E*)-1-[4-(4-Chlorophenyl)-3-phenyl-2-butenyl]pyrr olidine diphosphate (*Pyronil*). The diphosphate occurs as a white crystalline powder that is soluble to the extent of 10% in warm water. Pyrrobutamine was investigated originally as the hydrochloride salt, but the diphosphate was absorbed more readily and completely. Clinical studies indicate that it is long acting with a comparatively slow onset of action. The feeble antihistaminic properties of several analogs point to the importance of having a planar ArC=CH-CH_2N unit and a pyrrolidino group as the side chain tertiary amine.[14]



Pyrrobutamine Phosphate

Triprolidine Hydrochloride, USP. (E)-2-[3-(1-Pyrrolidinyl)-1-p-tolylpropenyl]pyridine monohydrochloride monohydrate (Actidil). Triprolidine hydrochloride occurs as a white crystalline powder having no more than a slight, but unpleasant, odor. It is soluble in water and in alcohol, and its solutions are alkaline to litmus.



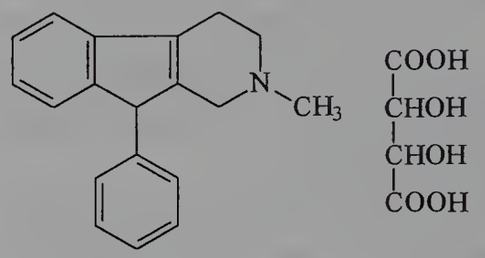
Triprolidine Hydrochloride

The activity is confined mainly to the geometric isomer in which the pyrrolidinomethyl group is trans to the 2-pyridyl group. Recent pharmacologic studies[30] confirm the high activity of triprolidine and the superlority of (E)- over corresponding (Z)-isomers as Hl-antagonists. At guinea pig ileum sites, the affinity of triprolidine for Hl-receptors was >1,000 times the affinity of its (Z)-partner. The relative potency of triprolidine is of the same order as that of dexchlorpheniramine. The peak effect occurs in ~3.5 hr after oral administration, and the duration of effect is ~12 hrs.

Phenindamine Tartrate, USP. 2,3,4,9-Tetrahydro-2-methyl-9-phenyl-1*H*-indeno[2,1-c; cbpyridine bitartrate. The hydrogen tartrate occurs as a creamy white powder, usually having a faint odor, and is sparingly soluble in water (1:40). A 2% aqueous solution has a *p*H of ~3.5. It is most stable in the *p*H range of 3.5 to 5.0 and is unstable in solutions of *p*H 7 or higher. Oxidizing substances or heat may cause isomerization to an inactive form. Structurally, phenindamine can be regarded as an unsaturated propylamine derivative in that the rigid ring system contains a distorted, trans alkene. Like the other commonly used antihistamines, it may produce drowsiness and sleepiness, but also it may cause a mildly stimulating action in some patients and insomnia when taken just before bedtime.[31]

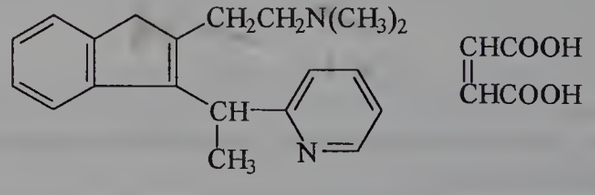
Usual adult dose: Oral, 25 mg/4–6 hr.

Dosage forms: Tablets.



Phenindamine Tartrate

Dimethindene Maleate. (±)2-[1-[2-[2-Dimethylamino)ethyl]inden-3-yl]ethy l]pyridine bimaleate (1:1) (*Forhistal Maleate*). The salt occurs as a white to off-white crystalline powder that has a characteristic odor and is sparingly soluble in water. This potent antihistaminic agent may be considered as a derivative of the unsaturated propylamines. The principal side effect is some degree of sedation or drowsiness. The antihistaminic activity resides mainly in the levorotatory isomer.[14]



Dimethindene Maleate

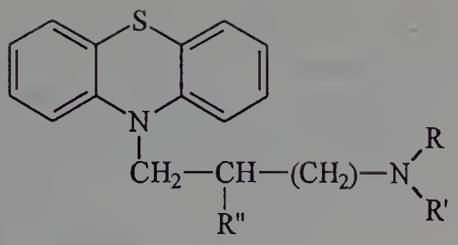


FIG. 21-11. General structure of the phenothiazines.

Phenothiazines

Beginning in the mid-1940s, several antihistaminic drugs have been discovered as a result of bridging the aryl units of agents related to the ethylenediamines. The search for effective antimalarials led to the investigation of phenothiazine derivatives in which the bridging entity is sulfur. In subsequent testing, the phenothiazine class of drugs was discovered to have not only antihistaminic activity, but also a pharmacologic profile of its own, considerably different from that of the ethylenediamines (Fig. 21-11). Thus began the era of the useful psychotherapeutic agent.[23,32]

The phenothiazine derivatives that display therapeutically useful antihistaminic actions contain a two- or three-carbon atom, branched alkyl chain between the ring system and terminal nitrogen atom. This differs significantly from the phenothiazine antipsychotic series in which an unbranched propyl chain is required. The phenothiazines with a three-carbon bridge between nitrogen atoms are more potent in vitro. Also, unlike the phenothiazine antipsychotics, the heterocyclic ring of the antihistamines is unsubstituted.[22]

The enantiomers of promethazine have been resolved and have similar antihistaminic and other pharmacologic properties as described below.[33] This is in contrast with studies of the pheniramines and carbinoxamine compounds in which the chiral center is closer to the aromatic feature of the molecule. Asymmetry appears to be of less influence on antihistaminic activity when the chiral center lies near the positively charged side chain nitrogen.

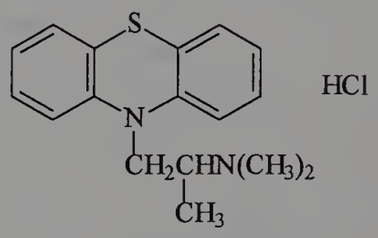
Promethazine, the parent member of this series, is moderately potent by present-day standards with prolonged action and pronounced sedative side effects. In addition to its antihistaminic action, it is a potent antiemetic, anticholingeric and sedating agent, and significantly potentiates the action of analgesic and sedative drugs.[23] The other members of this series display a similar pharmacologic profile and thus may cause drowsiness and so may impair the ability to perform tasks requiring alertness. Also concurrent administration of alcoholic beverages and other CNS depressants with the phenothiazines should be avoided.[23] In general, lengthening of the side chain and substitution of lipophilic groups in the 2-position of the aromatic ring results in compounds with decreased antihistaminic activity and increased psychotherapeutic properties.

While little pharmacokinetic data is available for the phenothiazine antihistamines, the metabolism of the close structural analogue promethazine has been studied in detail.[22] This compound undergoes mono and di-N-dealkylation, sulfur oxidation, aromatic oxidation at the 3-position to yield the phenol and N-oxidation. A number of these metabolites, particularly the phenol, may yield glucuronide conjugates. It is expected that the phenothiazine antihistamines would display similar metabolic profiles.

Promethazine Hydrochloride, USP. (±)10-[2-(Dimethylamino)propyl]phenothiazine monohydrochloride (*Phenergan*). The salt occurs as a white to faint yellow crystalline powder that is very soluble in water, in hot absolute alcohol, and in chloroform. Its aqueous solutions are slightly acid to litmus.

Usual adult dose: Oral, 12.5 mg/4–6 hr or 25 mg qd; IM or IV, 12.5–25 mg/4–6 hr.

Dosage forms: Syrup, tablets, injection, suppositories.

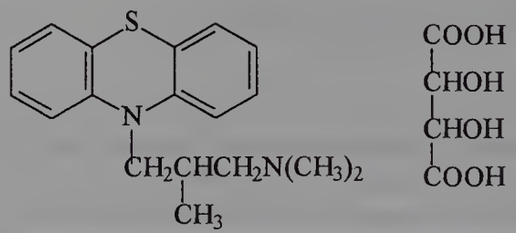


Promethazine Hydrochloride

Trimeprazine Tartrate, USP. (±)10-[3-(Di-methylamino)-2-methylpropyl]phenothiazine tartrate (*Temaril*). The salt occurs as a white to off-white crystalline powder that is freely soluble in water and soluble in alcohol. Its antihistaminic action is reported to be from 1.5 to 5 times that of promethazine. Clinical studies have shown it to have a pronounced antipruritic action. This action may be unrelated to its histamine-antagonizing properties.

Usual adult dose: Oral, 2.5 mg qid.

Dosage forms: Syrup and tablets.



Trimeprazine Tartrate

Methdilazine, USP. 10-[(1-Methyl-3-pyrrolidinyl)methyl]phenothiazine (*Tacaryl*). This compound occurs as a light tan crystalline powder that has a characteristic odor and is practically insoluble in water. Methdilazine, as the free base, is used in chewable tablets because its low solubility in water contributes to its tastelessness. Some local anesthesia of the buccal mucosa may be experienced if the tablet is chewed and not swallowed promptly.

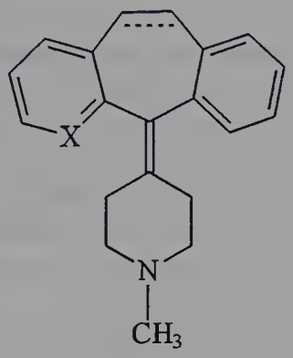
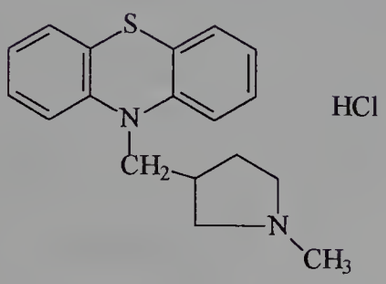


FIG. 21-12. General structure of the dibenzocycloheptenes/heptane.



Methdilazine
Methdilazine Hydrochloride

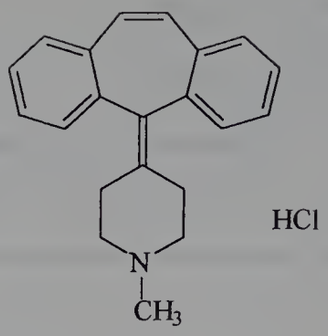
Methdilazine Hydrochloride, USP. 10-[(1-Methyl-3-pyrrolidinyl)methyl]phenothiazine monohydrochloride (*Tacaryl Hydrochloride*). The hydrochloride salt also occurs as a light tan crystalline powder having a slight characteristic odor. However, the salt is freely soluble in water and in alcohol.

The activity is similar to that of methdilazine and is administered orally for its antipruritic effect.

Dibenzocycloheptenes/Heptanes

The dibenzocycloheptene and heptane antihistamines may be regarded as phenothiazine analogues in which the sulfur atom has been replaced by an isosteric vinyl group (cyproheptadine) or a saturated ethyl bridge (azatadine), and the ring nitrogen replaced by an sp^2 carbon atom (Fig. 21-12). The two members of this are closely related in structure; azatadine is an aza (pyridyl) isostere of cyproheptadine in which the 10,11-double bond is reduced. The properties of each agent are detailed in the monographs below.

Cyproheptadine Hydrochloride, USP. 4-(5*H*-Dibenzo-[a,d]-cyclohepten-5-ylidene)-1-ethylpiperidine hydrochloride sesquihydrate (*Periactin*). The salt is slightly soluble in water and sparingly soluble in alcohol.



Cyproheptadine Hydrochloride

Cyproheptadine possesses both an antihistamine and an antiserotonin activity and is used as an antipruritic agent. Sedation is the most prominent side effect, and this is usually brief, disappearing after 3 or 4 days of treatment.

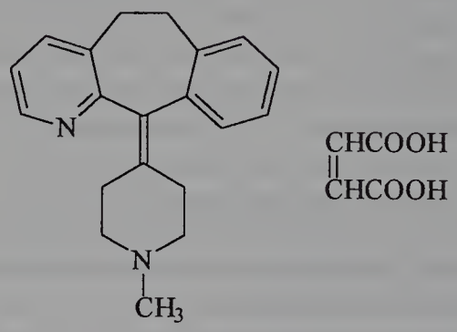
Usual adult dose: Oral, 4 mg tid or qid.

Dosage forms: Syrup and tablets.

Azatadine Maleate, USP. 6,11-Dihydro-11-(l-methyl-4-piperidylidene)-5*H*-benzo[5,6]cycloheptal[1,2-b]pyridine maleate (1:2) (*Optimine*). In early testing, azatadine exhibited more than three times the potency of chlorpheniramine in the isolated guinea pig ileum screen and more than seven times the oral potency of chlorpheniramine in protection of guinea pigs against a double lethal dose of intravenously administered histamine.[34] It is a potent, long-acting antihistaminic with antiserotonin activity. The usual dosage is 1 to 2 mg twice daily. Azatadine is available in 1-mg tablets.

Usual adult dose: Oral, 1–2 mg bid.

Dosage forms: Tablets.



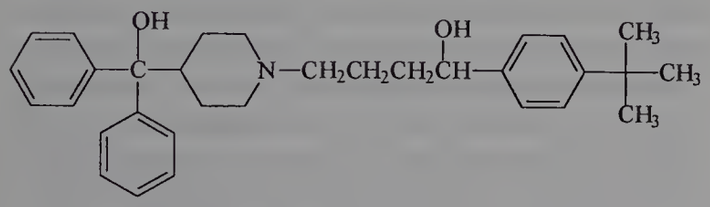
Azatadine Maleate

SECOND GENERATION H^1-ANTAGONIST DRUG CLASSES

The second generation antihistamines are more similar pharmacologically than structurally. As discussed earlier in this chapter, these compounds were developed as selective H_1-receptor antagonists with relatively high potency. Most of these compounds also produce prolonged antihistaminic effects as a result of slow dissociation from H_1-receptors, and

the formation of active metabolites with similar receptor binding profiles.[16] The second generation agents have little affinity for muscarinic, adrenergic or serotonergic receptors and therefore display a lower degree of side effects associated with antagonism at these receptors. But their affinities for these receptors is somewhat variable as indicated in the monographs below. Perhaps most importantly, all of these compounds are devoid of sedating effects at therapeutic concentrations due to poor CNS penetration, and possibly lowered affinities for central histaminic,[16] cholinergic and adrenergic receptors. While these compounds offer several advantages over the classical antihistamines, widespread use has revealed a number of therapeutic limitations. This is probably most true for terfenadine and astemizole which have been found to produce life-threatening arrhythmias when used concurrently with drugs that inhibit their metabolism. These drug interactions have been most evident with the imidazole antifungals ketoconazole, itraconazole and fluconazole, and the macrolides erythromycin, clarithromycin and troleandomycin which inhibit the metabolism of terfenadine and astemizole, resulting in elevated levels of the parent drugs which are proarrhythmic.[18] This adverse is evident by prolongation of QTc intervals. The chemical properties and pharmacological profiles of the individual second generation antihistamines, along with pharmacokinetic data and basic dosage information, is presented in the monographs that follow.

Terfenadine. Alpha-[4-(1,1-Dimethylethyl)phenyl]-4-(hydroxyd iphenylmethyl)-1-piperidinebutanol (*Seldane*) is a reduced butyrophenone derivative of an aminoalcohol-type anticholinergic agent. It occurs as a white to off-white crystalline powder that is soluble in alcohol and very slightly soluble in water.



Terfenadine

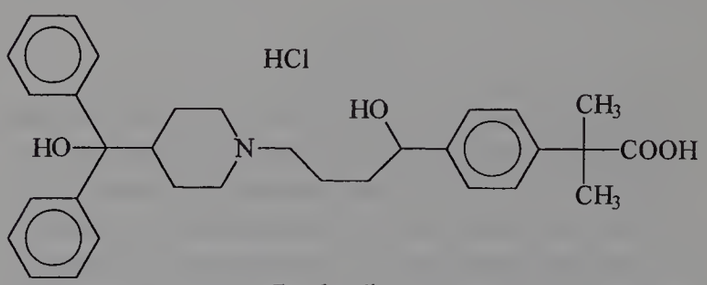
Terfenadine was developed during a search for new butyrophenone antipsychotic drugs as evident by the presence of the N-phenylbutanol substituent. It also contains a diphenylmethylpiperidine moiety analogous to that found in the piperazine antihistamines. Terfenadine is a selective, long-acting (>12 hr) H_1-antagonist with little affinity for muscarinic, serotonergic or adrenergic receptors. The histamine receptor affinity of this compound are believed to be related primarily to the presence of the diphenylmethylpiperidine moiety. The prolonged action results from very slow dissociation from these receptors.[18] The lack of anticholinergic, adrenergic, or serotonergic actions appears to be linked to the presence of the N-phenylbutanol substituent. This substituent also limits distribution of terfenadine to the CNS.[35,36]

Terfenadine is rapidly absorbed producing peak plasma levels in 1–2 hr. The drug undergoes significant first-pass metabolism, with the predominant metabolite being fexofenadine, an active metabolite resulting from methyl group oxidation. When drugs that inhibit this transformation, such as the imidazole antifungals and marolides, are used concurrently, terfenadine levels may rise to toxic levels, resulting in potentially fatal heart rhythm problems. Terfenadine is highly plasma protein bound (97%) and has a half-life of ~20 hr. Terfenadine is widely distributed in peripheral tissues, with highest concentrations in the liver. The major route of elimination of terfenadine and its metabolites is in the feces, and elimination is biphasic. The mean elimination half-life is 16–23 hr.[18]

Usual adult dose: Oral, 60 mg every 8 to 12 hr as needed.

Dosage form: Tablets.

Fexofenadine Hydrochloride. (+/−)-4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperinyl]butyl-α,α-dimethylbenzeneacetic acid (Allegra). This compound occurs as a white to off-white crystalline powder that is freely soluble in methanol and ethanol, slightly soluble in chloroform and water, and insoluble in hexane. This compound is marketed as a racemate and exists as a zwitterion in aqueous media at physiological pH.



Fexofenadine

Fexofenadine is a primary metabolite of terfenadine. It was developed based on studies that revealed when terfenadine's hepatic conversion to the fexofenadine was blocked by other drugs or disease, levels of the parent drug rise resulting in heart rhythm problems. Subsequent clinical trials demonstrated that fexofenadine was not only active and effective in allergic disorders, but less cardiotoxic than terfenadine. This led to the approval of fexofenadine as an alternative to relieve the symptoms of seasonal allergies.[37]

Fexofenadine is a selective peripheral H_1-receptor blocker that, like terfenadine, produces no clinically significant anticholinergic effects or alpha$_1$-adrenergic receptor blockade at therapeutic doses. No sedative or other CNS effects have been reported for this drug and animal studies indicate that fexofenadine does not cross the blood-brain barrier. In vitro studies also suggest that, unlike terfenadine, fexofenadine does not

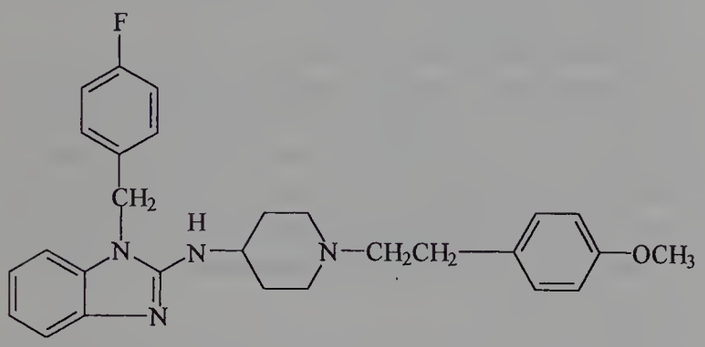
block potassium channels in cardiocytes. Furthermore in drug interaction studies no Prolongation of the QTc interval or related heart rhythm abnormalities were detected when administered concurrently with erythromycin or ketoconazole.[16,37]

Fexofenadine is rapidly absorbed after oral administration producing peak serum concentrations in ~2.5 hr Fexofenadine is 60% to 70% plasma protein bound. Unlike its parent drug, only 5% of the total dose of fexofenadine is metabolized. The remainder is excreted in the bile and urine and the mean elimination half-life is ~14 hr.[16,38]

Usual adult dose: Oral, 60 mg bid.

Dosage form: Capsules.

Astemizole, USP. 1-(4-Fluorobenzyl)-2-((1-(4-methoxyphenyl)-4-piperidyl)amino)benz imidazole (*Hismanal*). It is a white to slightly off-white powder that is insoluble in water, slightly soluble in ethanol and soluble in chloroform and methanol.



Astemizole

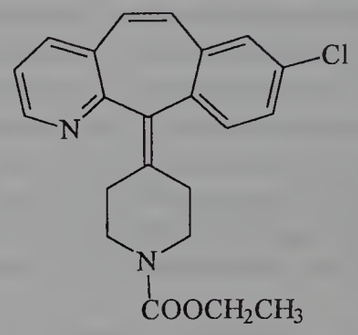
Astemizole was developed from a series of diphenylbutylpiperidine antihistamines in an effort to extend the duration of action.[16,39] During development, it was discovered that this compound produced little sedation or autonomic side effect. Astemizole is a selective and long-acting H_1-antagonist with little affinity for muscarinic, serotonergic, adrenergic receptors or H_2-receptors. The piperidino-aminobenzimidazole moiety appears to be required for H_1-receptor affinity, and contributes significantly to the persistent receptor binding that results in prolonged action. Astemizole is more potent and longer acting than terfenadine. It does not penetrate the CNS readily, thus sedation and other CNS side effects (dizziness, drowsiness, fatigue) are minimal. Astemizole also has no local anesthetic actions. It is used for seasonal allergic rhinitis and chronic urticaria. It has a slow onset of action (2 to 3 days).[40,41]

Astemizole is rapidly and completely absorbed orally and should be administered 1 hr before meals. Peak plasma levels are observed within 1 to 4 hr. Astemizole is widely distributed in peripheral tissues, with highest concentrations attained in the liver, pancreas, and adrenal glands. It undergoes extensive first-pass metabolism by processes, including aromatic hydroxylation, oxidative dealkylation, and glucuronidation. The main metabolites are desmethylastemizole, 6-hydroxy desmethylastemizole and norastemizole. The desmethyl metabolite has antihistaminic activity comparable to the parent drug and thus contributes to the prolonged duration of action. Astemizole is highly protein bound (96%) and has a plasma half-life of 1.6 days. The apparent half-life of the desmethyl metabolite ranges from 10 to 20 days, depending on frequency of dosing of the parent drug. The primary route of elimination is in the feces.[16]

Usual adult dose: Oral, 10 mg once daily.

Dosage form: Tablets.

Loratadine, USP. 4-(8-chloro-5,6-dihydro-11H-benzo [5,6] -cyclohepta [1,2-b] pyridin-11-ylidene-1-carboxylic acid ethyl ester. It is a white to off-white powder not soluble in water, but very soluble in acetone, alcohols, and chloroform.



Loratadine

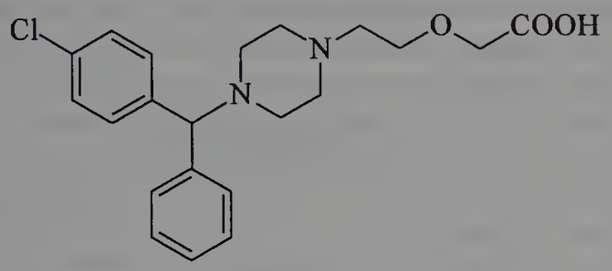
Loratadine is structurally related to the antihistamines azatadine and cyproheptadine. It differs from azatadine in that a neutral carbamate group has replaced the basic tertiary amino moiety, and the phenyl ring has been substituted with a chlorine atom. The replacement of the basic group with a neutral functionality is believed to preserve antihistaminic action while reducing CNS effects. Loratadine is also structurally related to a number of tricyclic antidepressants.[16,42]

Loratadine is a selective peripheral H_1-antagonist with a receptor binding profile like the other members of this series, except that it has more antiserotonergic activity. Thus it produces no substantial CNS or autonomic side effects. Loratadine displays potency comparable to astemizole and greater than terfenadine.[16,40]

Loratadine is rapidly absorbed after oral administration producing peak plasma levels in ~1.5 hr. This drug is extensively metabolized, primarily to the descarboethoxy metabolite, which retains some antihistaminic activity. Both the parent drug and metabolite have elimination half-lives ranging from 8–15 hr. The metabolite is excreted renally as a conjugate.

Usual adult dose: Oral, 10 to 40 mg daily.

Cetirizine, USP. [2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]etho xy]aceti c acid (*Zyrtec*). This compound is a racemic compound available as a white crystalline powder that is water soluble.



Cetirizine

Cetirizine is the primary acid metabolite of hydroxyzine resulting from complete oxidation of the primary alcohol moiety. This compound is zwitterionic and relatively polar and thus does not penetrate the blood-brain barrier readily. Prior to its introduction in the United States, cetirizine was one of the most widely prescribed H_1-antihistamines in Europe. It is highly selective in its interaction with various hormonal binding sites and highly potent terfenadine as well.[43,44]

The advantages of this compound appear to be once-daily dosing, a rapid onset of activity, minimized CNS effects, and a lack of clinically significant effects on cardiac rhythm when administered with imidazole antifungals and macrolide antibiotics. The onset of action is within 20 to 60 min in most patients. Cetirizine produces qualitatively different effects on psychomotor/psychophysical functions compared to the first generation antihistamines. However the most common adverse reaction associated with cetirizine is dose-related somnolence and thus patients should be advised that cetirizine may interfer with the performance of certain psychomotor/psychophysical activities. Other effects of this drug include fatigue, dry mouth, pharyngitis, and dizziness. Because the drug is primarily eliminated by a renal route, its adverse reactions may be more pronounced in individuals suffering from renal insufficiency. No cardiotoxic effects, such as QT prolongation, are observed with the new drug when used at its recommended or higher doses or when coadministered with imidazole antifungals and macrolide antibiotics. However, other typical drug interactions of H_1-antihistamines apply to cetirizine. Concurrent use of this drug with alcohol and other CNS depressant should be avoided.[43,44]

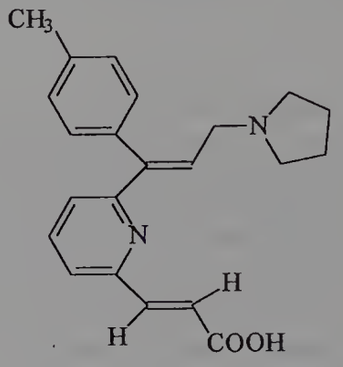
Dose proportional Cmax values are achieved within 1 hr of oral administration of cetirizine. Food slows the rate of cetirizine absorption but does not affect the overall extent. Consistent with the polar nature of this carboxylic acid drug, <10% of peak plasma levels has been measured in the brain. Cetirizine is not extensively metabolized and ~70% of a 10 mg oral dose is excreted in the urine (>80% as unchanged drug) and 10% recovered in the feces. The drug is highly protein bound (93%) and has a terminal half-life of 8.3 hr. The clearance of cetirizine is reduced in elderly subjects as well as in renally and hepatically impaired patients.[45]

Usual adult dose: Oral, 5–10 mg once daily.

Dosage form: Tablets.

Acrivastine, USP. (E,E)-3-[6-[1-(4-methylphenyl)-3-(1-pyrrolidinyl)-1-propenyl- 2-pyridiny l]-2-propenoic acid (*Semprex*). This is fixed combination product of the antihistamine acrivastine (8 mg) with the decongestant pseudoephedrine (60 mg). Acrivastine is an odorless, white to pale cream crystalline powder that is soluble in chloroform and alcohol, slightly soluble in water.

Acrivastine is an analogue of triprolidine containing a carboxyethenyl moiety at the 6-position of the pyridyl ring. Acrivastine shows antihistaminic potency and duration of action comparable to triprolidine. Unlike triprolidine, acrivastine does not display significant anticholinergic activity



Acrivastine

at therapeutic concentrations. Also, the enhanced polarity of this compound resulting from carboxyethenyl substitution limits BBB penetration and thus this compound produces less sedation than triprolidine.[42,46]

Limited pharmacokinetic data is available for this compound. Orally administered drug has a half-life of ~1.7 hr and a total body clearance of 4.4 ml/min/kg. The mean peak plasma concentrations are reported to vary widely, and the drug appears to penetrate the CNS poorly. The metabolic fate of acrivastine has not been reported.

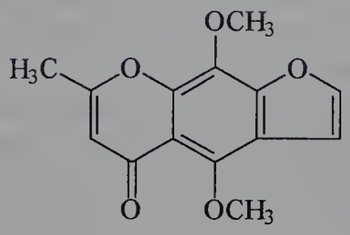
Usual adult dose: Oral, 8 mg/60 mg/3–4 times daily.

Dosage form: Tablets.

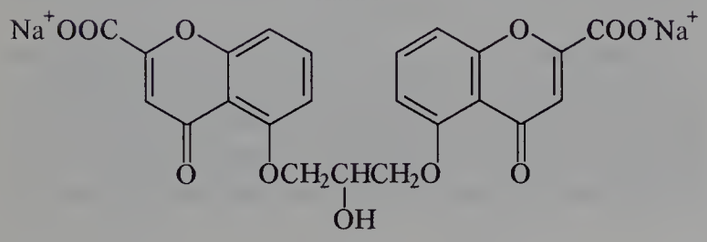
INHIBITION OF HISTAMINE RELEASE

The discovery of the bronchodilating activity of the natural product khellin led to the development of the bis(chromones) are compounds that inhibit the release of histamine and other mediators of inflammation. The first therapeutically significant member of this class was cromolyn sodium.[31,49] Further research targeting more effective agents resulting in the introduction of nedocromil more recently. The structures, chemical properties, pharmacologic profiles, as well as dos-

age data for these agents are provided in the monographs that follow.



Khellin



Cromolyn Sodium

Cromolyn Sodium, USP. Disodium 1,3-bis(2-carboxychromon-5-yloxy)-2-hydroxypropane (*Intal*). The salt is a hygroscopic, white, hydrated crystalline powder that is soluble in water (1::10). It is tasteless at first, but leaves a very slightly bitter aftertaste. The pK_a of cromolyn is 2.0. Cromolyn belongs to a completely novel class of compounds and bears no structural relationship to other commonly used antiasthmatic compounds. Unlike its naturally occurring predecessor (khellin), cromolyn is not a smooth-muscle relaxant or a bronchodilator. It has no intrinsic bronchodilator, antihistaminic, or antiinflammatory action.[47]

Cromolyn inhibits release of histamine, leukotrienes, and other potent substances from mast cells during allergic responses. Apparently, its action is on the mast cell after the sensitization stage but before the antigen challenge. It does not seem to interfere with the antigen-antibody reaction, but it seems to suppress the responses to this reaction.

Although growing evidence[29] indicates that the mechanism of action is not all mast cell related, the benefits of the drug in asthma are exclusively prophylactic. It is of no value after an asthmatic attack has begun (status asthmaticus). Cromolyn is also indicated for the prevention and treatment of the symptoms of allergic rhinitis. In order for cromolyn to be effective it must be administered at least 30 min prior to antigen challenge, and administered at regular intervals (see dosing information below). Overuse of cromolyn results in tolerance.

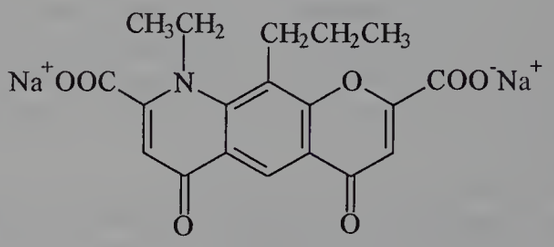
Usual adult dose: Intranasal, 5.2 mg (one metered spray) in each nostril three or four times daily at regular intervals; ophthalmic, one drop of a 2% to 4% solution four to six times daily.

Dosage forms: Cromolyn sodium for nasal insufflation USP; Cromolyn sodium nasal solution, USP; Cromolyn sodium ophthalmic solution

Nedocromil Sodium, USP. Disodium 9-ethyl-6,9-dihydro-4,6-dioxo-10-propyl-4H-pyrano[3,2-g]quinoline-2,8-dicarboxylate (Tilade). Nedocromil is structurally related to cromolyn and displays similar, but broader pharmacological actions. This drug prevents the release of inflammatory, chemotactic, and smooth muscle contracting mediators from the inflammatory cells implicated in asthma, including neutrophils, eosinophils, monocytes, platelets, and mast cells. Nedocromil also suppresses neuronal reflexes, including C-fiber response in the lung implicated with bronchoconstriction, and blocks the immunologic and nonimmunologic activation of mast cells. As a result of these actions, this drug inhibits not only the acute bronchoconstrictor response to inhaled irritants, but also the delayed asthmatic or inflammatory response. The superiority of nedocromil over cromolyn in the treatment of asthma has been established in an number of comparative clinical trials.

Usual adult dose: Intranasal, 14 mg (two inhalations) four times daily at regular intervals.

Dosage forms: Aerosol (metered-dose inhaler).



Nedocromil Sodium

HISTAMINE H_2-RECEPTOR ANTAGONISTS

Drugs whose pharmacological action primarily involves antagonism of the action of histamine at its H_2-receptors find therapeutic application in the treatment of acid-peptic disorders ranging from heartburn to peptic ulcer disease, Zollinger-Ellison syndrome, gastroesophageal reflux disease (GERD), acute stress ulcers, and erosions.[48,49]

PEPTIC ACID SECRETION

A characteristic feature of the mammalian stomach is its ability to secrete acid as part of its involvement in digesting food for absorption later in the intestine. The presence of acid and proteolytic pepsin enzymes, whose formation is facilitated by the low gastric pH, is generally assumed to be required for the hydrolysis of proteins and other foods.

The acid secretory unit of the gastric mucosa is the parietal (oxyntic) cell. Parietal cells contain a hydrogen ion pump, a unique H_3O^+-K^+–ATPase system that secretes H_3O^+ in exchange for the uptake of K^+ ion. Secretion of acid by gastric parietal (oxyntic) cells is regulated by the actions of various mediators at receptors located on the basolateral membrane, including histamine agonism of H_2-receptors

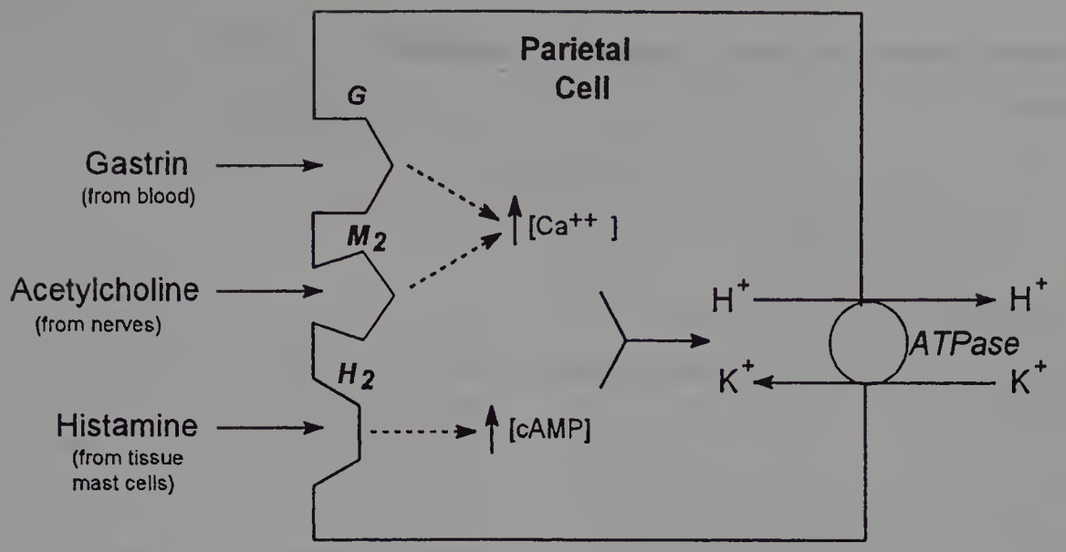


FIG. 21-13. *Simplified diagram of the gastric parietal cell illustrating the secretion of acid and its various hormonal regulators. (Adapted from Spencer, C.M. and Faulds, F.: Drugs 48:405, 1994.)*

(cellular), gastrin activity at G-receptors (blood), and acetylcholine at M_2-muscarinic receptors (neuronal) (Fig. 21-13).

PEPTIC ULCER DISEASE

Peptide ulcer disease (PUD) is a group of upper gastrointestinal tract disorders that result from the erosive action of acid and pepsin.[50] Duodenal ulcer (DU) and gastric ulcer (GU) are the most common forms, although PUD may occur in the esophagus or small intestine. Factors that are involved in the pathogenesis and recurrence of PUD include hypersecretion of acid and pepsin and GI infection by *Helicobacter pylori*, a gram-negative spiral bacterium. *H. pylori* has been found in virtually all patients with DU and ~75% of patients with GU. Some risk factors associated with recurrence of PUD include cigarette smoking, chronic use of ulcerogenic drugs (e.g., NSAIDs), male gender, age, alcohol consumption, emotional stress, and family history.

The goals of PUD therapy are to promote healing, relieve pain, and prevent ulcer complications and recurrences. Medications used to heal or reduce ulcer recurrence include antacids, histamine H_2-receptor antagonists, protective mucosal barriers, proton pump inhibitors, prostaglandins, and bismuth salt/antibiotic combinations.

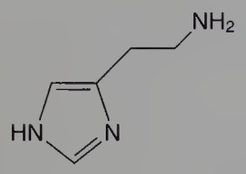
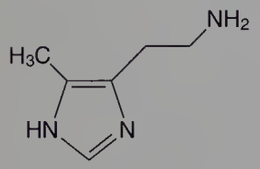
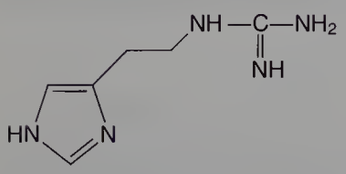
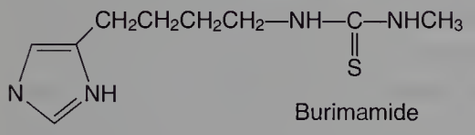
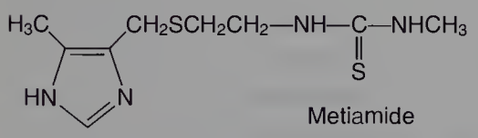
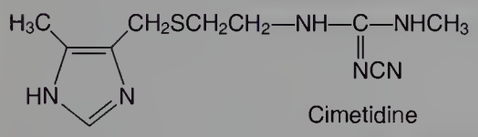
STRUCTURAL DERIVATION

A review of the characterization and development of histamine H_2-receptor antagonists reveals a classic medicinal chemistry approach to problem-solving.[51] Structural evolution of the first discovered, clinically useful H_2-antagonist, cimetidine, is depicted in Table 21-1. Methylation of the 5-position of the imidazole heterocycle of histamine produces a selective agonist at atrial histamine receptors (H_2). The guanidino analogue of histamine possesses a small degree of antagonist activity to the acid-secretory actions of histamine. Increasing the length of the side chain from two to four carbons coupled with replacement of the strongly basic guanidino group by the neutral methyl thiourea function leads to burimamide, the first antagonist to be developed lacking detectable agonist activity in laboratory assays. The low potency of burimamide is postulated to be related to its nonbasic, electron-releasing side chain which favors the nonpharmacophoric N^{π}-H imidazole tautomer compared to the basic, electron-withdrawing side chain in histamine, which predominantly presents the higher affinity N^{τ}-H imidazole tautomer to the receptor (Fig. 21-1). Insertion of an electronegative thioether function in the side chain in place of a methylene group favors the N^{τ}-tautomer and introduction of the 5-methyl group favors H_2-receptor selectivity leads to metiamide, a H_2-blocker of higher potency and oral bioavailability compared to burimamide. Toxicity associated with the thiourea structural feature is eliminated by replacing the thiourea sulfur with a cyano-imino function to produce cimetidine.

Introduction of cimetidine into human medicine revealed that it is an effective gastric antisecretory agent effective in promoting the healing of duodenal ulcers. However, cimetidine is not without a number of limitations. Because it is short-acting it requires a frequent dosing schedule in man and, in addition, its selectivity is poor. Cimetidine has antiandrogenic activity, which can lead to gynecomastia, and it inhibits the cytochrome P-450 mixed function oxygenase metabolizing enzyme system in the liver, an action which potentiates the effects of drugs whose clearance also depends upon biotransformation by this system. Cimetidine also causes confusional states in some elderly patients. Subse-

TABLE 21-1

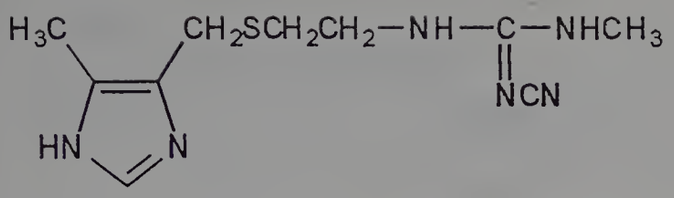
STRUCTURAL DERIVATION OF HISTAMINE H_2-RECEPTOR LIGANDS

Structural Modification	*Histamine Receptor Actions*
 Histamine	$H_1 \approx H_2$ receptor agonism
 4-Methylhistamine	$H_2 > H_1$ receptor agonism
 N^a-Guanylhistamine	Weak H_2-antagonist (partial agonist)
 Burimamide	Full H_2-antagonist of low potency and poor oral bioavailability
 Metiamide	Full H_2-antagonist of high potency, improved oral bioavailability, thiourea-toxicity
 Cimetidine	Full H_2-antagonist—high potency, good oral bioavailability, low toxicity

quent development of additional drugs of this class indicate that a great deal of structural latitude is available in the design of H_2-antagonists.[52]

Examination of the structural features of H_2-antagonists that came after cimetidine makes it obvious that the imidazole ring of histamine is not required for competitive antagonism of histamine at H_2-receptors. Other heterocycles may be used and may, in fact, enhance both potency and selectivity of H_2-receptor antagonism. However, if the imidazole ring is used, the Nτ-H tautomer should be the predominant species for maximal H_2-antagonist activity. The electronic effects of the ring substituents and side chain structural feature determine the tautomerism. Separation of the ring and the nitrogen group with the equivalent of a four-carbon chain appears to be necessary for optimal antagonist activity. The isosteric thioether link is present in the four agents currently marketed in the United States. The terminal nitrogen-containing functionality should be a polar, nonbasic substituent for maximal antagonist activity. Groups that are positively charged at physiologic *p*H appear to confer agonist activity. In general, antagonist activity varies inversely with the hydrophilic character of the nitrogen group. However, the hydrophilic group, 1,1-diaminonitroethene, found in ranitidine and nizatidine is an exception in that it is much more active than is predicted by relative solubility effects.

Cimetidine, USP. N''-Cyano-N-methyl-N′-[2-[[5-methylimidazol-4-yl)methyl]-thio]e thyl]-guanidine, Tagamet, is a colorless crystalline solid that is slightly soluble in water (1.14% at 37°C). The solubility is greatly increased with the addition of dilute acid to protonate the imidazole ring (apparent pK_a of 6.8). At *p*H 7, aqueous solutions are stable for at least 7 days. Cimetidine is a relatively hydrophilic molecule having an octanol/water partition coefficient of 2.5.



Cimetidine

Cimetidine reduces the hepatic metabolism of drugs biotransformed by the cytochrome P-450 mixed oxidase system delaying elimination and increasing serum levels of these drugs. Concomitant therapy of patients with cimetidine and drugs metabolized by hepatic microsomal enzymes, particularly those of low therapeutic ratio or in patients with renal or hepatic impairment, may require dosage adjustment. Table 21-2 provides a complication of drugs whose combination therapy with cimetidine may result in their increased pharmacologic effects or toxicity.[53] Antacids interfere with cimetidine absorption and should be administered at least one hour before or after a cimetidine dose.

Cimetidine has a weak antiandrogenic effect. Gynecomastia in patients treated for ≥ 1 month may occur.

Cimetidine exhibits high oral bioavailability (60% to 70%) and an plasma half-life of ~2 hr, which is increased in renal and hepatic impairment and in the elderly. Approximately 30% to 40% of a cimetidine dose is metabolized (S-oxidation, 5-CH_3 hydroxylation), and the parent drug and metabolites are eliminated primarily by renal excretion.

TABLE 21-2

CIMETIDINE DRUG INTERACTIONS

Benzodiazepines
Caffeine
Calcium channel blockers
Carbamazepine
Chloroquine
Labetalol
Lidocaine
Metoprolol
Metronidazole
Moricizine
Pentoxifylline
Phenytoin
Propafenone
Propranolol
Quinidine
Quinine
Sulfonylurea
Tacrine
Theophylline
Triamterene
Tricyclic antidepressants
Valproic acid
Warfarin

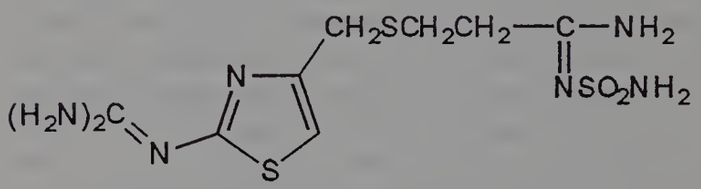
Usual adult oral dose:

- Duodenal ulcer—treatment: 800 to 1,200 mg once to four times a day with meals and at bedtime; maintenance: 400 mg once daily
- Benign gastric ulcer—800 to 1,200 once to four times daily
- Hypersecretory condition—1,200 to 2,400 mg four times daily
- Heartburn—200 mg (2 OTC tablets) up to twice daily

Usual pediatric dose: Oral, 20 to 40 mg (base) per kilogram of body weight four times a day, with meals and at bedtime.

Dosage forms: Tablet (200, 300, 400, 800 mg), liquid (300 mg/5 ml), injection (300 mg/2 and 50 ml).

Famotidine, USP. N′-(Aminosulfonyl)-3-[[[2[(diaminomethylene)-amino]-4-thiazolyl ; cb-methyl]thio]propanimidamide, Pepcid, which utilizes a thiazole bioisostere of the imidazole heterocycle, is a white to pale yellow crystalline compound that is very slightly soluble in water and practically insoluble in ethanol.



Famotidine

Famotidine is a competitive inhibitor of histamine H_2-receptors and inhibits basal and nocturnal gastric secretion as well as secretion stimulated by food and pentagastrin. Its current labeling indications are for the short-term treatment of duodenal and benign gastric ulcers, gastroesophogeal reflux disease (GERD), pathologic hypersecretory conditions (e.g., Zollinger-Ellison syndrome), and heartburn (OTC only).

No cases of gynecomastia, increased prolactin levels, or impotence have been reported, even at the higher dosage levels used in patients with pathologic hypersecretory conditions. Studies with famotidine in humans, in animal models, and in vitro have shown no significant interference with the disposition of compounds metabolized by the hepatic microsomal enzymes (e.g., cytochrome P-450 system).

Famotidine is incompletely absorbed (40% to 45% bioavailability). The drug is eliminated by renal (65% to 70%) and metabolic (30% to 35%) routes. Famotidine sulfoxide is the only metabolite identified in humans. The effects of food or antacid on the bioavailability of famotidine are not clinically significant.

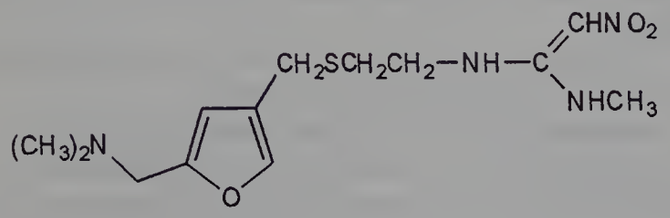
Usual adult oral dose:

- Duodenal ulcer—treatment: 40 mg once to twice a day at bedtime, maintenance: 20 mg once daily at bedtime
- Benign gastric ulcer—40 once daily

- Hypersecretory condition—80 to 640 mg four times daily,
- Heartburn—10 mg (1 OTC tablet) for relief or 1 hr before a meal for prevention

Dosage forms: Tablet (20 and 40 mg), oral suspension (40 mg/5 ml), injection (10 mg/ml).

Ranitidine, USP. N-[2-[[[5-[Dimethylaminomethyl]-2-furanyl]-methyl]thio]-ethyl] - N′-methyl - 2 - nitro-1,1-ethenediamine, Zantac, is an aminoalkyl furan derivative with pK_a values of 2.7 (side chain) and 8.2 (dimethylamino). It is a white solid. The hydrochloride salt is highly soluble in water.



Ranitidine

Bioavailability of an oral dose of ranitidine is ~50% to 60% and is not significantly affected by the presence of food. Some antacids may reduce ranitidine absorption and should not be taken within one hour of administration of the H_2-blocker. The plasma half-life of the drug is 2 to 3 hr, and it is excreted along with its metabolites in the urine. Three metabolites, ranitidine N-oxide, ranitidine S-oxide, and desmethyl ranitidine, have been identified. Ranitidine is only a weak inhibitor of hepatic cytochrome P-450 mixed function oxidase system.

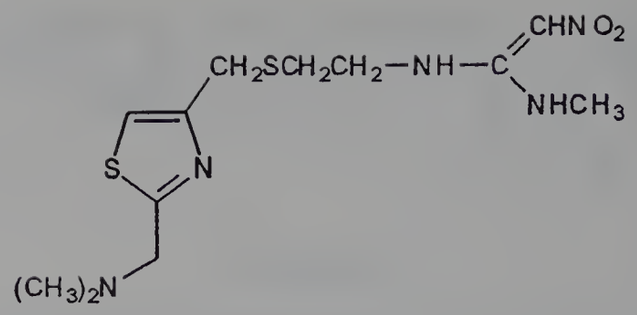
In addition to being available in a variety of dosage forms as the hydrochloride salt, ranitidine is also available as a bismuth citrate salt for use with the macrolide antibiotic clarithromycin in treating patients with an active duodenal ulcer associated with *H. pylori* infection. The eradication of *H. pylori* has been demonstrated to reduce the risk of duodenal ulcer recurrence.

Usual adult oral dose:

- Duodenal ulcer—treatment: 200 to 3,000 mg once to twice a day, maintenance: 150 mg once daily,
- Benign gastric ulcer—300 once daily,
- Hypersecretory condition—300 to 6,000 mg twice or more times daily,

Dosage forms: Tablets (150 and 300 mg of HCL salt), syrup (15 mg/ml as HCL salt), injection (0.5 and 25 mg/ml as HCL salt).

Nizatidine. N-[2-[[[2-[(Dimethylamino)methyl]-4-thiazolyl]-methyl]-thio]-ethyl] - N′-methyl - 2 - nitro-1,1-ethenediamine, Axid, is an off-white to buff colored crystalline solid that is soluble in water, alcohol and chloroform. The pK_a's of the drug in water are 2.1 (side chain) and 6.8 (dimethylamino).



Nizatidine

Nizatidine has excellent oral bioavailability (>90%). The effects of antacids or food on its bioavailability are not clinically significant. The elimination half-life is 1 to 2 hr. It is excreted primarily in the urine (90%) and mostly as unchanged drug (60%). Metabolites include nizatidine sulfoxide (6%), N-desmethylnizatidine (7%), and nizatidine N_2-oxide (dimethylaminomethyl function). Nizatidine has no demonstrable antiandrogenic action or inhibitory effects on cytochrome P-450-linked drug-metabolizing enzyme system.

Usual adult oral dose:

- Duodenal ulcer—treatment: 300 mg once to twice a day, maintenance: 150 mg once daily,
- Hypersecretory condition—150 mg twice daily,

Dosage forms: Capsules (150 and 300 mg).

OTHER ANTIULCER THERAPIES

Proton Pump Inhibitors

The final step in acid secretion in the parietal cell is the extrusion (''pumping'') of protons. The membrane pump, a H^+/K^+-ATPase, catalyzes the exchange of hydrogen ions for potassium ions. Inhibition of this proton pump acts beyond the site of action of second messengers, e.g., calcium ion and cyclic AMP, and is independent of the action of secretogogues histamine, gastrin, and acetylcholine. Thus, acid pump inhibitors block basal and stimulated secretion.

In 1972, a group of Swedish medicinal chemists discovered that certain pyridylmethyl benzimidazole sulfides possessed potent gastric acid (proton) pump inhibitory (PPI) activity.[54] The benzimidazole PPIs are not the active inhibitor of the H^+/K^+-ATPase but are transformed within the acid compartment of the parietal cell to an inhibitor molecule which reacts covalently with an essential thiol (SH) function on the enzyme. In vitro, the benzimidazoles are reversibly transformed in acidic media to a sulfenamide which can react

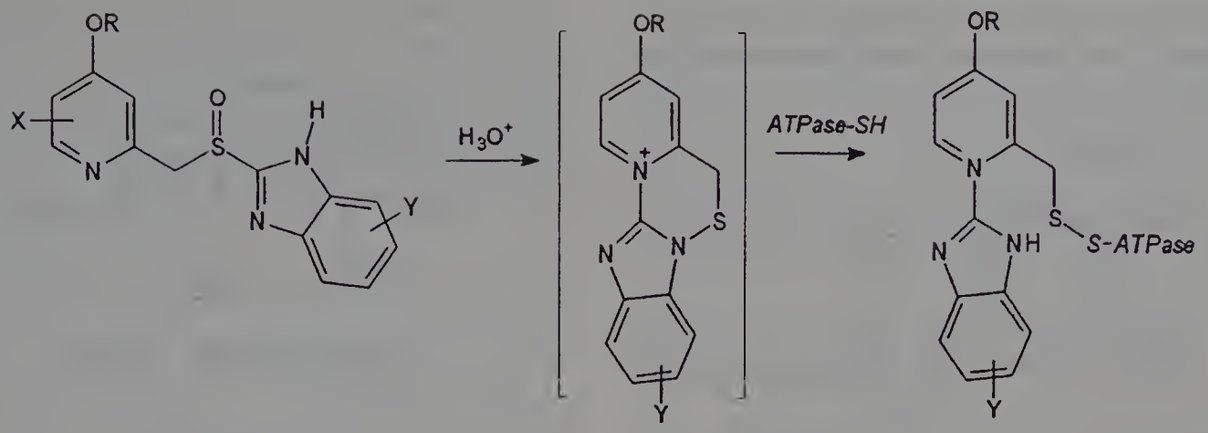
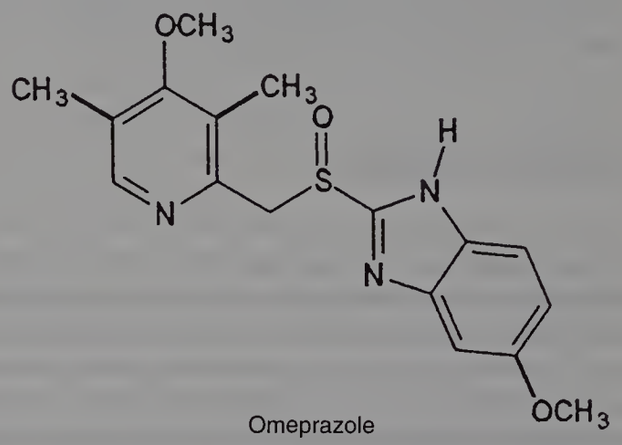


FIG. 21-14. *Mechanism of action of PPIs.*

with thiols to establish a disulfide link between inhibitor and pump enzyme (Fig. 21-14).[55]

The PPIs inhibit both basal and stimulated gastric acid secretion. Unlike the H_2-blockers, the PPIs inhibit daytime and nocturnal acid secretion regardless of whether they are administered in the morning or the evening. Gastric acid inhibition is also similar when these drugs are administered before or after a meal. The PPIs have no effect on postprandial digestive function or gastric emptying. The PPIs are more effective in the short term than the H_2-blockers in healing duodenal ulcers and erosive esophagitis and can heal esophagitis resistant to treatment with the H_2-blockers. In addition, the benzimidazole PPIs have antimicrobial activity against *H. pylori* and therefore possess efficacy in treating gastric ulcers or with one or more antimicrobials in eradicating infection by this organism. Two members of the benzimidazole class, omeprazole and lansoprazole, have been approved for marketing in the United States.

Omeprazole. 5-Methoxy-2(((4-methoxy-3,5-dimethyl-2-pyridinyl)-me thyl)sulfinyl)-lH-benzimidazole, Losec, is a white to off-white crystalline powder that has very slight solubility in water. Omeprazole is an amphoteric compound (pyridine N, pK_a 3.97, benzimidazole N-H, pK_a 8.70) and, consistent with the proposed mechanism of action of the substituted benzimidazoles, it is acid labile. Hence, the omeprazole product is formulated as delayed-release capsules containing enteric coated granules. The absolute bioavailability of orally administered omeprazole is 30% to 40% related to substantial first-pass biotransformation. The drug has a plasma half life of ~1 hr. The majority (77%) of an oral dose of omeprazole is excreted in the urine as metabolites having insignificant antisecretory activity (primarily hydroxy-omeprazole and its corresponding carboxylic acid salt). The drug has a plasma half-life of ~1 hr. The antisecretory actions of omeprazole persist for 24 to 72 hr, long after the drug has disappeared from plasma, an observation consistent with it suggested mechanism of action involving irreversible inhibition of the proton pump H^+/K^+-ATPase.[56]



Omeprazole

Omeprazole is approved for the treatment and reduction of risk of recurrence of duodenal ulcer, GERD, gastric ulcer, and pathological hypersecretory conditions.

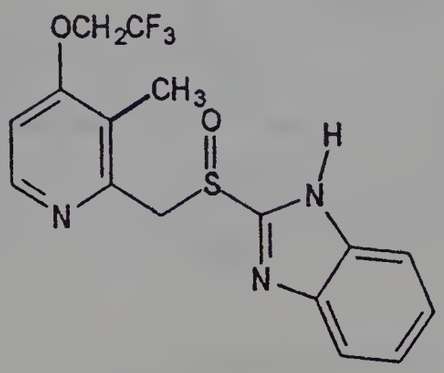
Usual adult dose: Oral, 20 mg once daily.

Dosage form: Delayed-release capsules containing 20 mg of omeprazole in enteric coated granules.

Lansoprazole. 2-[[[3-Methyl-4-(2,2,2-trifluoroethoxy)-2-py ridyl]methyl]sulfinyl]-benzimidazole, Prevacid, is a white to brownish-white, odorless, crystalline powder that is practically insoluble in water. Like omeprazole, lansoprazole is essentially a prodrug that, in the acidic biophase of the parietal cell, forms an active metabolite that irreversibly interacts with the target ATPase of the pump. Lansoprazole must be formulated as encapsulated enteric-coated granules for oral administration to protect the drug from the acidic environment of the stomach.

In the fasting state, ~80% of a dose of lansoprazole, compared to ~50% with omeprazole, reaches systemic circulation, where it is 97% bound to plasma proteins. The drug is

metabolized in the liver (sulfone and hydroxy metabolites) and excreted in bile and urine with a plasma half-life of ~1.5 hr.[57]



Lansoprazole

Usual adult dose: Daily oral dose administered before breakfast: duodenal ulcer, 15 mg once daily; erosive esophagitis, 30 mg; Zollinger-Ellison syndrome, 60 mg.

Dosage form: Delayed-release capsules containing 15 and 30 mg of lansoprazole in enteric-coated granules.

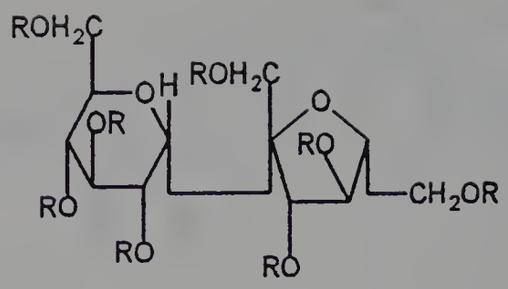
Chemical Complexation

The sulfate esters and sulfonate derivatives of polysaccharides and lignin form chemical complexes with the enzyme pepsin. These complexes have no proteolytic activity. Because polysulfates and polysulfonates are poorly absorbed from the gastrointestinal tract, specific chemical complexation appears to be a desirable mechanism of pepsin inhibition. Unfortunately, these polymers are also potent anticoagulants.

The properties of chemical complexation and anticoagulant action are separable by structural variation. In a comparison of selected sulfated saccharides of increasing number of monosaccharide units, from disaccharides through starch-derived polysaccharides of differing molecular size, three conclusions are supported by the data: (a) the anticoagulant activity of sulfated saccharide is positively related to molecular size; (b) anticoagulant activity is absent in the disaccharides; and (c) the inhibition of pepsin activity and the protection against experimentally induced ulceration is dependent on the degree of sulfation and not on molecular size.

The readily available disaccharide, sucrose, has been used to develop a useful antiulcer agent, sucralfate.

Sucralfate. 3,4,5,6-Tetra-(polyhydroxyaluminum)-α-D-glucopyranosyl sulfate-2,3,4,5-tetra-(polyhydroxyaluminum)-β-D-fructofuranosid e sulfate, Carafate, is the aluminum hydroxide complex of the octasulfate ester of sucrose. It is practically insoluble in water and soluble in strong acids and bases. It has a pK_a value of 0.43 to 1.19.



$R = SO_3[Al_2(OH)_5 . (H_2O)_2]$

Sucralfate

Sucralfate is minimally absorbed from the gastrointestinal tract and thus exerts its antiulcer effect through local rather than systemic action. It has negligible acid-neutralizing or buffering capacity in therapeutic doses.

Its mechanism of action has not been established. Studies suggest that sucralfate binds preferentially to the ulcer site to form a protective barrier that prevents exposure of the lesion to acid and pepsin. In addition, it adsorbs pepsin and bile salts. Either would be very desirable modes of action.

The product labeling states that the simultaneous administration of sucralfate may reduce the bioavailability of certain agents (e.g., tetracycline, phenytoin, digoxin, or cimetidine). It further recommends restoration of bioavailability by separating administration of these agents from that of sucralfate by two hours. Presumably sucralfate binds these agents in the gastrointestinal tract.

The most frequently reported adverse reaction to sucralfate is constipation (2.2%). Antacids may be prescribed as needed, but should not be taken within one-half hour before or after sucralfate.

Usual adult dose: Oral, 1 g four times a day on an empty stomach.

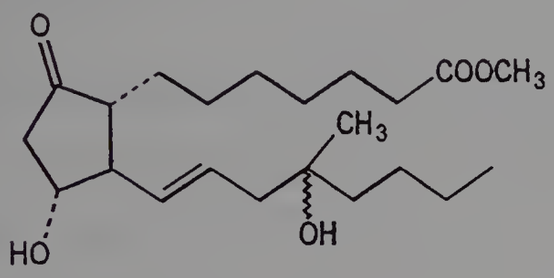
Dosage form: 1-g sucralfate tablets.

Prostaglandins

The prostaglandins are endogenous 20-carbon unsaturated fatty acids biosynthetically derived from arachidonic acid. These bioactive substances and their synthetic derivatives have been of considerable research and development interest as potential therapeutic agents because of their widespread physiologic and pharmacologic actions on the cardiovascular system, gastrointestinal smooth muscle, the reproductive system, the nervous system, platelets, kidney, the eye, etc.[58] Prostaglandins of the E, F, and I series are found in significant concentrations throughout the gastrointestinal tract. The gastrointestinal actions of the prostaglandins include inhibition of basal and stimulated gastric acid and pepsin secretion in addition to prevention of ulcerogen or irritant-induced gross mucosal lesions of the stomach and intestine (termed *cytoprotection*). The prostaglandins are capable of both

stimulation (PGFs) and inhibition (PGEs and PGIs) of intestinal smooth muscle contractility and accumulation of fluid and electrolytes in the gut lumen (PGEs). Therapeutic application of the natural prostaglandins in the treatment of gastrointestinal disorders is hindered by their lack of pharmacologic selectivity coupled with a less than optimal biodisposition profile.

Misoprostol. (±)-methyl 11α, 16-dihydroxy-16-methyl-9-oxoprost-13*E*-en-1-oate. This is a semisynthetic derivative of PGE_1 that derives some pharmacologic selectivity as well as enhanced biostability from its 16-methyl, 16-hydroxy structural features. Misoprostol exhibits both antisecretory and cytoprotectant effects characteristic of the natural prostaglandins along with a therapeutically acceptable biodisposition profile. While the antisecretory effects of misoprostol are thought to be related to its agonistic actions at parietal cell prostaglandin receptors, its cytoprotective actions are proposed to be related to increases in gastrointestinal mucus and bicarbonate secretion, increases in mucosal blood flow and/or prevention of back diffusion of H_3O^+ into the gastric mucosa.[59]



Misoprostol

Misoprostol is rapidly absorbed following oral administration and undergoes rapid deesterification to the pharmacologically-active free acid with a terminal half-life of 20 to 40 min.[60] Misoprostol is commonly used to prevent NSAID-induced gastric ulcers in patients at high risk of complications from a gastric ulcer such as elderly patients and patients with a history of ulcer. Misoprostol has also been used in treating duodenal ulcers unresponsive to histamine H_2-antagonists; however, the drug does not prevent duodenal ulcers in patients on NSAIDS. Misoprostol can cause miscarriage, often associated with potentially dangerous bleeding.

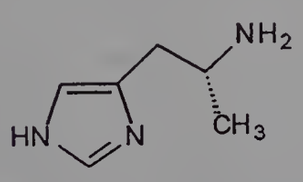
Usual adult dose: Oral, 200 mcg 4 times daily with food.

Dosage form: 100 and 200 mcg tablets.

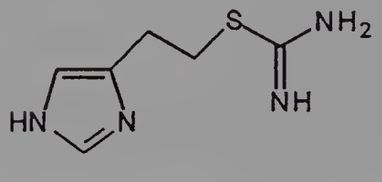
HISTAMINE H_3-RECEPTOR LIGANDS

The histamine H_3 receptor has been described to play a role as a general regulatory receptor system, modulating not only the release and synthesis of histamine but also the release of other neurotransmitters.[61] In order to characterize the physiologic and potential therapeutic roles of histamine H_3-receptors, medicinal chemists have been actively seeking structural features associated with selective agonism and antagonism of these sites. Structural alterations of histamine itself have resulted in development of the potent and selective H_3-agonist (*R*)-α-methylhistamine. Replacement of the amino group with other polar cationic groups has yielded the potent and selective H_3-agonist, imetit. Immepip, which has an aminobutylene chain incorporated in a piperidine ring, is comparable in agonist potency to (*R*)-α-methylhistamine (Fig. 21-15).

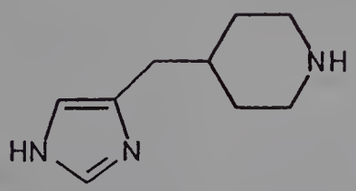
The classical H_1-antagonists are not very active at the H_3-receptor but several compounds that are active antagonists at the H_2-receptor, such as burimamide, display moderate H_3-antagonistic activity. Thioperamide, a cyclohexylthiourea derivative of immepip, is a potent, competitive H_3-antagonist both in vitro and in vivo. Thioperamide has become an important tool for the pharmacological characterization of possible H_3-receptor-mediated effects. Clobenpropit, a benzylthiourea homolog of histamine, is also a frequently used pharmacological tool for the study of histamine H_3-receptors (Fig. 21-16).



(*R*)-α–Methylhistamine



Imetit



Immepip

FIG. 21-15. *H_3-receptor agonists.*

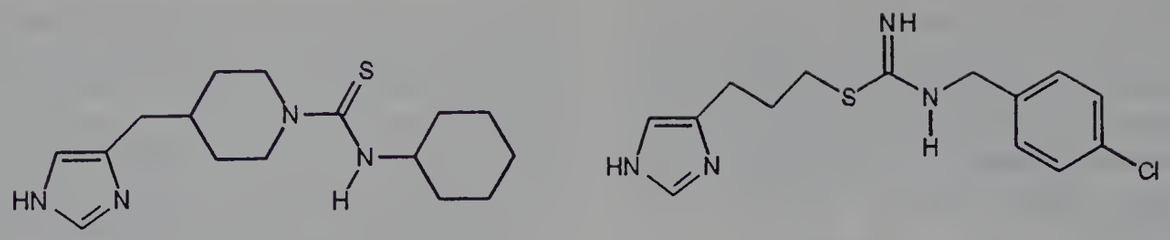


FIG. 21-16. *Histamine H_3-receptor antagonists.*

Studies with these experimental agents suggest that H_3-receptor agents might provide new therapeutics for CNS, airway, and gastrointestinal disorders.

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CHAPTER 22

Analgesic Agents

Robert E. Willette

The struggle to relieve pain began with the origin of humanity. Ancient writings, both serious and fanciful, dealt with secret remedies, religious rituals, and other methods of pain relief. Slowly, there evolved the present, modern era of synthetic analgesics.*

Tainter[1] has divided the history of analgesic drugs into four major eras, namely:

1. The period of discovery and use of naturally occurring plant drugs
2. The isolation of pure plant principles (e.g., alkaloids) from the natural sources and their identification with analgesic action
3. The development of organic chemistry and the first synthetic analgesics
4. The development of modern pharmacologic techniques, making it possible to undertake a systematic testing of new analgesics

The discovery of morphine's analgesic activity by Sertürner, in 1806, ushered in the second era. It continues today only on a small scale. Wöhler introduced the third era indirectly with his synthesis of urea in 1828. He showed that chemical synthesis could be used to make and produce drugs. In the third era, the first synthetic analgesics used in medicine were the salicylates. These originally were found in nature (methyl salicylate, salicin) and then were synthesized by chemists. Other early, synthesized drugs were acetanilid (1886), phenacetin (1887), and aspirin (1899).

These early discoveries were the principal contributions in this field until the modern methods of pharmacologic testing initiated the fourth era. The effects of small structural modifications on synthetic molecules now could be assessed accurately by pharmacologic means. This has permitted systematic study of the relationship of structure to activity during this era. The development of these pharmacologic testing procedures, coupled with the fortuitous discovery of meperidine by Eisleb and Schaumann,[2] has made possible the rapid strides in this field today.

The consideration of synthetic analgesics, as well as the naturally occurring ones, will be facilitated considerably by dividing them into two groups: morphine and related compounds, and the antipyretic analgesics.

It should be called to the reader's attention that there are numerous drugs that, in addition to possessing distinctive pharmacologic activities in other areas, may also possess analgesic properties. The analgesic property exerted may be a direct effect or may be indirect, but is subsidiary to some other more pronounced effect. Some examples of these, which are discussed elsewhere in this text, are sedatives (e.g., barbiturates); muscle relaxants (e.g., mephenesin, methocarbamol); tranquilizers (e.g., meprobamate), and others. These types will not be considered in this chapter.

MORPHINE AND RELATED COMPOUNDS

HISTORICAL PERSPECTIVE

The discovery of morphine early in the 19th century and the demonstration of its potent analgesic properties led directly to the search for more of these potent principles from plant sources. In tribute to the remarkable potency and action of morphine, it has remained alone as an outstanding and indispensable analgesic from a plant source.

It is only since 1938 that synthetic compounds rivaling it in action have been found, although many earlier changes made on morphine itself gave more effective agents.

Modifications of the morphine molecule will be considered under the following headings:

1. Early changes on morphine before the work of Small, Eddy, and their co-workers.

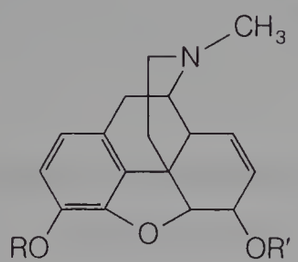
* An *analgesic* may be defined as a drug bringing about insensibility to pain without loss of consciousness. The etymologically correct term *analgetic* may be used in place of the incorrect but popular *analgesic*.

2. Changes on morphine initiated in 1929 by Small, Eddy, and co-workers[3] under the auspices of the Committee on Drug Addiction of the National Research Council and extending to the present time.
3. The researches initiated by Eisleb and Schaumann[2] in 1938, with their discovery of the potent analgesic action of meperidine, a compound departing radically from the typical morphine molecule.
4. The researches initiated by Grewe, in 1946, leading to the successful synthesis of the morphinan group of analgesics.

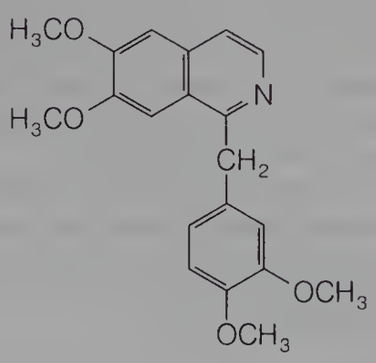
Early Morphine Modifications

Morphine is obtained from opium, which is the partly dried latex from incised unripe capsules of *Papaver somniferum*. Opium contains numerous alkaloids (as meconates and sulfates), of which morphine, codeine, noscapine (narcotine), and papaverine are therapeutically the most important, and thebaine, which has convulsant properties, but is an important starting material for many other drugs. Other alkaloids, such as narceine, also have been tested medicinally, but are not of great importance. The action of opium is principally due to its morphine content. As an analgesic, opium is not as effective as morphine because of its slower absorption, but it has a greater constipating action and, thus, is better suited for antidiarrheal preparations (e.g., paregoric). Opium, as a constituent of Dover's powders and Brown Mixture, also exerts a valuable expectorant action that is superior to that of morphine.

Two types of basic structures usually are recognized among the opium alkaloids [i.e., the *phenanthrene* (morphine) type and the *benzylisoquinoline* (papaverine) type] (see structures).



Phenanthrene Type
(Morphine, R & R' = H)



Benzylisoquinoline Type
(Papaverine)

The pharmacologic actions of the two types of alkaloids are dissimilar. The morphine group acts principally on the central nervous system as a depressant and stimulant, whereas the papaverine group has little effect on the nervous system, but has a marked antispasmodic action on smooth muscle.

Clinically, the depressant action of the morphine group is the most useful property, resulting in an increased tolerance to pain, a sleepy feeling, a lessened perception to external stimuli, and a feeling of well-being (euphoria). Respiratory depression, central in origin, is perhaps the most serious objection to this type of alkaloid, aside from its tendency to cause addiction. The stimulant action is well illustrated by the convulsions produced by certain members of this group (e.g., thebaine).

Before 1929, the derivatives of morphine that had been made were primarily the result of simple changes on the molecule, such as esterification of the phenolic or alcoholic hydroxyl group, etherification of the phenolic hydroxyl group, and similar minor changes. The net result was the discovery of some compounds with greater activity than morphine, but also with greater toxicities and addiction tendencies. No compounds were found that did not possess in some measure the addiction liabilities of morphine.*

Some of the compounds that were in common usage before 1929 are listed in Table 22-1, together with some other more recently introduced ones. All have the morphine skeleton in common.

Among the earlier compounds is codeine, the phenolic methyl ether of morphine, which also had been obtained from natural sources. It has survived as a good analgesic and cough depressant, together with the corresponding ethyl ether, which has found its principal application in ophthalmology. The diacetyl derivative of morphine, heroin, has been known for a long time; it has been banished for years from the United States and is being used in decreasing amounts in other countries. It is the most widely used illicit drug among narcotic addicts. Among the reduced compounds were dihydromorphine and dihydrocodeine and their oxidized congeners, dihydromorphinone (hydromorphone) and dihydrocodeinone (hydrocodone). Derivatives of the last two compounds possessing a hydroxyl group in position 14 are dihydrohydroxymorphinone, or oxymorphone, and dihydrohydroxycodeinone, or oxycodone. These represent the principal compounds that either had been on the market or had been prepared before the studies of Small, Eddy, and co-workers.† It is well to note that no really systematic effort had been made to investigate the structure-activity relationships in the molecule, and only the easily changed peripheral groups had been modified.

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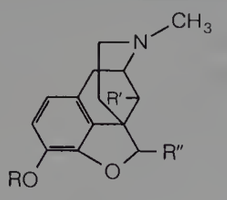
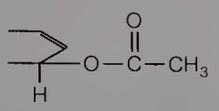
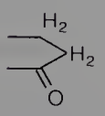
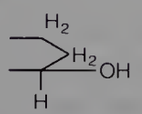
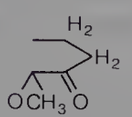
* The term *addiction liability*, or the preferred term *dependence liability*, as used in this text, indicates the ability of a substance to develop true addictive tolerance and physical dependence and/or to suppress the morphine abstinence syndrome after withdrawal of morphine from addicts.

† The only exception is oxymorphone; this was introduced in the United States in 1959, but is mentioned here because it obviously is closely related to oxycodone.

TABLE 22-1

SYNTHETIC DERIVATIVES OF MORPHINE



Compound Proprietary Name	*R*	*R'*	*R''*	*Principal Use*
Morphine	H	H		Analgesic
Codeine	CH_3	H	Same as above	Analgesic and to depress cough reflex
Ethylmorphine *Dionin*	C_2H_5	H	Same as above	Ophthalmology
Diacetylmorphine (heroin)	CH_3CO	H		Analgesic (prohibited in US)
Hydromorphone (dihydromorphinone) *Dilaudid*	H	H		Analgesic
Hydrocodone (dihydrocodeinone) *Dicodid*	CH_3	H	Same as above	Analgesic and to depress cough reflex
Oxymorphone (dihydrohydroxymorphinone)	H	OH	Same as above	Analgesic
Oxycodone (dihydrohydroxycodeinone)	CH_3	OH	Same as above	Analgesic and to depress cough reflex
Dihydrocodeine *Paracodin*	CH_3	H		Depress cough reflex
Dihydromorphine	H	H	Same as above	Analgesic
Methyldihydromorphinone *Metopon*	H	H		

Morphine Modifications Initiated by the Researches of Small and Eddy

The avowed purpose of Small, Eddy, and co-workers,[3] in 1929, was to approach the morphine problem from the standpoint that

1. It might be possible to separate chemically the addiction property of morphine from its other more salutary attributes. That this could be done with some addiction-producing compounds was shown by the development of the nonaddictive procaine from the addictive cocaine.
2. If it were not possible to separate the addictive tendencies from the morphine molecule, it might be possible to find other synthetic molecules without this undesirable property.

Proceeding on these assumptions, they first examined the morphine molecule in an exhaustive manner. As a starting point, it offered the advantages of ready availability, proven potency, and ease of alteration. In addition to its addictive tendency, it was hoped that other liabilities, such as respiratory depression, emetic properties, and gastrointestinal tract and circulatory disturbances, could be minimized or abolished as well. Because early modifications of morphine (e.g., acetylation or alkylation of hydroxyls, quaternization of the nitrogen, and so on) caused variations in the addictive potency, it was felt that the physiologic effects of morphine could be related, at least in part, to the peripheral groups.

It was not known if the actions of morphine were primarily a function of the peripheral groups or of the structural skeleton. This did not matter, however, because modification of the groups would alter activity in either case. These groups and the effects on activity by modifying them are listed in Table 22-2. The results of these and earlier studies[4] have not always shown quantitatively the effects of simple modifications on the analgesic action of morphine. However, they do indicate in which direction the activity is likely to go. The studies are far more comprehensive than Table 22-2 indicates, and the conclusions usually depend on more than one pair of compounds.

Unfortunately, these studies on morphine did not provide the answer to the elimination of addiction potentialities from these compounds. In fact, the studies suggested that any modification bringing about an increase in the analgesic activity caused a concomitant increase in addiction liability.

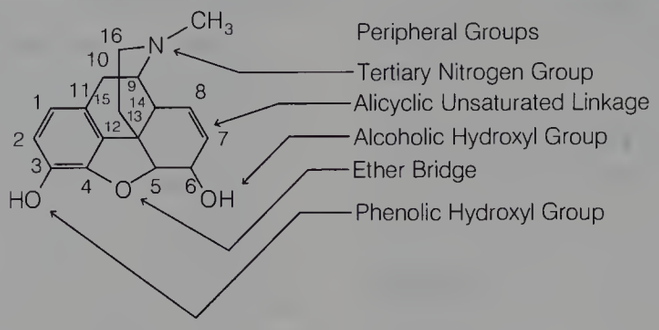
The second phase of the studies, engaged in principally by Mosettig and Eddy,[3] had to do with the attempted synthesis of substances with central narcotic and, especially, analgesic action. It is obvious that the morphine molecule contains in its makeup certain well-defined types of chemical structures. Among these are the phenanthrene nucleus, the dibenzofuran nucleus, and, as a variant of the latter, carbazole. These synthetic studies, although extensive and interesting, failed to provide significant findings and will not be discussed further in this text.

One of the more useful results of the investigations was the synthesis of 5-methyldihydromorphinone* (Table 22-1). Although it possessed addiction liabilities, it was found to be a very potent analgesic with a minimum of the undesirable

* The location of the methyl substituent was originally assigned to position 7.[5]

TABLE 22-2

SOME STRUCTURAL RELATIONSHIPS IN THE MORPHINE MOLECULE



Peripheral Groups of Morphine	*Modification (On Morphine Unless Otherwise Indicated)*	*Effects on Analgesic Activity* (Morphine or Another Compound as Indicated = 100)*
Phenolic hydroxyl	—OH → —OCH_3 (codeine)	15
	—OH → —OC_2H_5 (ethylmorphine)	10
	—OH → —OCH_2CH_2—N⟨ ⟩O (pholcodine)	1
Alcoholic hydroxyl	—OH → —OCH_3 (heterocodeine)	500
	—OH → —OC_2H_5	240
	—OH → —$OCOCH_3$	420
	—OH → =O (morphinone)	37
	†—OH → =O (dihydromorphine to dihydromorphinone)	600 (dihydromorphine vs dihydromorphinone)
	†—OH → =O (dihydrocodeine to dihydrocodeinone)	390 (dihydrocodeine vs dihydrocodeinone)
	†—OH → —H (dihydromorphine to dihydrodesoxymorphine-D)	1000 (dihydromorphine vs dihydrodesoxymorphine-D)
Ether bridge	‡=C—O—CH— → =C—OH HCH— (dihydrodesoxymorphine-D to tetrahydrodesoxymorphine)	13 (dihydrodesoxymorphine D vs tetrahydrodesoxymorphine)
Alicyclic unsaturated linkage	—CH=CH— → —CH_2CH_2— (dihydromorphine)	120
	†—CH=CH— → —CH_2CH_2— (codeine to dihydrocodeine)	115 (codeine vs dihydrocodeine)
Tertiary nitrogen	N—CH_3 → N—H (normorphine)	5
	N—CH_3 → N—CH_2CH_2—C_6H_5	1400
	§N—CH_3 → N—R	Reversal of activity (morphine antagonism); R = propyl, isobutyl, allyl, methallyl
	N—CH_3 → N^+(CH_3)(CH_3)Cl—	1 (strong curare action)
	Opening of nitrogen ring (morphimethine)	Marked decrease in action

(Continued)

TABLE 22-2 *Continued*

SOME STRUCTURAL RELATIONSHIPS IN THE MORPHINE MOLECULE

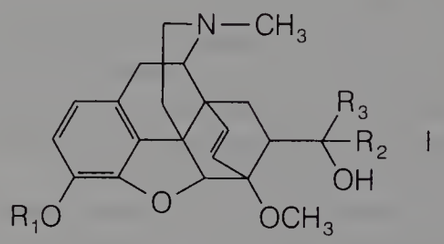
Peripheral Groups of Morphine	*Modification (On Morphine Unless Otherwise Indicated)*	*Effects on Analgesic Activity * (Morphine or Another Compound as Indicated = 100)*
Nuclear substitution	Substitution of:	
	$-NH_2$ (most likely at position 2)	Marked decrease in action
	—Cl or —Br (at position 1)	50
	—OH (at position 14 in dihydromorphinone)	250 (dihydromorphinone vs oxymorphone)
	—OH (at position 14 in dihydrocodeinone)	530 (dihydrocodeinone vs oxycodone)
	#$-CH_3$ (at position 6)	280
	#$-CH_3$ (at position 6 in dihydromorphine)	33 (dihydromorphine vs 6-methyldihydromorphine)
	#$-CH_3$ (at position 6 in dihydrodesoxy morphine D)	490 (dihydrodesoxymorphine D vs 6-methyldihydrodesoxymorphine)
	#$=CH_2$ (at position 6 in dihydrodesoxymorphine D)	600 (dihydrodesoxymorphine D vs-6-methylenedihydrodesoxymorphine)

side effects of morphine, such as emetic action and mental dullness.

Later, the high degree of analgesic activity demonstrated by morphine congeners in which the alicyclic ring is either reduced or methylated (or both) and the alcoholic hydroxyl at position 6 is absent has prompted the synthesis of related compounds possessing these features. These include 6-methyldihydromorphine and its dehydrated analogue, 6-methyl-Δ^6-desoxymorphine or methyldesorphine,[6] both of which have shown high potency. Also of interest were compounds reported by Rapoport and his co-workers:[7] morphinone; 6-methylmorphine; 6-methyl-7-hydroxy-, 6-methyl-, and 6-methylenedihydrodesoxymorphine. In analgesic activity in mice, the last-named compound was 82 times more potent, milligram for milligram, than morphine. Its therapeutic index (TI_{50}) was 22 times as great as that of morphine.[8]

The structure-activity relationships of 14-hydroxymorphine derivatives have been reviewed,[3] and several new compounds have been synthesized.[9] Of these, the dihydrodesoxy compounds possessed the highest degree of analgesic activity. Also, esters of 14-hydroxycodeine derivatives have shown very high activity.[10] For example, in rats, 14-cinnamyloxycodeinone was 177 times more active than morphine.

In 1963, Bentley and Hardy[11] reported the synthesis of a novel series of potent analgesics derived from the opium alkaloid thebaine. In rats the most active members of the series (I, $R_1 = H$, $R_2 = CH_3$, R_3 = isoamyl; and I, $R_1 = COCH_3$, $R_2 = CH_3$, $R_3 = n\text{-}C_3H_7$) were found to be several thousand times stronger than morphine.[12] These compounds exhibited marked differences in activity of optical isomers, as well as other interesting structural effects. It was postulated that the more rigid molecular structure might allow them to fit the receptor surface better. Extensive structural and pharmacologic studies have been reported.[13] Some of the *N*-cyclopropylmethyl compounds are the most potent antagonists yet discovered and have been studied very intensively.



I

As indicated in Table 22-2, replacement of the *N*-methyl group in morphine by larger alkyl groups not only lowers analgesic activity, but confers morphine antagonistic properties on the molecule (discussed later). In direct contrast to this effect, the *N*-phenethyl derivative has 14 times the analgesic activity of morphine. This enhancement of activity by *N*-aralkyl groups has wide application, as will be shown later.

Some of the morphine antagonists, such as nalorphine, are also strong analgesics.[14] The similarity of the ethylenic double bond and the cyclopropyl group has prompted the synthesis of *N*-cyclopropylmethyl derivatives of morphine and its derivatives.[15] This substituent usually confers strong narcotic antagonistic activity, with variable effects on analgesic potency. The dihydronormorphinone derivative had only moderate analgesic activity.

Morphine Modifications Initiated by the Eisleb and Schaumann Research

In 1938, Eisleb and Schaumann[2] reported the fortuitous discovery that a simple piperidine derivative, now known as meperidine, possessed analgesic activity. It was prepared as an antispasmodic, a property it shows as well. As the story is told, during the pharmacologic testing of meperidine in mice, it was observed to cause the peculiar erection of the tail known as the Straub reaction. Because the reaction is characteristic of morphine and its derivatives, the compound then was tested for analgesic properties and was about one-

fifth as active as morphine. This discovery led not only to the finding of an active analgesic but, far more important, it served as a stimulus to research workers. The status of research in analgesic compounds with an activity comparable with that of morphine was at a low ebb in 1938. Many felt that potent compounds could not be prepared, unless they were very closely related structurally to morphine. However, the demonstration of high potency in a synthetic compound that was related only distantly to morphine spurred the efforts of various research groups.[16,17]

The first efforts, naturally, were made upon the meperidine-type molecule in an attempt to enhance its activity further. It was found that replacement of the 4-phenyl group by hydrogen, alkyl, other aryl, aralkyl, and heterocyclic groups reduced analgesic activity. Placement of the phenyl and ester groups at the 4-position of 1-methylpiperidine also gave optimum activity. Several modifications of this basic structure are listed in Table 22-3.

Among the simplest changes to increase activity is the insertion of a *m*-hydroxyl group on the phenyl ring. It is in the same relative position as in morphine. The effect is more pronounced on the keto compound (Table 22-3, A-4) than on meperidine (A-1). Ketobemidone is equivalent to morphine in activity and was once widely used.

More significantly, Jensen et al.[18] discovered that replacement of the carbethoxyl group in meperidine by acyloxyl groups gave better analgesic, as well as spasmolytic, activity. The "reversed" ester of meperidine, the propionoxy compound (A-6), was the most active, being five times as active as meperidine. These findings were validated and expanded upon by Lee et al.[19] In an extensive study of structural modifications of meperidine, Janssen and Eddy[20] concluded that the propionoxy compounds were always more active, usually about twofold, regardless of what group was attached to the nitrogen.

Lee[21] had postulated that the configuration of the propionoxy derivative (A-6) more closely resembled that of morphine, with the ester chain taking a position similar to that occupied by C-6 and C-7 in morphine. His speculations were based on space models and certainly did not reflect the actual conformation of the nonrigid meperidine. However, he did arrive at the correct assumption that introduction of a methyl group into position 3 of the piperidine ring in the propionoxy compound would yield two isomers, one with activity approximating that of desomorphine and the other with less activity. One of the two diastereoisomers (A-7), betaprodine, has an activity in mice of about nine times that of morphine and three times that of A-6. Beckett et al.[22] have established it to be the *cis* (methyl/phenyl)-form. The *trans*-form, alphaprodine, is twice as active as morphine. Resolution of the racemates shows one enantiomer to have the predominant activity. In humans, however, the sharp differences in analgesic potency are not so marked. The *trans*-form is marketed as the racemate. The significance of the 3-methyl has been attributed to discrimination of the enantiotropic edges of these molecules by the receptor. This is even more dramatic in the 3-allyl and 3-propyl isomers, for which the α-*trans*-forms are considerably more potent than the β-isomers, indicating three-carbon substituents are not tolerated in the axial orientation. The 3-ethyl isomers are nearly equal in activity, further indicating that two or fewer carbons are more acceptable in the drug-receptor interaction.[23]

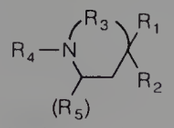
Until only the last few years it appeared that a small substituent, such as methyl, attached to the nitrogen was optimal for analgesic activity. This was believed to be true not only for the meperidine series of compounds, but also for all the other types. It is now well established that replacement of the methyl group by various aralkyl groups can increase activity markedly.[20] A few examples of this type of compound in the meperidine series are shown in Table 22-3. The phenethyl derivative (A-9) is seen to be about three times as active as meperidine (A-1). The *p*-amino congener, anileridine (A-10), is about four times more active. Piminodine, the phenylamino-propyl derivative (A-11), has 55 times the activity of meperidine in rats and, in clinical trials, is about five times as effective in humans as an analgesic.[24] The most active meperidine-type compounds to date are the propionoxy derivative (A-12), which is nearly 2000 times as active as meperidine, and the *N*-phenethyl analogue of betaprodine, which is over 2000 times as active as morphine.[22] Diphenoxylate (A-13), a structural hybrid of meperidine and methadone types, lacks analgesic activity although it reportedly suppresses the morphine abstinence syndrome in morphine addicts.[25,26] It is quite effective as an intestinal spasmolytic and is used for the treatment of diarrhea (Lomotil). Several other derivatives of it have been studied.[27] The related *p*-chloro analogue, loperamide (A-14), has been shown to bind to opiate receptors in the brain, but not to penetrate the blood-brain barrier sufficiently to produce analgesia.[28]

Another manner of modifying the structure of meperidine with favorable results has been the enlargement of the piperidine ring to the seven-membered hexahydroazepine (or hexamethylenimine) ring. As was true in the piperidine series, the most active compound was the one containing a methyl group on position 3 of the ring adjacent to the quaternary carbon atom in the propionoxy derivative, that is, 1,3-dimethyl-4-phenyl-4-propionoxy-hexahydroazepine, to which the name proheptazine has been given. In the study by Eddy and co-workers, previously cited, proheptazine was one of the more active analgesics included and had one of the highest addiction liabilities. The higher ring homologue of meperidine, ethoheptazine, was on the market. Although originally thought to be inactive,[29] it is less active than codeine as an analgesic in humans, but has the advantages of being free of addiction liability and having a low incidence of side effects.[30] It is no longer available.

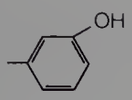
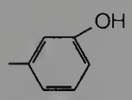
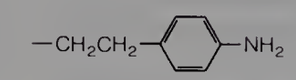
Contraction of the piperidine ring to the five-membered pyrrolidine ring has also been successful. The lower ring homologue of alphaprodine, prodilidene (A-16), is an effective analgesic, 100 mg being equivalent to 30 mg of codeine, but because of its potential abuse liability, it has not been marketed.[31]

TABLE 22-3

COMPOUNDS RELATED TO MEPERIDINE



(R_5 = H except in trimeperidine, where it is CH_3)

Compound	Structure				Name (If Any)	Analgesic Activity* (Meperidine = 1)
	R_1	R_2	R_3	R_4		
A-1	$-C_6H_5$	$-COOC_2H_5$	$-CH_2CH_2-$	$-CH_3$	Meperidine	1.0
A-2		$-COOC_2H_5$	$-CH_2CH_2$	$-CH_3$	Bemidone	1.5
A-3	$-C_6H_5$	$-COOCH(CH_3)_2$	$-CH_2CH_2-$	$-CH_3$	Properidine	15
A-4	$-C_6H_5$	$-C(=O)-C_2H_5$	$-CH_2CH_2-$	$-CH_3$		0.5
A-5		$-C(=O)-C_2H_5$	$-CH_2CH_2-$	$-CH_3$	Ketobemidone	6.2
A-6	$-C_6H_5$	$-O-C(=O)-C_2H_5$	$-CH_2CH_2-$	$-CH_3$		5
A-7	$-C_6H_5$	$-O-C(=O)-C_2H_5$	$-CH_2CH(CH_3)-$	$-CH_3$	Alphaprodine Betaprodine	5 14
A-8	$-C_6H_5$	$-O-C(=O)-C_2H_5$	$-CH_2CH(CH_3)-$	$-CH_3$ ($R_5 = CH_3$)	Trimeperidine	7.5
A-9	$-C_6H_5$	$-COOC_2H_5$	$-CH_2CH_2-$	$-CH_2CH_2C_6H_5$	Pheneridine	2.6
A-10	$-C_6H_5$	$-COOC_2H_5$	$-CH_2CH_2-$		Anileridine	3.5
A-11	$-C_6H_5$	$-COOC_2H_5$	$-CH_2CH_2-$	$-(CH_2)_3-NH-C_6H_5$	Piminodine	55†
A-12	$-C_6H_5$	$-O-C(=O)-C_2H_5$	$-CH_2CH_2-$	$-CH_2CH_2CH(C_6H_5)(O-C(=O)-C_2H_5)$		1880†
A-13	$-C_6H_5$	$-COOC_2H_5$	$-CH_2CH_2-$	$-CH_2CH_2C(C_6H_5)_2(CN)$	Diphenoxylate	None

(Continued)

TABLE 22-3 *Continued*

COMPOUNDS RELATED TO MEPERIDINE

Compound	Structure				Name (If Any)	Analgesic Activity* (Meperidine = 1)
	R_1	R_2	R_3	R_4		
A-14	$-C_6H_4$-*p*-Cl	$-OH$	$-CH_2CH_2-$	$-CH_2CH_2C(C_6H_5)_2$ | $CN(CH_3)_2$ || O	Loperamide	None
A-15	$-C_6H_5$	$-COOC_2H_5$	$-CH_2CH_2CH_2-$	$-CH_3$	Ethoheptazine	1
A-16	$-C_6H_5$	$-O-CC_2H_5$ || O	$-CH-$ | CH_3	$-CH_3$	Prodilidine	0.3
A-17	$-H$	$-N-CC_2H_5$ || O | C_6H_5	$-CH_2CH_2-$	$-CH_2CH_2C_6H_5$	Fentanyl	940
A-18	$-COOCH_3$	$-N-CC_2H_5$ || O | C_6H_5	$-CH_2CH-$ | CH_3	$-CH_2CH_2C_6H_5$	Lofentanil (R 34,995)	8400‡

A more unusual modification of the meperidine structure may be found in fentanyl (A-17), in which the phenyl and the acyl groups are separated from the ring by a nitrogen. It is a powerful analgesic, 50 times stronger than morphine in humans, with minimal side effects.[32] Its short duration of action makes it well suited for use in anesthesia.[33] It is marketed for this purpose in combination with a neuroleptic, droperidol. The *cis*-(−)-3-methyl analogue with an ester group at the 4-position, like meperidine (A-18) was 8,400 times more potent than morphine as an analgesic. In addition, it has shown the highest binding affinity to isolated opiate receptors of all other compounds tested.[28] Fentanyl and its 3-methyl and α-methyl analogues have found their way into the illicit drug market and are sold as substitutes for heroin. Because of their extreme potency, they have caused many deaths.

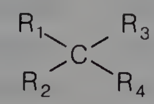
It should be recalled by the reader that when the nitrogen ring of morphine is opened, as in the formation of morphimethines, the analgesic activity virtually is abolished. On this basis, the prediction of whether a compound would or would not have activity without the nitrogen in a ring would be in favor of lack of activity or, at best, a low activity. The first report indicating that this was a false assumption was based on the initial work of Bockmuehl and Ehrhart[34] wherein they claimed that the type of compound represented by B-1 in Table 22-4 possessed analgesic as well as spasmolytic properties. The Hoechst laboratories in Germany followed up this lead during World War II by preparing the ketones corresponding to these esters. Some of the compounds they prepared with high activity are represented by formulas B-2 through B-7. Compound B-2 is the well-known methadone. In the meperidine and bemidone types, the introduction of a *m*-hydroxyl group in the phenyl ring brought about slight to marked increase in activity, whereas the same operation with the methadone-type compound brought about a marked decrease in action. Phenadoxone (B-8), the morpholine analogue of methadone, has been marketed in England. The piperidine analogue, dipanone, was once under study in this country after successful results in England.

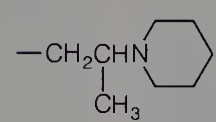
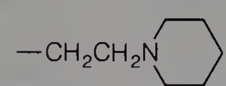
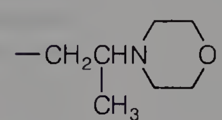
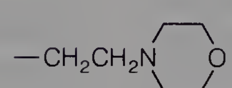
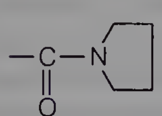
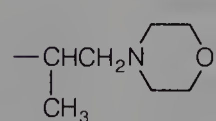
Methadone was first brought to the attention of American pharmacists, chemists, and allied workers by the Kleiderer report[35] and by the early reports of Scott and Chen.[36] Since then, much work has been done on this compound, its isomer known as isomethadone, and related compounds. The report by Eddy, Touchberry, and Lieberman[37] covers most of the points concerning the structure-activity relationships of methadone. It was demonstrated that the *levo*-isomer (B-3) of methadone (B-2) and the *levo*-isomer of isomethadone (B-4) were twice as effective as their racemic mixtures. It is also of interest that all structural derivatives of methadone demonstrated a greater activity than the corresponding structural derivatives of isomethadone. In other words, the superiority of methadone over isomethadone seems to hold, even through the derivatives. Conversely, the methadone series of compounds was always more toxic than the isomethadone group.

More extensive permutations, such as replacement of the propionyl group (R_3 in B-2) by hydrogen, hydroxyl, or acetoxyl, led to decreased activity. In a series of amide analogues of methadone, Janssen ahd Jageneau[38] synthesized racemoramide (B-12), which is more active than methadone. The (+)-isomer, dextromoramide (B-13), is the active isomer and has been marketed. A few of the other modifications that have been carried out, together with the effect on analge-

TABLE 22-4

COMPOUNDS RELATED TO METHADONE



Compound	Structure R_1	R_2	R_3	R_4	Name	Isomer, Salt	Analgesic Activity* (Methadone = 1)
B-1	$-C_6H_5$	$-C_6H_5$	—COO—Alkyl	$-CH_2CH_2N(CH_5)_2$		—	0.17
B-2	$-C_6H_5$	$-C_6H_5$	$-C(=O)-C_2H_5$	$-CH_2CH(CH_3)N(CH_3)_2$	Methadone	(±)-HCl	1.0
B-3	Same as in B-2				Levanone	(−)-bitartr.	1.9
B-4	$-C_6H_5$	$-C_6H_5$	$-C(=O)-C_2H_5$	$-CH(CH_3)CH_2N(CH_3)_2$	Isomethadone	(±)-HCl	0.65
B-5	$-C_6H_5$	$-C_6H_5$	$-C(=O)-C_2H_5$	$-CH_2CH_2N(CH_3)_2$	Normethadone	HCl	0.44
B-6	$-C_6H_5$	$-C_6H_5$	$-C(=O)-C_2H_5$		Dipanone	(±)-HCl	0.80
B-7	$-C_6H_5$	$-C_6H_5$	$-C(=O)-C_2H_5$		Hexalgon	HBr	0.50
B-8	$-C_6H_5$	$-C_6H_5$	$-C(=O)-C_2H_5$		Phenadoxone	(±)-HCl	1.4
B-9	$-C_6H_5$	$-C_6H_5$	$-CH(C_2H_5)-O-C(=O)CH_3$	$-CH_2CH(CH_3)N(CH_3)_2$	Alphacetylmethadol	α,(±)-HCl	1.3
B-10	Same as in B-9				Betacetylmethadol	β,(±)-HCl	2.3
B-11	$-C_6H_5$	$-C_6H_5$	$-COOC_2H_5$		Dioxaphetyl butyrate	HCl	0.25
B-12	$-C_6H_5$	$-C_6H_5$			Racemoramide	(+)-base	3.6
B-13	Same as in B-12				Dextromoramide	(+)-base	13
B-14	$-C_6H_5$	$-CH_2C_6H_5$	$O-C(=O)-C_2H_5$	$-CH(CH_3)CH_2N(CH_3)_2$	Propoxyphene	(+)-HCl	0.21

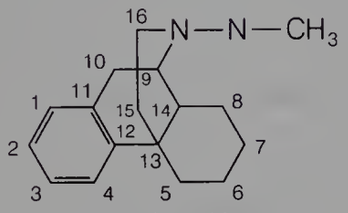
sic activity relative to methadone, are described in Table 22-4, which comprises most of the methadone congeners that are or were on the market. It can be assumed that much deviation in structure from these examples will result in varying degrees of activity loss.

Particular attention should be called to the two phenyl groups in methadone and the sharply decreased action resulting by removal of one of them. It is believed that the second phenyl residue helps to lock the $-COC_2H_5$ group of methadone in a position to simulate again the alicyclic ring of

morphine, even though the propionyl group is not a particularly rigid group. However, in this connection it is interesting to note that the compound with a propionoxy group in place of the propionyl group (R_3 in B-2) is without significant analgesic action.[17] In direct contrast with this is (+)-propoxyphene (B-14), which is a propionoxy derivative with one of the phenyl groups replaced by a benzyl group. In addition, it is an analogue of isomethadone (B-4), making it an exception to the rule. This compound is lower than codeine in analgesic activity, possesses few side effects, and has a limited addiction liability.[39] Replacement of the dimethylamino group in (+)-propoxyphene with a pyrrolidyl group gives a compound that is nearly three-fourths as active as methadone and possesses morphinelike properties. The (−)-isomer of alphacetylmethadol (B-9), known as LAAM, has been marketed as a long-acting substitute for methadone in the treatment of addicts.[40]

Morphine Modifications Initiated by Grewe

Grewe, in 1946, approached the problem of synthetic analgesics from another direction when he synthesized the tetracyclic compound that he first named morphan and then revised to *N*-methyl-morphinan. The relationship of this compound to morphine is obvious.



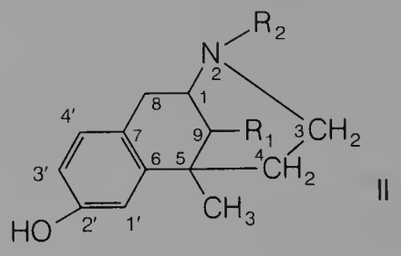
N-Methylmorphinan

N-Methylmorphinan differs from the morphine nucleus in the lack of the ether bridge between C-4 and C-5. Because this compound possesses a high degree of analgesic activity, it suggests the nonessential nature of the ether bridge. The 3-hydroxyl derivative of *N*-methylmorphinan (racemorphan) was on the market and had an intensity and duration of action that exceeded that of morphine. The original racemorphan was introduced as the hydrobromide and was the (±)-, or racemic, form as obtained by synthesis. Since then, realizing that the levorotatory form of racemorphan was the analgesically active portion of the racemate, the manufacturers have successfully resolved the (±)-form and have marketed the *levo* form as the tartrate salt (levorphanol). The *dextro* form has also found use as a cough depressant (see dextromethorphan). The ethers and acylated derivatives of the 3-hydroxyl form also exhibit considerable activity. The 2- and 4-hydroxyl isomers are, not unexpectedly, without value as analgesics. Likewise, the *N*-ethyl derivative is lacking in activity and the *N*-allyl compound, levallorphan, is a potent morphine antagonist.

Eddy et al.[41] have reported on an extensive series of *N*-aralkylmorphinan derivatives. The effect of the *N*-aralkyl substitution was more dramatic in this series than it was for morphine or meperidine. The *N*-phenethyl and *N*-*p*-aminophenethyl analogues of levorphanol are about 3 and 18 times, respectively, more active than the parent compound in analgesic activity in mice. The most potent member of the series was its *N*-β-furylethyl analogue, which was nearly 30 times as active as levorphanol or 160 times as active as morphine. The *N*-acetophenone analogue, levophenacylmorphan, was once under clinical investigation. In mice, it is about 30 times more active than morphine, and in humans a 2-mg dose is equivalent to 10 mg of morphine in its analgesic response.[42] It has a much lower physical-dependence liability than morphine.

The *N*-cyclopropylmethyl derivative of 3-hydroxymorphinan (cyclorphan) was reported to be a potent morphine antagonist, capable of precipitating morphine withdrawal symptoms in addicted monkeys, indicating that it is nonaddicting.[15] Clinical studies have indicated that it is about 20 times stronger than morphine as an analgesic, but has some undesirable side effects, primarily hallucinatory. However, the *N*-cyclobutyl derivative, butorphanol, possesses mixed agonist-antagonist properties and has been marketed as a potent analgesic.

Inasmuch as removal of the ether bridge and all the peripheral groups in the alicyclic ring in morphine did not destroy its analgesic action, May et al.[43] synthesized a series of compounds in which the alicyclic ring was replaced by one or two methyl groups. These are known as benzomorphan derivatives or, more correctly, as benzazocines. They may be represented by the following formula:

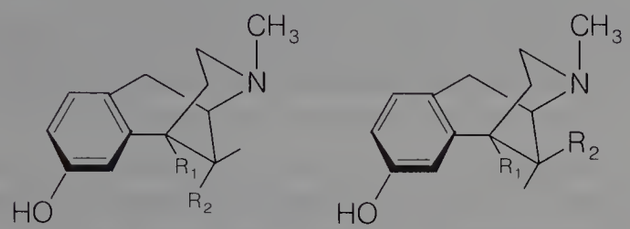


II

The trimethyl compound (II, $R_1 = R_2 = CH_3$) is about three times more potent than the dimethyl (II, $R_1 = H$, $R_2 = CH_3$). The *N*-phenethyl derivatives have almost 20 times the analgesic activity of the corresponding *N*-methyl compounds. Again, the more potent was the one containing the two ring methyls (II, $R_1 = CH_3$, $R_2 = CH_2CH_2C_6H_5$). Deracemization proved the *levo*-isomer of this compound to be more active, being about 20 times as potent as morphine in mice. The (±)-form, phenazocine, was on the market but was removed in favor of pentazocine.

May et al.[44] have demonstrated an extremely significant difference between the two isomeric *N*-methyl benzomorphans in which the alkyl in the 5-position is *n*-propyl (R_1) and the alkyl in the 9-position is methyl (R_2). These have been termed the α-isomer and the β-isomer and have the groups oriented as indicated. The isomer with the alkyl *cis* to the phenyl has been shown to possess analgesic activity (in mice) equal to that of morphine, but has little or no capac-

ity to suppress withdrawal symptoms in addicted monkeys. On the other hand, the *trans*-isomer has one of the highest analgesic potencies among the benzomorphans, but it is quite able to suppress morphine withdrawal symptoms. Further separation of properties is found between the enantiomers of the *cis*-isomer. The (+)-isomer has weak analgesic activity, but a high physical-dependence capacity. The (−)-isomer is a stronger analgesic, without the dependence capacity, and possesses antagonistic activity.[45] The same was true with the 5,9-diethyl and 9-ethyl-5-phenyl derivatives. The (−)-*trans*-5,9-diethyl isomer was similar, except it had no antagonistic properties. This demonstrates that it is possible to divorce analgesic activity comparable with morphine from addiction potential. That *N*-methyl compounds have shown antagonistic properties is of great interest as well. The most potent of these is the benzomorphan with an α-methyl and β-3-oxoheptyl group at position 9. The (−)-isomer shows greater antagonistic activity than naloxone and is still three times more potent than morphine as an analgesic.[46]



α-isomer (*cis*) β-isomer (*trans*)

An extensive series of the antagonist-type analgesics in the benzomorphans has been reported.[47] Of these, pentazocine (II, $R_1 = CH_3$, $R_2 = CH_2CH = C(CH_3)_2$) and cyclazocine (II, $R_1 = CH_3$, $R_2 = CH_2$—cyclopropyl) have been the most interesting. Pentazocine has about half the analgesic activity of morphine, with a lower incidence of side effects.[48] Its addiction liability is much lower, approximating that of propoxyphene.[49] It is currently available in parenteral and tablet form. Cyclazocine is a strong morphine antagonist, showing ~10 times the analgesic activity of morphine.[50] It was investigated as an analgesic and for the treatment of heroin addiction, but it was never marketed because of hallucinatory side effects.

It was mentioned previously that replacement of the *N*-methyl group in morphine by larger alkyl groups lowered analgesic activity. In addition, these compounds were found to counteract the effect of morphine and other morphinelike analgesics and are thus known as *narcotic antagonists*. The reversal of activity increases from ethyl, to propyl, to allyl, with the cyclopropylmethyl usually being maximal. This property was true not only for morphine, but with other analgesics as well. *N*-Allylnormorphine (nalorphine) was the first of these, but was taken off the market owing to side effects. Levallorphan, the corresponding allyl analogue of levorphanol, naloxone (*N*-allylnoroxymorphone), and naltrexone (*N*-cyclopropyl-noroxymorphone) are the three narcotic antagonists now on the market. Naloxone and naltrexone appear to be pure antagonists with no morphine- or nalorphinelike effects. They also block the effects of other antagonists. These drugs are used to prevent, diminish, or abolish many of the actions or the side effects encountered with the narcotic analgesics. Some of these are respiratory and circulatory depression, euphoria, nausea, drowsiness, analgesia, and hyperglycemia. They are thought to act by competing with the analgesic molecule for attachment at its, or a closely related, receptor site. The observation that some narcotic antagonists, which are devoid of addiction liability, are also strong analgesics has spurred considerable interest in them.[14] The *N*-cyclopropylmethyl compounds mentioned are the most potent antagonists, but appear to produce psychotomimetic effects and may not be useful as analgesics. However, one of these, buprenorphine, has shown an interesting profile and has been introduced in Europe and in the United States as a potent analgesic.[51] It is being studied as a possible treatment for narcotic addicts.[52]

Very intensive efforts were once under way to develop narcotic antagonists that can be used to treat narcotic addiction.[53] The continuous administration of an antagonist will block the euphoric effects of heroin, thereby aiding rehabilitation of an addict. The cyclopropylmethyl derivative of naloxone, naltrexone, has been marketed for this purpose. The oral dose of 100 to 150 mg three times a week is sufficient to block several usual doses of heroin.[54] Long-acting preparations are also under study.[55]

Much research, other than that described in the foregoing discussion, has been carried out by the systematic dissection of morphine to give several interesting fragments. These approaches have not yet produced important analgesics; therefore, they are not discussed in this chapter. However, the interested reader may find a key to this literature from the excellent review of Eddy,[4] Bergel and Morrison,[17] and Lee.[21]

STRUCTURE-ACTIVITY RELATIONSHIPS

Several reviews on the relationship between chemical structure and analgesic action have been published.[4,25,56–64] Only the major conclusions will be considered here, and the reader is urged to consult these reviews for a more complete discussion of the subject.

From the time Small et al. started their studies on the morphine nucleus to the present, there has been much light shed on the structural features connected with morphinelike analgesic action. In a very thorough study made for the United Nations Commission on Narcotics in 1955, Braenden et al.[57] found that the features possessed by all known morphine-like analgesics were as follows:

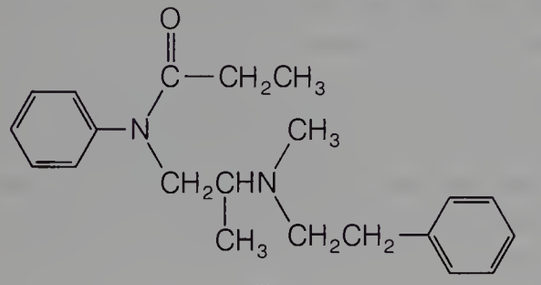
1. A tertiary nitrogen, the group on the nitrogen being relatively small
2. A central carbon atom, of which none of the valences is connected with hydrogen

3. A phenyl group or a group isosteric with phenyl, which is connected to the central carbon atom
4. A two-carbon chain separating the central carbon atom from the nitrogen for maximal activity

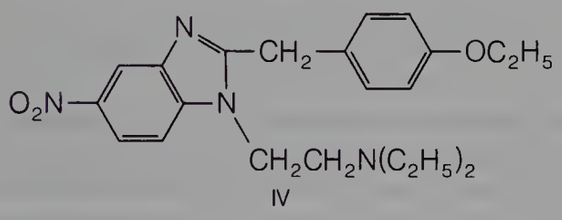
From the foregoing discussion it is evident that several exceptions to these generalizations may be found in the structures of compounds that have been synthesized in the last several years. Eddy[25] has discussed the more significant exceptions.

Relative to the first feature mentioned, extensive studies of the action of normorphine have shown that it possesses analgesic activity that approximates that of morphine. In humans, it is about one-fourth as active as morphine when administered intramuscularly, but it was slightly superior to morphine when administered intracisternally. On the basis of the last-mentioned effect, Beckett et al.[65] postulated that *N*-dealkylation was a step in the mechanism of analgesic action. This has been questioned.[66] Additional studies indicate that dealkylation does occur in the brain, although its exact role is not clear.[67] It is clear, from the previously discussed *N*-aralkyl derivatives, that a small group is not necessary.

Several exceptions to the second feature have been synthesized. In these series, the central carbon atom has been replaced by a tertiary nitrogen. They are related to methadone and have the following structures:



III



IV

Diampromide (III) and its related anilides have potencies that are comparable with those of morphine;[68] however, they have shown addiction liability and have not appeared on the market. The closely related cyclic derivative fentanyl (Table 22-3, A-17), is used in surgery. The benzimidazoles, such as etonitazene (IV), are very potent analgesics, but show the highest addiction liabilities yet encountered.[69]

Possibly an exception to feature 3, and the only one that has been encountered, may be the cyclohexyl analogue of A-6 (Table 22-3), which has significant activity.

Eddy[25] mentions two possible exceptions to feature 4 in addition to fentanyl.

As a consequence of the many studies on molecules of varying types that possess analgesic activity, it became increasingly apparent that activity was associated not only with certain structural features, but also with the size and the shape of the molecule. The hypothesis of Beckett and Casy[70] has dominated thinking for several years in the area of stereochemical specificity of these molecules. They initially noted that the more active enantiomers of the methadone- and thiambutene-type analgesics were related configurationally to (*R*)-alanine. This suggested to them that a stereoselective fit at a receptor could be involved in analgesic activity. To depict the dimensions of an analgesic receptor, they selected morphine (because of its semirigidity and high activity) to provide them with information on a complementary receptor. The features that were thought to be essential for proper receptor fit were

1. A basic center able to associate with an anionic site on the receptor surface
2. A flat aromatic structure, coplanar with the basic center, allowing for van der Waals bonding to a flat surface on the receptor site to reinforce the ionic bond.
3. A suitably positioned projecting hydrocarbon moiety forming a three-dimensional geometric pattern with the basic center and the flat aromatic structure.

These features were selected, among other reasons, because they are present in *N*-methyl-morphinan, which may be looked upon as a "stripped down" morphine [i.e., morphine without the characteristic peripheral groups (except for the basic center)]. Inasmuch as *N*-methylmorphinan possessed substantial activity of the morphine type, it was felt that these three features were the fundamental ones determining activity and that the peripheral groups of morphine acted essentially to modulate the activity.

In accord with the foregoing postulates, Beckett and Casy[70] proposed a complementary receptor site (Fig. 22-1) and suggested ways[71,72] in which the known active molecules could be adapted to it. Subsequent to their initial postu-

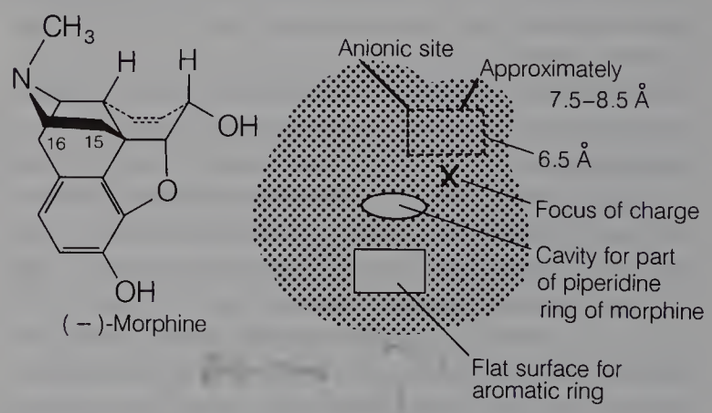
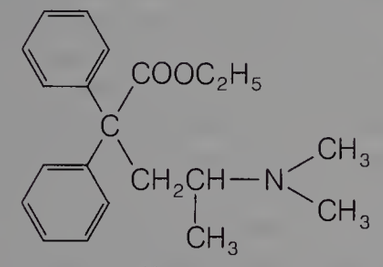


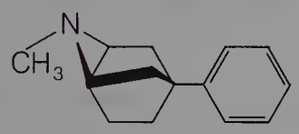
FIG. 22-1. *Diagram of the surface of the analgesic receptor site with the corresponding lower surface of the drug molecule. The three-dimensional features of the molecule are shown by the bonds: —, - - -, and ——, which represent in front of, behind, and in the plane of the paper, respectively. [Gourley, D. R. H.: In Jucker, E. (ed.). Prog. Drug Res. 7:36, 1964.]*

lates, it was demonstrated that natural (−)-morphine was related configurationally to methadone and thiambutene, a finding that lent weight to the hypothesis. Fundamental to their proposal was that such a receptor was essentially inflexible and that a lock-and-key-type situation existed. More recently, the unnatural (+)-morphine was synthesized and shown to be inactive.[73]

Although the foregoing hypothesis appeared to fit the facts quite well and was a useful hypothesis for several years, it now appears that certain anomalies exist that cannot be accommodated by it. For example, the more active enantiomer of α-methadol is not related configurationally to (*R*)-alanine, in contrast with the methadone and thiambutene series. This is also true for the carbethoxy analogue of methadone (V) and for diampromide (III) and its analogues. Another factor that was implicit in considering a proper receptor fit for the morphine molecule and its congeners was that the phenyl ring at the 4-position of the piperidine moiety should be in the axial orientation for maximum activity. The fact that structure VI has only an equatorial phenyl group, yet possesses activity equal to that of morphine, would seem to cast doubt on the necessity for axial orientation as a receptor-fit requirement.



V



VI

In view of the difficulty of accepting Beckett and Casy's hypothesis as a complete picture of analgesic-receptor interaction, Portoghese[74,76] has offered an alternative hypothesis. This hypothesis is based, in part, on the established ability of enzymes and other types of macromolecules to undergo conformational changes[77,78] on interaction with small molecules (substrates or drugs). The fact that configurationally unrelated analgesics can bind and exert activity is interpreted as meaning that more than one mode of binding may be possible at the same receptor. Such different modes of binding may be due to differences in positional or conformational interactions with the receptor. The manner in which the hypothesis can be adapted to the methadol anomaly is illustrated in Fig. 22-2. Portoghese,[74,76] after considering activity changes in various structural types (i.e., methadones, meperidines, prodines) as related to the identity of the *N*-substituent, noted that in certain series there was a parallelism in the direction of activity when identical changes in *N*-substituents were made. In others there appeared to be a nonparallelism.

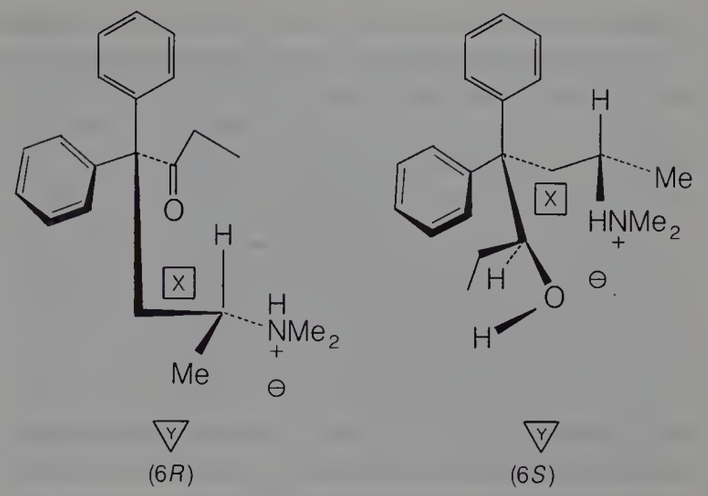


FIG. 22-2. *An illustration of how different polar groups in analgesic molecules may cause inversion in the configurational selectivity of an analgesic receptor. A hydrogen-bonding moiety, denoted by X and Y, represents a site that is capable of being hydrogen bonded.*

He has interpreted parallelism and nonparallelism, respectively, as being due to similar and to dissimilar modes of binding. As viewed by this hypothesis, although it is still a requirement that analgesic molecules be bound in a fairly precise manner, it nevertheless liberalizes the concept of binding in that a response may be obtained by two different molecules binding stereoselectively in two different precise modes at the same receptor. A schematic representation of such different possible binding modes is shown in Fig. 22-3. This representation will aid in visualizing the meaning of *similar* and *dissimilar* binding modes. If two different analgesiophores* bearing identical *N*-substituents are posi-

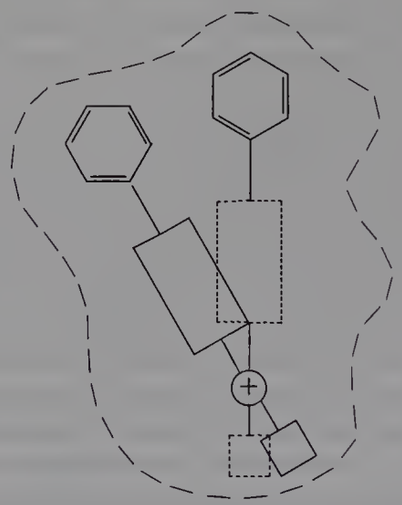


FIG. 22-3. *A schematic illustration of two different molecular modes of binding to a receptor. The protonated nitrogen is represented by ⊕. The square denotes an N-substituent. The anionic sites lie directly beneath ⊕.*

* The analgesic molecule less the *N*-substituent (i.e., the portion of the molecule giving the characteristic analgesic response).

tioned on the receptor surface such that the *N*-substituent occupies essentially the same position, a similar pharmacologic response may be anticipated. Thus, as one proceeds from one *N*-substituent to another, the response should likewise change, resulting in a parallelism of effect. On the other hand, if two different analgesiophores are bound to the receptor such that the *N*-substituents are not arranged identically, one may anticipate nonidentical responses on changing the *N*-substituent (i.e., a nonparallel response). From the preceding statements, as well as the diagram, it is not to be inferred that the analgesiophore necessarily will be bound in the identical position within a series. They do, however, suggest that, in series with parallel activities, the pairs being compared will be bound identically to produce the parallel effect. Interestingly, when binding modes are similar, Portoghese has been able to demonstrate the existence of a linear free-energy relationship. There is also the possibility that more than one receptor is involved.[74,76]

Considerable evidence is now available to demonstrate that multiple receptors exist. Martin has characterized and named these by responses from probe molecules [i.e., μ (mu) receptors for morphine-specific effects, σ (sigma) for cyclazocine, and κ (kappa) for ketocyclazocine].[79] Various combinations of these in different tissues could be responsible for the varying effects observed.[80,81]

Although this hypothesis is relatively new, it appears to embrace virtually all types of analgesic molecules presently known,* and it will be interesting to see whether it is of further general applicability as other molecules with activity are devised.

Another of the highly important developments in structure-activity correlations has been the development of highly active analgesics from the *N*-allyl-type derivatives that were once thought to be only morphine antagonists and devoid of analgesic properties. Serendipity played a major role in this discovery: Lasagna and Beecher,[82] in attempting to find some "ideal" ratio of antagonist (*N*-allylnormorphine, nalorphine) to analgesic (morphine) such as to maintain the desirable effects of morphine while minimizing the undesirable ones, discovered that nalorphine was, milligram for milligram, as potent an analgesic as morphine. Unfortunately, nalorphine has depersonalizing and psychotomimetic properties that preclude its use clinically as a pain reliever. However, the discovery led to the development of related derivatives such as pentazocine and cyclazocine. Pentazocine has achieved some success in providing an analgesic with low addiction potential, although it is not totally free of some of the other side effects of morphine. The pattern of activity in these and other *N*-allyl and *N*-cyclopropyl-methyl derivatives indicates that most potent antagonists possess psychotomimetic activity, whereas the weak antagonists do not. It is from this latter group that useful analgesics, such as pentazocine, butorphanol, and nalbuphine, have been found. The latter two possess *N*-cyclobutylmethyl groups.

What structural features are associated with antagonistlike activity has become uncertain. The *N*-allyl and dimethylallyl substituent does not always confer antagonist properties. This is true in the meperidine and thevinol series. Demonstration of antagonistlike properties by specific isomers of *N*-methyl benzomorphans has raised still further speculation. The exact mechanisms by which morphine and the narcotic antagonists act are not clearly defined, and a great amount of research is presently being carried on. Published reviews and symposia may be consulted for further discussions of these topics.[53,63–85]

A further problem also is demonstrated in the testing for analgesic activity. The analgesic activity of the antagonists was not apparent from animal testing, but was observed only in humans. Screening in animals can be used to assess the antagonistic action, which indirectly indicates possible analgesic properties in humans.[86]

It has been customary in the area of analgesic agents to attribute differences in their activities to structurally related differences in their receptor interactions. This rather universal practice continues, in spite of early warnings and recent findings. It now appears clear that much of the differences in relative analgesic potencies can be accounted for on the basis of pharmacokinetic or distribution properties.[84] For example, a definite correlation was found between the partition coefficients and the intravenous analgesic data for 17 agents of widely varying structures.[87] Usual test methods do not help define which structural features are related to receptor and which to distribution phenomena. Studies directed toward making this distinction have used the measurement of actual brain and plasma levels[88,89] or direct injection into the ventricular area,[87] the measurement of ionization potentials and partition coefficients,[90] and the application of molecular orbital theories and quantum mechanics.[85,91–93] These are providing valuable insight to the designing of new and more successful agents.

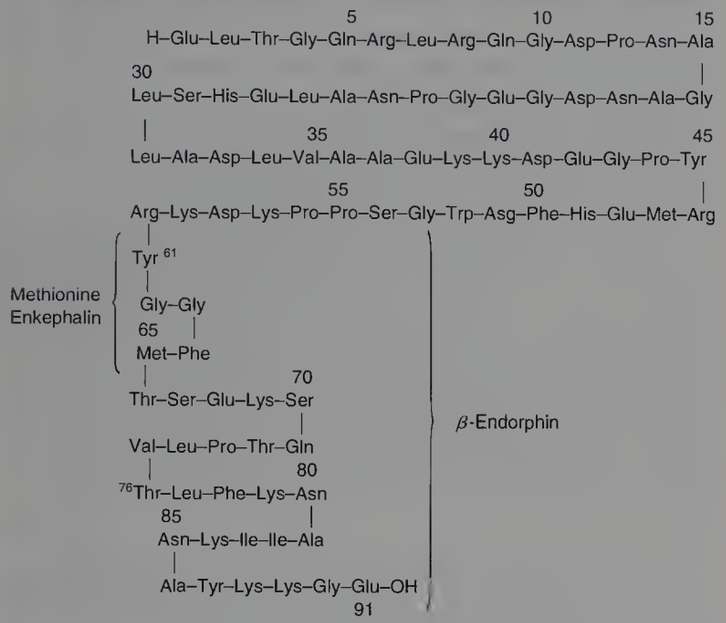
All of the foregoing work had strongly suggested for years the existence of specific binding sites or *receptors* in brain and other tissue. The demonstration of the high degree of steric and structural specificity in the action of the opiates and their antagonists led many investigators to search for such receptors.[94,95] Thus in 1971, Goldstein and co-workers demonstrated stereospecific binding in brain homogenates.[96] This was quickly followed by refinements and further discoveries by Simon, Terenius, and Snyder.[97–99] These receptor-binding studies have now become a routine assay for examining structure-activity relationships.

Endogenous Opioids

In addition to the binding studies, considerable attention continued on the use of in vitro models, in particular the isolated guinea pig ileum, rat jejunum, and mouse vas deferens.[100] While working with these preparations, Hughes was the first

* Two possible exceptions are 4-propionoxy-4-cyclohexyl-1-methylpiperidine and 1-tosyl-4-phenyl-4-ethylsulfone piperidine. (Helv. Chim. Acta 36:819, 1953.)

to discover the existence of an endogenous factor from pig brains that possessed opiatelike properties.[101] This factor, given the name *enkephalin*, consisted of two pentapeptides, called *methionine-*, or *metenkephalin*, and *leucine-*, or *leu-enkephalin*. These two enkephalins have subsequently been shown to exist in all animals, including humans, and to possess all morphinelike properties. It was also observed that they exist as segments of a pituitary hormone, the 91-amino acid β-lipotropin, which is cleaved selectivity to release specific segments that have now been found to have functions within the body. Thus, segment 61 to 65 is met-enkephalin, 61 to 76 is α-endorphin, 61 to 77 is γ-endorphin, and, probably the most important, 61 to 91 is β-endorphin.



β-Lipotropin, β-Endorphin, Methionine Enkephalin Relationships

The last endorphin (short for *end*ogenous mor*phine*) has 20 times the analgesic potency of morphine when injected into rat brain. It has also been shown that these substances can produce tolerance and dependence.

It is obvious that all of these techniques will lead to new concepts and understanding of the processes of analgesia, tolerance, and dependence. It is hoped that learning how these mechanisms operate will aid in the design and development of better analgesics.

PRODUCTS*

Morphine. This alkaloid was isolated first in 1803 by Derosne, but the credit for isolation generally goes to Sertürner (1803), who first called attention to the basic properties of the substance. Morphine, incidentally, was the first plant base isolated and recognized as such. Although intensive research was carried out on the structure of morphine, it was only in 1925 that Gulland and Robinson[105] postulated the currently accepted formula. The total synthesis of morphine finally was effected by Gates and Tschudi[106] in 1952, thereby confirming the Gulland and Robinson formula.

Morphine is obtained only from the opium poppy, *Papaver somniferum*, either from opium, the resin obtained by lancing the unripe pod, or from poppy straw. The latter process is being favored as it helps to eliminate illicit opium from which heroin is readily produced. It occurs in opium in amounts varying from 5% to 20% (*USP* requires not less than 9.5%). It is isolated by various methods, of which the final step is usually the precipitation of morphine from an acid solution by using excess ammonia. The precipitated morphine then is recrystallized from boiling alcohol.

The free alkaloid occurs as levorotatory, odorless, white, needlelike crystals possessing a bitter taste. It is almost insoluble in water (1:5,000,† 1:1,100 at boiling point), ether (1:6,250), or chloroform (1:1,220). It is somewhat more soluble in ethyl alcohol (1:210, 1:98 at boiling point). Because of the phenolic hydroxyl group, it is readily soluble in solutions of alkali or alkaline earth metal hydroxides.

Morphine is a monoacidic base and readily forms water-soluble salts with most acids. Thus, because morphine itself is so poorly soluble in water, the salts are the preferred form for most uses. Numerous salts have been marketed, but the ones in use are principally the sulfate and, to a lesser extent, the hydrochloride. Morphine acetate, which is freely soluble in water (1:2.5), but is relatively unstable, has been used to a limited extent in liquid antitussive combinations.

Many writers have pointed out the "indispensable" nature of morphine, based on its potent analgesic properties toward all types of pain. It is properly termed a narcotic analgesic. However, because it causes addiction so readily, it should be used only in those cases for whom other pain-relieving drugs prove to be inadequate. It controls pain caused by serious injury, neoplasms, migraine, pleurisy, biliary and renal colic, and numerous other causes. It often is administered as a preoperative sedative, together with atropine to control secretions. With scopolamine, it is given to obtain the so-called twilight sleep. This effect is used in obstetrics, but care is exercised to prevent respiratory depression in the fetus. It is noteworthy that the toxic properties of morphine are much more evident in young and old people.

Morphine Hydrochloride. This salt may be prepared by neutralizing a hot aqueous suspension of morphine with diluted hydrochloric acid and then concentrating the resultant solution to crystallization. It is no longer commercially available.

* In General Circular No. 253, March 10, 1960, the Treasury Department, Bureau of Narcotics, Washington, DC 20525 has published an extensive listing of narcotics of current interest in the drug trade. This listing will be much more extensive than the following monographic coverage of compounds primarily of interest to American pharmacists.

† In this chapter, a solubility expressed as 1:5,000 indicates that 1 g is soluble in 5,000 ml of the solvent at 25°C. Solubilities at other temperatures will be so indicated.

It occurs as silky, white, glistening needles or cubical masses or as a crystalline, white powder. The hydrochloride is soluble in water (1:17.5, 1:0.5 at boiling point), alcohol (1:52, 1:46 at 60°), or glycerin, but it is practically insoluble in ether or chloroform. Solutions have a pH of approximately 4.7 and may be sterilized by boiling.

Its uses are the same as those of morphine.

The usual oral and subcutaneous dose is 15 mg every four hours as needed, with a suggested range of 8 to 20 mg.

Morphine Sulfate, USP. This morphine salt is prepared in the same manner as the hydrochloride (i.e., by neutralizing morphine with diluted sulfuric acid).

It occurs as feathery, silky, white crystals, as cubical masses of crystals, or as a crystalline, white powder. Although it is a fairly stable salt, it loses water of hydration and darkens on exposure to air and light. It is soluble in water (1:16, 1:1 at 80°), poorly soluble in alcohol (1:570, 1:240 at 60°), and insoluble in chloroform or ether. Aqueous solutions have a *p*H of ~4.8 and may be sterilized by heating in an autoclave.

This common morphine salt is used widely in England by oral administration for the management of pain in cancer patients. It has largely replaced Brompton's mixture, a combination of heroin and cocaine in chloroform water used for cough. In the United States this preparation has become mistakenly popular, substituting morphine sulfate for the heroin. Moreover, Twycross has advised that the stimulant cocaine is contraindicated because it interferes with sleep.[107]

Codeine, USP. Codeine is an alkaloid that occurs naturally in opium, but the amount present is usually too small to be of commercial importance. Consequently, most commercial codeine is prepared from morphine by methylating the phenolic hydroxyl group. The methylation methods make use of reagents such as diazomethane, dimethyl sulfate, and methyl iodide. Newer methods are based on its synthesis from thebaine, which makes it possible to use *P. bracteatum* as a natural source (see above).

It occurs as levorotatory, colorless, efflorescent crystals or as a white crystalline powder. It is light-sensitive. Codeine is slightly soluble in water (1:120) and sparingly soluble in ether (1:50). It is freely soluble in alcohol (1:2) and very soluble in chloroform (1:0.5).

Codeine is a monoacidic base and readily forms salts with acids, the most important salts being the sulfate and the phosphate. The acetate and the methylbromide derivatives have been used to a limited extent in cough preparations.

The general pharmacologic action of codeine is similar to that of morphine but, as previously indicated, it does not possess the same degree of analgesic potency.

There are studies that indicate that 30 to 120 mg of codeine are considerably less efficient parenterally than 10 mg of morphine, and the usual side effects of morphine—respiratory depression, constipation, nausea, and such—are apparent. Codeine is less effective orally than parenterally, and it has been stated by Houde and Wallenstein[108] that 32 mg of codeine is about as effective as 650 mg of aspirin in relieving terminal cancer pain. However, it also has been recognized that combinations of aspirin and codeine act additively as analgesics, thus giving some support to the common practice of combining the two drugs.

Codeine has a reputation as an antitussive, depressing the cough reflex, and is used in many cough preparations. It is one of the most widely used morphinelike analgesics. It is considerably less addicting than morphine and, in the usual doses, respiratory depression is negligible, although an oral dose of 60 mg will cause such depression in a normal person. It is probably true that much of codeine's reputation as an antitussive rests on subjective impressions rather than on objective studies. The average 5-ml dose contains 10 mg of codeine. Several cough preparations containing codeine are available, with some that may be sold over-the-counter as exempt narcotic preparations. However, abuse or misuse of these preparations has led to their being placed on a prescription-only status in many states.

Codeine Phosphate, USP. This salt may be prepared by neutralizing codeine with phosphoric acid and precipitating the salt from aqueous solution with alcohol.

Codeine phosphate occurs as fine, needle-shaped, white crystals or as a white crystalline powder. It is efflorescent and is sensitive to light. It is freely soluble in water (1:2.5, 1:0.5 at 80°), but less soluble in alcohol (1:325, 1:125 at boiling point). Solutions may be sterilized by boiling.

Because of its high solubility in water, compared with the sulfate, this salt is used widely. It is often the only salt of codeine stocked by pharmacies and is dispensed, rightly or wrongly, on all prescriptions calling for either the sulfate or the phosphate.

Codeine Sulfate, USP. Codeine sulfate is prepared by neutralizing an aqueous suspension of codeine with diluted sulfuric acid and then effecting crystallization.

It occurs as white crystals, usually needlelike, or as a white crystalline powder. The salt is efflorescent and light-sensitive. It is soluble in water (1:30, 1:6.5 at 80°), much less soluble in alcohol (1:1,280), and insoluble in ether or chloroform.

This salt of codeine is prescribed frequently, but is not as suitable as the phosphate for liquid preparations. Solutions of the sulfate and the phosphate are incompatible with alkaloidal reagents and alkaline substances.

Ethylmorphine Hydrochloride. Dionin. This synthetic compound is analogous to codeine, but instead of being the methyl ether it is the ethyl ether. Ethylmorphine may be prepared by treating an alkaline alcoholic solution of morphine with diethyl sulfate. The hydrochloride is obtained from the free base by neutralizing it with diluted hydrochloric acid.

The systemic action of this morphine derivative is intermediate between those of codeine and morphine. It has analgesic qualities and sometimes is used for the relief of pain. As a depressant of the cough reflex, it is as effective as codeine and, consequently, is found in some commercial cough syrups. However, the chief use of this compound was in ophthalmology. By an irritant dilating action on vessels, it stimulates the vascular and lymphatic circulation of the

eye. This action is of value in chemosis (excessive edema of the ocular conjunctiva), and the drug is termed a *chemotic.*

Diacetylmorphine Hydrochloride. Heroin hydrochloride; diamorphine hydrochloride. Although heroin is two to three times more potent than morphine as an analgesic, its sale and use are prohibited in the United States because of its intense addiction liability. It is available in some European countries, where it has a limited use as an antitussive and as an analgesic in terminal cancer patients. Because of its superior solubility over morphine sulfate, arguments have been raised for its availability. However, the other more potent analgesics described here have significant advantages in being more stable and longer acting. It remains as one of the most widely used narcotics for illicit purposes and still places major economic burdens on society.

Hydromorphone. Dihydromorphinone. This synthetic derivative of morphine is prepared by the catalytic hydrogenation and dehydrogenation of morphine under acidic conditions, using a large excess of platinum or palladium.

The free base is similar in properties to those of morphine, being slightly soluble in water, freely soluble in alcohol, and very soluble in chloroform.

This compound, of German origin, was introduced in 1926. It is a substitute for morphine (five times as potent) but has approximately equal addicting properties and a shorter duration of action. It possesses the advantage over morphine of giving less daytime sedation or drowsiness. It is a potent antitussive and is often used for coughs that are difficult to control.

Hydromorphone Hydrochloride, USP. Dihydromorphinone hydrochloride (Dilaudid). Hydromorphone hydrochloride occurs as a light-sensitive, white crystalline powder that is freely soluble in water (1:3), sparingly soluble in alcohol, and practically insoluble in ether. It is used in about one-fifth the dose of morphine for any of the indications of morphine.

Hydrocodone Bitartrate, USP. Dihydrocodeinone bitartrate (Dicodid; Codone). This drug is prepared by the catalytic rearrangement of codeine or by hydrolyzing dihydrothebaine. It occurs as fine, white crystals or as a white crystalline powder. It is soluble in water (1:16), slightly soluble in alcohol, and insoluble in ether. It forms acidic solutions and is affected by light. The hydrochloride is also available.

Hydrocodone has a pharmacologic action midway between those of codeine and morphine, with 15 mg being equivalent to 10 mg of morphine in analgesic power. Although it possesses more addiction liability than codeine, it has been said to give no evidence of dependence or addiction with long-term use. Its principal advantage is in the lower frequency of side effects encountered with its use. It is more effective than codeine as an antitussive and is used primarily for this purpose. It is on the market in many cough preparations, as well as in tablet and parenteral forms. It has also been marketed in an ion-exchange resin complex form under the trade name of Tussionex. The complex releases the drug at a sustained rate and is said to produce effective cough suppression over a 10- to 12-hr period.

Hydrocodone is also marketed in combination with acetaminophen (e.g., Hydrocet, Vicodin, and Zydone) and with homatropine as Hycodan.

Although this drug found extensive use in antitussive formulations for many years, it has been placed under more stringent narcotic regulations.

Oxymorphone Hydrochloride, USP. (−)-14-Hydroxydihydromorphinone hydrochloride (Numorphan). Oxymorphone, introduced in 1959, is prepared by cleavage of the corresponding codeine derivative. It is used as the hydrochloride salt, which occurs as a white crystalline powder freely soluble in water and sparingly soluble in alcohol. In humans, oxymorphone is as effective as morphine in one-eighth to one-tenth the dosage, with good duration and a slightly lower frequency of side effects.[109] It has high addiction liability. It is used for the same purposes as morphine, such as control of postoperative pain, pain of advanced neoplastic diseases, as well as other types of pain that respond to morphine. Because of the risk of addiction, it should not be employed for relief of minor pains that can be controlled with codeine. It is also well to note that it has poor antitussive activity and is not used as a cough suppressant.

It may be administered orally, parenterally (intravenously, intramuscularly, or subcutaneously), or rectally, and for these purposes is supplied as a solution for injection (1.0 and 1.5 mg/ml), and in suppositories (2 and 5 mg).

Nalbuphine Hydrochloride. *N*-Cyclobutyl-methyl-14-hydroxy-*N*-nordihydromorphinone hydrochloride (Nubain). *N*-Cyclobutylmethylnoroxymorphone hydrochloride was introduced in 1979 as a potent analgesic of the agonist-antagonist type, with no to low abuse liability. It is somewhat less potent as an analgesic than its parent oxymorphone, but shares some of the antagonist properties of the closely related, but pure antagonists, naloxone and naltrexone. Nalbuphine hydrochloride occurs as a white to off-white crystalline powder that is soluble in water and sparingly soluble in alcohol. It is prepared from cyclobutylmethyl bromide and noroxycodone followed by cleavage of the *O*-methyl group.

This analgesic shows a very rapid onset with a duration of action of up to 6 hr. It has relatively low abuse liability, being judged to be less than that of codeine and propoxyphene. The injection is therefore available without narcotic controls, although caution is urged for long-term administration or use in emotionally disturbed patients. Abrupt discontinuation after prolonged use has given rise to withdrawal signs. Usual doses cause respiratory depression comparable with that of morphine, but no further decrease is seen with higher doses. It has fewer cardiac effects than pentazocine and butorphanol. The most frequent adverse effect is sedation, and, as with most other CNS depressants and analgesics, caution should be urged when administered to ambulatory patients who may need to drive a car or operate machinery.

Nalbuphine is marketed as an injectable (10 and 20

mg/ml). The usual dose is 10 mg administered subcutaneously, intramuscularly, or intravenously at 3- to 6-hr intervals, with a maximal daily dose of 160 mg.

Oxycodone Hydrochloride. Dihydrohydroxycodeinone hydrochloride. This compound is prepared by the catalytic reduction of hydroxycodeinone, the latter compound being prepared by hydrogen peroxide (in acetic acid) oxidation of thebaine. This derivative of morphine occurs as a white crystalline powder that is soluble in water (1 : 10) or alcohol. Aqueous solutions may be sterilized by boiling. Although this drug is almost as likely to cause addiction as morphine, it is sold in the United States in Percodan and several other products in combination with aspirin or acetominophen.

It is used as a sedative, an analgesic, and a narcotic. To depress the cough reflex, it is used in 3- to 5-mg doses and as an analgesic in 5- to 10-mg doses. For severe pain, a dose of 20 mg is given subcutaneously.

Dihydrocodeine Bitartrate. Dihydrocodeine is obtained by the reduction of codeine. The bitartrate salt occurs as white crystals that are soluble in water (1:4.5) and only slightly soluble in alcohol. Subcutaneously, 30 mg of this drug is almost equivalent to 10 mg of morphine as an analgesic, giving more prompt onset and negligible side effects. It has addiction liability. It is available in combination with aspirin or acetaminophen for pain and as a cough preparation (Cophene-S). The usual dose is 10 to 30 mg.

Normorphine. This drug may be prepared by *N*-demethylation of morphine.[110] In humans, by normal routes of administration, it is about one-fourth as active as morphine in producing analgesia, but has a much lower physical dependence capacity. Its analgesic effects are nearly equal by the intraventricular route. It does not show the sedative effects of morphine in single doses, but does so cumulatively. Normorphine suppresses the morphine abstinence syndrome in addicts, but after its withdrawal it gives a slow onset and a mild form of the abstinence syndrome.[111] It was once considered for possible use in the treatment of narcotic addiction.

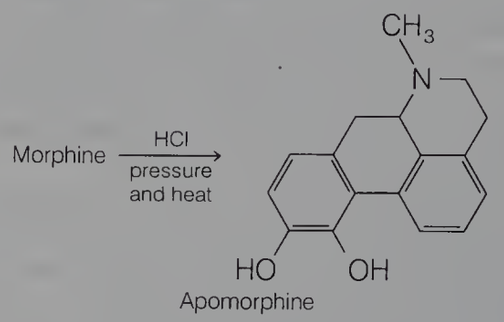
Concentrated Opium Alkaloids. These (Pantopon) consist of a mixture of the total alkaloids of opium. It is free of nonalkaloidal material, and the alkaloids are said to be present in the same proportions as they occur naturally. The alkaloids are in the form of the hydrochlorides, and morphine constitutes 50% of the weight of the material.

This preparation is promoted as a substitute for morphine, the claim being that it is superior to the latter because of the synergistic action of the opium alkaloids. This synergism is said to result in less respiratory depression, less nausea and vomiting, and an antispasmodic action on smooth muscle. According to several authorities, however, the superiority to morphine is overrated, and the effects produced are comparable with the use of an equivalent amount of morphine. The commercial literature suggests a dose of 20 mg of Pantopon to obtain the same effect as is given by 15 mg of morphine.

Solutions prepared for parenteral use may be slightly colored, a situation that does not necessarily indicate decomposition.

Tramadol Hydrochloride. (±)-*cis*-2-[(dimethylamino)methyl]-1-(*m*-methoxyphenyl)cyclohexanol hydrochloride (Ultram) represents a fragment of codeine's structure, consisting of the phenyl and cyclohexane rings. The drug possesses opioid activity, but has other analgesic activity that is not reversed by naloxone. The principle effect is attributed to the O-demethylated metabolite, which is six times more potent than the parent compound, an observation consistent with the differences between codeine and morphine. It produces significantly lower morphine-like side effects. It is available in tablet form for use in moderate to severe pain in a dose of 50 to 100 mg every 4 to 6 hr.

Apomorphine Hydrochloride, USP. When morphine or morphine hydrochloride is heated at 140° under pressure with strong (35%) hydrochloric acid, it loses a molecule of water and yields a compound known as apomorphine.



Apomorphine

The hydrochloride is odorless and occurs as minute, glistening, white or grayish white crystals or as a white powder. It is light-sensitive and turns green on exposure to air and light. It is sparingly soluble in water (1 : 50, 1 : 20 at 80°) and in alcohol (1 : 50) and is very slightly soluble in ether or chloroform. Solutions are neutral to litmus.

The change in structure from morphine to apomorphine causes a profound change in its physiologic action. The central depressant effects of morphine are much less pronounced, and the stimulant effects are enhanced greatly, thereby producing emesis by a purely central mechanism. It is administered subcutaneously to obtain emesis. It is ineffective orally. Apomorphine is one of the most effective, prompt (10 to 15 min), and safe emetics in use today. However, care should be exercised in its use because it may be depressant in already depressed patients. It is currently classified as an "orphan drug" for use in Parkinson's disease.

Meperidine Hydrochloride, USP. Ethyl 1-methyl-4-phenylisonipecotate hydrochloride; ethyl 1-methyl-4-phenyl-4-piperidinecarboxylate hydrochloride (Demerol Hydrochloride). This is a fine, white, odorless crystalline powder that is very soluble in water, soluble in alcohol, and sparingly soluble in ether. It is stable in the air at ordinary temperature, and its aqueous solution is not decomposed by a short period of boiling. The free base may be made by heating benzyl cyanide with bis(β-chloroethyl)methylamine, hydrolyzing to the corresponding acid and esterifying the latter with ethyl alcohol.[2]

Meperidine first was synthesized to study its spasmolytic character, but it was found to have analgesic properties in far greater degree. The spasmolysis is primarily due to a direct papaverinelike depression of smooth muscle and, also, to some action on parasympathetic nerve endings. In therapeutic doses, it exerts an analgesic effect that lies between those of morphine and codeine, but it shows little tendency toward hypnosis. It is indicated for the relief of pain in most patients for whom morphine and other alkaloids of opium generally are employed, but it is especially of value where the pain is due to spastic conditions of intestine, uterus, bladder, bronchi, and so on. Its most important use seems to be in lessening the severity of labor pains in obstetrics and, with barbiturates or tranquilizers, to produce amnesia in labor. In labor, 100 mg is injected intramuscularly as soon as contractions occur regularly, and a second dose may be given after 30 min if labor is rapid or if the cervix is thin and dilated ($\geq$ 2 to 3 cm). A third dose may be necessary an hour or two later, and at this stage a barbiturate may be administered in a small dose to ensure adequate amnesia for several hours. Meperidine possesses addiction liability. There is a development of psychic dependence in those individuals who experience a euphoria lasting for an hour or more. The development of tolerance has been observed, and it is significant that meperidine can be successfully substituted for morphine in addicts who are being treated by gradual withdrawal. Furthermore, mild withdrawal symptoms have been noted in certain persons who have become purposely addicted to meperidine. The possibility of dependence is great enough to put it under the federal narcotic laws. Nevertheless, it remains as one of the more widely used analgesics.

Alphaprodine Hydrochloride, USP.[21] (±)-1,3-Dimethyl-4-phenyl-4-piperidinol propionate hydrochloride. This compound is prepared according to the method of Ziering and Lee.[112]

It occurs as a white crystalline powder that is freely soluble in water, alcohol, and chloroform, but insoluble in ether.

The compound is an effective analgesic, similar to meperidine, and is of special value in obstetric analgesia. It appears to be quite safe for use in this capacity, causing little or no depression of respiration in either mother or fetus.

Anileridine, USP. Ethyl 1-(*p*-aminophenethyl)-4-phenylisonipecotate (Leritine). It is prepared by the method of Weijlard et al.[113] It occurs as a white to yellowish white crystalline powder that is freely soluble in alcohol, but only very slightly soluble in water. It is oxidized on exposure to air and light. The injection is prepared by dissolving the free base in phosphoric acid solution.

Anileridine is more active than meperidine and has the same usefulness and limitations. Its dependence capacity is less, and it is considered a suitable substitute for meperidine.

Anileridine Hydrochloride, USP. Ethyl 1-(*p*-aminophenethyl)-4-phenylisonipecotate dihydrochloride (Leritine Hydrochloride). It is prepared as cited for anileridine, except that it is converted to the dihydrochloride by conventional procedures. It occurs as a white or nearly white, crystalline, odorless powder that is stable in air. It is freely soluble in water, sparingly soluble in alcohol, and practically insoluble in ether and chloroform.

This salt has the same activity as that cited for anileridine.

Diphenoxylate Hydrochloride, USP. Ethyl 1-(3-cyano-3,3-diphenylpropyl)-4-phenylisonipecotate monohydrochloride (Lomotil; Lonox; Logen; Lomonate). It occurs as a white, odorless, slightly water-soluble powder with no distinguishing taste.

Although this drug has a strong structural relationship to the meperidine-type analgesics it has very little, if any, such activity itself. Its most pronounced activity is its ability to inhibit excessive gastrointestinal motility, an activity reminiscent of the constipating side effect of morphine itself. Investigators have demonstrated the possibility of addiction,[25,26] particularly with large doses, but virtually all studies using ordinary dosage levels show nonaddiction. Its safety is reflected in its classification as an exempt narcotic, with, however, the warning that it may be habit forming. To discourage possible abuse of the drug, the commercial product (Lomotil) once contained a subtherapeutic dose (25 μg) of atropine sulfate in each 2.5-mg tablet and in each 5 mL of the liquid, which contains a like amount of the drug. Atropine has now been removed because of unwarranted side effects.

It is indicated in the oral treatment of diarrheas resulting from a variety of causes. The usual initial adult dose is 5 mg, three or four times a day, with the maintenance dose usually being substantially lower and being individually determined. Appropriate dosage schedules for children are available in the manufacturer's literature.

The incidence of side effects is low, but the drug should be used with caution, if at all, in patients with impaired hepatic function. Similarly, patients taking barbiturates concurrently with the drug should be observed carefully, in view of reports of barbiturate toxicity under these circumstances.

Loperamide Hydrochloride, USP. 4-(4-Chlorophenyl)-4-hydroxy-*N,N*-dimethyl-α,α-diphenyl-1-piperidinebutanamide; 4-(4-*p*-chlorophenyl-4-hydroxypiperidino)-*N,N*-dimethyl-2, 2-diphenylbutyramide hydrochloride (Imodium). This hybrid of a methadonelike and meperidine molecule is closely related to diphenoxylate, being more specific, potent, and longer acting. It acts as an antidiarrheal by a direct effect on the circular and longitudinal intestinal muscles. After oral administration it reaches peak blood levels within four hours and has a very long plasma half-life (40 hours). Tolerance to its effects has not been observed.[114] Although it has shown minimal central nervous system effects, it has been controlled under schedule V.

Loperamide is available as 2-mg capsules (Loperamide hydrochloride capsules, USP) for treatment of acute and chronic diarrhea. Dosage recommended is 4 mg initially, with 2 mg after each loose stool for a maximum of 16 mg/day.

Ethoheptazine Citrate. Ethyl hexahydro-1-methyl-4-phenyl-1*H*-azepine-4-carboxylate citrate; 1-methyl-4-carbe-

thoxy-4-phenylhexamethylenimine citrate (Zactane Citrate). It is effective orally against moderate pain in doses of 50 to 100 mg, with minimal side effects. Parenteral administration is limited because of central stimulating effects. It appears to have no addiction liability, but toxic reactions have occurred with large doses. A double-blind study in humans rated 100 mg of the hydrochloride salt equivalent to 30 mg of codeine and found that the addition of 600 mg of aspirin increased analgesic effectiveness.[29] In another study, 150 mg was found to be equal to 65 mg of propoxyphene, both being better than placebo.[115] It was once available as a 75-mg tablet and in combination with 600 mg of aspirin (Zactirin).

Fentanyl Citrate, USP. *N*-(1-Phenethyl-4-piperidyl)-propionanilide citrate (Sublimaze). This compound occurs as a crystalline powder, soluble in water (1:40) and methanol, and sparingly soluble in chloroform.

This novel anilide derivative has demonstrated analgesic activity 50 times that of morphine in humans.[32] It has a very rapid onset (4 mins) and short duration of action. Side effects similar to those of other potent analgesics are common—in particular, respiratory depression and bradycardia. It is used primarily as an adjunct to anesthesia. For use as a neuroleptanalgesic in surgery, it is available in combination with the neuroleptic droperidol (Innovar). It is also available as a transdermal release system (Duragesic) for management of chronic pain. It has dependence liability.

Alfentanil Hydrochloride. N-[1-[2-(4-Ethyl-5-oxo-2-tetrazolin-1-yl)-ethyl]- 4-(methoxymethyl)-4-piperidyl]propionanilide mono hydrochloride (Alfenta) is closely related to fentanyl. It is a potent analgesic used as a primary anesthetic or for as an adjunct in the maintenance of anesthesia. It shares the same properties and side effects as fentanyl. It is available as an injection (0.5 mg/ml).

Methadone Hydrochloride, USP. 6-(Dimethylamino)-4,4-diphenyl-3-heptanone hydrochloride (Dolophine Hydrochloride). It occurs as a bitter, white crystalline powder. It is soluble in water, freely soluble in alcohol and chloroform, and insoluble in ether.

Methadone is synthesized in several ways. The method of Easton et al.[116] is noteworthy in that it avoids the formation of the troublesome isomeric intermediate aminonitriles.[34,116] The analgesic effect and other morphinelike properties are exhibited chiefly by the (−)-form. Aqueous solutions are stable and may be sterilized by heat for intramuscular and intravenous use. Like all amine salts, it is incompatible with alkali and salts of heavy metals. It is somewhat irritating when injected subcutaneously.

The toxicity of methadone is three to ten times greater than that of morphine, but its analgesic effect is twice that of morphine and ten times that of meperidine. It has been placed under federal narcotic control because of its high addiction liability.

Methadone is a most effective analgesic, used to alleviate many types of pain. It can replace morphine for the relief of withdrawal symptoms. It produces less sedation and narcosis than does morphine and appears to have fewer side reactions in bedridden patients. In spasm of the urinary bladder and in the suppression of the cough reflex, methadone is especially valuable.

The *levo*-isomer, levanone, is said not to produce euphoria or other morphinelike sensations and has been advocated for the treatment of addicts.[117] Methadone itself is being used quite extensively in addict treatment, although not without some controversy.[118] It will suppress withdrawal effects and is widely used to maintain former heroin addicts during this rehabilitation. Large doses are often used to "block" the effects of heroin during treatment.

The use of methadone in treating addicts is subject to FDA regulations that require special registration of physicians and dispensers. Methadone is available, however, for use as an analgesic under the usual narcotic requirements.

Levomethadyl Acetate Hydrochloride. *l*-α-Acetylmethadol; (−)-α-6-(Dimethylamino)-4,4-diphenyl-3-heptyl acetate hydrochloride; methadyl acetate; LAAM. It occurs as a white crystalline powder that is soluble in water, but dissolves with some difficulty. It is prepared by hydride reduction of (+)-methadone followed by acetylation.

Of the four possible methadol isomers, the (3*S*,6*S*)-isomer LAAM has the unique characteristic of producing long-lasting narcotic effects. Extensive metabolism studies have shown that this is due to its *N*-demethylation to give (−)-α-acetylnormethadol, which is more potent than its parent, LAAM, and possesses a long half-life.[119] This is further accentuated by its demethylation to the dinor metabolite, which has similar properties.[119,120]

Because of the need to administer methadone daily, which leads to inconvenience to the maintenance patient and illicit diversion, the long-acting LAAM was actively investigated as an addict-maintenance drug to replace methadone. Generally, an 80- to 100-mg dose three times a week is sufficient for routine maintenance.[40,121] The drug has been approved for use by FDA and is marketed as ORLAAM in solution form (10 mg/mL).

It is of interest to note that the racemate of the normetabolite, noracylmethadol, was once studied in the clinic as a potential analgesic.[122]

Propoxyphene Hydrochloride, USP. (2*S*,3*R*)-(+)-4(Dimethylamino)-3-methy l-1,2-diphenyl-2-butanol proportionate hydrochloride (Darvon; Dolene; Doxaphene. This drug was introduced into therapy in 1957. It may be prepared by the method of Pohland and Sullivan.[123] It occurs as a bitter, white crystalline powder that is freely soluble in water, soluble in alcohol, chloroform, and acetone, but practically insoluble in benzene and ether. It is the α-(+)-isomer, the α-(−)-isomer and β-diastereoisomers being far less potent in analgesic activity. The α-(−)-isomer, *levo*-propoxyphene, is an effective antitussive (see below).

In analgesic potency, propoxyphene is approximately equal to codeine phosphate and has a lower frequency of side effects. It has no antidiarrheal, antitussive, or antipyretic effect, thus differing from most analgesic agents. It is able to suppress the morphine abstinence syndrome in addicts,

but has shown a low level of abuse because of its toxicity. It is not very effective in deep pain and appears to be no more effective in minor pain than aspirin. Its widespread use in dental pain seems justified, since aspirin is reported to be relatively ineffective. It has been classified as a narcotic and controlled under federal law. It does give some euphoria in high doses and has been abused. It has been responsible for numerous overdosage deaths. Refilling of the drug should be avoided if misuse is suspected.

It is available in several combination products with aspirin or acetaminophen (e.g., Wygesic).

Propoxyphene Napsylate, USP. (+)-α-4-Dimethylamino-3-methyl-1,2-diphenyl-2-butanol propionoate (ester) 2-naphthylenesulfonate (salt) (Darvon-N). It is very slightly soluble in water, but soluble in alcohol, chloroform, and acetone.

The napsylate salt of propoxyphene was introduced shortly before the patent on Darvon expired. As an insoluble salt form it is claimed to be less prone to abuse because it cannot be readily dissolved for injection, and upon oral administration gives a slower, less pronounced peak blood level.

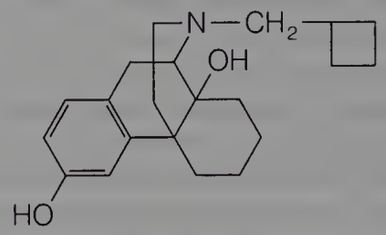
Because of its mild narcoticlike properties it was once investigated as an addict-maintenance drug to be used in place of methadone. It was hoped that it would offer the advantage of providing an easier withdrawal and serve as an addict-detoxification drug. Unfortunately, toxicity at higher doses has limited this application.

It is available in combination with acetaminophen (e.g., Propocet, Darvocet-N).

Levorphanol Tartrate, USP. (−)-3-Hydroxy-*N*-methylmorphinan bitartrate (Levo-Dromoran). The basic studies in the synthesis of this type of compound were made by Grewe, as pointed out earlier. Schnider and Grüssner synthesized the hydroxymorphinans, including the 3-hydroxyl derivative, by similar methods. The racemic 3-hydroxy-*N*-methylmorphinan hydrobromide (racemorphan, (±)-Dromoran) was the original form in which this potent analgesic was introduced. This drug is prepared by resolution of racemorphan. It should be noted that the *levo* compound is available in Europe under the original name, Dromoran. As the tartrate, it occurs in the form of colorless crystals. The salt is sparingly soluble in water (1:60) and is insoluble in ether.

The drug is used for the relief of severe pain and is in many respects similar in its actions to morphine, except that it is from six to eight times as potent. The addiction liability of levorphanol is as great as that of morphine and, for that reason, caution should be observed in its use. It is claimed that the gastrointestinal effects of this compound are significantly less than those experienced with morphine. Naloxone is an effective antidote for over-dosage. Levorphanol is useful for relieving severe pain originating from a multiplicity of causes (e.g., inoperable tumors, severe trauma, renal colic, biliary colic). In other words, it has the same range of usefulness as morphine and is considered an excellent substitute. It is supplied in ampules, in multidose vials, and in the form of oral tablets. The drug requires a narcotic form.

Butorphanol Tartrate, USP. 17-(Cyclobutyl-methyl)-morphinan-3,14-diol D-(−)-tartrate; (−)-*N*-cyclobutyl-methyl-3,14-dihydroxymorphinan bitartrate (Stadol). This potent analgesic occurs as a white crystalline powder soluble in water and sparingly soluble in alcohol. It is prepared from the dihydroxy-*N*-normorphinan obtained by a modification of the Grewe synthesis. It is the cyclobutyl analogue of levorphanol and levallorphan, being equally as potent as the former as an analgesic



Butorphanol

and somewhat less active as an antagonist than the latter.

The onset and duration of action of the drug is comparable with that of morphine, but it has the advantages of showing a maximal ceiling effect on respiratory depression and a greatly reduced abuse liability. The injectable form was marketed without narcotic controls; however, this product was considered for placement in schedule IV because of reported misuse and lack of recognition of its potential abuse liability. The drug has also been used illegally in the doping of race horses.

Butorphanol shares the adverse hemodynamic effects of pentazocine, causing increased pressure in specific arteries and on the heart work load. It should, therefore, be used with caution and only with patients hypersensitive to morphine for the treatment of myocardial infarction or other cardiac problems. Other adverse effects include a high incidence of sedation and, less frequently, nausea, headache, vertigo, and dizziness.[124]

It is available as a parenteral for intramuscular and intravenous administration in a dose of 1 or 2 mg every 3 to 4 hr, with a maximal single dose of 4 mg. It is also available as a nasal spray (Stadol NS).

Buprenorphine Hydrochloride. 21-Cyclopropyl-7α-[(*S*)-1-hydroxy-1,2,2- trimethylpropyl]-6,14-*endo*-ethanol-6,7,8,14-tetrahydrooripavine hydrochloride (Buprenex) is a rapidly-acting, centrally-acting analgesic in the class of agonist-antagonists. It is ~30 times more potent than morphine. It is available for treating moderate to severe pain as a parenteral for intramuscular or intravenous administration in a dose of 0.3 mg every 6 hrs. The drug has been investigated for use in treating opioid addiction.

Dezocine. (−)-13β-Amino-5,6,7,8,9,10,11α,12-octahydro-5α-methyl-5,11-methanobenzocyclodecen-3-ol (Dalgan) is a synthetic agonist-antagonist analgesic, with an unusual structure. It is similar to morphine in analgesic potency and duration. It produces fewer side effects, due to its antago-

nist activity, with reported minimal dependence capacity. It is available for treating moderate to severe pain as a parenteral for intramuscular or intravenous administration in a dose of 5 to 20 mg every 3 to 6 hrs.

Nalbuphine Hydrochloride. 17-(Cyclobutylmethyl)-4,5α-epoxymorphinan-3 ,6α,14-triol hydrochloride (Nubain) is a combination of the oxymorphine nucleus and the nitrogen substituent of butorphanol. It is a potent analgesic of the agonist-antagonist class, similar to morphine in potency, but with an abuse potential rated less than that of codeine. It is useful in treating moderate to severe pain and is available for parenteral use with a usual dose of 10 mg per 70 kg every 3 to 6 hrs.

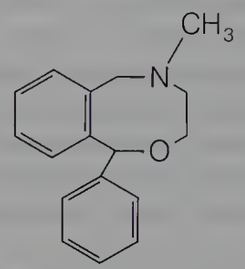
Pentazocine, USP. 1,2,3,4,5,6-Hexahydro-*cis*-6,11-dimethyl-3-(3-methyl-2-b utenyl)-2,6-methano-3-benzazocin-8-ol; *cis*-2-dimethylallyl-5,9-dimethyl-2′-hydroxy-6,7-benzomorphan (Talwin). It occurs as a white crystalline powder that is insoluble in water and sparingly soluble in alcohol. It forms a poorly soluble hydrochloride salt, but is readily soluble as the lactate.

Pentazocine in a parenteral dose of 30 mg or an oral dose of 50 mg is about as effective as 10 mg of morphine in most patients. There is some evidence that the analgesic action resides principally in the (−)-isomer, with 25 mg being approximately equivalent to 10 mg of morphine sulfate.[125] Occasionally, doses of 40 to 60 mg may be required. Pentazocine's plasma half-life is ~3.5 hr.[126] At the lower dosage levels, it appears to be well tolerated, although some degree of sedation occurs in about one-third of those persons receiving it. The incidence of other morphinelike side effects is as high as with morphine and other narcotic analgesics. In patients who have been receiving other narcotic analgesics, large doses of pentazocine may precipitate withdrawal symptoms. It shows an equivalent or greater respiratory depressant activity. Pentazocine has given rise to a few cases of possible dependence liability. It has been placed under control, and its abuse potential should be recognized and close supervision of its use maintained. Levallorphan cannot reverse its effects, although naloxone can, and methylphenidate is recommended as an antidote for overdosage or excessive respiratory depression.

Pentazocine as the lactate is available in injection form containing the base equivalent of 30 mg/ml, buffered to pH 4 to 5. It should not be mixed with barbiturates. Tablets of 50 mg (as the hydrochloride in combination with nalorone to prevent abuse) are also available for oral administration. It is also available in combination with aspirin (Talwin Compound) and with acetaminophen (Talacen).

Methotrimeprazine, USP. (−)-10-[3-(Dimethylamino)-2-methylpropyl]-2-methoxyphenothi a zine (Levoprome). This phenothiazine derivative, closely related to chlorpromazine, possesses strong analgesic activity. An intramuscular dose of 15 to 20 mg is equal to 10 mg of morphine in humans. It has not shown any dependence liability and appears not to produce respiratory depression. The most frequent side effects are similar to those of phenothiazine tranquilizers, namely, sedation and orthostatic hypotension. These often result in dizziness and fainting, limiting the use of methotrimeprazine to nonambulatory patients. It is to be used with caution along with antihypertensives, atropine, and other sedatives. It shows some advantage in patients for whom addiction and respiratory depression are problems.[127]

Nefopam. 3,4,5,6-Tetrahydro-5-methyl-1-phenyl-1*H*-2,5-benzoxazocine; 5-methyl-1-phenyl-3,4,5,6-tetrahydro-[1*H*]-2,5-benzoxazo cine (Acupan). This rather novel analgesic represents a departure from traditional structure-activity relationships, but shows activity comparable with that of codeine. It gives very rapid onset owing to rapid absorption, with 60 mg giving pain relief comparable with 600 mg of aspirin. Side effects were minimal.[128]



Nefopam

NARCOTIC ANTAGONISTS

Nalorphine Hydrochloride, USP. *N*-Allylnormorphine hydrochloride. This morphine derivative may be prepared according to the method of Weijlard and Erickson.[110] It occurs in the form of white or practically white crystals that slowly darken on exposure to air and light. It is freely soluble in water (1 : 8), but is sparingly soluble in alcohol (1 : 35), and is almost insoluble in chloroform and ether. The phenolic hydroxyl group confers water solubility in the presence of fixed alkali. Aqueous solutions of the salt are acid, having a pH of ~5.

Nalorphine has a direct antagonistic effect against morphine, meperidine, methadone, and levorphanol. However, it has little antagonistic effect toward barbiturate or general anesthetic depression.

Perhaps one of the most striking effects is on the respiratory depression accompanying morphine overdosage. The respiratory minute volume is quickly returned to normal by intravenous administration of the drug. However, it does have respiratory depressant activity itself, which may potentiate the existing depression. It affects circulatory disturbances in a similar way, reversing the effects of morphine. Other effects of morphine are affected similarly. It was observed that morphine addicts, when treated with the drug, exhibited certain of the withdrawal symptoms associated with abstinence from morphine. Thus, it was used as a diagnostic test agent to determine narcotic addiction. Administration of nalorphine along with morphine may occasionally prevent or minimize the development of dependence on morphine. It has strong analgesic properties, but it is not accept-

able for such use owing to the high incidence of undesirable psychotic effects. Because of these properties and the availability of alternate antagonists, it was withdrawn from the market.

Levallorphan Tartrate, USP. 17-(2-Propenyl)-morphinan-3-ol tartrate; (−)-*N*-allyl-3-hydroxymorphinan bitartrate (Lorfan). This compound occurs as a white or practically white, odorless crystalline powder. It is soluble in water (1:20), sparingly soluble in alcohol (1:60), and practically insoluble in chloroform and ether. Levallorphan resembles nalorphine in its pharmacologic action, being about five times more effective as a narcotic antagonist. It has also been useful in combination with analgesics such as meperidine, alphaprodine, and levorphanol to prevent the respiratory depression usually associated with these drugs (Table 22-5).

Naloxone Hydrochloride, USP. 4,5-Epoxy-3,14-dihydroxy-17-(2-propenyl)morphinan-6-one hydrochloride; *N*-allyl-14-hydroxynordihydromorphinone hydrochloride (Narcan). *N*-Allylnoroxymorphone hydrochloride is presently on the market as the agent of choice for treating narcotic overdosage. It lacks not only the analgesic activity shown by other antagonists, but also all of the other agonist effects. It is almost seven times more active than nalorphine in antagonizing the effects of morphine. It shows no withdrawal effects after long-term administration. The duration of action is ~4 hrs. It was briefly investigated for the treatment of heroin addiction. With adequate doses of naloxone, the addict does not receive any effect from heroin. It is given to an addict only after a detoxification period. Its long-term usefulness is currently limited because of its short duration of action, thereby requiring large oral doses. Long-acting and alternative antagonists are available (Table 22-5).

Cyclazocine. 3-(Cyclopropylmethyl)-1,2,3,4,5,6-hexahydro-6,11-di methyl-2,6-methano-3-benzazocin-8-ol; *cis*-2-cyclopropylmethyl-5,9-dimethyl-2′-hydroxy-6,7-benzomorpha n is a potent narcotic antagonist that has shown analgesic activity in humans in 1-mg doses. It once was investigated as a clinical analgesic. It does possess hallucinogenic side effects at higher doses which has limited its usefulness as an analgesic. It was studied similarly to naloxone in the treatment of narcotic addiction. By voluntary treatment with cyclazocine, addicts are deprived of the euphorogenic effects of heroin. Its dependence liability is lower, and the effects of withdrawal develop more slowly and are milder. Tolerance develops to the side effects of cyclazocine, but not to its antagonist effects.[129] The effects are long-lasting and are not reversed by other antagonists such as nalorphine. It has not been marketed.

Naltrexone. 17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-d ihydroxymorphinan-6-one; *N*-cyclopropylmethyl-14-hydroxynordihydromorphinone; *N*-cyclopropylmethylnoroxymorphone; EN-1639. This naloxone analogue has been marketed as the preferred agent for treating former opiate addicts. Oral doses of 50 mg daily or 100 mg three times weekly are sufficient to "block" or protect a patient from the effects of heroin. Its metabolism,[130] pharmacokinetics,[131] and pharmacology[132] have been intensely studied because of the tremendous governmental interest in developing new agents for the treatment of addiction.[54]

It is available as 50-mg tablets (ReVia) for use in treating narcotic addiction. It has also shown promise for suppressing craving in the treatment of alcoholism and is available for that use.

Sustained-release or depot dosage forms of naltrexone continue to be investigated to avoid the recurrent decision on the part of the former addict of whether or not a protecting dose of antagonist is needed.[55,133]

Nalmefene Hydrochloride. 17-(Cyclopropylmethyl)-4,5α-epoxy-6-methylenemorphinan-3,14-diol hydrochloride (Revex) is the 6-methylene analog of naloxone. It is the latest pure antagonist to be introduced for use in reversing the effects of opioid agonists. It is longer acting than naltrexone and is used for the same indications. It is available as an injection (0.1 and 1.0 mg/ml).

There are several other narcotic antagonists that have been investigated (e.g., diprenorphine[134] and oxilorphan).[135]

ANTITUSSIVE AGENTS

Cough is a protective, physiologic reflex that occurs in health as well as in disease. It is very widespread and commonly ignored as a mild symptom. However, in many conditions it is desirable to take measures to reduce excessive coughing. It should be stressed that many etiologic factors cause this reflex; and in a case where a cough has been present for an extended period or accompanies any unusual symptoms, the person should be referred to a physician. Cough preparations are widely advertised and often sold indiscriminately; hence, it is the obligation of the pharmacist to warn the public of the inherent dangers.

Among the agents used in the symptomatic control of cough are those that act by depressing the cough center located in the medulla. These have been termed *anodynes, cough suppressants*, and *centrally acting antitussives*. Until recently, the only effective drugs in this area were members of the narcotic analgesic agents. The more important and widely used ones are morphine, hydromorphone, codeine, hydrocodone, methadone, and levorphanol, which were discussed in the foregoing section.

In recent years, several compounds have been synthesized that possess antitussive activity without the addiction liabilities of the narcotic agents. Some of these act in a similar manner through a central effect. In a hypothesis for the initiation of the cough reflex, Salem and Aviad[136] proposed that bronchodilatation is an important mechanism for the relief of cough. Their hypothesis suggests that irritation of the mucosa initially causes bronchoconstriction, and this, in turn, excites the cough receptors.

Chappel and von Seemann[137] have pointed out that most antitussives of this type fall into two structural groups. The larger group has structures that bear a resemblance to metha-

TABLE 22-5

NARCOTIC ANTAGONISTS

Name Proprietary Name	*Preparations*	*Usual Adult Dose**	*Usual Dose Range**	*Usual Pediatric Dose**
Levallorphan tartrate NF *Lorfan*	Levallorphan tartrate injection NF	IV, 1 mg, repeated twice at 10- to 15-min intervals, if necessary	500 μg–2 mg, repeated, if necessary	0.05–0.1 mg in neonates to decrease respiratory depression
Naloxone hydrochloride USP *Narcan*	Naloxone hydrochloride injection USP	Parenteral, 400 μg, repeated at 2- to 3-min intervals as necessary		0.01 mg as above

done. The other group has large, bulky substituents on the acid portion of an ester, usually connected by means of a long, ether-containing chain to a *tertiary* amino group. The notable exceptions are benzonatate and sodium dibunate. Noscapine could be considered as belonging to the first group.

Many of the cough preparations sold contain various other ingredients in addition to the primary antitussive agent. The more important ones include antihistamines, useful when the cause of the cough is allergic, although some antihistaminic drugs (e.g., diphenhydramine) have a central antitussive action as well; sympathomimetics, which are quite effective owing to their bronchodilatory activity, the most useful being ephedrine, methamphetamine, phenylpropanolamine, homarylamine, isoproterenol, and isoöctylamine; parasympatholytics, which help to dry secretions in the upper respiratory passages; and expectorants. It is not known if these drugs potentiate the antitussive action, but they usually are considered as adjuvant therapy.

The more important drugs in this class will be discussed in the following section. For a more exhaustive coverage of the field, the reader is urged to consult the excellent review of Chappel and von Seemann.[137]

PRODUCTS

Some of the narcotic antitussive products have been discussed previously with the narcotic analgesics. Others are discussed below (Table 22-6).

Noscapine, USP. (−)-Narcotine (Tusscapine). This opium alkaloid was isolated, in 1817, by Robiquet. It is isolated rather easily from the drug by ether extraction. It makes up 0.75% to 9% of opium.

Noscapine occurs as a fine, white or practically white crystalline powder that is odorless and stable in the presence of light and air. It is practically insoluble in water, freely soluble in chloroform, and soluble in acetone and benzene. It is only slightly soluble in alcohol and ether.

With the discovery of its unique antitussive properties, the name of this alkaloid was changed from narcotine to noscapine. It was realized that it would not meet with widespread acceptance as long as its name was associated with the narcotic opium alkaloids. The name ''noscapine'' was probably selected because a precedent existed in the name of (±)-narcotine, namely ''gnoscopine.''

Although noscapine had been used therapeutically as an antispasmodic (similar to papaverine), antineuralgic, and antiperiodic, it had fallen into disuse. It had also been used in malaria, migraine, and other conditions in the past in doses of 100 to 600 mg. Newer methods of testing for antitussive compounds were responsible for revealing the effectiveness of noscapine as an antitussive. In addition to its central action, it exerts bronchodilatation effects.

Noscapine is an orally effective antitussive, approximately equal to codeine in effectiveness. It is free of the side effects usually encountered with the narcotic antitussives and, because of its relatively low toxicity, may be given in larger doses to obtain a greater antitussive effect. Although

TABLE 22-6

ANTITUSSIVE AGENTS

Name Proprietary Name	*Preparations*	*Usual Adult Dose**	*Usual Dose Range**
Dextromethorphan hydrobromide USP *Romilar*	Dextromethorphan hydrobromide syrup USP	15–30 mg qd to qid	
Levopropoxyphene napsylate USP *Novrad*	Levopropoxyphene napsylate capsules USP Levopropoxyphene napsylate oral suspension USP	50–100 mg of levopropoxyphene, as the napsylate, every 4 hr	
Benzonatate USP *Tessalon*	Benzonatate capsules USP	100 mg tid	100–200 mg

it is an opium alkaloid, it is devoid of analgesic action and addiction liability. It was once available in various cough preparations (e.g., Conar).

Dextromethorphan Hydrobromide, USP. (+)-3-Methoxy-17-methyl-9 α,13α, 14α-morphinan hydrobromide (Romilar). This drug is the *O*-methylated (+)-form of racemorphan left after the resolution necessary in the preparation of levorphanol. It occurs as practically white crystals, or as a crystalline powder, possessing a faint odor. It is sparingly soluble in water (1:65), freely soluble in alcohol and chloroform, and insoluble in ether.

It possesses the antitussive properties of codeine, without the analgesic, addictive, central depressant, and constipating features. Ten milligrams is suggested as being equivalent to a 15-mg dose of codeine in antitussive effect.

It affords an opportunity to note the specificity exhibited by very closely related molecules. Here, the (+)- and (−)-forms both must attach to receptors responsible for the suppression of cough reflex, but the (+)-form is apparently in a steric relationship, such that it is incapable of attaching to the receptors involved in analgesic, constipative, addictive, and other actions exhibited by the (−)-form. It has largely replaced many older antitussives, including codeine, in prescription and nonprescription cough preparations.

Benzonatate, USP. 2,5,8,11,14,17,20,23,26-Nonaoxaoctacosan-28-yl *p*-(butylamino)benzoate (Tessalon). This compound was introduced in 1956. It is a pale yellow, viscous liquid, insoluble in water and soluble in most organic solvents. It is chemically related to *p*-aminobenzoate local anesthetics, except that the aminoalcohol group has been replaced by a methylated polyethylene glycol group.

Benzonatate is said to possess both peripheral and central activity in producing its antitussive effect. It somehow blocks the stretch receptors thought to be responsible for cough. Clinically, it is not as effective as codeine, but produces far fewer side effects and has a very low toxicity. It is available in 100-mg capsules (''perles'').

Caramiphen Edisylate. 2-Diethylaminoethyl 1-phenylcyclopentane-1-carboxylate ethanedisulfonate. Caramiphen occurs in the form of water- and alcohol-soluble crystals. The antitussive activity of this compound is less than that of codeine. It has been shown to have both central and bronchodilator activity. The frequency of side effects is lower than with the narcotic antitussives. It is currently marketed as a combination under the tradenames of Tuss-Ornade, both in a liquid form and in a sustained-release form, and as Tussogest, Tuss-Allergine, and others.

Carbetapentane Citrate. 2-[2-(Diethylamino)-ethoxy]-ethyl 1-phenylcyclopentanecarboxylate citrate. This salt is a white, odorless, crystalline powder that is freely soluble in water (1:1), slightly soluble in alcohol, and insoluble in ether. It is similar to caramiphen chemically and is said to be equivalent to codeine as an antitussive. Introduced in 1956, it is well tolerated and has a low frequency of side effects. It is available as a pediatric suspension (2.5 mg/5 ml) or as capsules (20 mg; Cophene-X).

The tannate is also available (Rynatuss) as a 60-mg tablet and is said to give a more sustained action.

ANTI-INFLAMMATORY ANALGESICS

The growth of this group of analgesics was related closely to the early belief that the lowering or ''curing'' of fever was an end in itself. Drugs bringing about a drop in temperature in feverish conditions were considered to be quite valuable and were sought after eagerly. The decline of interest in these drugs coincided more or less with the realization that fever was merely an outward symptom of some other, more fundamental, ailment. However, during the use of the several antipyretics, it was noted that some were excellent analgesics for the relief of minor aches and pains. These drugs have survived to the present time on the basis of the analgesic, rather than the antipyretic, effect. Although these drugs are still widely utilized for the alleviation of minor aches and pains, they are also employed extensively in the symptomatic treatment of rheumatic fever, rheumatoid arthritis, and osteoarthritis. The dramatic effect of salicylates in reducing the inflammatory effects of rheumatic fever is time-honored, and, even with the development of the corticosteroids, these drugs are still of great value in this respect. It has been reported that the steroids are no more effective than the salicylates in preventing the cardiac complications of rheumatic fever.[138]

The analgesic drugs that fall into this category have been disclaimed by some as not deserving the term ''analgesic'' because of the low order of activity in comparison with the morphine-type compounds. Indeed, Fourneau has suggested the name *antalgics* to designate this general category and, in this way, to make more emphatic the distinction from the narcotic or so-called true analgesics. Two of the principal features distinguishing these minor analgesics from the narcotic analgesics are the low activity for a given dose and that a higher dosage does not give any significant increase in effect.

Considerable research has continued in an effort to find new nonsteroidal anti-inflammatory drugs (NSAID). Long-term therapy with the corticosteroids is often accompanied by various side effects. Efforts to discover new agents have been limited, for the most part, to structural analogues of active compounds owing to a lack of knowledge about the causes and mechanisms of inflammatory diseases.[139] Although several new agents have been introduced for use in rheumatoid arthritis, aspirin remains as one of the most widely used drugs for this purpose.

Of considerable interest is the observation that prostaglandins play a major role in the inflammatory process.[140] Of particular significance are reports that drugs, such as aspirin and indomethacin, inhibit prostaglandin synthesis in several tissues.[141] Furthermore, almost all classes of nonsteroidal anti-inflammatory drugs strongly inhibit the conversion of arachidonic acid into prostaglandin E_2(PGE_2). This has been

shown to occur at the stage of conversion of arachidonic acid, released by the action of phospholipase A on damaged tissues, to the cyclic endoperoxides, PGG_2 and PGH_2, by prostaglandin synthetase. These are known to cause vasoconstriction and pain. They, in turn, are converted in part to PGE_2 and $PGF_{2\alpha}$, which can cause pain and vasodilatation. This effect of the nonsteroidal anti-inflammatory drugs parallels their relative potency in various tests and is stereospecific.[142] The search for specific inhibitors of prostaglandin synthesis has opened a new area of research in this field.

Discussion of these drugs will be facilitated by considering them in their various chemical categories.

SALICYLIC ACID DERIVATIVES

Historically, the salicylates were among the first of this group to achieve recognition as analgesics. Leroux, in 1827, isolated salicin, and Piria, in 1838, prepared salicylic acid. Following these discoveries, Cahours (1844) obtained salicylic acid from oil of wintergreen (methyl salicylate); and Kolbe and Lautermann (1860) prepared it synthetically from phenol. Sodium salicylate was introduced in 1875 by Buss, followed by the introduction of phenyl salicylate by Nencki, in 1886. Aspirin, or acetylsalicylic acid, was first prepared in 1853 by Gerhardt, but remained obscure until Felix Hoffmann discovered its pharmacologic activities in 1899. It was tested and introduced into medicine by Dreser, who named it *aspirin* by taking the *a* from acetyl and adding it to *spirin*, an old name for salicylic or spiric acid, derived from its natural source of spirea plants.

The pharmacology of the salicylates and related compounds has been reviewed extensively by Smith.[144,145] Salicylates, in general, exert their antipyretic action in febrile patients by increasing heat elimination of the body through the mobilization of water and consequent dilution of the blood. This brings about perspiration, causing cutaneous dilatation. This does not occur with normal temperatures. The antipyretic and analgesic actions are believed to occur in the hypothalamic area of the brain. It is also thought by some that the salicylates exert their analgesia by their effect on water balance, reducing the edema usually associated with arthralgias. Aspirin has been shown to be particularly effective for this.

For an interesting account of the history of aspirin and a discussion of its mechanisms of action, the reader should consult an article on the subject by Collier,[146] as well as the reviews by Smith,[144,145] and Nickander et al.[143]

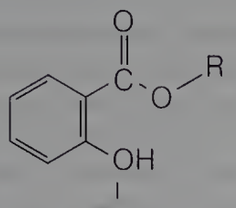
The possibility of hypoprothrombinemia and concomitant capillary bleeding in conjunction with salicylate administration accounts for the inclusion of menadione in some salicylate formulations. However, there is some doubt about the necessity for this measure. A more serious aspect of salicylate medication has been the possibility of inducing hemorrhage from direct irritative contact with the mucosa. Alvarez and Summerskill have pointed out a definite relationship between salicylate consumption and massive gastrointestinal hemorrhage from peptic ulcer.[147] Barager and Duthie,[148] on the other hand, in an extensive study found no danger of increase in anemia or in development of peptic ulcer. Levy[149] has demonstrated with the use of radiolabeled iron that bleeding does occur following administration of aspirin. The effects varied with the formulation. It is suggested by Davenport[150] that back diffusion of acid from the stomach is responsible for capillary damage.

Because of these characteristics of aspirin, it has been extensively studied as an antithrombotic agent in the treatment and prevention of clinical thrombosis.[151] It is thought to act by its selective action on the synthesis of the prostaglandin-related thromboxane and prostacyclin, which are the counterbalancing factors involved in platelet aggregation and are released when tissue is injured. Although aspirin has now been approved for the prevention of transient ischemic attacks, indicators of an impending stroke, it is not recommended for patients who have suffered heart attacks.[152]

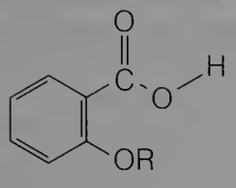
The salicylates are readily absorbed from the stomach and the small intestine, being quite dependent on the pH of the media. Absorption is considerably slower as the pH rises (more alkaline) because of the acidic nature of these compounds and the necessity for the presence of undissociated molecules for absorption through the lipoidal membrane of the stomach and the intestines. Therefore, buffering agents administered at the same time in *excessive* amounts will decrease the rate of absorption. In small quantities, their principal effect may be to aid in the dispersion of the salicylate into fine particles. This would help to increase absorption and decrease the possibility of gastric irritation by the accumulation of large particles of the undissolved acid and their adhesion to the gastric mucosa. Levy and Haves[153] have shown that the absorption rate of aspirin and the incidence of gastric distress were a function of the dissolution rate of its particular dosage form. A more rapid dissolution rate of calcium and buffered aspirin was believed to account for faster absorption. They also established that significant variations exist in dissolution rates of different nationally distributed brands of plain aspirin tablets. This may account for some of the conflicting reports and opinions concerning the relative advantages of plain and buffered aspirin tablets. Lieberman et al.[154] have also shown that buffering is effective in raising the blood levels of aspirin. In a measure of the antianxiety effect of aspirin by means of electroencephalograms (EEG), differences between buffered, brand name, and generic aspirin preparations were found.[155]

Potentiation of salicylate activity by virtue of simultaneous administration of *p*-aminobenzoic acid or its salts has been the basis for the introduction of numerous products of this kind. Salassa and coworkers have shown this effect to be due to the inhibition both of salicylate metabolism and of excretion in the urine.[156] This effect has been proved amply, provided that the ratio of 24 g of *p*-amino-benzoic acid to 3 g of salicylate per day is observed. However, there is no strong evidence to substantiate any significant elevation of plasma salicylate levels when a smaller quantity of *p*-aminobenzoic acid is employed.

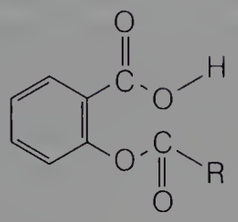
The derivatives of salicylic acid are of two types [I and II (a,b)]:



I



IIa



IIb

Type I represents those that are formed by modifying the carboxyl group (e.g., salts, esters, or amides). Type II (a and b) represents those that are derived by substitution on the hydroxyl group of salicylic acid. The derivatives of salicylic acid were introduced in an attempt to prevent the gastric symptoms and the undesirable taste inherent in the common salts of salicylic acid. Hydrolysis of type I takes place to a greater extent in the intestine, and most of the type II compounds are absorbed unchanged into the bloodstream (see aspirin).

Compounds of Type I

The alkyl and aryl esters of salicylic acid (type I) are used externally, primarily as counterirritants, where most of them are well absorbed through the skin. This type of compound is of little value as an analgesic.

A few inorganic salicylates are used internally when the effect of the salicylate ion is intended. These compounds vary in their irritation of the stomach. To prevent the development of pink or red coloration in the product, contact with iron should be avoided in their manufacture.

Sodium Salicylate, USP. This may be prepared by the reaction, in aqueous solution, between 1 mole each of salicylic acid and sodium bicarbonate; upon evaporating to dryness, the white salt is obtained.

Generally, the salt has a pinkish tinge or is a white microcrystalline powder. It is odorless or has a faint, characteristic odor, and it has a sweet, saline taste. It is affected by light. The compound is soluble in water (1 : 1), alcohol (1 : 10), and glycerin (1 : 4).

In solution, particularly in the presence of sodium bicarbonate, the salt will darken on standing (see salicyclic acid). This darkening may be lessened by the addition of sodium sulfite or sodium bisulfite. Also, a color change is lessened by using recently boiled distilled water and dispensing in amber-colored bottles. Sodium salicylate forms a eutectic mixture with antipyrine and produces a violet coloration with iron or its salts. Solutions of the compound must be neutral or slightly basic to prevent precipitation of free salicylic acid. However, the USP salt forms neutral or acid solutions.

This salt is the one of choice for salicylate medication and usually is administered with sodium bicarbonate to lessen gastric distress, or it is administered in enteric-coated tablets. The use of sodium bicarbonate[157] is ill-advised, because it decreases the plasma levels of salicylate and increases the excretion of free salicylate in the urine.

Sodium Thiosalicylate. Asproject; Rexolate; Tusal. This is the sulfur or thio analogue of sodium salicylate. It is more soluble and better absorbed, thereby requiring lower dosages. It is recommended for gout, rheumatic fever, and muscular pains in doses of 100 to 150 mg every 3 to 6 hr for 2 days, and then 100 mg once or twice daily. It is available only for injection.

Magnesium Salicylate, USP. Original Doan's Mobidin; Magan. This is a sodium-free salicylate preparation that may be used in conditions in which sodium intake is restricted. It is claimed to produce less gastrointestinal upset. The dosage and indications are the same as for sodium salicylate.

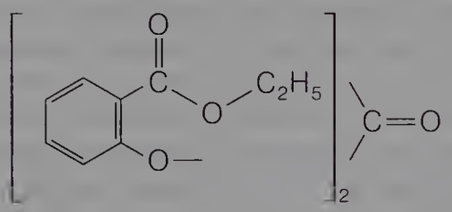
Choline Salicylate. Arthropan. This salt of salicylic acid is extremely soluble in water. It is claimed to be absorbed more rapidly than aspirin, giving faster peak blood levels. It is used in conditions for which salicylates are indicated in a recommended dose of 870 mg to 1.74 g four times daily. It is also available in combination with magnesium salicylate (Trilisate).

Other salts of salicylic acid that have found use are those of ammonium, lithium, and strontium. They offer no distinct advantage over sodium salicylate.

Carbethyl Salicylate. Ethyl salicylate carbonate (Sal-Ethyl Carbonate) is an ester of ethyl salicylate and carbonic acid, and thus is a combination of a type I and type II compound.

It occurs as white crystals, insoluble in water and in diluted hydrochloric acid, slightly soluble in alcohol or ether and readily soluble in chloroform or acetone. The insolubility tends to prevent gastric irritation and makes it tasteless.

In action and uses it resembles aspirin and gives the antipyretic and analgesic effects of the salicylates. It was once marketed as a powder, tablet, and a tablet containing aminopyrine.



Carbethyl Salicylate

Salol Principle

Nencki introduced salol in 1886 and by so doing presented to the science of therapy the "Salol Principle." In salol, two

toxic substances (phenol and salicylic acid) were combined into an ester that, when taken internally, will slowly hydrolyze in the intestine to give the antiseptic action of its components. This type of ester is referred to as a "full salol" or "true salol" when both components of the ester are active compounds. Examples are guaiacol benzoate, β-naphthol benzoate, and salol.

This Salol Principle can be applied to esters of which only the alcohol or the acid is the toxic, active or corrosive portion, and this type is called a "partial salol."

Examples of a partial salol containing an active acid are ethyl salicylate and methyl salicylate. Examples of a partial salol containing an active phenol are creosote carbonate, thymol carbonate, and guaiacol carbonate.

Although a host of the salol-type compounds have been prepared and used to some extent, none is presently very valuable in therapeutics, and all are surpassed by other agents.

Phenyl Salicylate. Salol. Phenyl salicylate occurs as fine, white crystals or as a white crystalline powder with a characteristic taste and a faint, aromatic odor. It is insoluble in water (1:6700), slightly soluble in glycerin, soluble in alcohol (1:6), ether, chloroform, acetone, or fixed and volatile oils.

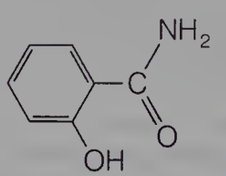
Damp or eutectic mixtures form readily with many organic materials, such as thymol, menthol, camphor, chloral hydrate, and phenol.

Salol is sold in combination with methenamine and atropine alkaloids as a urinary tract antiseptic and analgesic (e.g., Cystrea, Lanased, Renalgin, and Urised).

Salol is insoluble in the gastric juice but is slowly hydrolyzed in the intestine into phenol and salicylic acid. Because of this fact, coupled with its low melting point (41°C to 43°C), it has been used in the past as an enteric coating for tablets and capsules. However, it is not efficient as an enteric-coating material, and its use has been superseded by more effective materials.

It also is used externally as a sun filter (10% ointment) for sunburn prevention (Rayderm).

Salicylamide. *o*-Hydroxybenzamide. This is a derivative of salicylic acid that has been known for almost a century and has found renewed interest. It is readily prepared from salicyl chloride and ammonia. The compound occurs as a nearly odorless, white crystalline powder. It is fairly stable to heat, light, and moisture. It is slightly soluble in water (1:500), soluble in hot water, alcohol (1:15), and propylene glycol, and sparingly soluble in chloroform and ether. It is freely soluble in solutions of alkalies. In alkaline solution with sodium carbonate or triethanolamine, decomposition takes place, resulting in a yellow to red precipitate.



Salicylamide

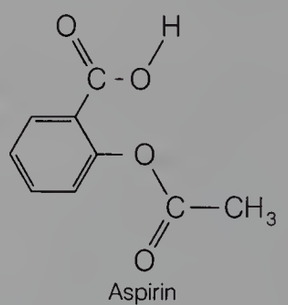
Salicylamide is said to exert a moderately quicker and deeper analgesic effect than does aspirin. Long-term studies on rats revealed no untoward symptomatic or physiologic reactions. Its metabolism is different from that of other salicylic compounds, and it is not hydrolyzed to salicylic acid.[144] Its analgesic and antipyretic activity is probably no greater than that of aspirin, and possibly less. However, it can be used in place of salicylates and is particularly useful for those patients for whom there is a demonstrated sensitivity to salicylates. It is excreted much more rapidly than other salicylates, which probably accounts for its lower toxicity and, thus, does not permit high blood levels.

The dose for simple analgesic effect may vary from 300 mg to 1 g administered three times daily; but for rheumatic conditions the dose may be increased to 2 to 4 g, three times a day. However, gastric intolerance may limit the dosage. The usual period of this higher dosage should not extend beyond three to six days. It is available in several combination products (e.g., Saleto, BC Tablets).

Aspirin, USP. Acetylsalicylic acid (Aspro; Empirin). Aspirin was introduced into medicine by Dreser in 1899. It is prepared by treating salicylic acid, which was first prepared by Kolbe in 1874, with acetic anhydride.

The hydrogen atom of the hydroxyl group in salicylic acid has been replaced by the acetyl group; this also may be accomplished by using acetyl chloride with salicylic acid or ketene with salicylic acid.

Aspirin occurs as white crystals or as a white crystalline powder. It is slightly soluble in water



Aspirin

(1:300) and soluble in alcohol (1:5), choloroform (1:17), and ether (1:15). Also, it dissolves easily in glycerin. Aqueous solubility may be increased by using acetates or citrates of alkali metals, although these are said to decompose it slowly.

It is stable in dry air, but in the presence of moisture, it slowly hydrolyzes into acetic and salicylic acids. Salicylic acid will crystallize out when an aqueous solution of aspirin and sodium hydroxide is boiled and then acidified.

Aspirin itself is sufficiently acid to produce effervescence with carbonates and, in the presence of iodides, to cause the slow liberation of iodine. In the presence of alkaline hydroxides and carbonates, it decomposes, although it does form salts with alkaline metals and alkaline earth metals. The presence of salicylic acid, formed upon hydrolysis, may be confirmed by the formation of a violet color upon the addition of ferric chloride solution.

Aspirin is not hydrolyzed appreciably on contact with weakly acid digestive fluids of the stomach, but on passage into the intestine is subjected to some hydrolysis. However, most of it is absorbed unchanged. The gastric mucosal irritation of aspirin has been ascribed by Garrett[158] to salicylic acid formation, the natural acidity of aspirin, or the adhesion of undissolved aspirin to the mucosa. He has also proposed the nonacidic anhydride of aspirin as a superior form for oral administration. Davenport[150] concludes that aspirin causes an alteration in mucosal cell permeability, allowing back diffusion of stomach acid which damages the capillaries. A number of proprietaries (e.g., Bufferin) employ compounds, such as sodium bicarbonate, aluminum glycinate, sodium citrate, aluminum hydroxide, or magnesium trisilicate, to counteract this acid property. One of the better antacids is dihydroxyaluminum aminoacetate, USP. Aspirin has been shown to be unusually effective when prescribed with calcium glutamate. The more stable, nonirritant calcium acetylsalicylate is formed, and the glutamate portion (glutamic acid) maintains a *p*H of 3.5 to 5.

Preferably, dry dosage forms (i.e., tablets, capsules, or powders) should be used, since aspirin is somewhat unstable in aqueous media. In tablet preparations, the use of acid-washed talc has been shown to improve the stability of aspirin.[159] Also, it has been found to break down in the presence of phenylephrine hydrochloride.[160] Aspirin in aqueous media will hydrolyze almost completely in less than one week. However, solutions made with alcohol or glycerin do not decompose as quickly. Citrates retard hydrolysis only slightly. Some studies have indicated that sucrose tends to inhibit hydrolysis. A study of aqueous aspirin suspensions has indicated that sorbitol exerts a pronounced stabilizing effect.[161] Stable liquid preparations are available that use triacetin, propylene glycol, or a polyethylene glycol. Aspirin lends itself readily to combination with many other substances, but tends to soften and become damp with methenamine, aminopyrine, salol, antipyrine, phenol, or acetanilid.

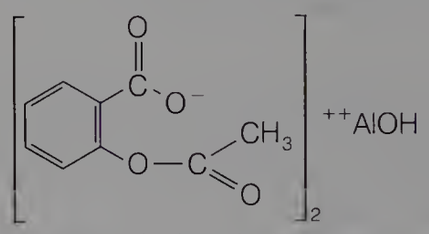
Aspirin is one of the most widely used compounds in therapy and, for many years, was not associated with untoward effects. Allergic reactions to aspirin are now commonly observed. Asthma and urticaria are the most common manifestations and, when they occur, are extremely acute and difficult to relieve. Like sodium salicylate, it has been shown to cause congenital malformations when administered to mice.[162] Pretreatment with sodium pentobarbital or chlorpromazine resulted in a significant lowering of these effects.[163] Similar effects have been attributed to the consumption of aspirin in women, and its use during pregnancy should be avoided. However, other studies indicate that no untoward effects are seen. The reader is urged to consult the excellent review by Smith for an account of the pharmacologic aspects of aspirin.[144,145]

Practically all salts of aspirin, except those of aluminum and calcium, are unstable for pharmaceutic use. These salts appear to have fewer undesirable side effects and to induce analgesia faster than aspirin.

A timed-release preparation of aspirin is available. It does not appear to offer any advantages over aspirin, except for bedtime dosage.

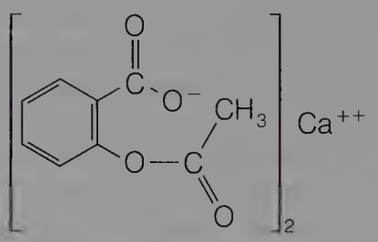
Aspirin is used as an antipyretic, analgesic, and antirheumatic, usually in powder, capsule, suppository, or tablet form. Its use in rheumatism has been reviewed, and it is said to be the drug of choice over all other salicylate derivatives.[164,165] There is some anesthetic action when applied locally, especially in powder form in tonsilitis or pharyngitis, and in ointment form for skin itching and certain skin diseases. In the usual dose, 52% to 75% is excreted in the urine, in various forms, in a period of 15 to 30 hr. It is believed that analgesia is due to the unhydrolyzed acetylsalicylic acid molecule.[144–146]

Aluminum Aspirin. Hydroxybis(salicylato)aluminum diacetate. This salt of aspirin may be prepared by thoroughly mixing aluminum hydroxide gel, water, and acetylsalicylic acid, maintaining the temperature below 65°. Aluminum aspirin occurs as a white to off-white powder or granules and is odorless or has only a slight odor. It is insoluble in water and organic solvents, is decomposed in aqueous solutions of alkali hydroxides and carbonates, and is not stable above 65°C. It offers the advantages of being free of odor and taste and possesses added shelf-life stability. It is available in a flavored form for children (Dulcet).



Aluminum Aspirin

Calcium Acetylsalicylate. Soluble aspirin; calcium aspirin. This compound is prepared by treating acetylsalicylic acid with calcium ethoxide or methoxide in alcohol or acetone solution. It is readily soluble in water (1:6), but only sparingly soluble in alcohol (1:80). It is more stable in solution than aspirin and is used for the same conditions.



Calcium Acetylsalicylate

Calcium aspirin is marketed also as a complex salt with urea, calcium carbaspirin (Calurin), which is claimed to give more rapid salicylate blood levels and to be less irritating than aspirin, although no clear advantage has been shown.

The usual dose is 500 mg to 1.0 g.

Salsalate. Salicylsalicylic acid (Amigesic; Disalcid) is the ester formed between two salicylic acid molecules to which it is hydrolyzed following absorption. It is said to cause less gastric upset than aspirin because it is relatively

insoluble in the stomach and is not absorbed until it reaches the small intestine. Limited clinical trails[166–168] suggest that it is as effective as aspirin and that it may have fewer side effects.[169] The recommended dose is 325 to 1,000 mg two or three times a day. It is available only on prescription.

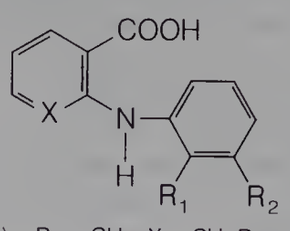
4-Aminosalicylic Acid. Once available as the sodium salt (Tubasal, P.A.S. Sodium) for use as an analgesic, the free acid is being offered as an orphan drug in the treatment of ulcerative colitis (Pamisyl, Rezipas).

Flufenisal. Acetyl-5-(4-fluorophenyl)salicylic acid; 5′-fluoro-4-hydroxy-3-biphenylcarboxylic acid acetate (Dolobid). Over the years several hundred analogues of aspirin have been made and tested to produce a compound that was more potent, longer acting, and with less gastric irritation. By the introduction of a hydrophobic group in the 5-position, flufenisal appears to meet these criteria. In animal tests it is at least four times more potent. In humans, it appears to be about twice as effective with twice the duration.[170] Like other aryl acids it is highly bound to plasma protein as its deacylated metabolite. It is marketed in tablets (250 and 500 mg) for treating mild to moderate pain and rheumatoid and osteoarthritis.

N-ARYLANTHRANILIC ACIDS

One of the early advances in the search for nonnarcotic analgesics was centered in the *N*-arylanthranilic acids. Their outstanding characteristic is that they are primarily nonsteroidal anti-inflammatory agents and, secondarily, that some possess analgesic properties.

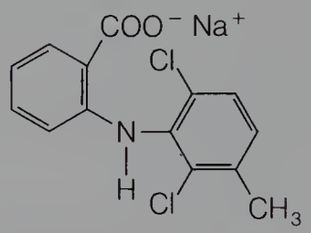
Mefenamic Acid. *N*-2,3-Xylylanthranilic acid (Ponstel) occurs as an off-white crystalline powder that is insoluble in water and slightly soluble in alcohol. It appears to be the first genuine antiphlogistic analgesic discovered since aminopyrine. Because it is believed that aspirin and aminopyrine owe their general purpose analgesic efficacy to a combination of peripheral and central effects,[171] a wide variety of arylanthranilic acids were screened for antinociceptive (analgesic) activity if they showed significant anti-inflammatory action. It has become evident that the combination of both effects is a rarity among these compounds. The mechanism of analgesic action is believed to be related to its ability to block prostaglandin synthetase. No relationship to lipid-plasma distribution, partition coefficient, or pK_a has been noted. The interested reader, however, will find additional information on antibradykinin and anti-UV erythema activities of these compounds, together with speculations on a receptor site, in the literature.[172]



(a) $R_1 = CH_3$, X = CH, R_2 = H
(b) R_1 = H, $R_2 = CF_3$, X = CH

It has been shown[173] that mefenamic acid in a dose of 250 mg is superior to 600 mg of aspirin as an analgesic and that doubling the dose gives a sharp increase in efficacy. A study[174] examining this drug relative to gastrointestinal bleeding indicated that it has a lower incidence of this side effect than has aspirin. Diarrhea, drowsiness, and headache have accompanied its use. The possibility of blood disorders has prompted limitation of its administration to seven days. It is not recommended for children or during pregnancy. It has been approved for use in the management of primary dysmenorrhea, which is thought to be caused by excessive concentrations of prostaglandins and endoperoxides.

Meclofenamate Sodium. Meclomen. This is sodium *N*-(2,6-dichloro-*m*-tolyl)anthranilate.



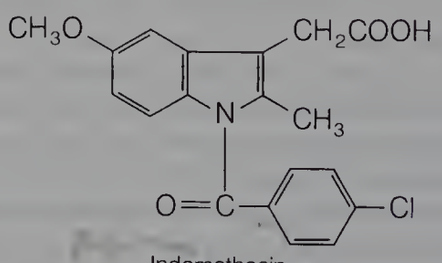
Meclofenamate Sodium

This drug is available in 50- and 100-mg capsules for use in the treatment of acute and chronic rheumatoid arthritis. The most significant side effects are gastrointestinal, including diarrhea.

ARYLACETIC ACID DERIVATIVES

This group of anti-inflammatory agents has received the most intensive attention for new clinical candidates. As a group they have the characteristic of showing high analgesic potency in addition to their anti-inflammatory activity.

Indomethacin, USP. 1-(*p*-Chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid (Indocin) occurs as a pale yellow to yellow-tan crystalline powder that is soluble in ethanol and acetone and practically insoluble in water. It is unstable in alkaline solution and sunlight. It shows polymorphism, one form melting at ~155°C and the other at ~162°C. It may occur as a mixture of both forms with a melting range between these melting points.



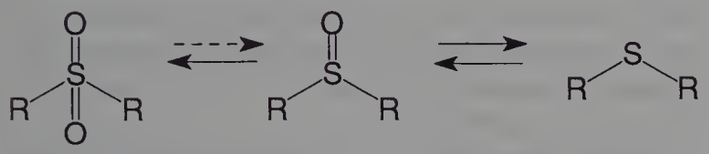
Indomethacin

Since its introduction in 1965, it has been widely used as an anti-inflammatory analgesic in rheumatoid arthritis, spondylitis, and osteoarthritis, and to a lesser extent in gout. Although both its analgesic and anti-inflammatory activities have been well established, it appears to be no more effective than aspirin.[175]

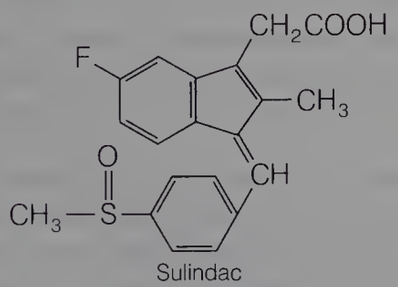
The most frequent side effects are gastric distress and headache. It has also been associated with peptic ulceration, blood disorders, and possible deaths. The side effects appear to be dose-related and sometimes can be minimized by reducing the dose. It is not recommended for use in children because of possible interference with resistance to infection. As do many other acidic compounds, it circulates bound to blood protein, requiring caution in the concurrent use of other protein-binding drugs.

Indomethacin is recommended only for those patients by whom aspirin cannot be tolerated, and in place of phenylbutazone in long-term therapy, for which it appears to be less hazardous than corticosteroids or phenylbutazone.

Sulindac, USP. (*Z*)-5-Fluoro-2-methyl-1-[[*p* -(methylsulfinyl)phenyl]methylene]-1*H*-indene-3-acetic acid (Clinoril) occurs as yellow crystals soluble in alkaline but insoluble in acidic solutions. The drug reaches peak blood levels within 2 to 4 hr and undergoes a complicated, reversible metabolism shown as follows:

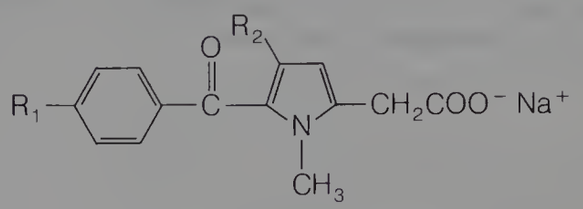


The parent sulfinyl has a plasma half-life of 8 hr, with that of the active sulfide metabolite being 16.4 hr. The more polar and inactive sulfoxide is virtually the sole form excreted. The long half-life is due to extensive enterohepatic recirculation.[176] In in vitro studies, only the sulfide species inhibits prostaglandin synthetase. Although these forms are highly protein bound, the drug does not appear to affect binding of anticoagulants or hypoglycemics. Coadministration of aspirin is contraindicated because it considerably reduces the sulfide blood levels.


Sulindac

Careful monitoring of patients with a history of ulcers is recommended. Gastric bleeding, nausea, diarrhea, dizziness, and other adverse effects have been noted, but with a lower frequency than with aspirin. Sulindac is recommended for rheumatoid arthritis, osteoarthritis, and ankylosing spondolitis in 150- to 200-mg dose, twice daily.[177] It is available as tablets (150 and 200 mg).

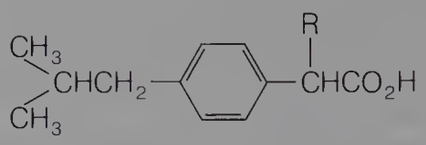
Tolmetin Sodium, USP. 1-Methyl-5-(*p*-toluoyl)pyrrole-2-acetate dihydrate sodium; McN-2559 (Tolectin) is an arylacetic acid derivative with a pyrrole as the aryl group. This drug is rapidly absorbed with a relatively short plasma half-life (1 hr). It is recommended for use in the management of acute and long-term rheumatoid arthritis. It shares similar, but less frequent, adverse effects with aspirin. It does not potentiate coumarinlike drugs nor alter the blood levels of sulfonylureas or insulin. As with other drugs in this class, it is known to inhibit prostaglandin synthetase and lower PGE blood levels.


Tolmetin: $R_1 = CH_3$, $R_2 = H$
Zomepirac: $R_1 = Cl$, $R_2 = CH_3$

Available as tablets (200 mg), a dose of 400 mg three times daily, with a maximum of 2,000 mg, is recommended. Clinical trials indicate a usual daily dose of 1200 mg is comparable in relief to 3.9 g of aspirin and 150 mg of indomethacin per day.[178]

Zomepirac Sodium, USP. 5-(4-Chlorobenzoyl)-1,4-dimethyl-1*H*-pyrrole-2-acetate dihydrate sodium; McN-2783 (Zomax) is the chloro analogue of tolmetin. It shows significantly longer plasma levels (7 hr),[179] thereby requiring less frequent dosing. In pain relief, 25 to 50 mg is reported to give relief equivalent to 650 mg of aspirin. In a study on cancer patients, oral doses of 100 to 200 mg were as effective as moderate parenteral doses of morphine.[180] This drug was marketed briefly in the United States in 100-mg tablets, but it was abruptly withdrawn by the manufacturer after reports of severe anaphylactoid reactions.

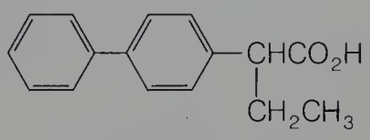
Ibuprofen, USP. 2-(4-Isobutylphenyl)propionic acid (Motrin; Advil; Nuprin). This arylacetic acid derivative was introduced into clinical practice following extensive clinical trials. It appears comparable to aspirin in the treatment of rheumatoid arthritis, with a lower incidence of side effects.[181] It has also been approved for use in primary dysmenorrhea.


Ibufenac R = H
Ibuprofen R = CH_3

Of interest in this series of compounds is that it was noted that potency was enhanced by introduction of the α-methyl group on the acetic acid moiety. The precursor ibufenac (R = H), which was abandoned owing to hepatotoxicity, was less potent. Moreover, it was found that the activity resides in the (*S*)-(+)-isomer, not only in ibuprofen; but throughout the arylacetic acid series. Furthermore, it is these isomers that are the more potent inhibitors of prostaglandin synthetase.[142] The recommended dosage is 400 mg. Ibuprofen is also available over-the-counter as 200-mg tablets.

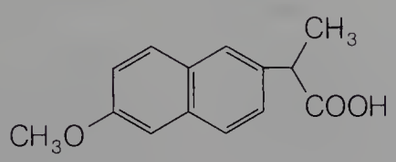
Namoxyrate. 2-(4-Biphenyl)butyric acid dimethylami-

noethanol salt (Namol) is another phenylacetic acid derivative under investigation. Namoxyrate shows high analgesic activity, being about seven times that of aspirin and nearly as effective as codeine. It has high antipyretic activity, but appears to be devoid of anti-inflammatory activity. These effects are peripheral. The dimethylaminoethanol increases its activity by increasing intestinal absorption. The ester of these two components is less active.[182]



Namoxyrate

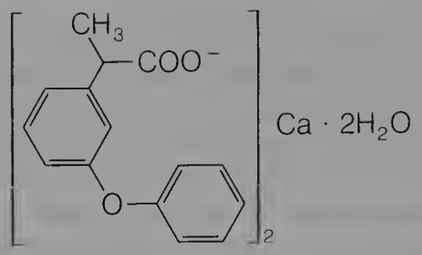
Naproxen, USP. (+)-6-Methoxy-α-methyl-2-naphthaleneacetic acid (Anaprox; Naprosyn) occurs as white to off-white crystals that are sparingly soluble in acidic solutions, freely soluble in alkaline solutions, and highly soluble in organic or lipidlike solutions. After oral administration, it is well absorbed, giving peak blood levels in 2 to 4 hr and a half-life of 13 hr. A steady-state blood level is usually achieved following four to five doses. This drug is very highly protein bound and displaces most protein-bound drugs. Dosages of these must be adjusted accordingly.



Naproxen

Naproxen is recommended for use in rheumatoid and gouty arthritis. It shows good analgesic activity, 400 mg being comparable to 75 to 150 mg of oral meperidine and superior to 65 mg propoxyphene and 325 mg of aspirin plus 30 mg of codeine. A 220- to 330-mg dose was comparable to 600 mg of aspirin alone. It has been reported to produce dizziness, drowsiness, and nausea, with infrequent mentions of gastrointestinal tract irritation. Similarly to aspirin, it inhibits prostaglandin synthetase and prolongs blood-clotting time. It is not recommended for pregnant or lactating women or in children under 16.[185] It is also available over the counter as 200-mg tablets (Aleve).

Fenoprofen Calcium, USP. α-Methyl-3-phenoxybenzeneacetic acid dihydrate calcium (Nalfon) occurs as a white crystalline powder that is slightly soluble in water, soluble in alcohol, and insoluble in benzene. It is rapidly absorbed orally, giving peak blood levels within 2 hr and has a short plasma half-life (3 hr). It is highly protein bound similar to the other acylacetic acids, and caution must be exercised when used concurrently with hydantoins, sulfonamides, and sulfonylureas. It shares many of the adverse effects common to this group of drugs, with gastrointestinal bleeding, ulcers, dyspepsia, nausea, sleepiness, and dizziness reported at a lower incidence than with aspirin. It inhibits prostaglandin synthetase.[183]



Fenoprofen

Available as capsules (200 and 300 mg) and tablets (600 mg), it is recommended for rheumatoid arthritis and osteoarthritis in divided doses four times a day for a maximum of 3200 mg/day. It should be taken at least 30 min before or 2 hr after meals. It is not yet recommended for the management of acute flare-ups. Doses of 2.4 g/day have been shown to be comparable to 3.9 g/day of aspirin in arthritis. For pain relief, 400 mg gave similar results to 650 mg of aspirin.[184]

Ketoprofen. *m*-Benzoylhydratropic acid (Orudis) is closely related to fenoprofen in structure and properties. It has demonstrated a low incidence of side effects and has been approved for sale over-the-counter (Orudis KT, Actron). It is available as capsules and tablets (25 and 50 mg), with a recommended daily dose of 150 to 300 mg divided into three or four doses.

Flurbiprofen, USP. (±)-2-(2-Fluoro-4-biphenylyl)propionic acid (Ansaid, Ocufen) is another hydratropic acid analog that is used in the acute or long-term management of rheumatoid arthritis and osteoarthritis. It is available as tablets (50 and 100 mg) with a recommended dose of 200 to 300 mg divided into two, three, or four times daily.

Diclofenac Potassium and Sodium. Sodium [*o*-(2,6-dichloroanilino)phenyl]acetate (Voltaren) is indicated for acute and chronic treatment of rheumatoid arthritis, osteoarthrits, and ankylosing spondylitis. The potassium salt (Cataflam), which is faster acting, is indicated for the management of acute pain and primary dysmenorrhea. The sodium salt is available as delayed release tablets (25, 50, and 75 mg) with a recommended daily dose of 100 to 200 mg in divided doses. The potassium salt is available as a tablet (50 mg) with a recommended dose of 50 mg three times daily.

Nabumetone. 4-(6-Methoxy-2-naphthyl)-2-butanone (Relafen) serves as a prodrug to its active metabolite, 6-Methoxy-2-naphthylacetic acid. Similar to the other arylacetic acid drugs, it is used in the acute or chronic management of rheumatoid arthritis and osteoarthritis. It is available as tablets (500 and 750 mg), with a recommended single daily dose of 1,000 mg.

Ketorolac Tromethamine. (±)-Benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-car boxylic acid compound with 2-amino-2-(hydroxymethyl)-1,3-propanediol (Toradol) is a potent NSAID analgesic indicated for the treatment of moderately severe, acute pain. Because of a number of potential side effects, its administration should not exceed 5 days. Treatment is usually initiated by intravenous (30 mg) or intramuscular (60 mg) administration, with analgesia maintained by

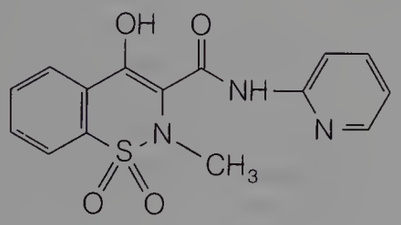
an initial oral doses of 20 or 30 mg, followed by 10 mg every 4 to 6 hr.

Etodolac. 1,8-Diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*] indole-1-acetic acid (Lodine) possesses an indole ring as the aryl portion of this group of NSAID drugs. It shares many of the properties of this group and is indicated for the acute and long-term management of pain and osteoarthritis. It is available as capsules (200 and 300 mg), with a recommended daily dose of 800- to 1,200-mg divided doses.

Oxaprozin. 4,5-Diphenyl-2-oxazolepropionic acid (Daypro) differs from the other members of this group in that it is an arylpropionic acid derivative. It shares the same properties and side effects of other members in this group. It is indicated for the acute and long-term management of osteoarthritis and rheumatoid arthritis, administered as a single 1,200-mg dose. It is available as 600-mg caplets.

Several other aryl acetic acid are under evaluation for use as analgesics of in various rheumatoid conditions. These include pirprofen, prodolic acid, bucloxic acid, alclofenac, and fenclofenac.

Piroxicam, USP. 4-Hydroxy-2-methyl-*N*-2-pyridyl-2*H*-1,2-benzoth iazine-3- carboximide 1,1-dioxide; CP-16,171 (Feldene) represents a class of acidic inhibitors of prostaglandin synthetase, although it does not antagonize PGE_2 directly.[186] This drug is very long acting with a plasma half-life of 38 hr, thereby requiring a dose of only 20 to 30 mg once daily. It is reported to give similar results to 25 mg of indomethacin or 400 mg of ibuprofen three times a day.[187,188]



Piroxicam

Several other arylacetic acid derivatives have been under clinical evaluation. These include ketoprofen, alclofenac, fenclofenac, pirprofen, and prodolic and bucloxic acids. Although only early reports are available, many of these appear to show superiority over indomethacin and aspirin. Diclofenac Sodium, 2-[2,6-dichlorophenyl)amino]benzeneacetic acid monosodium salt (Volteran) has recently been introduced on the United States market as 75-mg tablets. The reader may consult the reviews of Evens and Scherrer and Whitehouse for further details.

ANILINE AND *p*-AMINOPHENOL DERIVATIVES

The introduction of aniline derivatives as analgesics is based on the discovery by Cahn and Hepp, in 1886, that aniline (C-1) and acetanilid (C-2) (Table 22-7) both have powerful antipyretic properties. The origin of this group from aniline has led to their being called "coal tar analgesics." Acetanilid was introduced by these workers because of the known toxicity of aniline itself. Aniline brings about the formation of methemoglobin, a form of hemoglobin that is incapable of functioning as an oxygen carrier. The acyl derivatives of aniline were thought to exert their analgesic and antipyretic effects by first being hydrolyzed to aniline and the corresponding acid, following which the aniline was oxidized to *p*-aminophenol (C-3). This is then excreted in combination with glucuronic or sulfuric acid.

The aniline derivatives do not appear to act upon the brain cortex; the pain impulse appears to be intercepted at the hypothalamus, wherein also lies the thermoregulatory center of the body. It is not clear if this is the site of their activity because most evidence suggests that they act at peripheral thermoreceptors. They are effective in the return to normal temperature of feverish individuals. Normal body temperatures are not affected by the administration of these drugs.

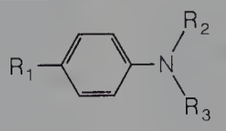
It is notable that, of the antipyretic analgesic group, the aniline derivatives show little if any antiinflammatory activity.

Table 22-7 shows some of the types of aniline derivatives that have been made and tested in the past. In general, any type of substitution on the amino group that reduces its basicity results also in a lowering of its physiologic activity. Acylation is one type of substitution that accomplishes this effect. Acetanilid (C-2) itself, although the best of the acylated derivatives, is toxic in large doses, but when administered in analgesic doses, it is probably without significant harm. Formanilid (C-4) is readily hydrolyzed and too irritant. The higher homologues of acetanilid are less soluble and, therefore, less active and less toxic. Those derived from aromatic acids (e.g., C-5) are virtually without analgesic and antipyretic effects. One of these, salicylanilide (C-6), is used as a fungicide and antimildew agent. Exalgin (C-7) is too toxic.

The hydroxylated anilines (*o, m, p*), better known as the aminophenols, are quite interesting from the standpoint of being considerably less toxic than aniline. The *para* compound (C-3) is of particular interest from two standpoints: namely, it is the metabolic product of aniline, and it is the least toxic of the three possible aminophenols. It also possesses a strong antipyretic and analgesic action. However, it is too toxic to serve as a drug and, for this reason, there were numerous modifications attempted. One of the first was the acetylation of the amine group to provide *N*-acetyl-*p*-aminophenol (acetaminophen) (C-8), a product that retained a good measure of the desired activities. Another approach to the detoxification of *p*-aminophenol was the etherification of the phenolic group. The best known of these are anisidine (C-9) and phenetidine (C-10), which are the methyl and ethyl ethers, respectively. However, it became apparent that a free amino group in these compounds, although promoting a strong antipyretic action, was also conducive to methemoglobin formation. The only exception to the preceding was for compounds in which a carboxyl group or sulfonic acid group had been substituted on the benzene nucleus. In these

TABLE 22-7

SOME ANALGESICS RELATED TO ANILINE



Compound	Structure			Name
	R_1	R_2	R_3	
C-1	—H	—H	—H	Aniline
C-2	—H	—H	—C(=O)—CH_3	Acetanilid
C-3	—OH	—H	—H	*p*-Aminophenol
C-4	—H	—H	—C(=O)—H	Formanilid
C-5	—H	—H	—C(=O)—C_6H_5	Benzanilid
C-6	—H	—H	—C(=O)—C_6H_4—OH	Salicylanilide (not an analgesic, but is an antifungal agent)
C-7	—H	—CH_3	—C(=O)—CH_3	Exalgin
C-8	—OH	—H	—C(=O)—CH_3	Acetaminophen
C-9	—OCH_3	—H	—H	Anisidine
C-10	—OC_2H_5	—H	—H	Phenetidine
C-11	—OC_2H_5	—H	—C(=O)—CH_3	Phenacetin
C-12	—OC_2H_5	—H	—C(=O)—$CHCH_3$(OH)	Lactylphenetidin
C-13	—OC_2H_5	—H	—C(=O)—CH_2NH_2	Phenocoll
C-14	—OC_2H_5	—H	—C(=O)—CH_2OCH_3	Kryofine
C-15	—OC(=O)—CH_3	—H	—C(=O)—CH_3	*p*-Acetoxyacetanilid taceanilid
C-16	—OC(=O)—C_6H_4—OH	—H	—C(=O)—CH_3	Phenetsal
C-17	—OCH_2CH_2OH	—H	—C(=O)—CH_3	Pertonal

compounds, however, the antipyretic effect also had disappeared. The foregoing considerations led to the preparation of the alkyl ethers of *N*-acetyl-*p*-aminophenol of which the ethyl ether was the best and is known as phenacetin (C-11). The methyl and propyl homologues were undesirable from the standpoint of causing emesis, salivation, diuresis, and other reactions. Alkylation of the nitrogen with a methyl group has a potentiating effect on the analgesic action but, unfortunately, has a highly irritant action on mucous membranes.

The phenacetin molecule has been modified by changing the acyl group on the nitrogen with sometimes beneficial results. Among these are lactylphenetidin (C-12), phenocoll (C-13), and kryofine (C-14). None of these, however, is in current use.

Changing the ether group of phenacetin to an acyl type of derivative has not always been successful. *p*-Acetoxyacetanilid (C-15) has about the same activity and disadvantages as the free phenol. However, the salicyl ester (C-16) exhibits a diminished toxicity and an increased antipyretic activity. Pertonal (C-17) is a somewhat different type in which glycol has been used to etherify the phenolic hydroxyl group. It is very similar to phenacetin. None of these is currently on the market.

Relative to the fate in humans of the types of compounds just discussed, Brodie and Axelrod[189] point out that acetanilid and phenacetin are metabolized by two different routes. Acetanilid is metabolized primarily to *N*-acetyl-*p*-aminophenol, acetaminophen, and only a small amount to aniline, which they showed to be the precursor of phenylhydroxylamine, the compound responsible for methemoglobin formation. Phenacetin is mostly de-ethylated to acetaminophen, whereas a small amount is converted by deacetylation to *p*-phenetidine, also responsible for methemoglobin formation. With both acetanilid and phenacetin, the metabolite acetaminophen formed is believed to be responsible for the analgesic activity of the compounds.

Acetanilid. Antifebrin; phenylacetamid. This is the monoacetyl derivative of aniline, prepared by heating aniline and acetic acid for several hours.

It can be recrystallized from hot water and occurs as a stable, white crystalline compound. It is slightly soluble in water (1:190) and easily soluble in hot water, acetone, chloroform, glycerin (1:5), alcohol (1:4), or ether (1:17). It is available only in powdered form.

Acetanilid is a neutral compound and will not dissolve in either acids or alkalies.

It is prone to form eutectic mixtures with aspirin, antipyrine, chloral hydrate, menthol, phenol, pyrocatechin, resorcinol, salol, thymol, or urethan.

It is definitely toxic in that it causes formation of methemoglobin, affects the heart, and may cause skin reactions and a jaundiced condition. Nevertheless, in the doses used for analgesia, it is a relatively safe drug. However, it is recommended that it be administered in intermittent periods, no period exceeding a few days.[190]

The analgesic effect is selective for most simple headaches and for the pain associated with many muscles and joints. The usual dose is 200 mg.

Several compounds related to acetanilid have been synthesized in attempts to find a better analgesic, as previously indicated. They have not become particularly important in the practice of medicine, for they have little to offer over acetanilid. The physical and chemical properties are also much the same. Eutectic mixtures are formed with many of the same compounds.

Phenacetin, USP. Acetophenetidin; *p*-acetophenetidide may be synthesized in several steps from *p*-nitrophenol.

It occurs as stable, white, glistening crystals, usually in scales, or a fine, white crystalline powder. It is odorless and slightly bitter. It is very slightly soluble in water (1:1,300), soluble in alcohol (1:15), and chloroform (1:15), but only slightly soluble in ether (1:130). It is sparingly soluble in boiling water (1:85).

In general, properties and incompatibilities, such as decomposition by acids and alkalies, it is similar to acetanilid. Phenacetin forms eutectic mixtures with chloral hydrate, phenol, aminopyrine, pyrocatechin, or pyrogallol.

It was once used widely as an analgesic and antipyretic, having essentially the same actions as acetanilid. It should be used with the same cautions because the toxic effects are the same as those of acetaminophen, the active form to which it is converted in the body. Some feel there is little justification for its continued use,[189] and it is presently restricted to prescription use only and is available only in powdered form. In particular, a suspected nephrotoxic action[191] has been the basis for the present warning label requirements by the FDA (i.e., "This medication may damage the kidneys when used in large amounts or for a long period of time. Do not take more than the recommended dosage, nor take regularly for longer than 10 days without consulting your physician.") Some recent evidence suggests that phenacetin may not cause nephritis to any greater degree than aspirin, with which it has been most often combined.[192] However, it has been strongly indicated as being carcinogenic in rats and associated with tumors in abusers of phenocetin.[193,194] It has been removed from many combination products and replaced either with additional aspirin (e.g., Anacin) or with acetaminophen.

Acetaminophen, USP. *N*-Acetyl-*p*-aminophenol; 4′-hydroxyacetanifide (Datril; Tempra; Tylenol). This may be prepared by reduction of *p*-nitrophenol in glacial acetic acid, acetylation of *p*-aminophenol with acetic anhydride or ketene, or from *p*-hydroxy-acetophenone hydrazone. It occurs as a white, odorless, slightly bitter crystalline powder. It is slightly soluble in water and ether, soluble in boiling water (1:20), alcohol (1:10), and sodium hydroxide T.S.

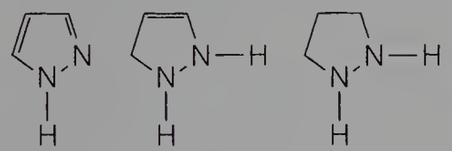
Acetaminophen has analgesic and antipyretic activities comparable with those of acetanilid and is used in the same conditions. Although it possesses the same toxic effects as acetanilid, they occur less frequently and with less severity; therefore, it is considered somewhat safer to use. However,

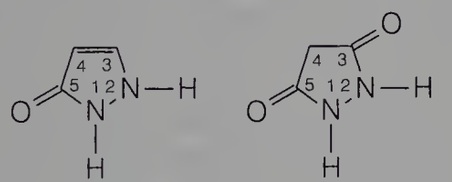
the same cautions should be applied. The required FDA warning label reads: "Warning: Do not give to children under 3 years of age or use for more than ten days unless directed by a physician."[195]

It is available in several nonprescription forms and, also, is marketed in combination with aspirin and caffeine (Trigesic).

PYRAZOLONE AND PYRAZOLIDINEDIONE DERIVATIVES

The simple doubly unsaturated compound containing two nitrogen and three carbon atoms in the ring, and with the nitrogen atoms neighboring, is known as pyrazole. The reduction products, named as are other rings of five atoms, are pyrazoline and pyrazolidine. Several pyrazoline substitution products are used in medicine. Many of these are derivatives of 5-pyrazolone. Some can be related to 3,5-pyrazolidinedione.

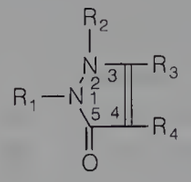




Ludwig Knorr, a pupil of Emil Fischer, while searching for antipyretics of the quinoline type, in 1884, discovered the 5-pyrazolone now known as antipyrine. This discovery initiated the beginnings of the great German drug industry that dominated the field for 40 years. Knorr, although at first mistakenly believing that he had a quinoline-type compound, soon recognized his error, and the compound was interpreted correctly as being a pyrazolone. Within two years, the analgesic properties of this compound became apparent when favorable reports began to appear in the literature, particularly with reference to its use in headaches and neuralgias. Since then, it has retained some of its popularity as an analgesic, although its use as an antipyretic has declined steadily. Since its introduction into medicine, there have been >1,000 compounds made in an effort to find others with a more potent analgesic action combined with less toxicity. Many modifications of the basic compound have been made. The few derivatives and modifications on the market are listed in Tables 22-8 and 22-9. Phenylbutazone, although analgesic itself, was originally developed as a solubilizer for the insoluble aminopyrine. It is now being used for the relief of many forms of arthritis, in which capacity it has more than an analgesic action in that it also reduces swelling and spasm by an anti-inflammatory action.

TABLE 22-8

DERIVATIVES OF 5-PYRAZOLONE



Compound Proprietary Name	R_1	R_2	R_3	R_4
Antipyrine *Phenazone*	$-C_6H_5$	$-CH_3$	$-CH_3$	$-H$
Aminopyrine *Amidopyrine*	$-C_6H_5$	$-CH_3$	$-CH_3$	$-N(CH_3)_2$
Dipyrone *Methampyrone*	$-C_6H_5$	$-CH_3$	$-CH_3$	$-NCH_2SO_3Na$ $\vert$ CH_3

Antipyrine, USP. 2,3-Dimethyl-1-phenyl-3-pyrazolin-5-one; phenazone (Felsol). This was one of the first important drugs to be made (1887) synthetically.

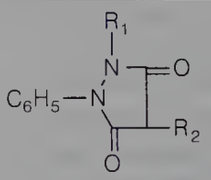
Antipyrine and many related compounds are prepared by the condensation of hydrazine derivatives with various esters. Antipyrine itself is prepared by the action of ethyl acetoacetate on phenylhydrazine and subsequent methylation.

It consists of colorless, odorless crystals or a white powder, with a slightly bitter taste. It is very soluble in water, alcohol, or chloroform, less so in either, and its aqueous solution is neutral to litmus paper. However, it is basic in nature, which is due primarily to the nitrogen at position 2.

Locally, antipyrine exerts a paralytic action on the sensory and the motor nerves, resulting in some anesthesia and vasoconstriction, and it also exerts a feeble antiseptic effect. Systemically, it causes results that are very similar to those of acetanilid, although they are usually more rapid. It is readily absorbed after oral administration, circulates freely and is excreted chiefly by the kidneys without having been changed chemically. Any abnormal temperature is reduced rapidly

TABLE 22-9

DERIVATIVES OF 3,5-PYRAZOLIDINEDIONE



Compound Proprietary Name	R_1	R_2
Phenylbutazone *Azolid, Butazolidin*	$-C_6H_5$	$-C_4H_9$ (n)
Oxyphenbutazone *Oxalid, Tandearil*	$-C_6H_4(OH)$ (p)	$-C_4H_9$ (n)

by an unknown mechanism, usually attributed to an effect on the serotonin-mediated thermal regulatory center of the nervous system. It has a higher degree of anti-inflammatory activity than aspirin, phenylbutazone, and indomethacin. It also lessens perception to pain of certain types, without any alteration in central or motor functions, which differs from the effects of morphine. Very often it produces unpleasant and possibly alarming symptoms, even in small or moderate doses. These are giddiness, drowsiness, cyanosis, great reduction in temperature, coldness in the extremities, tremor, sweating, and morbilliform or erythematous eruptions; with very large doses there are asphyxia, epileptic convulsions, and collapse. Treatment for such untoward reactions must be symptomatic. It is probably less likely to produce collapse than acetanilid and is not known to cause the granulocytopenia that sometimes follows aminopyrine.

The great success of antipyrine in its early years led to the introduction of a great many derivatives, especially salts with a variety of acids, but none of these has any advantage over the parent compound. Currently in use is the compound with chloral hydrate (Hypnal).

Aminopyrine. Amidopyrine; aminophenazone; 2,3-dimethyl-4-dimethylamino-1-phenyl-3-pyrazolin-5-one. It is prepared from nitrosoantipyrine by reduction to the 4-amino compound followed by methylation.

It consists of colorless, odorless crystals that dissolve in water and the usual organic solvents. It has about the same incompatibilities as antipyrine.

It has been employed as an antipyretic and analgesic, as is antipyrine, but is somewhat slower in action. However, it seems to be much more powerful, and its effects last longer. The usual dose is 300 mg for headaches, dysmenorrhea, neuralgia, migraine, and other like disorders, and it may be given several times daily in rheumatism and other conditions that involve continuous pain.

One of the chief disadvantages of therapy with aminopyrine is the possibility of producing agranulocytosis (granulocytopenia). It has been shown that this is caused by drug therapy with a variety of substances, including mainly aromatic compounds, but particularly with aminopyrine; indeed, several fatal cases have been traced definitely to this drug. The symptoms are a marked fall in leukocytes, absence of granulocytes in the blood, fever, sore throat, ulcerations on mucous surfaces, and prostration, with death in most cases of secondary complications. The treatment is merely symptomatic with penicillin to prevent any possible superimposed infection. The condition seems to be more or less an allergic reaction, because only a certain small percentage of those who use the drug are affected, but great caution must be observed to avoid susceptibility. Many countries have forbidden or greatly restricted its administration, and it has fallen more or less into disfavor.

Dipyrone. Methampyrone. This occurs as a white, odorless crystalline powder possessing a slightly bitter taste. It is freely soluble in water (1:1.5) and sparingly soluble in alcohol.

It is used as an analgesic, an antipyretic, and an antirheumatic. The recommended dose is 300 mg to 1 g orally and 500 mg to 1 g intramuscularly or subcutaneously. It is available as an injectable for veterinary use.

Phenylbutazone, USP. 4-Butyl-1,2-diphenyl-3,5-pyrazolidinedione (Azolid; Butazolidin; Phenylzone-A). This drug is a white to off-white, odorless, slightly bitter powder. It has a slightly aromatic odor and is freely soluble in ether, acetone, and ethyl acetate, very slightly soluble in water, and is soluble in alcohol (1:20).

The principal usefulness of phenylbutazone was in the treatment of the painful symptoms associated with gout, rheumatoid arthritis, psoriatic arthritis, rheumatoid spondylitis, and painful shoulder (peritendinitis, capsulitis, bursitis, and acute arthritis of the joint). Because of its many unwelcome side effects, this drug was not generally considered to be the drug of choice and was reserved for trial in those patients who do not respond to less toxic drugs. It is no longer available.

Oxyphenbutazone, USP. 4-Butyl-1-(*p*-hydroxyphenyl)-2-phenyl-3,5-pyrazoli dinedione (Oxalid; Tandearil). This drug is metabolite of phenylbutazone and has the same effectiveness, indications, side effects, and contraindications. Its only apparent advantage is that it causes acute gastric irritation less frequently.

The pharmacology of these and other analogues has been reviewed extensively.[196]

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CHAPTER 23

Steroids and Therapeutically Related Compounds

Dwight S. Fullerton

Steroids are widely distributed throughout the plant and animal kingdoms, and are formed by identical or nearly identical biosynthetic pathways in both plants and animals. Furthermore, because of their relatively rigid chemical structures, the steroids usually have easily predictable physical and chemical properties.

However, the similarity among the steroids ends with their fundamental chemical properties. The steroids have little in common therapeutically, except that as a group they are the most extensively used drugs in modern medicine. The major therapeutic classes of steroids are illustrated in Fig. 23-1. The fact that minor changes in steroid structure can cause extensive changes in biologic activity has been a continual source of fascination for medicinal chemists and pharmacologists for some three decades.

No one has captured the excitement and fascination of steroid drugs better than Rupert Witzmann in his 1981 book *Steroids—Keys to Life*.[1] Witzmann vividly describes the drama of "the greatest [chemical and biologic research] attack on a single group of substances that the world has ever seen." His book is highly recommended for further reading.

In this chapter, we consider the steroids used in modern medicine. Some nonsteroidal compounds that have similar therapeutic uses are also discussed (e.g., nonsteroidal inotropic agents for treatment of heart failure, the diethylstilbestrol estrogens, the nonsteroidal chemical contraceptive agents, ovulation stimulants, and gonadotropin-releasing hormone (GnRH) analogues).

Many textbooks with reviews of steroids and their pharmacology have recently been published.[1–5] Additional reviews on particular classes of steroids are cited in subsequent sections.

STEROID RECEPTORS

Over the last decade, substantial progress has been made in understanding steroid receptors, and the relationship of their structure to their pharmacology.[6] Advances in recombinant DNA studies, gene cloning, use of specific monoclonal antibodies against specific steroid receptors, receptor purification, nuclear magnetic resonance (NMR), and x-ray crystallography have all contributed to this progress. This section provides an overview of steroid hormone receptors, cell surface receptors and cholesterol regulation, and membrane-bound receptors for cardiac glycosides.

STEROID HORMONE RECEPTORS[6–15]

Steroid hormones regulate tissue-specific gene expression. (See the overview and drawings in the 1995 *Scientific American Science and Medicine* review by David Smith.[6]) The steroid hormones exhibit remarkable tissue selectivity, even though their structural differences are relatively minor. Estrogens such as estradiol (see Fig. 23-1) increase uterine cell proliferation, for example, but not that of the prostate. Androgens such as testosterone do the reverse, but neither androgens nor estrogens affect stomach epithelium.

Steroid receptors are proteins. Several other proteins are required for steroid receptors to be activated; chaperone proteins, for example, help twist and turn the receptor proteins into the right three-dimensional shape. Together, the steroid hormone receptor and associated proteins (Fig. 23-2, 23-3) make up the mature receptor complex.

The actions of steroid hormones are all the more extraordinary considering how tiny they are relative to their receptors. Estradiol, testosterone, and hydrocortisone are all tiny relative to the size of their receptors and receptor complexes.

Structure of Steroid Hormone Receptors

The complement DNAs (cDNAs) of all the major types have been cloned, giving the complete amino acid sequence of each. Although their three-dimensional structures are not yet

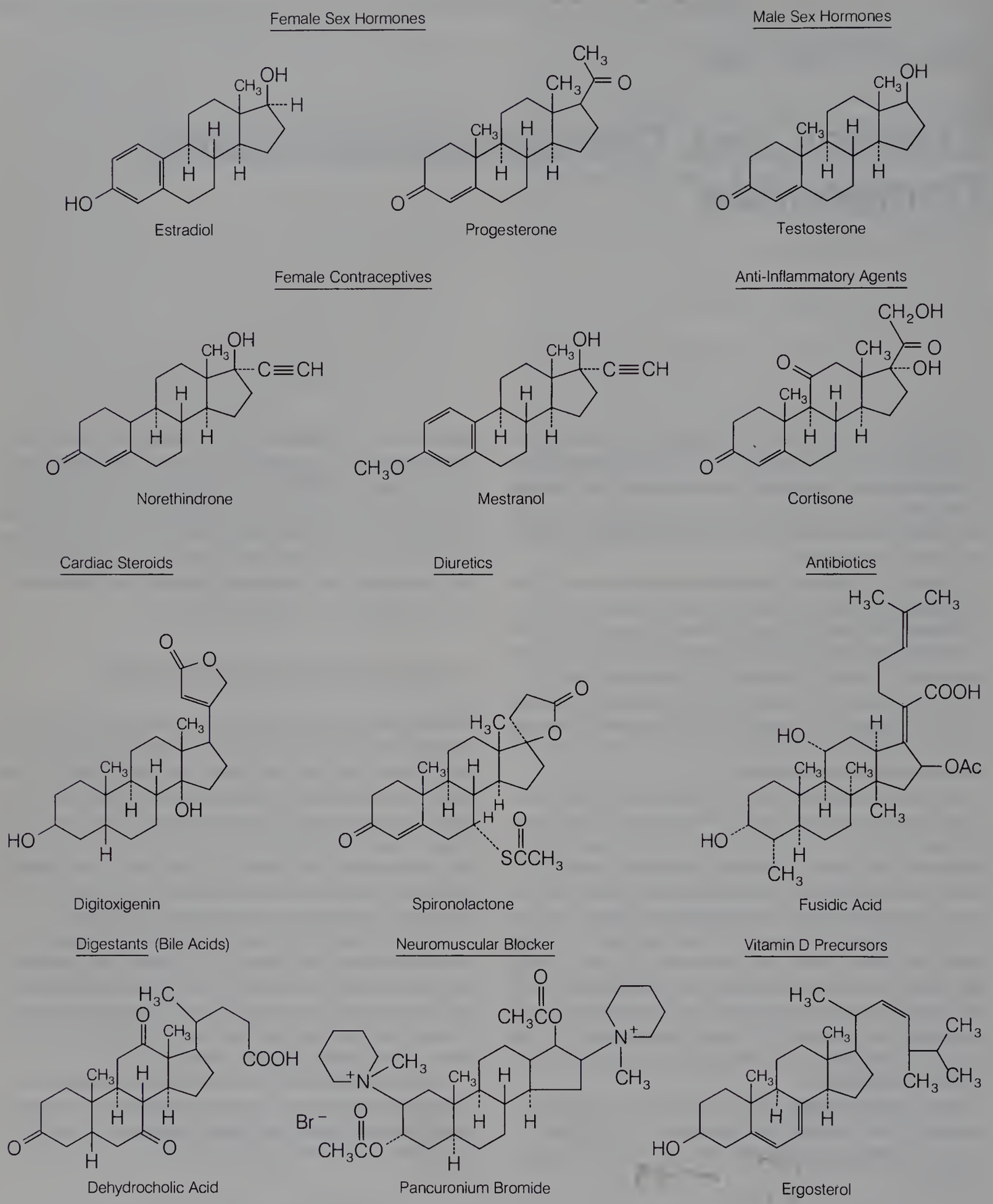


FIG. 23-1. *Representative examples of primary therapeutic classes of steroids.*

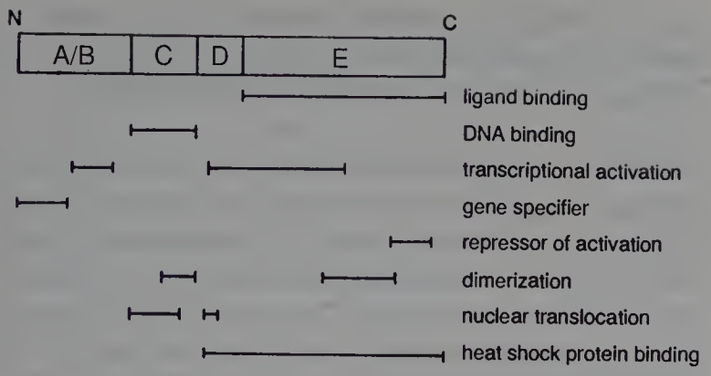


FIG. 23-2. *The steroid hormone receptor: functional domains. (Modified from Power et al.[7]) The length of the receptor, primarily the domain, varies with the particular hormone, e.g., estrogen receptor, glucocorticoid receptor.*

delineated, the functional role of each part is well known (see Fig. 23-2):

1. *N-terminal (''A/B'') domain:* Once the steroid receptor complex has bound to the target gene(s), this domain (also called the A/B modulator domain) activates the hormone response elements adjacent to the genes. The hormone response elements are on the DNA adjacent to the target gene. They comprise about 12 to 18 base pair DNA sequences, and consist of two ''half sites'' that are separated by a variable spacer. In the nucleus, steroid hormone receptor complexes exist as dimers; a monomer steroid hormone receptor complex binds to each half site. The nucleotide sequence and spacing between the half sites are essential for the specificity of the various steroid hormone complexes. After the two monomers bind to each half site, if the fit is correct (for example, that an estrogen steroid receptor complex has bound to an estrogen-activated gene), transcription is initiated.
2. *DNA binding (''C'') domain:* This short section is made up of about 70 amino acids, organized into two ''fingers'' each coordinated with a zinc ion. The three-dimensional structures of the zinc fingers has been explored with two-dimensional NMR. The zinc fingers are responsible for dimerization of the receptor as well as DNA recognition and binding.
3. *Hinge (''D'') domain:* This region appears to be involved with nuclear localization and transport (translocation) of the steroid/receptor complex into the nucleus.
4. *C-terminal ''ligand binding'' (''E'') domain:* The C-terminal domain includes about 250 amino acids. This section has the steroid hormone binding site(s), and is also involved with receptor dimerization, binding to chaperone proteins (discussed below), and in some cases, repressing (''silencing'') particular genes.

Structure of Steroid Hormone Receptor Complexes (Fig. 23-3)

Steroid hormone receptor complexes include (1) the steroid hormone receptor itself, and (2) typically about eight or

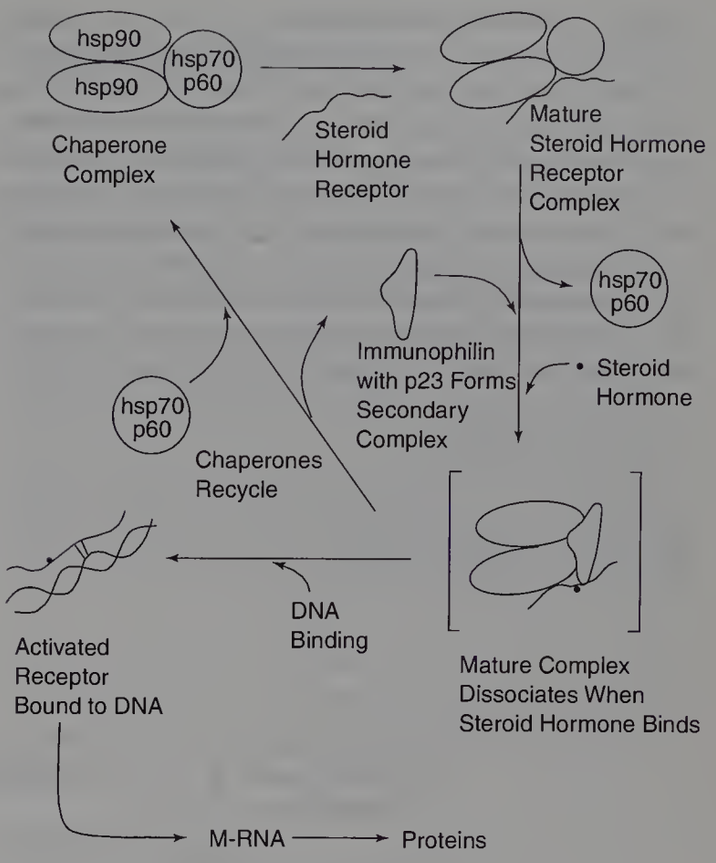


FIG. 23-3. *Steroid hormone activation of genes.*

more other peptides, predominantly chaperone (heat shock) proteins.[6,7] These chaperone proteins are produced in response to elevated temperature. Their role is to ''chaperone'' the correct conformation and folding of complex proteins, which is otherwise much more difficult as temperatures increase. At normal physiological temperatures, the chaperone proteins assist the proper folding of large proteins such as steroid hormone receptors. Without the chaperones, the steroid hormone binding site on the receptor does not have the proper folding and conformation for optimal steroid binding. Several ''passes'' are often needed by the chaperone proteins to achieve the proper conformation.

When the steroid hormone receptor binds to the chaperone complex, a ''mature'' steroid hormone receptor complex results (see Fig. 23-3). Recycling of the chaperone complex, however, happens continuously. With the assistance of a secondary chaperone complex, the steroid hormone binds to the receptor, and the mature receptor complex disassociates. The receptor is then phosphorylated, dimerized, and transported into the nucleus. There, the zinc fingers on the steroid hormone receptor bind to the target gene(s) in the DNA. Gene activation or suppression results.

CELL SURFACE RECEPTORS

The discovery of the cholesteryl ester low-density lipoprotein (LDL) receptor in 1973, by Michael S. Brown and

Joseph L. Goldstein, led to a virtual explosion of research worldwide. For their extraordinary contributions, Brown and Goldstein were awarded the Nobel Prize for Medicine in 1985. The research effort with cell surface receptors continues unabated to this day—particularly with the T4 cell surface receptor for the human immunodeficiency viruses (HIV), which cause the acquired immune deficiency syndrome (AIDS). Cell surface receptors, illustrated in Fig. 23-4 for transport of cholesterol by LDL receptors,[16,17] are now known to be responsible for the transport of numerous large molecules into cells.[18] Included are a wide variety of hormones such as insulin, triiodothyronine (T_3), second messengers, and even nutrients.

Although it is generally believed that most steroid hormones enter cells by passive diffusion, a body of data is emerging that suggests that there may be cell surface receptors for these molecules as well.[19]

LOW-DENSITY LIPOPROTEIN RECEPTORS AND THEIR ROLES IN CHOLESTEROL REGULATION AND ATHEROSCLEROSIS

After discovering the LDL receptor in 1973, Brown and Goldstein unraveled the genetic basis for hypercholesterolemia and resulting heart disease.[16,17] As described in their vividly illustrated 1984 review in *Scientific American*,[16] Brown and Goldstein showed how LDL receptor biosynthesis, cholesterol biosynthesis, and cholesterol metabolism are intimately intertwined in the regulation of cholesterol (see Fig. 23-4).

The LDL particles bind to LDL receptors (step 1) contained in coated pits on the cell surface. The coated pits are composed primarily of a polypeptide called "clathrin" that can spontaneously form these pitlike structures. It has been

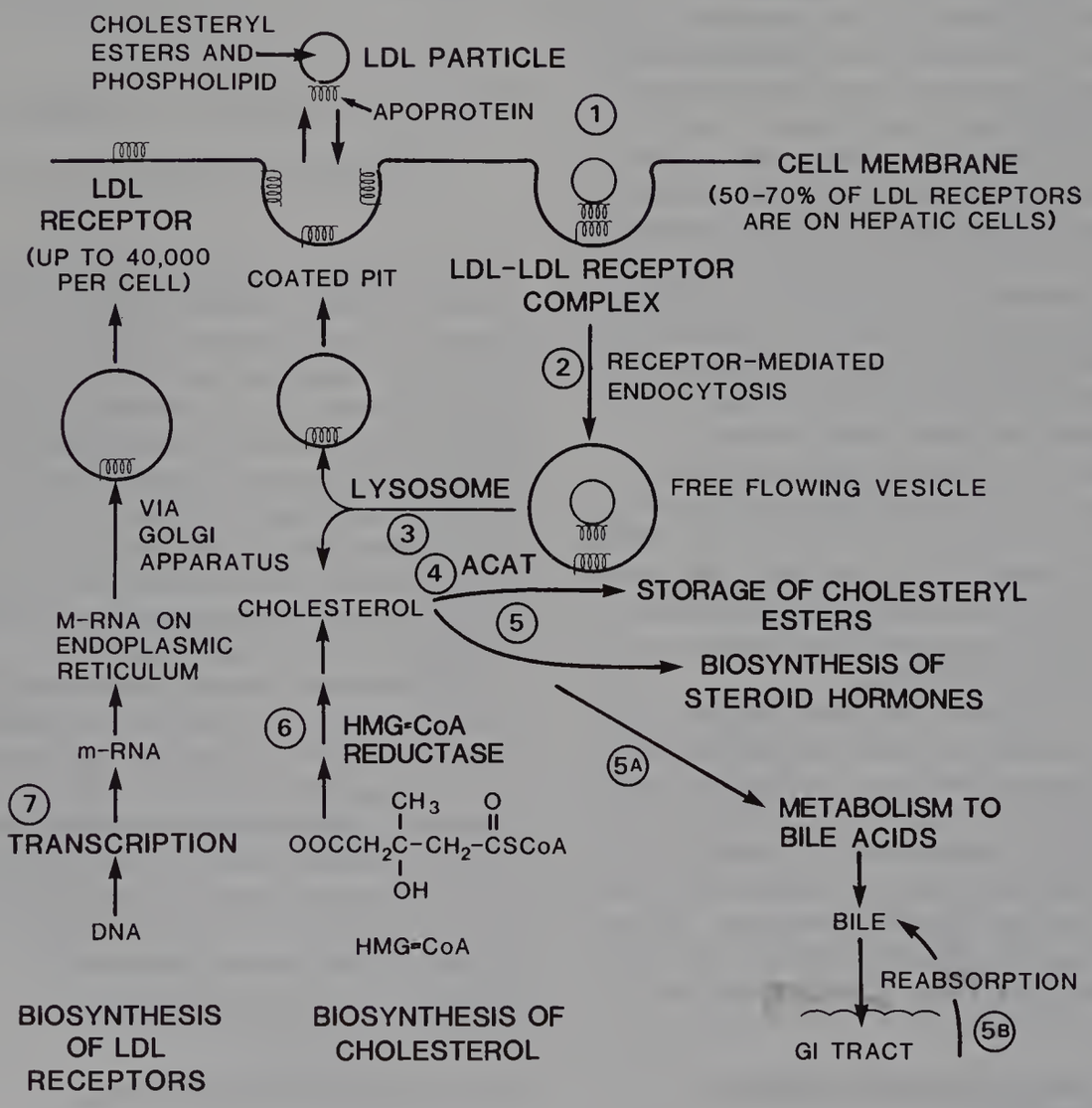


FIG. 23-4. *Cholesterol regulation pathways.*

estimated that at least 1500 to 3000 coated vesicles can form per minute per cell, making up about 2% of the cell surface.

The coated pits "pinch" off after the LDL–LDL receptor complex has formed, a process of receptor-mediated endocytosis (step 2) that leaves free-floating vesicles that transport the LDL inside the cell. Most large molecules circulating in the bloodstream are now known to enter cells by this process, including insulin, triiodothyronine (T_3), and other steroids.[17,18] The LDL is separated from the receptor and delivered to a lysosome filled with digestive enzymes (step 3). The LDL receptor is recycled and delivered to the cell surface. The LDL particle itself is degraded, releasing free cholesterol.

An excess of cholesterol in the cell results in a number of processes to reduce the amount. Acyl–coenzyme A (CoA): cholesterol acyltransferase (ACAT) is activated (step 4) to attach fatty acids to the cholesterol, forming cholesterol esters that are stored as droplets in the cell. Hydroxymethylglutaryl (HMG)-CoA reductase, a key rate-limiting regulatory enzyme in cholesterol biosynthesis, is inhibited (step 6) to thereby reduce the cell's own biosynthesis of cholesterol.

In addition, the transcription of the LDL receptor gene is inhibited (step 7), reducing the number of LDL receptors available on the cell surface to transport cholesterol into the cell. There is also evidence that reabsorption of bile acids is reduced from the gastrointestinal tract, increasing the metabolism of cholesterol inside the cell to provide enough bile acids for digestion.

Understanding the role of LDL receptors in cholesterol regulation has resulted in a better understanding of the processes that result in atherosclerosis. In particular, familial hypercholesterolemia is a genetic form of atherosclerosis that affects about 1 : 500 people in the United States. Individuals with this genetic disease have LDL levels about twice the normal level. The disease results from a defective gene for LDL receptors.

MEMBRANE-BOUND RECEPTORS

One important class of steroid drugs, the cardiac steroids, has a membrane-bound receptor. These steroids never enter the cell. The cardiac steroid receptor is the enzyme Na^+, K^+–adenosine triphosphatase (ATPase).[20–27] As described in the section on cardiac steroids, these drugs inhibit the Na^+, K^+–ATPase, resulting in a small net increase in intracellular Ca^{2+} ions. Membrane-bound Na^+, K^+–ATPase has had its entire amino acid sequence determined through recombinant DNA techniques.[28] Fullerton et al.[20,22,29] and Repke et al.[27,30] have located the receptor site for cardiac glycosides—the position on the Na^+, K^+–ATPase receptor at which they actually bind.

X-RAY CRYSTALLOGRAPHY AND STEROID FIT AT THE RECEPTOR

Medicinal chemists traditionally have assumed that there could be no relationship between the conformations of rigid molecules in crystals and their preferred conformations in solution with receptors. However, it is now clear from x-ray crystallography studies of steroids, prostaglandins, thyroid compounds, and many other drug classes that this technique can be a powerful tool in understanding drug action and in designing new drugs.[31–34] The ways in which x-ray crystallography and computer graphics revealed how cardiac steroids interact with their receptors are discussed later in this chapter. The relationship is simple: steroid drugs usually do not have a charge and, as a result, are held to their receptors by relatively weak forces of attraction (see Chap. 2). The same is true for steroid molecules as they "pack" into crystals. In both events, the binding energy is too small to hold any but low-energy conformations. In short, the steroid conformation observed in steroid crystals often is the same or very similar to that at the receptor.

MANY RECEPTORS PER CELL

There are many more than just a single receptor per cell in tissues sensitive to a particular steroid. This is probably not surprising to those who have used Avogadro's number to determine how many molecules are taken in a single dose of any drug. There are typically only 10^3 to 10^4 steroid hormone receptors per cell. However, for steroids that are not hormones, the number may be considerably larger.

For example, there are as many as 5.2 million digitalis receptor sites in a cell of a very digitalis steroid-sensitive tissue, such as cat ventricle, but there are many fewer in a less sensitive tissue (e.g., 100,000 per guinea pig atrium cell).[35]

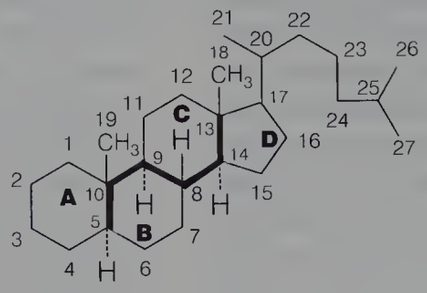
Changes in steroid receptors and the number of receptors during development and puberty[36] and aging[37] have recently been reported, giving new insight into the roles of steroid hormones during life.

STEROID NOMENCLATURE, STEREOCHEMISTRY, AND NUMBERING

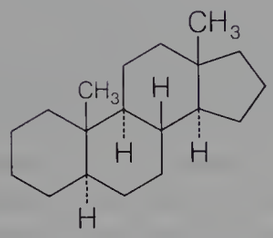
As shown in Fig. 23-5, nearly all steroids are named as derivatives of cholestane, androstane, pregnane, or estrane. The standard system of numbering is illustrated with 5α-cholestane.

The absolute stereochemistry of the molecule and any substituents is shown with solid (β) and dashed (α) bonds. Most

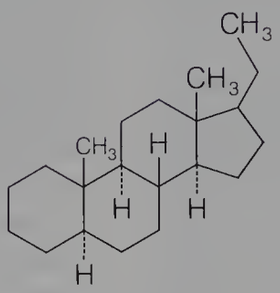
Numbering and Primary Steroid Names



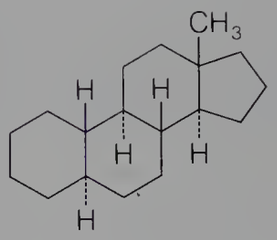
5α-Cholestane



5α-Androstane

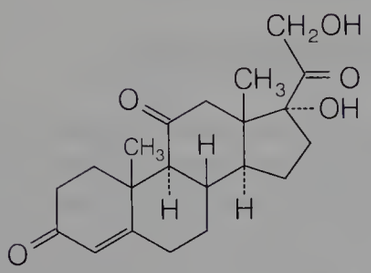


5α-Pregnane

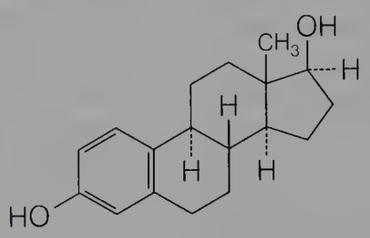


5α-Estrane

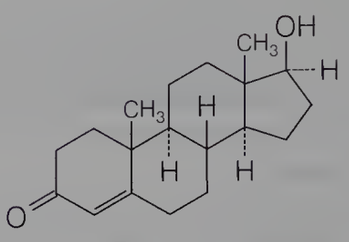
Examples of Common and Systematic Names



Cortisone
(17α,21-Dihydroxy-4-pregnene-3, 11,20-trione)



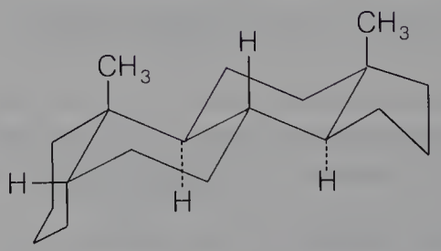
17β-Estradiol
(Estra-1,3,5(10)-triene-3,17β-diol)



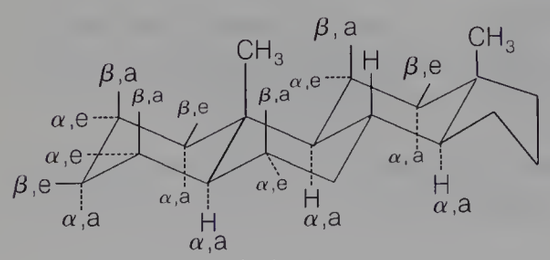
Testosterone
(17β-Hydroxyandrost-4-en-3-one)

FIG. 23-5. *Steroid nomenclature and numbering.*

carbons have one β-bond and one α-bond, with the β-bond lying closer to the "top" or C-18 and C-19 methyl side of the molecule. Both α- and β-substituents may be axial or equatorial. This system of designating stereochemistry can best be illustrated using 5α-androstane.

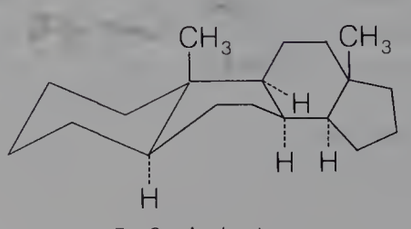


5β-Androstane



5α-Androstane

a = axial
e = equatorial
α = alpha bond
β = beta bond

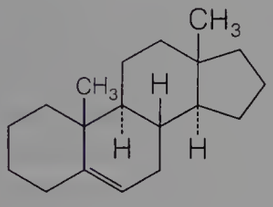


5α,8α-Androstane

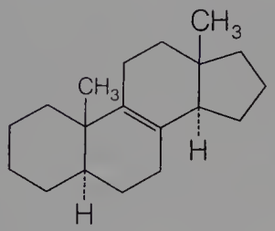
The stereochemistry of the H at C-5 is always indicated in the name. Stereochemistry of the other H atoms is not indicated unless different from 5α-cholestane. Changing the stereochemistry of any of the ring juncture or backbone carbons (shown in Fig. 23-5 with a heavy line on 5α-cholestane) greatly changes the shape of the steroid.

Because of the immense effect that "backbone" stereochemistry has upon the shape of the molecule, the International Union of Pure and Applied Chemistry (IUPAC) rules[38] require the stereochemistry at all backbone carbons to be clearly shown. That is, all hydrogens along the backbone must be drawn. When the stereochemistry is not known, a wavy line is used in the drawing, and the Greek letter xi (ξ) instead of α or β is used in the name. Methyls are always drawn as CH_3. Some authors also draw hydrogens at C-17.

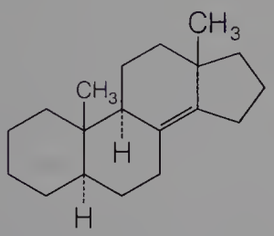
The position of double bonds can be designated in any of the various ways shown below. Double bonds from C-8 may go toward C-9 or C-14, and those from C-20 may go toward C-21 or C-22. In such cases, both carbons are indicated in the name if the double bond is not between sequentially numbered carbons.



5-Androstene or
Δ^5-Androstene or
Androst-5-ene



5α-Androst-8-ene or
5α-Δ^8-Androstene

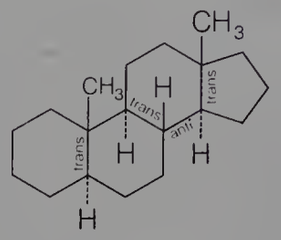


5α-Androst-8(14)-ene or
5α-$\Delta^{8(14)}$-Androstene

These principles of modern steroid nomenclature are applied to naming several common steroid drugs shown in Fig. 23-5.

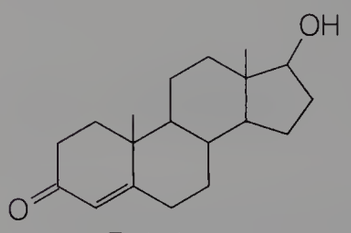
Such common names as "testosterone" and "cortisone" are obviously much easier to use than the long systematic names. However, substituents must always have their position and stereochemistry clearly indicated when common names are used (e.g., 17α-methyltestosterone, 9α-fluorocortisone).

The terms *cis* and *trans* are occasionally used in steroid nomenclature to indicate the backbone stereochemistry between rings. For example, 5α-steroids are A/B *trans*, and 5β-steroids are A/B *cis*. The terms *syn* and *anti* are used analogously to *trans* and *cis* for indicating stereochemistry in bonds connecting rings (e.g., the C-9:C-10 bond that connects rings A and C). The use of these terms is indicated below.

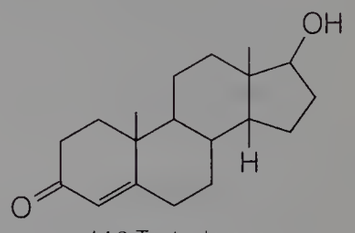


Other methods of indicating steroid stereochemistry and nomenclature occur in the early medical literature, but these methods are now seldom used.

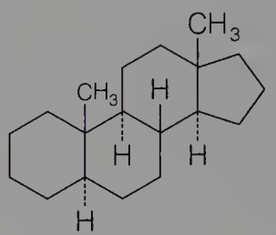
Steroid drawings sometimes appear with lines drawn instead of methyls (CH_3) (even though incorrect by IUPAC rules[38]), and backbone stereochemistry is not indicated unless different from 5α-androstane, as follows.



Testosterone

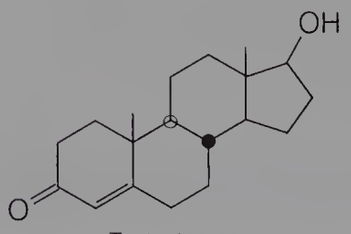


14β-Testosterone

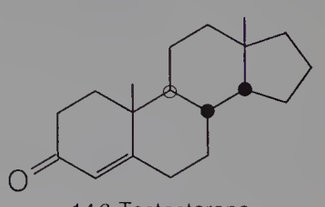


5α-Androstane

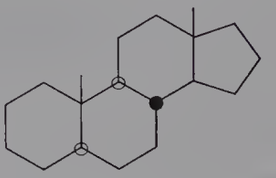
Finally, circles were sometimes used to indicate α-hydrogens and dark dots to indicate β-hydrogens.



Testosterone



14β-Testosterone



5α-Androstane

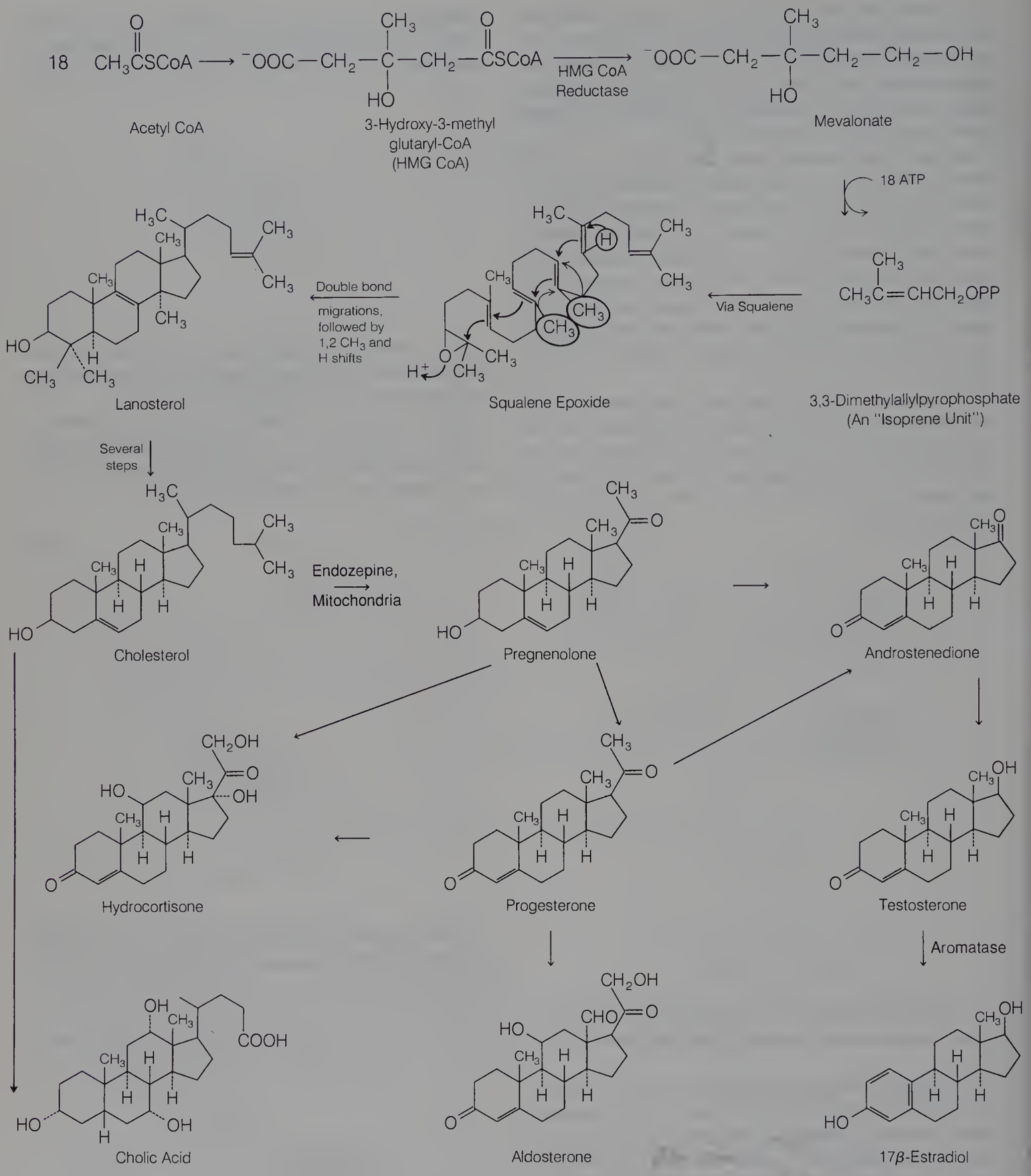


FIG. 23-6. *A schematic outline of the biosynthesis of steroids.*

STEROID BIOSYNTHESIS

Steroid hormones in mammals are biosynthesized from cholesterol, which in turn is made in vivo from acetyl-coenzyme A (acetyl-CoA). About 1 g of cholesterol is biosynthesized per day in humans, and an additional 300 mg is provided in the diet. (The possible roles of cholesterol and diet in atherosclerosis will be discussed later.) A schematic outline of these biosynthetic pathways is shown in Fig. 23-6.

The trigger for conversion of cholesterol to steroid hormones may have been discovered.[39] Endozepine is a peptide isolated from the adrenals and other steroid-forming organs. It has been found to increase the conversion of cholesterol to pregnenolone, and in addition binds to γ-aminobutyric acid (GABA) receptors in the central nervous system. (Initially it was considered an endogenous Valium or benzodiazepine, and was named accordingly.) Further research is needed to delineate its exact role in steroid biosynthesis.

HYDROXYMETHYLGLUTARYL-CoA REDUCTASE INHIBITORS

HMG-CoA reductase converts 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) to mevalonate, a key step in the biosynthesis of cholesterol (see Fig. 23-6). For several decades, inhibition of this enzyme has been envisioned as a likely approach to reducing hypercholesterolemia. This approach gained momentum with the discoveries of Brown and Goldstein about the overall processes of cholesterol regulation in the cell. Mevastatin (Compactin), isolated from cultures of *Penicillium citrinum* and *P. brevicompactum* in the early 1970s, was found to be a very effective inhibitor of HMG-CoA reductase. A few years later, the methyl analogue (named lovastatin) was isolated from cultures of *Monascus ruber* and *Aspergillus terreus* (Fig. 23-7).

Today, four HMG-CoA inhibitors are in widespread clinical use (Fig. 23-7). Lovastatin, for example, reduces LDL cholesterol up to 41% in patients with genetic hypercholesterolemia. Administration of bile acid sequestrants, such as colestipol (Colestid), with lovastatin reduces LDL levels even more than with lovastatin alone. Other drugs used to treat hypercholesterolemia are discussed in Chap. 18.

Lovastatin, United States Pharmacopeia (USP), 2-methylbutanoic acid, 1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)-ethyl]-1-naphthaleneyl ester (Mevacor), was the first HMG-CoA reductase inhibitor available clinically. Only about 30% is absorbed from the gastrointestinal tract, and much of this amount is metabolized in the first pass through the liver.

There are three other HMG-CoA inhibitors:

1. Pravastatin sodium, (3R,5R)-7-(1S,2S,6S,8S,8aR)-1,2,6,7,8,8a-hexahydro-6-hydroxy-2-methyl-8-[(S)-2-methylbutyryloxy]-1-naphthyl]-3,5-dihydroxyheptanoate (Pravachol): the only HMG-CoA reductase inhibitor that has been shown to have positive effects in reducing the risk for myocardial infarction.[40] (The study population included hypercholesterolemic patients without clinical evidence or history of significant coronary artery disease.) About 35% of a dose is absorbed, with absolute bioavailability of 17%; the half-life is 77 hours. The use of pravastatin in cirrhotic patients should be carefully monitored, because AUC can vary 18-fold, and peak values 47-fold.
2. Simvastatin (1S,2S,6R,8S,8aR)-1,2,3,7,8,8a-Hexahydro-3,7-dimethyl-8-[2-[(2R,4R)-tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl]ethyl]-1-naphthyl 2,2-dimethyl butyrate (Zoloft).
3. Fluvastatin [R*,S*-(E)]-3-(4-fluorophenyl)-1-(1-methylethyl)-1H-indol-2-yl]-3,5-dihydroxy-6-heptenoic acid (Lescol): less potent than any of the other three HMG-CoA inhibitors.

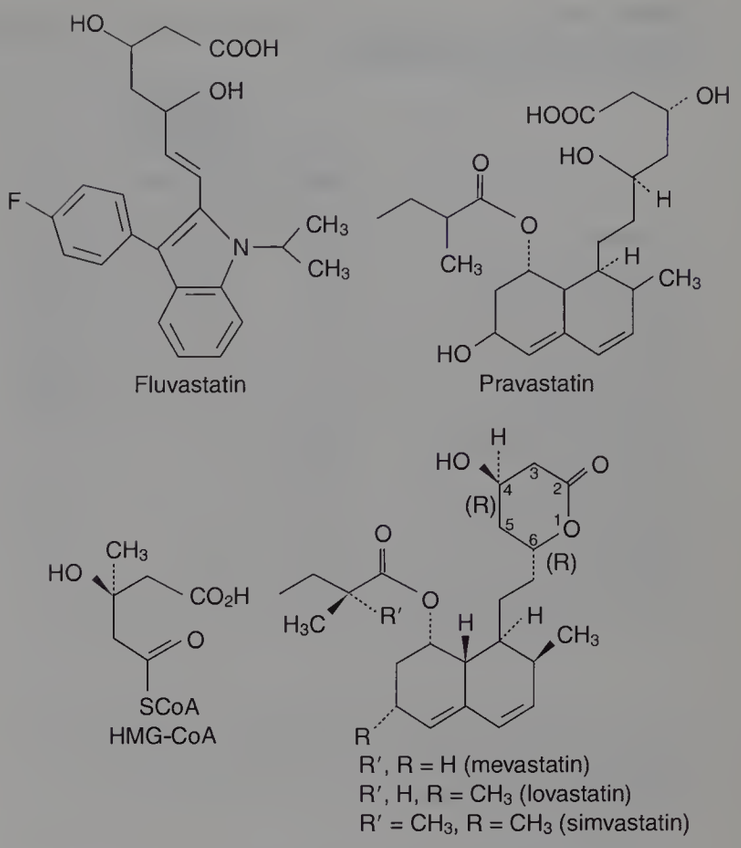


FIG. 23-7. *HMG-CoA and HMG-CoA reductase inhibitors.*

INHIBITION OF OTHER STEPS IN CHOLESTEROL BIOSYNTHESIS

The success of lowering plasma cholesterol with HMG-CoA reductase inhibitors has led to the design of inhibitors of other steps in cholesterol biosynthesis. Inhibitors of cytoplasmic *S*-acetyl coenzyme A synthetase (ACAT) (which activates acetate, a step essential for the cholesterol biosynthetic pathway), mevalonate-5-pyrophosphate decarboxyl-

TABLE 23-1

SOLUBILITIES OF STEROIDS

	Solubility (g/100 mL)		
	$CHCl_3$	EtOH	H_2O
Cholesterol	22	1.2	Insoluble
Testosterone	50	15	Insoluble
Testosterone propionate	45	25	Insoluble
Dehydrocholic acid	90	0.33	0.02
Estradiol	1.0	10	Insoluble
Estradiol benzoate	0.8	8	Insoluble
Betamethasone	0.1	2	Insoluble
Betamethasone acetate	10	3	Insoluble
Betamethasone $NaPO_4$ salt	Insoluble	15	50
Hydrocortisone	0.5	2.5	0.01
Hydrocortisone acetate	1.0	0.4	Insoluble
Hydrocortisone $NaPO_4$ salt	Insoluble	1.0	75
Prednisolone	0.4	3	0.01
Prednisolone acetate	1.0	0.7	Insoluble
Prednisolone $NaPO_4$ salt	0.8	13	25

ase (one of the enzymes that convert mevalonate to 3,3-dimethyl pyrophosphate), and squalene synthetase are among those being studied.

CHEMICAL AND PHYSICAL PROPERTIES OF STEROIDS

With few exceptions, the steroids are white crystalline solids. They may be in the form of needles, leaflets, platelets, or amorphous particles depending on the particular compound, the solvent used in crystallization, and skill and luck of the chemist. As the steroids have 17 or more carbon atoms, it is not surprising that they tend to be water-insoluble. Addition of hydroxyl or other polar groups (or decreasing carbons) increases water solubility slightly as expected. Salts are the most water-soluble. Examples are shown in Table 23-1.

CHANGES TO MODIFY PHARMACOKINETIC PROPERTIES OF STEROIDS

As with many other compounds described in previous chapters, the steroids can be made more lipid-soluble or more water-soluble simply by making suitable ester derivatives of hydroxyl groups. Derivatives with increased lipid solubility are often made to decrease the rate of release of the drug from intramuscular injection sites (i.e., in depot preparations). More lipid-soluble derivatives also have improved skin absorption properties, and thus are preferred for dermatologic preparations. Derivatives with increased water solubility are needed for intravenous preparations. As hydrolyzing enzymes are found throughout mammalian cells, especially in the liver, converting hydroxyl groups to esters does not significantly modify the activity of most compounds.

Some steroids are particularly susceptible to rapid metabolism after absorption or rapid inactivation in the gastrointestinal tract before absorption. Often a simple chemical modification can be made to decrease these processes and, thereby, increase the drug's half-life—or make it possible to be taken orally.

Examples of common chemical modifications are illustrated in Fig. 23-8. Drugs such as testosterone cyclopentylpropionate and methylprednisolone sodium succinate (which are converted in the body to more active drugs) are called *prodrugs*.

Counsell and co-workers[41] have given particular attention to the tissue distribution of steroids and the implication of such in drug design. For example, it has long been known that cholesterol is found in the highest concentration in the adrenal gland; therefore, [^{131}I]19-iodocholesterol has been used therapeutically for the diagnosis of various adrenal cortical diseases.[41] Radioactive steroids, for many years, have been recognized as binding most selectively to tissues that respond to them; consequently, labeled steroids have been used for many receptor and tissue studies.

Drugs with high affinity for the adrenal glands or other hormone-synthesizing tissues also have been studied as potential blockers of biosynthetic pathways (e.g., to block the biosynthesis of cholesterol in hyperlipidemia and heart disease, or the biosynthesis of excessive hormones from cholesterol in adrenal gland diseases).

GONADOTROPINS

The gonadotropins are peptides that in vivo and in therapy are closely related to the steroids estrogens, progesterone, and testosterone. As shown in Figs. 23-9 through 23-12, they control ovulation, spermatogenesis, and development of sex organs, and they maintain pregnancy. Included are the following:

- Gonadotropin-releasing hormone (GnRH).
- Luteinizing hormone (LH).
- Follicle-stimulating hormone (FSH).
- Menotropins, also called human menopausal gonadotropin (hMG), a purified preparation of FSH and LH obtained from the urine of postmenopausal women.
- Chorionic gonadotropin (CG; hCG is human gonadotropin), a glycopeptide produced by the placenta. Its pharmacologic actions are essentially the same as LH.

LUTEINIZING-RELEASING HORMONE ROLES IN MALES AND FEMALES

The hypothalamus releases GnRH, a decapeptide that stimulates the anterior pituitary to secrete LH and FSH in males and in females. This peptide controls and regulates both male and female reproduction (see Figs. 23-9 and 23-10). Its isola-

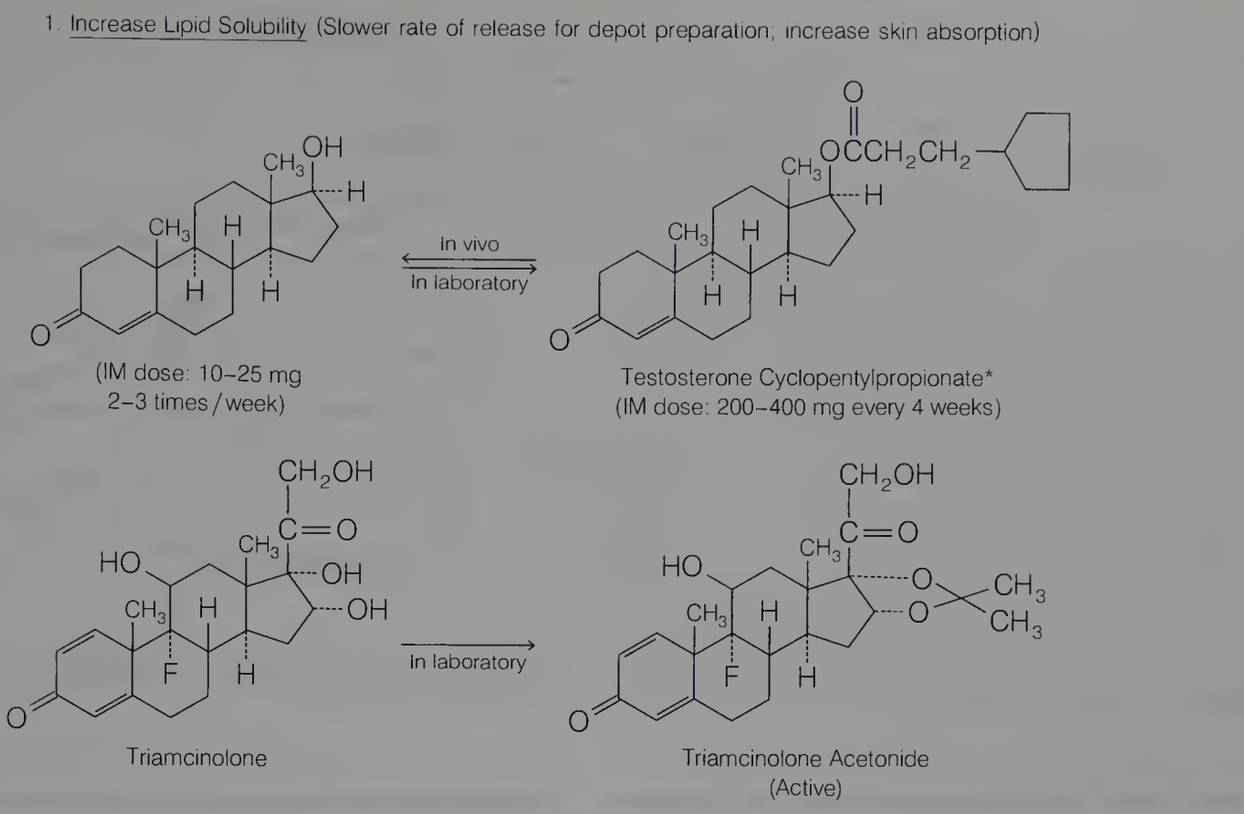


FIG. 23-8. *Common steroid modifications to alter therapeutic utility; *, prodrug.*

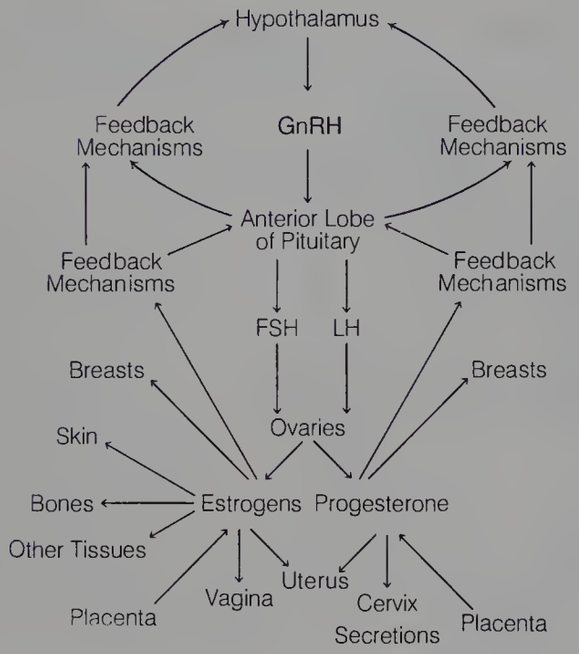


FIG. 23-9. Regulation of ovulation in females by LH-RH (GnRH).

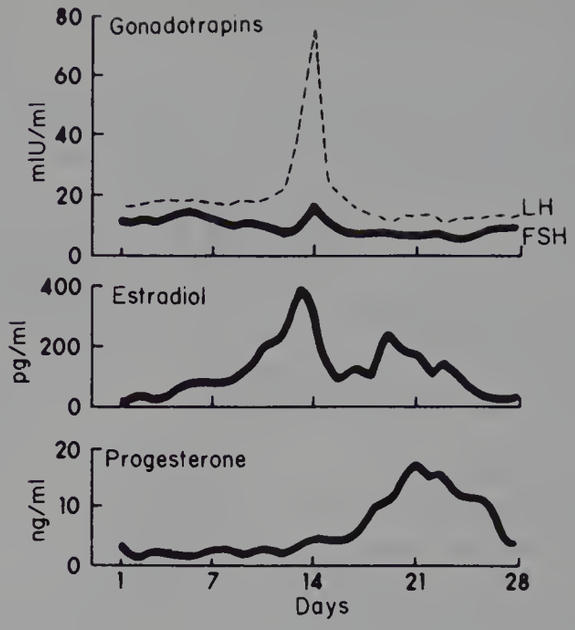


FIG. 23-11. Hormone changes in the normal menstrual cycle.

tion, purification, and structure determination were achieved in the early 1970s by two groups headed by Guillemin and Schalley, an accomplishment that earned them the Nobel Prize in 1977. GnRH is a decapeptide (10 amino acids):

Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2

Because of its simple structure and key roles in reproduction, there has been an enormous amount of interest in development of analogues as medicinal agents. Over 3000 GnRH analogues have now been synthesized and studied.[42–47]

THE PITUITARY GONADOTROPINS—LH AND FSH

The pituitary gonadotropins LH and FSH, their structures, genes, receptors, biological roles, and their regulation (including by negative feedback actions of steroid hormones) have been intensively studied.[48–50] FSH, LH, CG, and TSH (thyroid-stimulating hormone) all are peptide dimers with similar α-subunits within a species, but different β-subunits. Each peptide unit bears one or more carbohydrate chains.

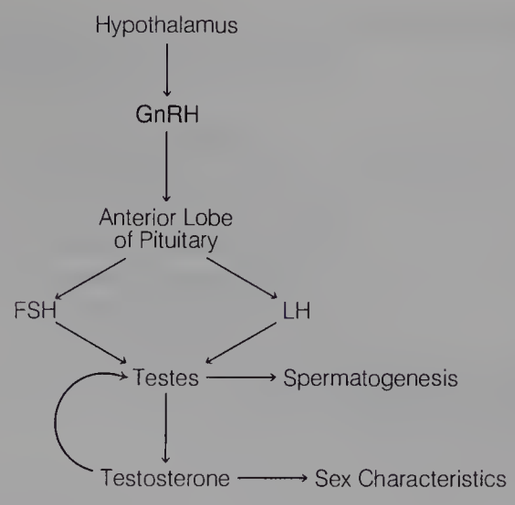


FIG. 23-10. Regulation of spermatogenesis in males.

In females, LH and FSH regulate the menstrual cycle (see Figs. 23-9 and 23-11). At the start of the cycle (with day 1 being the first day of menstruation), plasma concentrations of estradiol and other estrogens (see Fig. 23-11) and progesterone are low. FSH and LH stimulate several ovarian follicles to enlarge and begin developing more rapidly than others. After a few days, only one follicle continues the development process to the release of a mature ovum. The granulosa cells of the maturing follicles begin secreting estrogens, which then cause the uterine endometrium to thicken. Vaginal and cervical secretions increase. Gonadotropins and estrogen reach their maximum plasma concentrations at about the 14th day of the cycle. The release in LH causes the follicle to break open, releasing a mature ovum. Under the stimulation of LH, the follicle changes into the corpus luteum, which begins secreting progesterone as well as estrogen.

The increased concentrations of estrogens and progesterone regulate the hypothalamus and the anterior pituitary by a feedback inhibition process such that GnRH, LH, and FSH production is diminished. The result is that further ovulation is inhibited. As described later in this chapter, this is the primary mechanism by which the steroid birth control products inhibit ovulation.

If fertilization does not occur by about day 25, the corpus luteum begins to degenerate, slowing down its production of hormones. The concentrations of estrogens and progesterone become too low to maintain the vascularization of the endometrium, and menstruation results.

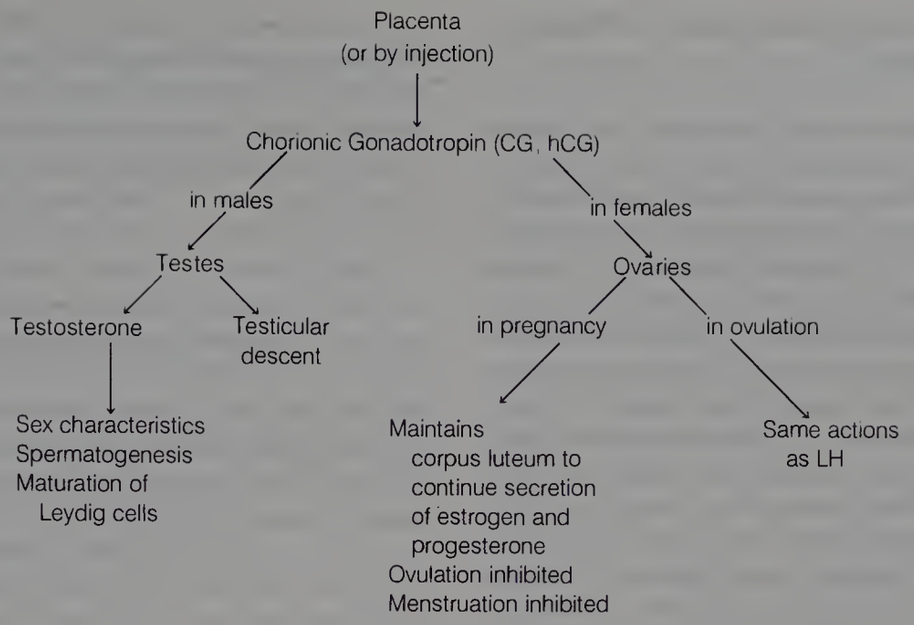


FIG. 23-12. *Natural and therapeutic roles of chorionic gonadotropin (CG) in females and males.*

In males (see Fig. 23-10), LH stimulates testosterone synthesis by the testes, and together testosterone and LH promote spermatogenesis (sperm production) and development of the testes. Testosterone is also essential for development of secondary sex characteristics in males. (Sharpe[50] concludes that FSH is probably necessary for quantitative maintenance of spermatogenesis, but artificially high levels of testosterone may overcome the requirement.)

HUMAN CHORIONIC GONADOTROPIN ROLES IN MALES AND FEMALES

Human gonadotropin (hCG) was originally commercially obtained from the urine of pregnant women, but more recently has been produced by recombinant DNA cloning techniques.[51] It is made up of two subunits (α and β), with the α-subunit structurally identical to the α-subunits of LH and FSH. The pharmacologic actions of hCG are essentially the same as LH. In females during pregnancy, the hCG secreted by the placenta maintains the corpus luteum to continue secretion of estrogen and progesterone, with the results that ovulation and menstruation are inhibited.

THERAPEUTIC APPLICATIONS—hCG, hMG, LH

Pregnancy Tests Based on Urine Analysis for Human Chorionic Gonadotropin

Tests for Clinical Laboratory Professional Use. Bioassays for hCG were first developed in 1928. Since then, a wide variety of agglutination, antibody, and radioimmunoassay test kits have been developed for professional use. They continue to be the mainstay of pregnancy diagnosis used in hospitals and for general clinical use.

The great sensitivity of new radioimmunoassays (RIAs) for the β-subunit of hCG has made these β-hCG tests particularly useful for diagnosis of ectopic pregnancies. Ectopic pregnancies generally do not produce enough hCG to consistently result in positive tests by other analytical methods.

Home Pregnancy Test Kits[52–54]. The nonradiological methods in professional use for hCG have been adopted for home use. Today, a wide range of products is available (marketed under trade names including Acu-Test, Advance, Answer, Conceive Daisy 2, e.p.t., Fact Plus One-Step Clear Blue Easy). Several (including Advance) are based on assay of the α- or β-hCG subunit. One study[54] used three brands (Daisy 2, e.p.t., and Answer) and showed that the predictive value of positive test results (all positive test results minus false-positive results) were generally in the range of 78% to 84%, and the predictive value of negative test results (all negative test results minus pregnancies the tests did not detect) were in the range of 60%.

Recent reviews[52,54] have summarized the causes of false-positive results (including menstrual irregularities, menopause, and hCG-producing tumors, and a variety of drugs—phenothiazines, methadone, and penicillin among others) and of false-negative results (technical errors, reagent outdating, test taken too early after the missed period, and too dilute urine samples).

In short, the convenience and predictive value of positive tests has resulted in home test kits' wide popularity and use. However, because of the incidence of some false-positive and false-negative test results, it must be emphasized that a physician should be consulted after positive results as well as after negative results with an overdue menstrual cycle.[52,54]

Ovulation Tests Based on Analysis of Urinary Luteinizing Hormone[52,54]

Numerous techniques have been claimed to predict ovulation, ranging from measurement of basal body temperature (BBT) to transvaginal ultrasound imaging, estradiol assays, and assays of gonadotropins in serum and urine. These tests are used for a variety of diagnoses, including to assist in predicting ovulation initially evaluated as infertile. RIA analyses have recently been developed for the LH plasma and urinary surge that takes place midcycle. More recently, home test kits have been used on nonradiologic enzymatic immunoassay (EIA) that uses the LH monoclonal antibody (marketed under trade names including First Response, Ovukit, and *QT*est for Ovulation). Studies have shown an 88% to 94% accuracy in predicting ovulation when urine samples were taken between 11:00 A.M. and 3:00 P.M., or 5:00 P.M. and 10:00 P.M. The home test kits also differ in sensitivity, such that with one kit, the apparent LH surge seemed to occur up to 5 days before the actual day of ovulation.[55]

Chorionic Gonadotropin with Menotropins to Treat Infertility

Human chorionic gonadotropin is used with menotropins (hMG) to induce spermatogenesis in men with primary or secondary hypogonadotropic hygonadism. hCG is used in women to induce ovulation in patients for whom the anovulation is the result of pituitary insufficiency. Thyroid, adrenal, pituitary, or ovarian tumors may also be the cause of anovulation and should be ruled out before hCG therapy. The most common side effect in women is temporary ovarian enlargement that lasts up to 2 to 3 weeks.

THERAPEUTIC APPLICATIONS—GONADOTROPINS

Gonadotropin agonists and antagonists have been widely studied for a wide range of therapeutic applications, ranging from contraception to shrinking of uterine fibroids prior to a hysterectomy.[42–47] However, thus far only the GnRH agonists goserelin, leuprolide, and nafarelin have been approved for clinical use, and for a very limited scope of conditions. No GnRH antagonists have been approved.

GnRH Agonists and Antagonists as Contraceptives—Promise and Problems. GnRH analogues were widely viewed as certain alternatives for steroid birth control products for women. An immense amount of research on GnRH agonists and antagonists as contraceptives has been completed. However, significant problems and side effects remain as serious barriers.

In vivo, GnRH production is pulsatile, leading to FSH and LH production and ovulation. When given continuously in women, GnRH agonists initially increase FSH and LH but then suppress FSH and LH and ovulation. Estrogen production can be quite variable, leading to irregular bleeding. If estrogen production is significantly inhibited, osteoporosis and bone loss can result. Estrogens could be given supplementally, but concern about the safety of oral estrogens would make such products difficult to market. Further, the much higher cost of GnRH analogues (relative to steroid oral contraceptives) would also limit their use.

GnRH antagonists greatly reduce FSH and LH without the lag period observed with GnRH agonists. Ideally these products could be effective contraceptives. However, the antagonists have tended to cause histamine release, largely related to positively charged D-amino acids in the antagonists.

When given in men, GnRH agonists produce "chemical castration" but have not produced a consistent blockade of spermatogenesis due to incomplete FSH suppression. There is usually a significant drop in serum testosterone levels, leading to decreased libido and impotence. As a result, if GnRH agonists are ever marketed, they will almost certainly have to include an androgen supplement.

Induction of Ovulation. Pulsatile pretreatment of GnRH agonists for 10 to 14 days allows egg collection from ovaries without the LH-induced rupture of ovarian follicles that normally occurs. This has allowed uninterrupted collection of eggs prior to in vitro fertilization procedures.

Clomiphene, a potent antiestrogen discussed later in this chapter, increases GnRH production in the hypothalamus, inducing ovulation in 80% of patients.

Management of Endometriosis. GnRH agonists have been helpful in treating young women with endometriosis, reducing pain as well as the endometrial lesions themselves. However, bone density decreases after several months of treatment, making GnRH treatment generally short term in duration.

Shrinking Uterine Fibroids, Controlling Excessive Uterine Bleeding. Uterine fibroids grow in response to estrogens and progesterone during the menstrual cycle as well as drug therapy. Since GnRH agonists decrease estrogen and progesterone production, these drugs can be effective in shrinking uterine fibroids and controlling excessive uterine bleeding. Typically such GnRH therapy is given to allow a vaginal hysterectomy (rather than a more invasive abdominal hysterectomy). GnRH agonists are also given to shrink intracavity fibroids in preparation for a hysteroscopic myomectomy. These agents are generally not used long term because of concern of loss of bone density secondary to a decrease in estrogen levels.

Treatment of Children with Precocious Puberty. GnRH agonists have been successfully used to treat premature puberty in young children. Menstruation is stopped, early development of secondary sex characteristics blocked or decreased, and a decrease in the velocity of growth (which can lead to short stature as adults) are all achieved. Unlike with adults, bone loss and other long-term side effects have not been observed as a result of treatment.

Prostate Cancer. Continuous dosage of GnRH agonists (unlike the natural pulsatile release of GnRH in vivo) greatly

decreases gonadal function, the medicinal equivalent of castration of men. The cause is receptor desensitization, also known as receptor downregulation. The resultant drop in testosterone production has been shown to significantly aid the treatment of prostatic cancer, especially when given with an antiandrogen such as flutamide.

Chorionic Gonadotropin or GnRH to Cause Testicular Descent in Prepubertal Males

Chorionic gonadotropin treatment for young boys is used to treat prepubertal cryptorchidism not caused by anatomic obstruction. Cryptorchidism occurs with about 0.8% of boys. Internasal therapy has also been very effective in treatment, with nearly an 80% success rate.[56]

Products

Goserelin acetate, 3-[5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-(3-O-*tert*-butyl)-D-seryl-L-leucyl-L-arginyl-L-prolyl]carbazamide acetate (Zoladex), is a GnRH agonist, available in the United States only as a 3.6-mg subcutaneous implant.

Leuprolide acetate, 5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-N-ethyl-L-prolinamide acetate (Leupron), is a GnRH agonist, used for the full range of indications for gonadotropins. In the United States, it is available both in intramuscular (IM) and subcutaneous injectable dosage forms.

Nafarelin, 5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-3-(2-naphthyl)-D-al anyl-L-leucyl-L-arginyl-L-prolylglycinamide acetate hydrate (Synarel nasal spray), is available as a nasal spray, used for the range of indications described for gonadotropins.

SEX HORMONES

Although the estrogens and progesterone are usually called female sex hormones and testosterone is called a male sex hormone, it should be noted that all these steroids are biosynthesized in both males and females. For example, an examination of the biosynthetic pathway in Fig. 23-6 reveals that progesterone serves as a biosynthetic precursor to cortisone and aldosterone and, to a lesser extent, to testosterone and the estrogens. Testosterone is one of the precursors of the estrogens. However, the estrogens and progesterone are produced in much larger amounts in females, as is testosterone in males. These hormones play profound roles in reproduction, in the menstrual cycle, and in giving women and men their characteristic physical differences.

A larger number of synthetic or semisynthetic steroids having biologic activities similar to those of progesterone have been made, and these are commonly called progestins. Several nonsteroidal compounds also have estrogenic activity. Although the estrogens and progestins have had their most extensive use as chemical contraceptive agents for women, their wide spectrum of activity has given them many therapeutic uses in both women and men.

Testosterone has two primary kinds of activities—androgenic (or male physical characteristic–promoting) and anabolic (or muscle-building). Many synthetic and semisynthetic androgenic and anabolic steroids have been prepared. Much interest has focused on the preparation of anabolic agents (e.g., for use in aiding recovery from debilitating illness or surgery). However, the androgenic agents do have some therapeutic usefulness in women (e.g., in the palliation of certain sex organ cancers).

In summary, it can be said that although many sex hormone products have their greatest therapeutic uses in either women or in men, nearly all have some uses in both sexes. Nevertheless, the higher concentrations of estrogens and progesterone in women and of testosterone in men cause the development of the complementary reproductive systems and characteristic physical differences of women and men.

ESTROGENS

Estrogen Receptors

The general mechanism by which steroid hormones lead to gene expression has been described earlier in this chapter (see also Fig. 23-3). Estrogen receptors have received special attention.[7,57–62] The contributions of O'Malley and co-workers[57,60] and his personal reflections of 30 years of research provide interesting reading.

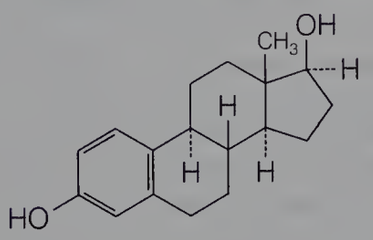
Structural Classes

As shown in Fig. 23-13, there are three structural classes of estrogens: human estrogens, equine estrogens, and diethylstilbestrol derivatives. (Each class is summarized in the sections that follow.) These estrogens are found in humans and most other mammals. As will be discussed later in this chapter, estrone and estriol are metabolites of estradiol in vivo (Fig. 23-14). Estrone is about one-third as active as estradiol, and estriol about one-sixtieth as active. The addition of a 17α-alkyl group blocks metabolism to estrone. Ethinyl estradiol is therefore very effective orally, whereas estradiol itself is not. They and their derivatives, such as ethinyl estradiol, all are produced semisynthetically from natural precursors such as diosgenin or cholesterol.

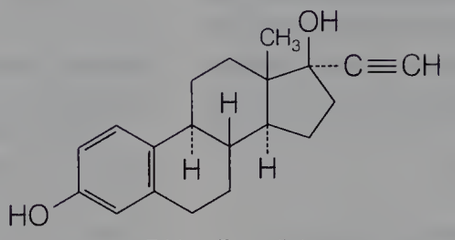
Equine Estrogens (Conjugated Estrogens, Esterified Estrogens)

Equine estrogens are estradiol metabolites originally obtained from the urine of horses, especially pregnant mares.

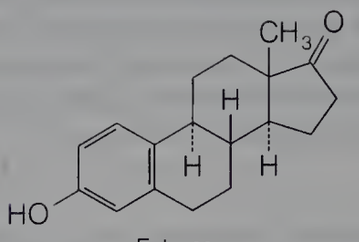
1. Human Estrogens and Derivatives



Estradiol
(IM and implantation pellets)

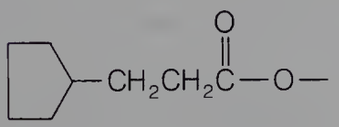


Ethinyl Estradiol
(Oral)

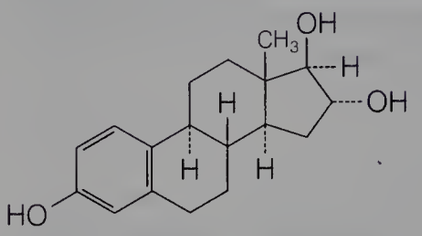


Estrone
(IM, vaginal, topical)

Esters for IM
- Estradiol 3-benzoate
- Estradiol 3,17β-dipropionate
- Estradiol 17β-cyclopentylpropionate



Ethers for oral use
- Ethinyl Estradiol 3-methylether (Mestranol)
- Ethinyl Estradiol 3-cyclopentyl ether (Quinestrol)

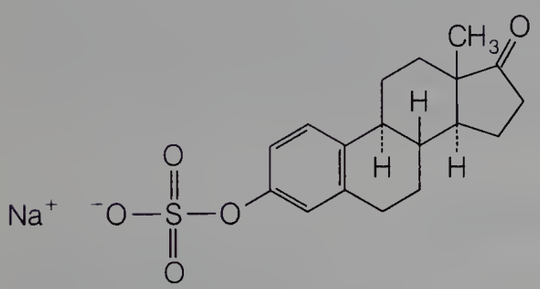


Estriol
(Oral)

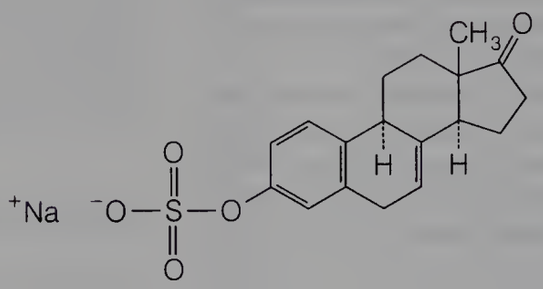
2. Equine Estrogens (Oral, IM, topical, vaginal)

(a) Conjugated Estrogens: 50–65% Sodium Estrone Sulfate
20–35% Sodium Equilin Sulfate
plus nonestrogenic compounds

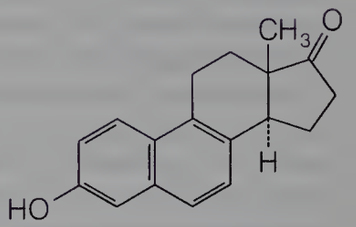
(b) Esterified Estrogens: 70–85% Sodium Estrone Sulfate
6.5–15% Sodium Equilin Sulfate
plus nonestrogenic compounds



Estrone Sodium Sulfate



Equilin Sodium Sulfate



Equilenin

Other salt available
Piperazine Estrone Sulfate

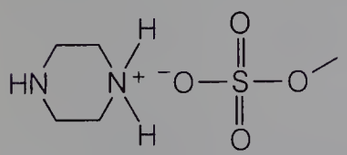
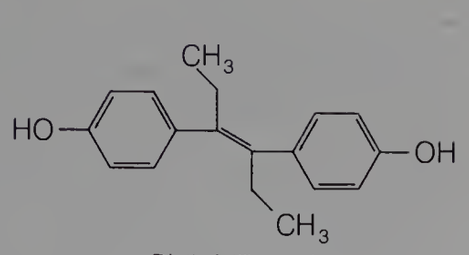
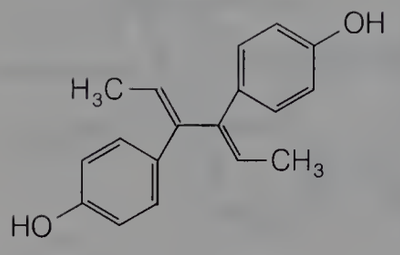


FIG. 23-13. *Natural and synthetic estrogens.*

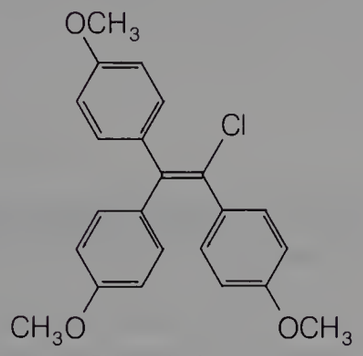
3. Synthetic Estrogens (Oral, IM, topical, vaginal)



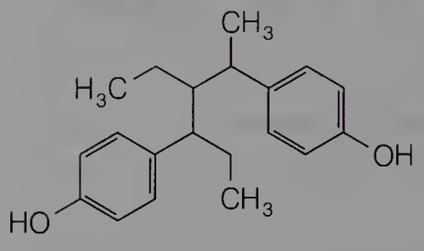
Diethylstilbestrol



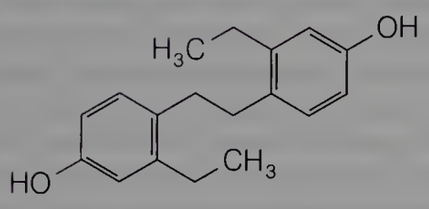
Dienestrol



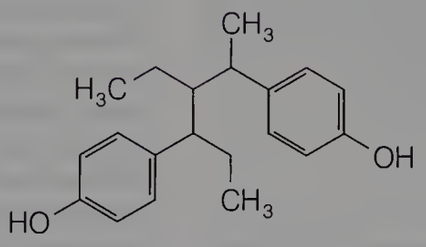
Chlorotrianisene



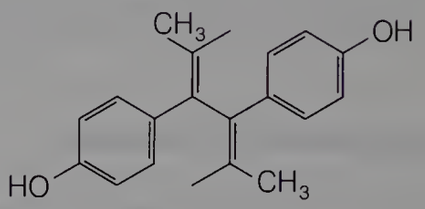
Benzestrol



Inactive

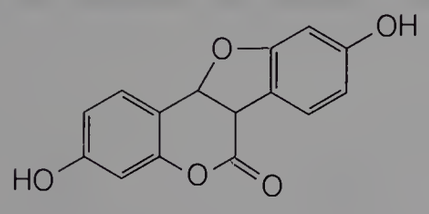


Benzestrol
Active

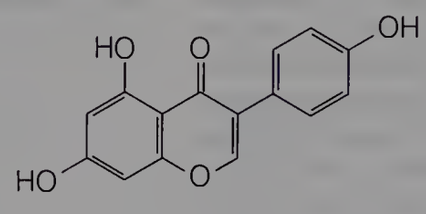


Dienestrol
Active

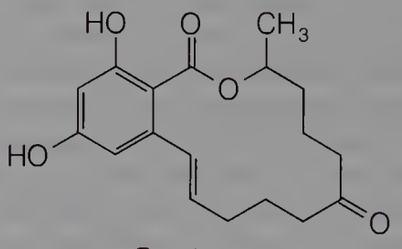
4. Estrogens from Plants



Coumestrol
Active



Genistein
Active



Zearalenone
Active

FIG. 23-13. *Continued.*

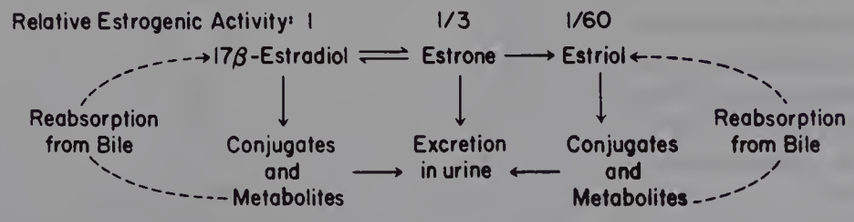


FIG. 23-14. *Interconversion and metabolism of natural estrogens.*

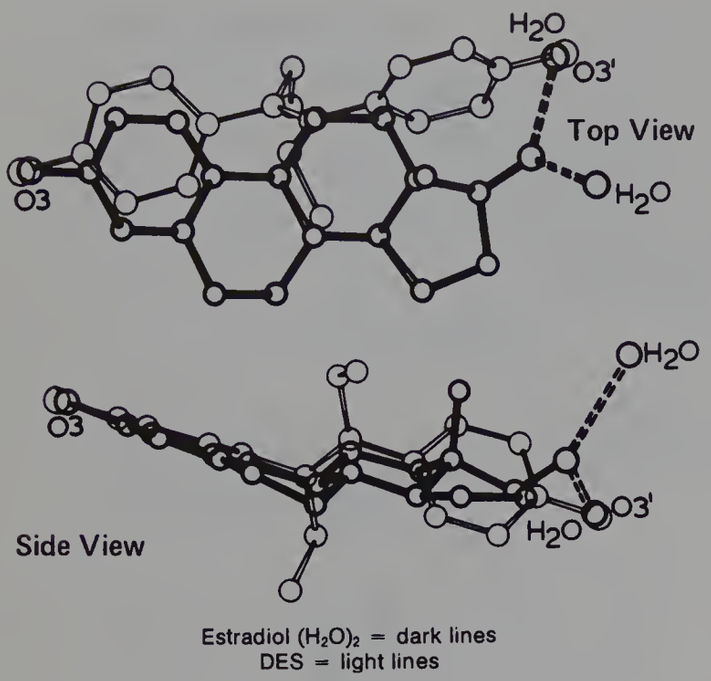


FIG. 23-15. *Computer graphics. Superposition of estradiol* $(H_2O)_2$ (dark lines) *with DES* (light lines). *(Courtesy of Medical Foundation of Buffalo, Inc.)*

Today, they are produced semisynthetically from estrogen intermediates synthesized from diosgenin and other natural precursors. Little or no equilin and equilenin are produced in humans. Equine estrogens are largely mixtures of estrone sodium sulfate and equilin sodium sulfate.

Diethylstilbestrol Derivatives

At first glance, it might be surprising that nonsteroidal molecules, such as diethylstilbestrol (DES), could have the same activity as estradiol or other estrogens. However, DES can be viewed as a form of estradiol with rings B and C open, and a six-carbon ring D. The activity of DES analogues was explained in 1946 by Schuler.[63] He proposed that the distance between the two DES phenol OH groups was the same as the 3-OH to 17-OH distance of estradiol; therefore, they could both fit the same receptor. Modern medicinal chemists have shown the OH-to-OH distance in DES in actually 12.1 Å, and 10.9 Å in estradiol. However, in water solution (or plasma), estradiol has two water molecules hydrogen bound to the 17-OH. If one of the two water molecules is included in the distance measurement, there is a perfect fit with the two OH groups of DES (Fig. 23-15). This suggests that water may have an important role for estradiol in its receptor site. It is now generally accepted that the estrogens must have a phenolic moiety for binding, but some investigators propose that the receptor may be flexible enough to accommodate varying differences between the two key hydroxyls. Wiese and co-workers[64] used computer modeling to study ten conformations of DES. All ten were found to have significant structural similarities with estradiol. They propose that the estrogen receptor may be able to recognize any of the ten DES conformations.

Thousands of DES analogues have been synthesized, and from them emerged many products including dienestrol, chlorotrianisene, and benzestrol. As long as the OH-to-OH distance relationship is maintained, significant estrogenic activity is usually found in the DES derivative. Without the central double bond and two ethyl or other alkyl groups, the molecule loses all its rigidity and shape, the OH-to-OH distance is not fixed, and activity is abolished (Fig. 23-16). Reduction of the double bond of DES results in two diastereomers of hexestrol. The *meso* form is active because the OH-to-OH distance is maintained. However, in the *threo* isomer, there is steric repulsion of the two ethyl groups. The two phenol groups rotate to relieve the repulsion, the OH-to-OH distance is changed, and consequently, the *threo* isomer is inactive (see Fig. 23-16).

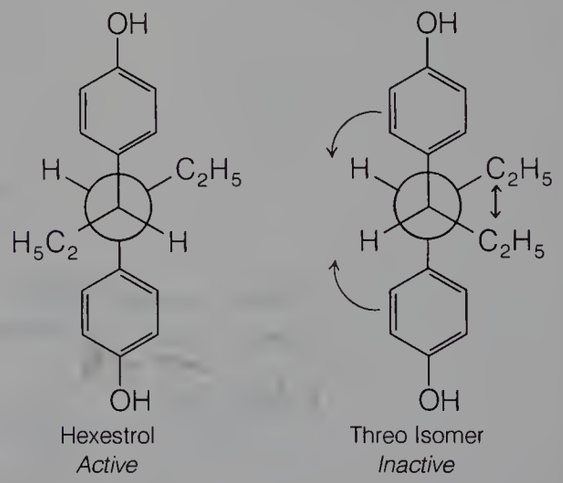


FIG. 23-16. *Importance of conformation and rigidity in estrogen activity. Examples of synthetic estrogens and estrogens from plants.*

Biosynthetic Sources

The estrogens are normally produced in relatively large quantities in the ovaries and placenta, in lower amounts in the adrenal glands, and in trace quantities in the testes. About 50 to 350 μg/day of estradiol are produced by the ovaries (especially the corpus luteum) during the menstrual cycle. During the first months of pregnancy, the corpus luteum produces larger amounts of estradiol and other estrogens, whereas the placenta produces most of the circulating hormone in late pregnancy. During pregnancy, the estrogen blood levels are up to 1000 times higher than during the menstrual cycle.

AROMATASE INHIBITORS

Aromatase[65–68] is a cytochrome P-450 enzyme complex that catalyzes the conversion of androstenedione and testosterone to estradiol (Figs. 23-17 and 23-18). The complex is made up of reduced nicotinamide adenine dinucleotide phosphate (NADPH)–cytochrome P-450 reductase, and cytochrome P-450 hemoprotein. In the first two steps, the C19 CH_3 is stereospecifically oxidized to CH_2OH, and then to CHO. In the final aromatization step, the C-19 carbon is oxidatively cleaved to formate. A hydride shift, proton transfer, and free radical pathways have been proposed, with *cis* elimination of the 1B and 2B hydrogens. In premenopausal women, aromatase is primarily found in ovaries, but in postmenopausal women, aromatase is largely in muscle and adipose tissue.

Some types of breast cancer and other cancers have been found to be (to varying degrees) estrogen dependent. Treatment of these conditions therefore focuses on decreasing estrogen, either by oophorectomy or by drug therapy. [The question of whether estrogens, especially postmenopausal estrogens, can increase the incidence of breast cancer is less well answered (discussed later in this chapter).] Two approaches have emerged for drug design:

1. Aromatase inhibitors (e.g., anastrozole)—Because the aromatase reaction is unique in steroid biosynthetic pathways, it would be anticipated that aromatase inhibitors would be very specific in their pharmacology (estrogen biosynthesis blockade).
2. Antiestrogens (e.g., tamoxifen)—estrogen receptor inhibitors. These compounds are discussed later in this chapter.

Examples of aromatase inhibitors are shown in Fig. 23-18; not all have been tested in humans. The first to be marketed in the United States is the nonsteroid anastrozole (Arimidex), and others are anticipated to be released in the near future. Anastrozole has been approved by the Food and Drug Administration (FDA) to treat advanced breast cancer in postmenopausal women with disease progression following tamoxifen therapy. Unfortunately, only about 15% of patients respond to the drug for more than 12 months. The drug is only effective when the cancer cells have been shown to be "estrogen receptor (ER) positive," meaning that they respond and proliferate in the presence of estrogen.

Currently available inhibitors block estrogen production by 50% to 80%. Probably the longest-studied steroid analogue is 4-hydroxyandrestenedione (4-OHA), which has been marketed in the United Kingdom for treatment of breast cancer. Although initially thought to be a completely reversible inhibitor, it is now known that 4-OHA is an enzyme activated irreversible ("suicide") inhibitor of armatase.

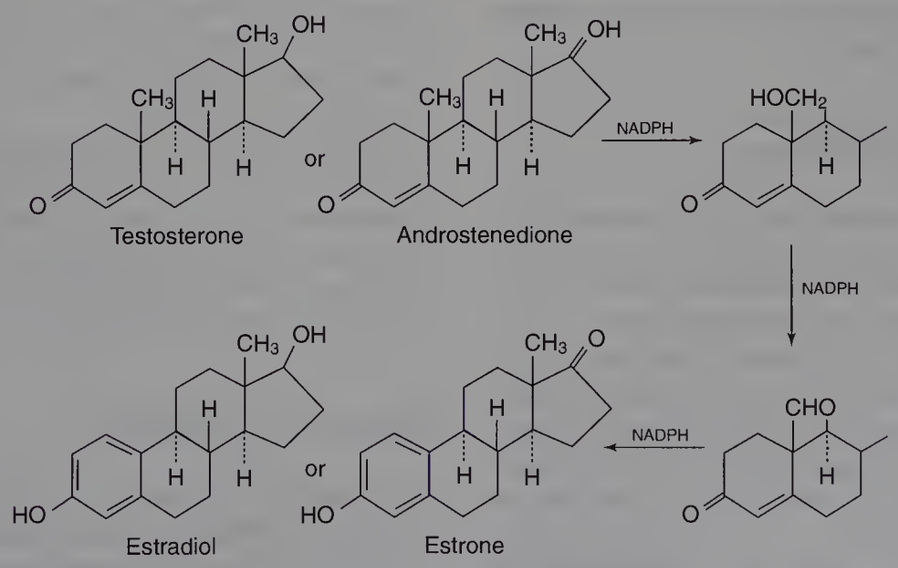


FIG. 23-17. *Aromatization of androgens to estrogens by aromatase.*

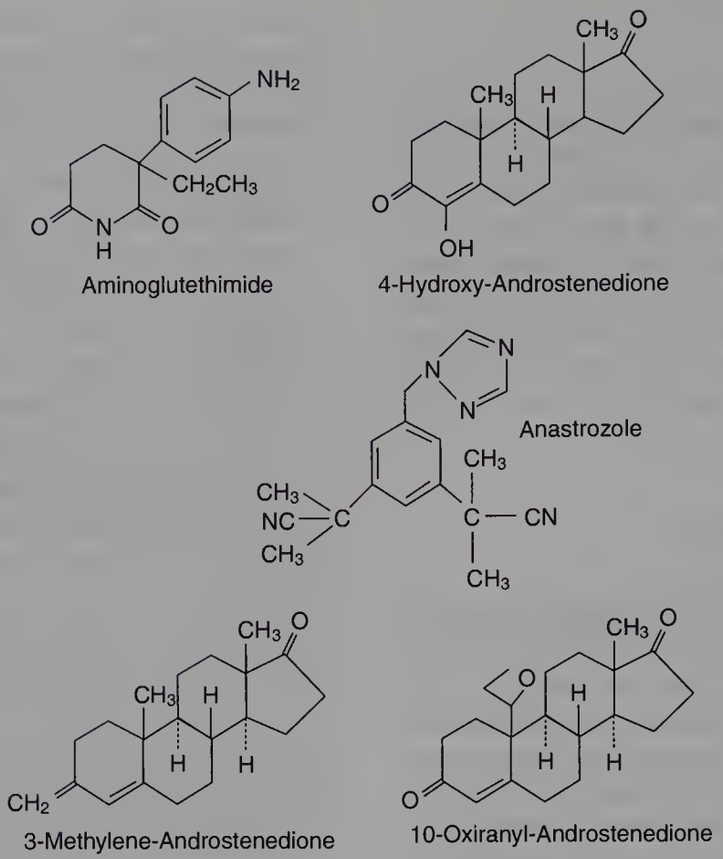


FIG. 23-18. *Examples of aromatase inhibitors.*

Aromatase Inhibitor Products

Anastrozole, USP, tetramethyl-5-(1H-1,2,4-triazol-1-ylmethyl)-1,3-benzenediacetonitrile, is the first aromatase inhibitor released in the United States. It is indicated for postmenopausal patients with advanced breast cancer who have had disease progression following tamoxifen therapy. Patients who did not respond to tamoxifen therapy rarely respond to anastrozole. It can cause fetal harm for pregnant women, and is contraindicated.

Biologic Activities of Estrogen

In addition to having important roles in the menstrual cycle (described earlier), the estrogens and, to a lesser extent, progesterone are largely responsible for the development of secondary sex characteristics in women at puberty.

The estrogens cause a proliferation of the breast ductile system, and progesterone stimulates development of the alveolar system. The estrogens also stimulate the development of lipid and other tissues that contribute to breast shape and function. Pituitary hormones and other hormones are also involved. Fluid retention in the breasts during the later stages of the menstrual cycle is a common effect of the estrogens. Interestingly, the breast engorgement that occurs after childbirth (stimulated by prolactin, oxytocin, and other hormones) can be suppressed by administration of estrogen—probably owing to feedback inhibition of the secretion of pituitary hormones.

The estrogens directly stimulate the growth and development of the vagina, uterus, and fallopian tubes and, in combination with other hormones, play a primary role in sexual arousal and in producing the body contours of the mature woman. Pigmentation of the nipples and genital tissues and growth stimulation of pubic and underarm hair (possibly with the help of small amounts of testosterone) are other results of estrogen action.

The physiologic changes at menopause emphasize the important roles of estrogens in the young woman. Breast and reproductive tissues atrophy, the skin loses some of its suppleness, coronary atherosclerosis and gout become potential health problems for the first time, and the bones begin to lose density because of decreased mineral content.

Metabolism of Estrogens

The metabolism of natural and synthetic (e.g., mestranol) estrogens has been reviewed in detail.[69]

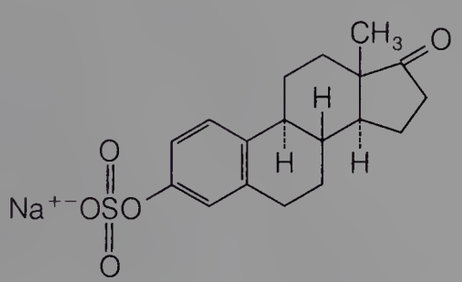
The three primary estrogens in women are 17β-estradiol, estrone, and estriol. Although 17β-estradiol is produced in

greatest amounts, it is quickly oxidized (see Fig. 23-14) to estrone, the estrogen found in highest concentration in the plasma. Estrone, in turn, is oxidized to estriol, the major estrogen found in human urine. During pregnancy, the placenta produces large amounts of estrone. However, in both pregnant and nonpregnant women, the three primary estrogens are also metabolized to small amounts of other derivatives (e.g., 2-hydroxyestrone, 2-methoxyestrone, and 16β-hydroxy-17β-estradiol). Only about 50% of therapeutically administered estrogens (and their various metabolites) are excreted in the urine during the first 24 hours. The remainder is excreted into the bile and reabsorbed; consequently, several days are required for complete excretion of a given dose. The primary metabolic path for ethinyl estradiol is 2-hydroxylation, followed by conversion to the 2- and 3-methyl ethers. Mestranol is 3-demethylated to ethinyl estradiol, its active metabolite.

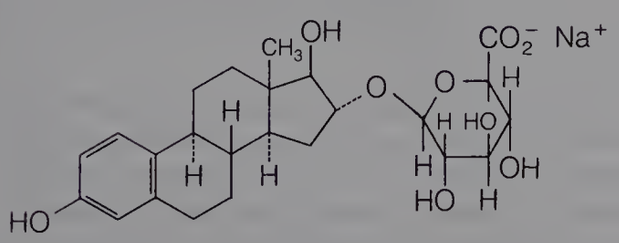
Metabolism of estrone is influenced by dietary fat. Low-fat diets promote the conversion of estrone to 2-hydroxyestrone rather than 16-hydroxyestrone (and then to estriol), whereas high-fat diets promote the opposite.

Conjugation appears to be very important in estrogen transport and metabolism. Although the estrogens are unconjugated in the ovaries, in the plasma and other tissues significant amounts of conjugated estrogens may predominate. Most of the conjugation takes place in the liver.

The primary estrogen conjugates found in plasma and urine are the combination of estrogen with glucuronic acid and, to a lesser degree, with sulfate. The conjugates are called glucuronides and sulfates, respectively. As sodium salts, they are quite water-soluble. The sodium glucuronide of estriol and the sodium sulfate ester of estrone are shown below:



Sodium Sulfate Ester of Estrone



Sodium Glucuronide of Estriol

Therapeutic Uses—Estrogens and Related Compounds

Birth Control. The greatest use of estrogens is for inhibition of ovulation, used in combination with progestins. Steroidal birth control agents containing estrogens are discussed in the section on chemical contraception, later in this chapter.

Osteoporosis Prevention and Treatment.[70–80] Osteoporosis is an enormous public health problem, responsible for approximately 1.5 million fractures in the United States each year. Included are 650,000 vertebral fractures and 250,000 hip fractures. The direct and indirect costs of osteoporosis have been estimated at $10 billion annually in the United States alone. (The well-illustrated booklet by Remagen is especially recommended for an overview of osteoporosis and its pathophysiology.)

A review of the process of bone remodeling will help in understanding the roles of estrogens, bisphosphonates, and calcitonin in treating osteoporosis. Bones are constantly breaking down (resorption) and undergoing formation. Osteoclasts facilitate bone resorption (breakdown), while osteoblasts cause bone formation. These two processes are normally in balance. But in osteoporosis, the rate of bone resorption is faster than the rate of bone formation; this imbalance most commonly occurs in the first 10 years of menopause. Long-term therapy with corticosteroids, calcitonin deficiency, physical inactivity, poor diet (shortage of calcium and/or vitamin D), and genetic factors can all contribute to osteoporosis and its severity. (Corticosteroids inhibit DNA synthesis of the precursors of osteoblasts, and change the sensitivity of osteoblasts to parathyroid hormone. Corticosteroids also increase the excretion of calcium and phosphate in the urine.) Calcitonin, a polypeptide hormone produced by the thyroid, inhibits the function of osteoclasts, and thereby inhibits bone resorption. Parathyroid hormone in low doses stimulates osteoblasts to form new bone. Estrogen increases the calcium sensitivity of the C cells in the thyroid, leading to increased formation of calcitonin. Estrogen also stimulates intestinal calcium absorption, and increases the synthesis of vitamin D (needed for intestinal absorption of calcium).

In menopause, the ovaries produce less estrogen. (Adipose tissue, however, continues to produce estrogen, so that obese women are less prone to estrogen deficiency and osteoporosis.) With less estrogen,

- calcitonin secretion by the thyroid is decreased; and,
- intestinal calcium absorption is decreased.

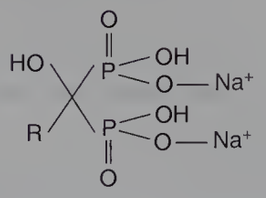
Combined with other factors (decreased exercise, which leads to bone loss, and decreased dietary calcium, phosphate, and magnesium), osteoporosis often results.

The prevention and treatment of osteoporosis have received much attention in the medical literature and lay press during the 1990s:

1. Premenopause: Good diet and exercise have been widely

shown to be essential for young women to decrease the risk of osteoporosis later in life.

2. Postmenopause:
   a. Estrogens: Estrogens taken after menopause (often with a supplemental progestin) have been unequivocally shown to greatly decrease the incidence and severity of osteoporosis, especially when combined with good nutrition and exercise. As discussed in the sections that follow, the risk of heart disease has also been shown to be reduced with women taking postmenopausal estrogens. However, conflicting data have suggested that postmenopausal estrogens may or may not increase the risk of breast cancer. The result has been a much publicized dilemma for postmenopausal women: ''Estrogens are sure to reduce my risk of osteoporosis, but risk of breast cancer might be increased. What should I do?''
   b. Bisphosphonates (etidronate, alendronate, pamidronate, Fig. 23-19): Bisphosphonates have been shown to inhibit bone resorption by alteration of osteoblast and osteoclast activity, or by reduction in the number of osteoclasts. Although estrogens remain the first line of osteoporosis treatment and prevention for most postmenopausal women, the bisphosphonates and calcitonin (see below) have been useful second-line agents. Alendronate is 200 to 1000 times more active in inhibiting bone resorption than etidronate and appears to increase bone mass density by about 4% to 7%. Pamidronate is about 10 times less potent than alendronate. None of the bisphosphonates to date has been shown to be more effective than estrogens in increasing bone mass density or in reducing the incidence of fractures.
   c. Calcitonin: Calcitonin is a naturally occurring peptide hormone produced by the parafollicular cells of the thyroid. Available by injection and by nasal inhalation, calcitonin has been used to treat both osteoporosis and Paget's disease. (In Paget's, bone growth and resorption are both accelerated, resulting in bone deformities.) Although human calcitonin has been produced by recombinant DNA techniques, salmon calcitonin is the most potent, and the form most used in therapy. Initial formulations of salmon calcitonin required IM or subcutaneous injection, resulting in a relatively high rate of noncompliance in patients. More recently, a nasal spray formulation has been developed and found to be a more acceptable alternative for patients who are not candidates for estrogen replacement therapy. Salmon calcitonin is indicated for women greater than 5 years postmenopause, and for whom estrogen therapy is contraindicated or not tolerated. Salmon calcitonin has been shown to increase bone mass density by 2% to 3%.



R = CH_3 Etidronate (Didronel)
R = $CH_2CH_2CH_2NH_2$ Alendronate (Fosamax)
R = $CH_2CH_2NH_2$ Pamidronate (Aredia)

FIG. 23-19. *Bisphosphonates for osteoporosis.*

Postmenopausal Estrogens in Lowering Risk of Heart Disease.[80–89] The benefits of estrogen or estrogen/progestin combinations in reducing heart disease risk factors have been clearly established. The Postmenopausal Estrogen/Progestin Interventions (PEPI) trial[88] showed, for example, a 50% reduction in heart disease risk with patients taking estrogen unopposed by progestin. Studies have also shown beneficial effects for women taking estrogen combined with progestin. It has been suggested that high-density lipoprotein cholesterol (HDL-C) is the best predictor of heart disease in women. Up to half of the observed cardiovascular benefit of estrogen may be the result of its increase of HDL-C, with or without added progestin.

Estrogens and Breast Cancer.[80–89] Two major studies published in 1995 reached different conclusions concerning the relationship of estrogens and the risk of breast cancer. Stanford and co-workers[82] studied 537 women in the Seattle area and concluded that women taking estrogens were not at risk compared to controls not taking estrogens. In contrast, the Nurses' Health Study[81] showed a significant increase in breast cancer among postmenopausal women taking estrogen alone or estrogen plus progestin. Thus, postmenopausal women feel significant uncertainty in weighing the benefits of estrogens for prevention and treatment of osteoporosis with an unresolved impact on breast cancer.

Treatment of Vasomotor Symptoms of Menopause and Atrophic Vaginitis (Kraurosis Vulvae). Estrogens have been very useful in treating the ''hot flashes'' associated with early menopause, as well as atrophic vaginitis and other vaginal symptoms of inadequate estrogen production. However, there is no evidence that they result in a more youthful appearance, nor that they help in the emotional symptoms, such as depression, sometimes associated with the onset of menopause.

Treatment of Estrogen Deficiency from Ovarian Failure or After Oophorectomy. Estrogen therapy, usually with a progestin, is common in cases of ovarian failure and after an oophorectomy.

Treatment of Advanced, Inoperable Breast Cancer in Men and Postmenopausal Women, and of Advanced, Inoperable Prostate Cancer in Men. Estrogens are used to treat inoperable breast cancer in men and in postmenopausal women, but estrogen therapy can actually stimulate existing breast cancers in premenopausal women. The antiestrogen tamoxifen is reported to have fewer side effects; hence, it

is usually preferred. Estrogens also are often used to treat inoperable prostate cancer.

DES Babies. During the late 1930s through the early 1950s, it was believed that DES treatment could help those pregnant women who tended to miscarry to have full-term pregnancies. Not only was the belief incorrect, it was subsequently reported that daughters of women who had taken DES during pregnancy (''DES babies'') had a high risk of vaginal or cervical cancer.[90–92] However, continuing studies of over 2000 women exposed to DES in utero revealed very few cases of cancer through early 1988, although a high percentage of the women had vaginal epithelial changes.[90,93,94] There have been no increases in breast disease among DES-exposed women, when compared with women who have not been exposed.

Products—Estrogens

Estrogens are commercially available in a wide variety of dosage forms: oral tablets, vaginal creams and foams, transdermal patches, and IM dosage preparations.

Dosages and Dosage Cycles. The general guideline for estrogen dosage is to use the lowest effective dose possible, and only for the duration in which it is truly needed. Estrogens are generally administered on a cyclic basis, typically one tablet daily for 3 weeks, followed by one week without any estrogen. Dosage cycles for contraceptive products containing estrogens are discussed later in the chapter.

Estrone, USP. 3-Hydroxyestra-1,3,5(10)-trien-17-one is only one-third as active as its natural precursor, estradiol (see Fig. 23-14). As the salt of its 3-sulfate ester, estrone is the primary ingredient in conjugated estrogens USP and esterified estrogens USP. Although originally obtained from the urine of pregnant mares (about 10 mg/L), estrone is now prepared from the Mexican yam, discussed later in this chapter. Assay is usually by ultraviolet spectroscopy, using the maximum absorption 280 nm (EtOH). Radioimmunoassay procedures are also available for assay of estrone in plasma.

Piperazine estrone sulfate, USP: 3-Sulfoxy-estra-1,3,5 (10)-trien-17-one piperazine salt. All the estrone 3-sulfate salts have the obvious pharmaceutic advantage of increased water solubility (as one would predict from Table 23-1) and better oral absorption. Acids convert the salts to the free 3-sulfate esters, and, in addition, cause some hydrolysis of the ester. This does not seem to adversely affect absorption, but precipitation of the free sulfate esters in acidic pharmaceutic preparations should be avoided. The dibasic piperazine molecule acts as a buffer, giving it somewhat greater stability.

Conjugated estrogens, USP, and esterified estrogens, USP: Conjugated estrogens contain 50% to 65% of sodium estrone sulfate and 20% to 35% of sodium equilin sulfate (based on the total estrogen content of the product). Esterified estrogens have an increased amount of sodium estrone sulfate, 70% to 85%, often synthetically prepared from diosgenin and added to the urine extract. Although most commonly used to treat postmenopausal symptoms, the conjugated estrogens and esterified estrogens are used for the entire range of indications described previously.

The bioequivalency of the first conjugated estrogen to be marketed (Premarin) and generic products introduced in the late 1960s has been the subject of considerable discussion.[95] In 1990, the FDA concluded that the ''B'' generics were not therapeutically equivalent to Premarin and withdrew them as candidates for substitution.[96]

Estradiol, USP. Estra-1,3,5(10)-triene-3,17β-diol is the most active of the natural steroid estrogens (see Fig. 23-14). Although its 17β-OH group is vulnerable to bacterial and enzymatic oxidation to estrone (see Fig. 23-14), it can be temporarily protected as an ester or permanently protected by adding a 17α-alkyl group (giving 17α-ethinyl estradiol and the 3-methyl ether, mestranol, the most commonly used estrogen in oral contraceptives). 3-Esters increase the duration of activity. These derivatives illustrate the principles of steroid modification shown in Fig. 23-8. The increased oil solubility of the 3- and 17β-esters (relative to estradiol) permits the esters to remain in oil at the injection site for extended periods. Transdermal estradiol products became available in 1986, with studies showing that they are as effective as oral estrogens for treating menopausal symptoms. The commercially available estradiol esters are the following:

Estradiol benzoate, USP
Estradiol valerate, USP
Estradiol cypionate, USP

Ethinyl estradiol, USP. 17α-Ethinyl estradiol has the great advantage over other estradiol products in that it is orally active. It is equal to estradiol in potency by injection, but 15 to 20 times more active orally. The 3-methyl ether of ethinyl estradiol is mestranol, USP, widely used in oral contraceptives. Mestranol is metabolized to ethinyl estradiol, with an oral dose of about 50 μg of mestranol providing an estrogenic action approximately equivalent to 35 μg of oral ethinyl estradiol. Quinestrol, the 3-cyclopentyl ether of estradiol, is a prodrug of ethinyl estradiol—inactive until dealkylated in vivo. Whereas about 60% of an oral dose of ethinyl estradiol is metabolized in the intestinal mucosa and during the first pass through the liver, most of an oral dose of quinestrol survives. The cyclopentyl group also enhances lipid solubility and, thereby, lipid storage. The overall result is that after initial loading in daily dosages for a week, quinestrol is typically taken just once weekly.

Estriol, USP, possesses estrogenic activity and is reported to be orally active.

Diethylstilbestrol, USP. α,α'-Diethyl-(*E*)-4,4′-stilbenediol, DES, is the most active of the nonsteroidal estrogens (see Estrogen Structural Classes, above), having about the

same activity as estrone when given intramuscularly. The *cis*-isomer has only one-tenth the activity of the *trans*. The *trans*-isomer is also well absorbed orally and slowly metabolized; consequently, it has been a popular estrogen for many medical purposes (see Therapeutic Uses, above). Side effects can be serious. The diphosphate ester, *diethylstilbestrol diphosphate, USP*, is used only for cancer of the prostate and is available for intravenous use. However, it has been reported that there may be an increased incidence of deaths from cardiovascular causes in men who received 5 mg of DES daily for prolonged periods. The diphosphate salt has great water solubility, as one would predict from Table 23-1. Diethylstilbestrol was extensively used in low doses as an aid to fatten cattle. Because DES has been implicated in cancer (albeit in higher doses), the United States Congress and the FDA began action in September 1975 to ban DES in animal feed.

Note: All stilbene derivatives, such as DES and dienestrol, are light-sensitive and must be kept in light-resistant containers.

Dienestrol, USP. 4,4′-(1,2-Diethylidene-1,2-ethanediyl)-bisphenol has about the same activity as DES when taken orally. The cream is used to treat atrophic vaginitis.

Benzestrol, USP. 4,4′-(1,2-Diethyl-3-methyl-1,3-propanediyl)bisphenol, when drawn like DES in Fig. 23-13, resembles DES. Yet it has no double bonds such as DES and dienestrol have to keep the phenolic groups in a *trans* spatial arrangement. However, the adjacent ethyl groups do not prefer eclipsed conformation (much higher in energy than *trans*), thereby helping keep the phenolic groups *trans*. Benzestrol is used for all the usual indications for estrogens (see Therapeutic Uses, above).

Chlorotrianisene, USP. Chlorotris-(*p*-methoxyphenyl)-ethylene is more active orally than by injection and is thought to be converted to a more active form hepatically. When given by injection, it is quite a weak estrogen. It has good lipid solubility and is slowly released from lipid tissues, thereby giving it a relatively longer duration of action. The fat storage can also delay its onset of action.

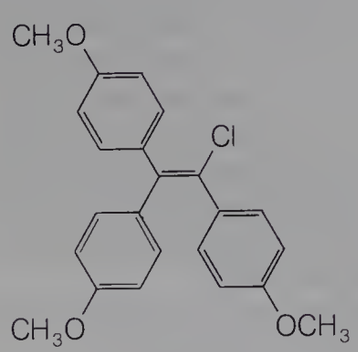
Estrogens from plants: Several natural plant substances that differ from DES in structure are also potent estrogens. These include genistein, from a species of clover; coumestrol, found in certain legumes; and zearalenone, from a *Fusarium* fungus. These and others have antifertility activity, as reviewed by Briggs and Christie.[97] There has also been a concern that environmental estrogens could contribute to the high incidence of breast cancer (for an overview, the 1995 *Scientific American* review of Davis and Bradlow[98] is recommended).

ANTIESTROGENS AND RELATED DRUGS

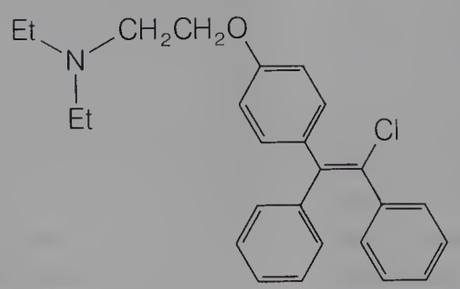
Whereas estrogens have been very important in chemical contraception and prevention and treatment of osteoporosis, estrogen antagonists (antiestrogens; Fig. 23-20) have been of great interest as ovulation stimulants and for treatment of estrogen-dependent breast cancers. Tumor biopsies have shown estrogen receptor to be present in about 60% of primary breast cancers, and most are responsive to estrogen blockade. Unfortunately, most of these estrogen receptor–related breast cancers also develop resistance to antiestrogen therapy within 5 years. In contrast, only about 6% of nonmalignant breast tissues have significant estrogen receptor present. The two antiestrogens used clinically today are clomiphene and tamoxifen.

The structural similarities of clomiphene and tamoxifen with nonsteroidal estrogens such as chlorotrianisene raise an important question: Why are some of these compounds estrogens, and why are some estrogens antagonists? In fact, both clomiphene and tamoxifen have some agonist activity, but they can effectively block H estradiol from binding. Tamoxifen, for example, has estrogenic effects on liver, bone, and the cardiovascular system.

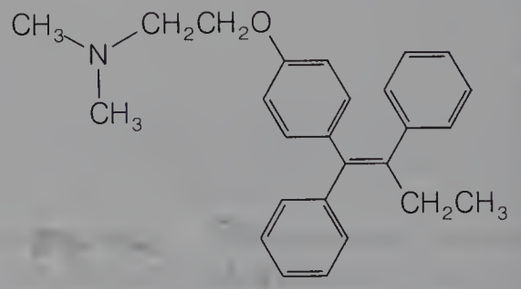
Hardcastle and co-workers[99] have gained important insights. They have found that there are two transactivation factors (TAF1 and TAF2) that are triggered by the estrogen receptor complex when bound to DNA, thus initiating tran-



Chlorotrianisene
(Estrogen)



Clomiphene
(Antiestrogen)



Tamoxifen
(Antiestrogen)

FIG. 23-20. *Representative antiestrogens showing structural similarity with the estrogen chlorotrianisene.*

scription. When triggered by moving into the proper conformation, TAF1 and TAF2 can displace a repressor complex to allow transcription. These investigators propose that antiestrogens, even though bound to the estrogen receptor complex, cannot trigger TAF1 sufficiently to displace the transcription receptor.

DES analogues such as clomiphene and tamoxifen that can bind to the estrogen receptor have been studied by x-ray crystallography by Duax and co-workers.[34] The phenyl A ring of antiestrogens is typically in the same position and conformation as the A ring of estradiol. They propose therefore that the A ring is responsible for binding. The bulky dialkyl aminoethyl side groups may not be able to trigger TAF1.

Tamoxifen

Tamoxifen is the primary drug used today to treat primary breast cancers that are estrogen receptor dependent.[99–102] Side effects are generally mild (unlike with clomiphene). With premenopausal women with metastatic disease, tamoxifen is an alternative and adjuvant with oophorectomy, ovarian irradiation, and mastectomy.

Two clinical dilemmas have resulted with tamoxifen. (The excellent reviews of Sylvester[100] and Ross and Whitehead[101] are recommended for a detailed clinical overview.) First, tamoxifen increases the incidence of endometrium polyps, hyperplasia, and carcinoma. However, the risk of endometrial cancer resulting from tamoxifen has been shown to be much less than the "modest but highly significant reductions in morbidity and mortality of breast cancer."[101]

The second dilemma focuses on the use of tamoxifen in helping prevent breast cancer in women at especially high risk (based on factors such as the number of immediate relatives with breast cancer). The question largely focuses on the risk of endometrial cancer for women who currently are breast cancer free. The Breast Cancer Prevention Trial (BCPT) of the National Cancer Institute was begun in 1992 to provide answers.

Clomiphene

Clomiphene's therapeutic application as an ovulation stimulant results from its ability to increase GnRH production by the hypothalamus. The mechanism is presumably a blocking of feedback inhibition of ovary-produced estrogens. The increased GnRH, in turn, leads to increased LH and FSH production, maturation of the ovarian follicle, and ovulation (as described earlier in this chapter, see Fig. 23-9). In tests with experimental animals, for example, clomiphene has no effect in the absence of a functioning pituitary gland.

Multiple births occur about 10% of the time with patients taking clomiphene, and birth defects with 2% to 3% of live newborns. Vasomotor "hot flashes" occur about 10% of the time, and abnormal enlargement of the ovaries about 14%. Abdominal discomfort should immediately be discussed with the physician.

Other drugs used for induction of ovulation include hMG and other GnRH pulsatile injections. hMG should be used with caution because ovarian enlargement is quite common. Multiple births occur in up to 20% of the cases, and pregnancies followed by spontaneous abortions occur in 20% to 30% of the cases.

In general, it is strongly recommended that product literature or detailed general references such as *Facts and Comparisons* or the *Hospital Formulary* be consulted before dispensing either clomiphene citrate or hMG.

Clomiphene citrate, USP. 2-[4-(2-Chloro-1,2-diphenylethenyl)phenoxy]-*N*,*N*-diethylethanamine (Clomid) is given to stimulate ovulation in the usual dosage of 50 mg daily for 5 days starting on the fifth day of the menstrual cycle. If ovulation does not occur, the dose is increased to 100 mg daily for 5 days in the next cycle. The patient should be warned to report any visual disturbances or abdominal pain to the physician. If menstruation does not occur at the end of the first full cycle following treatment, pregnancy tests should be conducted before additional clomiphene is taken. A careful physical examination before treatment is recommended, especially to determine the possible presence of ovarian cysts, because ovarian enlargement sometimes occurs.

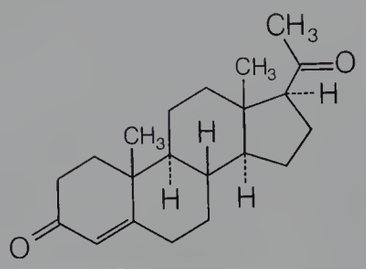
Tamoxifen citrate, USP. 2-[4-(1,2-Diphenyl-1-butenyl)-phenoxy]-*N*, *N*-dimethylethanamine (Novaldex) is an antiestrogen used to treat early and advanced breast carcinoma in postmenopausal women. In trials of tamoxifen as a postsurgical adjuvant treatment of early breast cancer, disease-free survival is consistently prolonged. Antiestrogenic and estrogenic side effects can include hot flashes, nausea, vomiting, platelet reduction, and (in patients with bone metastases) hypercalcemia. As with all triphenylethylene derivatives, it should be protected from light.

PROGESTINS

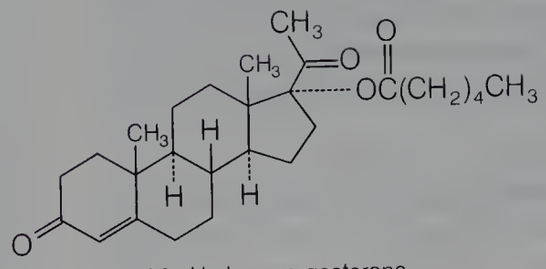
STRUCTURAL CLASSES

Progestins are compounds that have biologic activities similar to progesterone. They include two structural classes: progesterone and derivatives, and testosterones and 19-nortestosterones (Fig. 23-21). Progesterone is not orally effective; with a plasma half-life of only about 5 minutes, it is almost completely metabolized in one passage through the liver. Adding 17α-alkyl groups slows metabolism of the 20-one, whereas a 6-methyl or 6-chloro group enhances activity and reduces metabolism. Medroxyprogesterone acetate is a particularly potent example (Table 23-2).

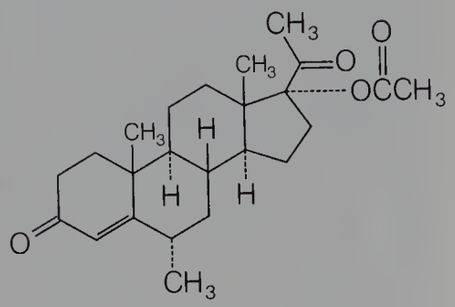
1. Progesterones and Derivatives



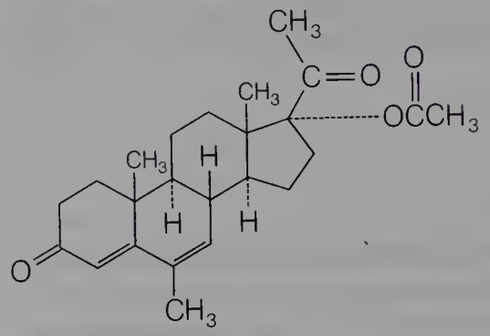
Progesterone
(IM, vaginal/gel)



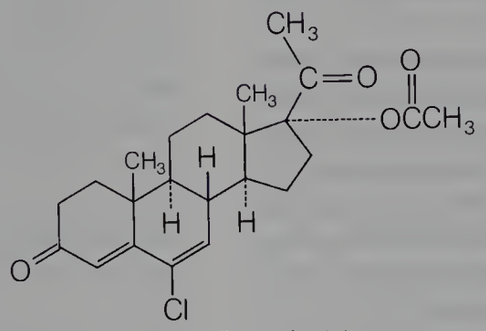
17α-Hydroxyprogesterone
Caproate (IM)



Medroxyprogesterone
Acetate (Oral, IM)

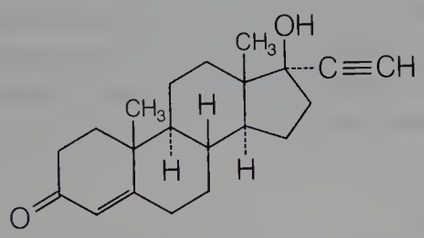


Megestrol Acetate

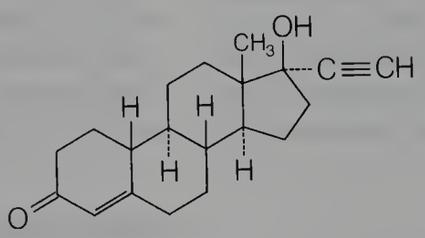


Chlormadinone Acetate

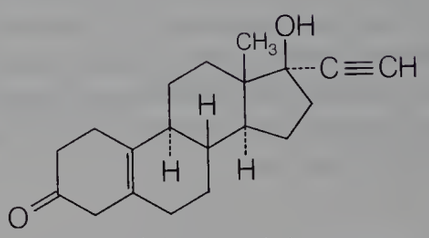
2. Testosterones and 19-Nortestosterone Derivatives



Ethisterone
(Oral)



Norethindrone
(Oral)



*Norethynodrel
(Oral)

Desobestrel

Norgestimate

FIG. 23-21. *Natural and synthetic progestins. (*Available only in contraceptive products.)*

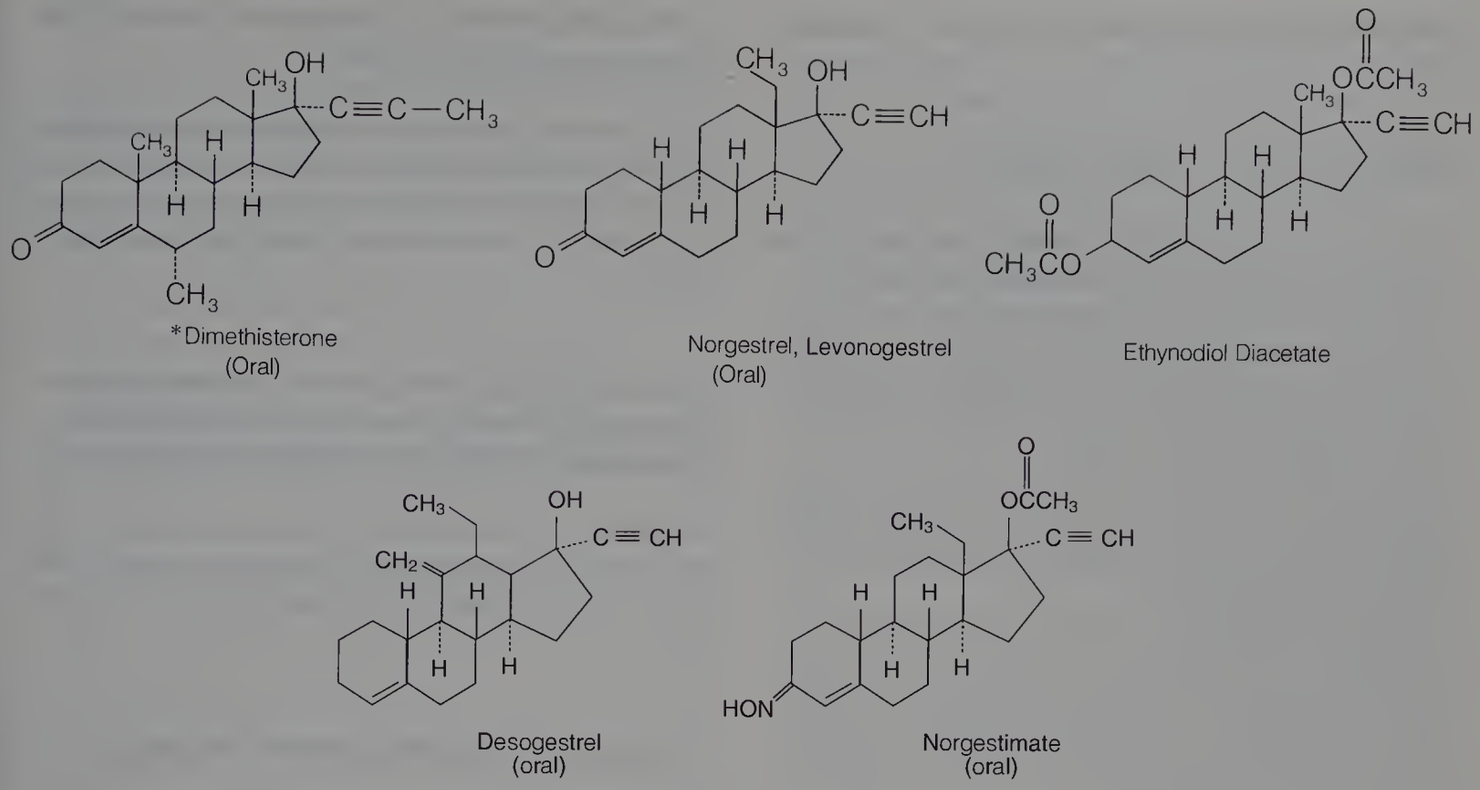


FIG. 23-21. *Continued.*

Duax and co-workers[33,34] have studied the structural requirements of the progesterone receptor in detail. They conclude that the progesterone 4-en-3-one ring A is a key to binding, but only when it is in a conformation quite different from that of testosterone or the glucocorticoids. Their reviews contain stereo drawings that show the requirement conformations in three dimensions.

Although the 19-nortestosterones do have androgenic side effects, their primary activity, nevertheless, is progestational. Addition of 17α-alkyl groups to testosterone blocks oxidation at C-17. However, 17α-methyltestosterone has only half the androgenic activity of parenterally administered testosterone, and the 17α-ethyl analogue is nearly inactive. Adding the electron density of a triple bond, as in 17α-ethinyl, causes a marked increase in progestational activity and, simultaneously, blocks metabolic or bacterial oxidation to the corresponding 17-ones. Thus, by adding a 17α-ethinyl or propinyl group to testosterone, one can simultaneously decrease anabolic activity, promote good progestational activity, and have an orally active compound as well. Table 23-2 illustrates the relative progestational activity of a number of progestins.

The 19-nor derivatives also have marked ovulation-inhibiting activity, which does not necessarily parallel progestational activity. The endometrial proliferation (Clauberg-McPhail) test is most often used to evaluate progestational activity, whereas antiovulation activity is determined by examining treated female rabbits for ovulation rupture points in their ovaries.

TABLE 23-2

COMPARATIVE PROGESTATIONAL ACTIVITY OF SELECTED PROGESTINS

	Relative Oral Activity	*Activity SC*
Progesterone	(nil)	1
17α-Ethinyltestosterone (ethisterone)	1	0.1
17α-Ethinyl-19-nortestosterone (norethindrone)	5–10	0.5–1
Norethynodrel	0.5–1	0.05–1
17α-Hydroxyprogesterone caproate	2–10	4–10
Medroxyprogesterone acetate	12–25	50
19-Norprogesterone		5–10
Norgestrel		3
Dimethisterone	12	

(Data from Salhanick, H. A., et al.: Metabolic Effects of Gonadal Hormones and Contraceptive Steroids. New York, Plenum Press, 1969.)

BIOSYNTHETIC SOURCES

Progesterone is produced in the ovaries, testes, and adrenal glands. Much of the progesterone that is synthesized is immediately converted to other hormonal intermediates and is not secreted. (Refer to the biosynthetic pathway, Fig. 23-6.) The corpus luteum secretes the most progesterone, 20 to 30 mg/day during the last or "luteal" stage of the menstrual

cycle. Normal men secrete about 1 to 5 mg of progesterone daily.

METABOLISM OF PROGESTINS

As noted at the beginning of this section, progesterone has a half-life of about only 5 minutes when taken orally.

As shown in Fig. 23-6, progesterone can be biotransformed to many other steroid hormones and, in that sense, it has numerous metabolic products. However, the principal excretory product of progesterone metabolism is 5β-pregnane-3α,20-diol and its conjugates.

The metabolism of progesterone is extremely rapid and, therefore, it is not effective orally. That fact has been a major stimulus in the development of the 19-nortestosterones with progesterone-like activity.

The great advantage of testosterone and 19-nortestosterone analogues is that, with a 17α-ethinyl group, these compounds are orally active. The 17-OH cannot be metabolized to the corresponding 17-ones that have little or no activity. Their 5-enes are metabolized to 5α-analogues; however, the metabolites are still quite active.

BIOLOGIC ACTIVITIES OF THE PROGESTINS

Crowley[103] has captured the fascination and wonder of progesterone in a succinct overview in the *New England Journal of Medicine*:

> Progesterone is a unique reproductive hormone. In women who are not pregnant, it is the chief secretory product. . . of the corpus luteum—itself a curious endocrine organ which is programmed for demise within a fortnight unless it is "rescued" from this fate by a fertilized ovum. When pregnancy occurs, hCG contributes to the persistence of the corpus luteum, which secretes the progesterone required for the maintenance of early pregnancy. . . . Progesterone's principal target organs are the uterus, breast, and brain. [Actions include] differentiation of the estrogen-primed, growing endometrium, and induction of protein secretion; [in breast tissue], differentiation of estrogen-prepared ductal tissue and support [of] lactation. Progesterone's influence on the CNS is poorly understood, but appears to have diverse effects on the hypothalamic–pituitary axis, respiratory center, and perhaps cortical function. . . . Its thermogenic effect, also centrally mediated, leads to an increase in basal body temperature . . . [but] a considerable amount remains to be learned. . . .[103]

THERAPEUTIC USES

Birth Control

The largest use of the progestins, as of the estrogens, is for inhibition of ovulation. Steroidal birth control agents are discussed in the section on chemical contraception, below.

To Reduce the Risk of Endometrial Cancer from Postmenopausal Estrogens

As discussed in the section on therapeutic uses of estrogens, several studies have suggested that the combination of a progestin with an estrogen may significantly reduce the risk of endometrial cancer in women taking postmenopausal estrogens.

Secondary Amenorrhea and Functional Uterine Bleeding Caused by Insufficient Progesterone Production or Estrogen-Progesterone Imbalance

Progestins have been used very effectively to treat secondary amenorrhea, functional uterine bleeding, and related menstrual disorders caused by hormonal deficiency or imbalance.

Treatment of Inoperable Endometrial Cancer

Surgery is usually the first treatment of endometrial or breast cancer, but in advanced cases for whom surgery would not be possible, progestins have provided palliative treatment.

Progestins for Premenstrual Syndrome (PMS)

There have been claims that progesterone may reduce the effects of PMS. However, the *Medical Letter* has concluded that the claims are not supported by medical data.[104]

Progestins and Pregnancy—A Warning

Progestins have been used to prevent habitual abortions, but the FDA has strongly warned against the use of steroids during pregnancy.[105] Large doses of progestins have also been given as a test for pregnancy, but the FDA warning would discourage this practice as well.

SIDE EFFECTS

Progestin therapy may cause menstrual irregularities, such as spotting or amenorrhea. Weight gain and acne have been associated with testosterone and 19-nortestosterone analogues, in part because of their slight androgenic effects. In combination with estrogens in oral contraceptives, the progestin components may contribute to the thromboembolic disorders primarily attributed to estrogens. As noted in the section on oral contraceptives, the frequency of these disorders in nonsmokers younger than 35 years is negligible.

PRODUCTS

The progestins are primarily used in oral contraceptive products for women, and they are also used to treat several gynecologic disorders: dysmenorrhea, endometriosis, amenorrhea, and dysfunctional uterine bleeding. Estrogens are given simultaneously in most of these situations.

The doses appropriate for the various foregoing indications can vary substantially, and detailed manufacturers' literature or general references should be consulted before advising physicians.

Progesterone, USP. Pregn-4-en-3,20-dione is so rapidly metabolized that it is not particularly effective orally, being only one-twelfth as active as intramuscularly. It can also be very irritating when given intramuscularly. Buccally it is only slightly more active than orally. As discussed later in this chapter (Commercial Production), progesterone was originally obtained from animal ovaries, but was prepared in ton quantities from diosgenin in the 1940s. This marked the start of the modern steroid industry, a fascinating history discussed later in this chapter. The discovery of 19-nortestosterones with progesterone activity made synthetically modified progestins of tremendous therapeutic importance.

Progesterone (and all other steroid 4-ene-3-ones) is light-sensitive and should be protected from light.

Hydroxyprogesterone Caproate, USP. 17-Hydroxypregn-4-ene-3,20-dione hexanoate is much more active and longer-acting than progesterone (see Table 23-2), probably because the 17α-ester function hinders reduction to the 20-ol. It is given only intramuscularly. The hexanoate ester greatly increases oil solubility, allowing it to be slowly released from depot preparations, as one would predict from Fig. 23-8.

Medroxyprogesterone Acetate, USP. 17-Hydroxy-6α-methylpregn-4-ene-3,20-dione acetate adds a 6α-methyl group to the 17α-hydroxyprogesterone structure to greatly decrease the rate of reduction of the 4-ene-3-one system. The 17α-acetate group also decreases reduction of the 20-one, just as with the 17α-caproate. Medroxyprogesterone acetate is very active orally (see Table 23-2) and has such a long duration of action intramuscularly that it cannot be routinely used intramuscularly for treating many menstrual disorders.

Norethindrone, USP, and Norethynodrel, USP. 17α-Ethinyl-19-nortestosterone, and its $\Delta^{5(10)}$ isomer, respectively, might appear at first glance to be subtle copies of each other. One would predict that the $\Delta^{5(10)}$ double bond would isomerize in the stomach's acid to the Δ^{3} position. In fact, however, the two drugs were simultaneously and independently developed; hence, neither can be considered a copy of the other. Furthermore, norethindrone is about ten times more active than norethynodrel (see Table 23-2), indicating that isomerization is not as facile in vivo as one might predict. Although they are less active than progesterone when given subcutaneously, they have the important advantage of being orally active. The discovery of the potent progestin activity of 17α-ethinyltestosterone (ethisterone) and 19-norprogesterone preceded the development of these potent progestins. All are orally active, with the 17α-ethinyl group blocking oxidation to the less active 17-one. The rich electron density of the ethinyl group and the absence of the 19-methyl group greatly enhance progestin activity. Both compounds have become of great importance as progestin ingredients of oral contraceptives, although norethindrone, USP, and norethindrone acetate, USP, are widely employed for all the usual indications of the progestins. Because these compounds retain the key feature of the testosterone structure—the 17β-OH—it is not surprising that they possess some androgenic side effects. The related compounds, norgestrel, USP, and levonorgestrel, USP, have an ethyl group instead of the C-13-methyl, but have similar biologic properties. Norgestrel is used only in oral contraceptives. Levonorgestrel is used both in oral combination birth control products, as well as in Silastic implants that provide contraception for up to 5 years. These 19-nortestosterone derivatives will be discussed in the later section on chemical contraceptives.

Megestrol Acetate, USP. 17-Hydroxy-6-methylpregna-4,6-diene-3,20-dione acetate (Megace) is a progestin used primarily for the palliative management of recurrent, inoperable, or metastatic endometrial or breast carcinoma.

Desogestrel, USP. (17α)-13-ethyl-11-methylene-18,19-dinorpregn-4-en-20-yn-17-ol (Desogen) is a 19-nortestosterone analogue with good progestin activity. As with the other progestins, it is orally active and used in combination with an estrogen in oral contraceptives.

Norgestimate, USP. (17α)-17-(acetyloxy)-13-ethyl-18,19-dinorpregn-4-en-20yn- 3-one oxime (Cyclen, Tri-Cyclen) is a 19-nortestosterone, 3-oxime, orally active, and used with an estrogen in oral contraceptive products.

CHEMICAL CONTRACEPTIVE AGENTS

The world's increasing population is a major concern, vastly exceeding fuel and food supplies in many parts of the world.

Political, cultural, and research cost barriers have enormously complicated the development of contraceptive agents in modern times. The reviews by Djerassi,[106] former Research Director of Syntex and inventor of norethindrone, and by Lednicer[107] are important reading. (Their "insider's viewpoint" of the research competition during the 1950s and 1960s to develop steroid products is especially interesting.)

The need for birth control was most dramatically and effectively expressed from 1910 to 1950 by Margaret Sanger.[108–110] This remarkable American woman, founder of Planned Parenthood in 1916, made birth control information generally available in the United States. She made the medical profession better aware of the needs of women, and

also raised the funds necessary for the early research on oral contraceptives.

During the 1940s and 1950s, great progress was made in the development of intravaginal spermicidal agents. However, the most notable achievement in chemical contraception came in the early 1960s with the development of oral contraceptive agents—"the pill." Since that time, a number of postcoital contraceptives and abortifacients have been developed. Hormone-releasing intrauterine devices and Silastic implants are now available. However, progress has been much slower in the development of male contraceptive agents.

In the following pages, each of these approaches to chemical contraception are discussed. Individual compounds have already been discussed with the estrogens and progestins.

Several excellent reviews on the mechanisms of action of birth control agents, and birth control in general, have recently been published. The Syntex guide for pharmacists, *Methods of Birth Control*,[111] is particularly recommended for the classroom. *Contraceptive Technology*, published annually by Irvington Publishers, provides a comprehensive overview of recent advances in contraceptive use and development.[112] The choice of "which oral contraceptive" has been recently reviewed.[113,114] Future developments, and the impact of politics and economics have also been discussed in depth.[115–117]

OVULATION INHIBITORS AND RELATED HORMONAL CONTRACEPTIVES

History[106,107,110]

In the 1930s, several research groups found that injections of progesterone inhibited ovulation in rats, rabbits, and guinea pigs.[118–121] Kurzrok, Albright, and Sturgis, in the early 1940s, are generally credited with the concept that estrogens, progesterone, or both could be used to prevent ovulation in women.[122–124] In 1965, Pincus[125] reported that progesterone given from day 5 to day 25 of the menstrual cycle would inhibit ovulation in women. During this time, Djerassi et al.[126] of Syntex, and Colton[127] of G.D. Searle and Co. reported the synthesis of norethindrone and norethynodrel. These progestins possessed very high progestational and ovulation-inhibiting activity. Most of the synthetic work was made possible by the development of the Birch reduction by Arthur J. Birch in 1950, and used by Birch to synthesize 19-nortestosterone itself.[128]

Extensive animal and clinical trials conducted by Pincus, Rock, and Garcia confirmed, in 1956, that Searle's norethynodrel and Syntex's norethindrone were effective ovulation inhibitors in women. In 1960 Searle marketed Enovid (a mixture of norethynodrel and mestranol), and in 1962 Ortho marketed Ortho Novum (a mixture of norethindrone and mestranol) under contract with Syntex. Norethynodrel and norethindrone have remained the most extensively used progestins in oral contraceptives, but several other useful agents have been developed. These will be discussed in the sections that follow.

Therapeutic Classes and Mechanism of Action

The ovulation inhibitors and modern hormonal contraceptives fall into several major categories (Table 23-3), each with its own mechanism of contraceptive action. Individual compounds have been discussed with the estrogens and progestins in the previous section.

Combination Tablets: Mechanism of Action. Although, as noted earlier, Sturgis and Albright recognized in the early 1940s that either estrogens or progestins could inhibit ovulation, it was subsequently found that combinations were highly effective. Some problems, such as breakthrough (midcycle) bleeding, were also reduced by the use of a combination of progestin and estrogen.

Although all the details of the process are still not completely understood, it is now believed that the combination tablets suppress the production of LH or FSH, or both, by a feedback-inhibition process (see Fig. 23-9). Without FSH or LH, ovulation is prevented. The process is similar to the natural inhibition of ovulation during pregnancy, caused by the release of estrogens and progesterone from the placenta and ovaries. An additional effect comes from the progestin in causing the cervical mucus to become very thick, providing a barrier for the passage of sperm through the cervix. However, because pregnancy is impossible without ovulation, the contraceptive effects of thick cervical mucus or alterations in the lining of the uterus (to decrease the probability of implantation of a fertilized ovum) would appear to be quite secondary. Nevertheless, occasionally ovulation may occur, and thus the alterations of the cervical mucus and the endometrium may actually serve an important contraceptive function (especially, perhaps, when the patient forgets to take one of the tablets). During combination drug treatment, the endometrial lining develops sufficiently for withdrawal bleeding to occur about 4 or 5 days after taking the last active tablet of the series (see Table 23-3).

Monophasic (Fixed) Combinations. The monophasic combinations of a progestin and estrogen contain the same amount of drug in each active tablet (see Table 23-3). As discussed later in this chapter, the trend in prescribing has been toward lower doses of estrogen. However, as estrogen levels are reduced, breakthrough bleeding (or "spotting") becomes an annoying side effect for some patients at early to midcycle. Spotting after midcycle or amenorrhea appears to be related to too little progestin relative to the estrogen. The biphasic and triphasic combinations were developed to solve these breakthrough bleeding problems in some patients.

TABLE 23-3

COMPARISON OF STEROID CONTRACEPTIVE REGIMENS

1. Combination—Monophasic

Products are available in 20 (21)- or 28-day dispensers and refills. The first tablet is taken on the fifth day after menstruation has started, or on the first Sunday after or on which menstruation has started. The 28-day dispensers contain seven inert (or Fe^{2+}-containing) tablets of a different color, taken daily after the 20 or 21 days of active tablets. With 20 (21)-day regimens, if menstruation does not occur, the next cycle of 20 (21) tablets begins on the eighth day after the last active tablet was taken. Doses of active tablets are shown below.

Brand	*Progestin*	*Estrogen*
Leven	Levonorgestrel, 0.15 mg	Ethinyl estradiol, 0.03 mg
Desogen	Desogenestrel, 0.15 mg	Estinyl estradiol, 0.03 mg
Ortho-cept	Desogenestrel, 0.15 mg	Estinyl estradiol, 0.03 mg
Loestrin 1/20	Norethindrone acetate, 1 mg	Ethinyl estradiol, 0.02 mg
Loestrin 1.5/30	Norethindrone acetate, 1.5 mg	Ethinyl estradiol, 0.03 mg
Lo/Ovral	Norgestrel, 0.3 mg	Ethinyl estradiol, 0.03 mg
Nordette	Norethindrone acetate, 1.5 mg	Ethinyl estradiol, 0.03 mg
Ortho-Cycline	Norgestimate, 0.25 mg	Ethinyl estradiol, 0.035 mg
Ovcon-35	Norethindrone, 0.4 mg	Ethinyl estradiol, 0.035 mg
Brevicon	Norethindrone, 0.5 mg	Ethinyl estradiol, 0.035 mg
Modicon	Norethindrone, 0.5 mg	Ethinyl estradiol, 0.035 mg
Genora 0.5/35	Norethindrone, 0.5 mg	Ethinyl estradiol, 0.035 mg
Nelova 0.5/35E	Norethindrone, 0.5 mg	Ethinyl estradiol, 0.035 mg
Demulen 1/35	Ethynodiol diacetate, 1 mg	Ethinyl estradiol, 0.035 mg
Ortho-Novum 1/35	Norethindrone, 1 mg	Ethinyl estradiol, 0.035 mg
Genora 1/35	Norethindrone, 1 mg	Ethinyl estradiol, 0.035 mg
Nelova 1/35E	Norethindrone, 1 mg	Ethinyl estradiol, 0.035 mg
Norinyl 1 + 35	Norethindrone, 1 mg	Ethinyl estradiol, 0.035 mg
Demulen 1/50	Ethynodiol diacetate, 1 mg	Ethinyl estradiol, 0.05 mg
Ovral	Norgestrel, 0.5 mg	Ethinyl estradiol, 0.05 mg
Norinyl 1 + 50	Norethindrone, 1 mg	Mestranol, 0.05 mg
Ortho-Novum 1/50	Norethindrone, 1 mg	Mestranol, 0.05 mg
Genora 1/50	Norethindrone, 1 mg	Mestranol, 0.05 mg
Nelova 1/50M	Norethindrone, 1 mg	Mestranol, 0.05 mg
Ovcon-50	Norethindrone, 1 mg	Ethinyl estradiol, 0.05 mg
Enovid 5 mg	Norethynodrel, 5 mg	Mestranol, 0.075 mg

2. Combination—Biphasic

Products are available in 21- or 28-day dispensers and refills. They are taken on the same schedule of 21 days plus 7 days of no (or inert) tablets as with the foregoing monophasics. Doses of active tablets are shown below.

Brand	*Progestin and Estrogen*
Ortho-Novum 10/11	10 days: Norethindrone, 0.5 mg, and ethinyl estradiol, 0.035 mg then 11 days: Norethindrone, 1 mg, and ethinyl estradiol, 0.035 mg
Nelova 10/11	10 days: Norethindrone, 0.5 mg, and ethinyl estradiol, 0.035 mg then 11 days: Norethindrone, 1 mg, and ethinyl estradiol, 0.035 mg
Jenest-28	7 days: Norethindrone, 0.5 mg, and ethinyl estradiol, 0.035 mg then 14 days: Norethindrone, 1 mg, and ethinyl estradiol, 0.035 mg

3. Combination—Triphasic

Products are available in 21- or 28-day dispensers and refills. They are taken on the same schedule of 21 days plus 7 days of no (or inert) tablets as with the foregoing monophasics. Doses of active tablets are shown below.

Brand	*Progestin and Estrogen*
Ortho 7/7/7	first 7 days: Norethindrone, 0.5 mg, and ethinyl estradiol, 0.035 mg next 7 days: Norethindrone, 0.75 mg, and ethinyl estradiol, 0.035 mg next 7 days: Norethindrone, 1 mg, and ethinyl estradiol, 0.035 mg
Ortho-Tricycline	first 7 days: Norgestimate, 0.18 mg, and ethinyl estradiol, 0.035 mg next 7 days: Norgestimate, 0.215 mg, and ethinyl estradiol, 0.035 mg next 7 days: Norgestimate, 0.25 mg, and ethinyl estradiol, 0.035 mg
Tri-Norinyl	first 7 days: Norethindrone, 0.5 mg, and ethinyl estradiol, 0.035 mg next 9 days: Norethindrone, 1 mg, and ethinyl estradiol, 0.035 mg next 5 days: Norethindrone, 0.5 mg, and ethinyl estradiol, 0.035 mg
Triphasil	first 6 days: Levonorgestrel, 0.05 mg, and ethinyl estradiol, 0.03 mg next 5 days: Levonorgestrel, 0.075 mg, and ethinyl estradiol, 0.04 mg next 10 days: Levonorgestrel, 0.125 mg, and ethinyl estradiol, 0.03 mg
Tri-Levlen	Same as Triphasil

(Continued)

TABLE 23-3 *Continued*

4. Progestin Only

An active tablet is taken each day of the year.

Brand	*Progestin*	*Dose*	*Dosage Cycle*
Micronor	Norethindrone	0.35 mg	Continuous daily—28 days
Nor-Q.D.	Norethindrone	0.35 mg	Continuous daily—42 days
Ovrette	Norgestrel	0.075 mg	Continuous daily—28 days

5. Injectable Depot Hormonal Contraceptives

Brand	*Progestin*	*Dosage Cycle*
Depo-Provera	Medroxyprogesterone acetate	150 mg/mo 150 mg Q 3 mo

6. Once-a-Month Oral Combination Contraceptive (Not Available in United States)

Progestin	*Estrogen*	*Dosage Cycle*
Norethindrone acetate 3-Cyclopentyl enol ether (quinestrol)	Ethinyl estradiol 3-Cyclopentyl ether (quingestanol)	1 tablet/mo

7. Hormone-Releasing Implants and IUDs

Brand	*Drug*	*Dosage Cycle*
Progestasert	Progesterone-releasing IUD	38 mg dose in IUD lasts 1 yr
Norplant	6 Silastic capsules with 36 mg levonorgestrel all 6 capsules are inserted subdermal in the middle upper arm	Contraceptive efficacy lasts for 5 years if the implants are not removed
(in trials)	Intravaginal Silastic rings containing progestins	Under study by Upjohn

8. Progesterone Antagonists (In Clinical Trials)

Brand	*Drug*	*Dosage Cycle*
Mifepristone	RU 486	50 mg once a month at midcycle

Biphasic and Triphasic (Variable) Combinations. As illustrated in Fig. 23-11, in the natural menstrual cycle, progesterone plasma concentrations peak late in the cycle. The higher estrogen/progesterone ratio early in the cycle is believed to assist in development of the endometrium. The higher progesterone concentration later contributes to proliferation of the endometrium and a resultant "normal" volume of menstrual flow. The biphasic and triphasic combinations attempt to mimic this variation in estrogen/progestin levels, and thereby to reduce the incidence of spotting associated with low-dose monophasic combinations. With proper selection of patients, the goal has been achieved; but in other patients, the incidence of spotting has not appreciably decreased.

How Safe?

The safety of the "pill" (typically meaning fixed-dose combinations) has been one of the most intensively discussed subjects in the press. Earlier studies, based largely on the earlier products that contained high doses of estrogen, showed an alarming incidence of thromboembolic disease. The results of these findings have been that (1) the sequential contraceptive products with their high doses of estrogen have been removed from American markets; (2) many combination contraceptives containing less than 0.050 mg estrogen per dose have recently been marketed (see Table 23-3); (3) progestin only or minipill products have appeared (see Table 23-3); and (4) a few groups of women have been identified who should definitely not take oral contraceptives (e.g., women who are moderate to heavy smokers, women with a history of breast cancer in their immediate family).

Several studies have shown that the risk of thromboembolism with preparations containing less than 0.05 mg estrogen per dose is less than with those containing 0.05 mg or more. As a result, nearly all oral contraceptives with more than 0.05 mg of estrogen have been removed from the market.

The actual incidence of "pill-induced" cardiovascular death for nonsmoking young women is quite small. In the United States since adoption of the "pill" in the 1960s, deaths of reproductive-aged women from cardiovascular dis-

ease have declined much more steeply than those for men of the same-aged group.[129] Indeed, the risk of death from myocardial infarction for men (who obviously do not take the estrogen/progestin contraceptives) is significantly higher.

The risk of death from myocardial infarction for "pill users" aged 30 to 39 is about 1.8:100,000 nonsmokers and 13.0:100,000 for heavy smokers (15 or more cigarettes per day).

Progestin Only (Minipill). The estrogen component of sequential and combination oral contraceptive agents has been related to some side effects, with thromboembolism being a concern. One solution to this problem has been to develop new products with decreased estrogen content. In the "minipill," there is no estrogen at all.

Although higher doses of progestin are known to suppress ovulation, minipill doses of progestin are not sufficient to suppress ovulation in all women. Some studies have indicated that an increase in the viscosity of the cervical mucus (or sperm barrier) could account for much of the contraceptive effect. Low doses of progestin have also been found to increase the rate of ovum transport and to disrupt implantation. There is a good probability that most, or all, of these factors contribute to the overall contraceptive effect of the minipill. The incidence of pregnancy with the minipill is slightly higher than with combination products.

Depo-Provera. Medroxyprogesterone acetate IM injection (Depo-Provera) provides contraception for 3 months after a single 150-mg IM dose. This contraceptive method is used by over 15 million women worldwide. Most women experience some irregular bleeding or spotting, and often experience small weight gain. Fertility returns for most women within the first 12 months after discontinuance of Depo-Provera. Contraception typically continues for a few weeks beyond the 3-month term, giving patients a short grace period if the subsequent IM dose is delayed.

Once-a-Month and Once-a-Week Oral Contraceptives. The advantages of a once-a-month oral contraceptive are obvious, and good progress has been made in the development of such drugs. However, development of a long-acting, fertility-regulating agent is a difficult, costly, and time-consuming process. A small oral dose of ethinyl estradiol 3-cyclopentyl ether (quinestrol) and norethindrone acetate 3-cyclopentyl enol ether (quingestanol) is effective in humans when given once a month. However, full contraceptive protection is not achieved until the second month's dose has been taken. After that time, contraceptive efficiency is reported to be excellent.

Progesterone IUD. The low progestin doses of the minipill seem to have a direct effect on the uterus and associated reproductive tract. Therefore, it would seem possible to lower the progestin dose even more if the drug was released in the reproductive tract itself.

In 1964, Folkman and Long[130] showed that chemicals can be released by diffusion through the walls of a silicone rubber capsule at a constant rate. A particularly attractive silicone rubber was found to be Silastic (Dow), which was nontoxic and apparently nonallergenic. During initial studies, capsules made of Silastic and containing estrogens or progestins, or both, were implanted subcutaneously in the forearms of women patients. The result has been the levonorgestrel implant (Norplant), described in the next section.

Similar studies were conducted with uterine-implanted Silastic capsules containing low doses of progestins. It was envisioned that progestin-containing intrauterine devices (IUDs) would have some particular advantages over other IUDs. First, the progestin should decrease uterine contractility (thus decreasing the number of IUDs ejected). Second, it should decrease the vaginal bleeding sometimes associated with IUDs. Additional studies are in progress to evaluate these predictions.

The Progestasert IUD (Progesterone Intrauterine Contraceptive System, USP) has 38 mg of microcrystalline progesterone dispersed in silicone oil. The dispersion is contained in a flexible polymer in the approximate shape of a T. The polymer acts as a membrane to permit 65 μg of progesterone to be slowly released into the uterus each day for one year. Contrary to prediction, the progesterone-containing IUD has had some of the therapeutic problems of other IUDs, including a relatively low patient continuation rate, some septic abortions, and some perforations of uterus and cervix. Clinical studies have produced the following data on Progestasert, expressed as events per 100 women through 12 months of use.

	Parous	Nulliparous
Pregnancy	1.9	2.5
Expulsion	3.1	7.5
Medical removals	12.3	16.4
Continuation rate	79.1	70.9

Norplant. As described in the previous section, Silastic copolymer has been found to be an effective carrier for progestin-releasing products. Levonorgestrel in six flexible closed Silastic capsules (Norplant), implanted in the forearm, provides contraception for up to 5 years. The capsules are each 34 mm long, and 2.4 mm in diameter. Norplant is implanted within the first 7 days of the patient's menses, in part to make sure she is not pregnant. Contraceptive efficacy is very high. Most women experience some changes in menstrual bleeding, ranging from irregular cycles to prolonged bleeding or amenorrhea; weight gain is also experienced by some patients. The old capsules must be removed (a minor, but sometimes painful surgical procedure) before new ones are inserted. In mid-1995 several popular news magazines reported that a significant number of patients were deciding not to continue Norplant and lawyers were quickly filing lawsuits (for example, "The Norplant Backlash: Is It Dangerous, or Are Lawyers Exploiting It?" *Newsweek*, November 27, 1995). The product continues to be used by many

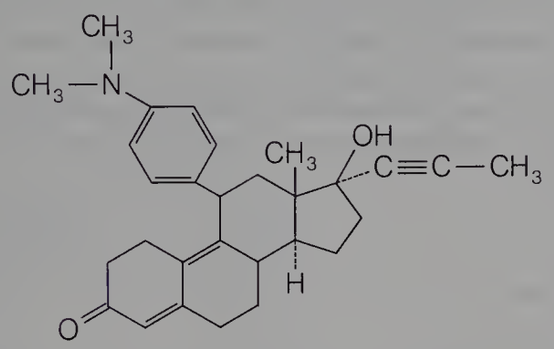
women, offering the convenience of long-term contraception with low risk.

Biodegradable Sustained-Release Systems. A very interesting and exciting approach to fertility control uses biodegradable polymers and microparticles to release the estrogen or progestin. The microparticles can be injected through regular needles. Release of the active drug occurs by erosion, diffusion, and cleavage of covalent bonds between the drug and polymer. Polymer matrices that have been investigated for these purposes include caprolactone, glutamic acid, lactic acid, and glycolic acid polymers.

OTHER METHODS OF CHEMICAL CONTRACEPTION

RU 486

A completely new drug design approach to oral contraceptives for women, progesterone antagonists, has received considerable attention. The first to be studied in clinical trials is RU 486 {mifepristone; 11β-[*p*-(dimethylamino)phenyl]-17β-hydroxy-17-(1-propynyl)estra-4,9-dien-3-one}.



RU 486

As summarized in *Science* in 1989,[131] and *Scientific American* in 1990,[132]

> For a drug not yet a decade old, . . . RU 486 is causing quite a ruckus. . . . There is one thing no one argues about: RU 486 taken in conjunction with prostaglandins is an extremely effective method of terminating pregnancy within the first 9 weeks of gestation. And that could change the context of the debate over abortion and birth control. . . . It is not surprising, then, that RU 486 is viewed with alarm by anti-abortion groups.

France in mid-1990 was the only country in which RU 486 was widely available. Typically a prostaglandin E_1 or E_2 (PGE_1 or PGE_2) analogue has been used with the RU 486.

RU 486 binds strongly with both the glucocorticoid receptor and with the progesterone receptor. The story behind its design and development is fascinating reading.

When progesterone binds to its receptor, heat shock protein is released from the receptor and thereby opens the progesterone-receptor complex to DNA binding. However, when RU 486 binds to the progesterone receptor, heat shock protein is not released; therefore, no transcription of the DNA can occur. Alternatively, RU 486 may induce a conformational change in the progesterone receptor so that it does not fit its DNA site.

As shown in Fig. 23-11, progesterone levels peak late in the menstrual cycle. Progesterone performs a variety of roles in maintaining secretory endometrium, and in inhibiting contractility of the uterus. Blocking these actions of progesterone results in breakdown of the endometrium, and detachment of the fertilized ovum (or embryo).

In addition to birth control applications, a variety of other therapeutic uses are also possible for RU 486, including treatment of progesterone sensitive cancers, and for Cushing's syndrome (overproduction of glucocorticoids—discussed later in this chapter).

Postcoital Contraceptives

A variety of compounds have been found to be effective as postcoital contraceptives in animal studies. They range from steroid estrogens, such as DES, to compounds that bear little or no structural similarity to steroid hormones. Most act by an alteration of the mechanisms of fertilized ovum transport and implantation in the uterus.

Although the antiprogestin RU 486 (see Table 23-3) may be approved for clinical use as a postcoital contraceptive, the most widely used agent today is the combination of norgestrel and ethinyl estradiol (Ovral). Two Ovral tablets are taken within 72 hours of unprotected intercourse, followed by another two tablets 12 hours later. Some patients experience nausea, which is usually mild. The treatment is successful with about 90% of patients in preventing pregnancy. It must be emphasized, however, that this treatment is intended only for use in short-term emergency situations.

Diethylstilbestrol has also been used as a postcoital contraceptive. However, the high estrogen doses (25 mg twice daily for 5 consecutive days) are a significant concern—both for the patient and (if pregnancy results in spite of the treatment) for the unborn fetus (see section on estrogen side effects). As with Ovral, treatment must begin no later than 72 hours after unprotected intercourse, and preferably within 24 hours.

Abortifacients

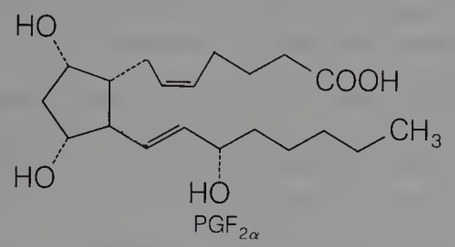
History records many different compounds that have been tried as abortifacients—everything from plant extracts to rusty nail water. Many chemicals have been very effective with animals, including metabolites, cytotoxic agents, 5-hydroxytryptamine, monoamine oxidase inhibitors, androgens, and others. Usually, these same compounds also have been

found to be toxic or mutagenic or to cause severe hemorrhaging along with the abortion.

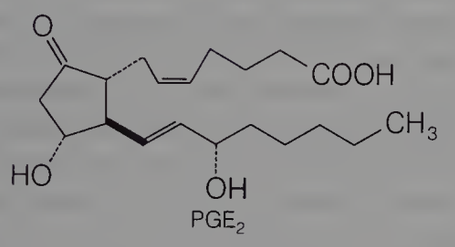
However, two prostaglandins have been approved by the FDA to induce second trimester abortions:

$PGF_{2\alpha}$—Carboprost (Hemabate)
PGE_2—Dinoprostone (Prostin E_2)

$PGF_{2\alpha}$ and PGE_2 concentrations significantly increase in amniotic fluid before normal labor and childbirth.



$PGF_{2\alpha}$



PGE_2

Good surgical support is essential with $PGF_{2\alpha}$ and PGE_2 because some clinicians report a high incidence of incomplete abortions that require dilatation and curettage. Furthermore, in those women in whom the placenta is retained, severe hemorrhage requiring transfusion may result. The drug is approved only for intra-amniotic injection in the second trimester. Suction is a common (and probably safer) method of clinical abortion during the first trimester, and saline-induced abortions are sometimes used during the second trimester. However, the saline method has been associated with disseminated intravascular coagulation in the patient, a problem not reported with $PGF_{2\alpha}$ or PGE_2. (It should be noted that the β-adrenergic agonist ritodrine is used for the opposite effect—inhibiting preterm labor contractions.)

Spermicides

"As early as the 19th Century B.C., the Egyptians were mixing honey, natron [sodium carbonate], and crocodile dung to form a vaginal contraceptive paste. . . . During the middle ages, rock salt and alum were frequently used as vaginal contraceptives."[133] The history of spermicidal agents is indeed a long one. Modern spermicidal agents, or "vaginal contraceptives," fall into three categories: nonionic surfactants, bactericides, and acids.

The FDA Advisory Panel on Nonprescription Contraceptive Products[134] found only three spermicides to be generally safe and effective: nonoxynol-9, octoxynol-9, and menfegol (Table 23-4). Other agents, including PEG 600 monolaurate and laureth-10S, were not classified because of insufficient data at the time the study was completed. Phenylmercuric acetate and phenylmercuric nitrate were found to be generally not safe and effective.

TABLE 23-4

EXAMPLES OF COMMONLY USED SPERMICIDES

(In jellies, creams, suppositories, foaming tablets, aerosol foams, and soluble films — not all are available in the United States)

1. SAFE AND EFFECTIVE

Surface-active agents (also somewhat bactericidal)

a. $CH_3-(CH_2)_7-CH_2-C_6H_4-(OCH_2CH_2)_9OH$

Nonoxynol-9, USP
(nonylphenoxypolyethoxyethanol)
(in Delfen, Immolin, Emko, Because, Encare Oval)

b. $CH_3C(CH_3)_2CH_2C(CH_3)_2C_6H_4O(CH_2CH_2O)_9H$

Octoxynol-9

2. NOT SAFE AND / OR EFFECTIVE

Bactericides

a. $C_6H_5HgOCCH_3$ (O)
Phenylmercuric acetate
(Lorophyn)

b. Others: benzethonium chloride, methylbenzethonium chloride, phenylmercuric borate

Acids

a. Boric acid
b. Others: tartaric acid, phenols, etc.

A 1987 study by Louik and co-workers[135] has confirmed several earlier reports showing that there is no increase in the overall frequency of birth defects in association with the use of spermicides. The study included use of spermicides during the first trimester of pregnancy.

In addition to the inherent spermicidal properties of the active agent, the efficiency of spermicidal products depends on many more factors. They must be inserted high into the vagina (usually with an applicator). They must (perhaps inconveniently) be used just before intercourse and reused if intercourse is to be repeated.

Furthermore, the formulation of these contraceptive products becomes almost as important as the active spermicidal agent itself. The product's formulation must permit diffusion into the cervix because some spermatozoa may be released directly into it. The product must also have a reasonable

stability in the vagina so that enough active spermicide remains after intercourse. Finally, the ideal vaginal contraceptive must be nontoxic and nonirritating to both partners.

The primary action of the surface-active agents is to reduce the surface tension at the sperm cell surface and cause a lethal osmotic imbalance. They may also inhibit fructose metabolism. The bactericides also may alter the surface properties of the sperm cells, and after penetrating the cell membrane they can disrupt metabolic processes. The acidic agents cause direct damage to the surface of the sperm cell membranes by denaturation of cell protein material. Examples of common spermicidal agents are shown in Table 23-4.

There are five primary types of vaginal contraceptive products containing spermicides: (1) creams, jellies, and pastes that are squeezed from a tube or applicator; (2) vaginal suppositories; (3) vaginal sponges; (4) foams (from aerosol pressurized containers or tablets); and (5) soluble films. Often, the vaginal contraceptives are used in combination with another contraceptive method (e.g., diaphragm, or "rhythm" method, or condom). The soluble films, primarily used in Europe, are transparent, water-soluble films that are impregnated with a spermicidal agent and then inserted into the vagina before intercourse.

Future Prospects

Unfortunately, little progress has been achieved in the development of chemical contraceptives for men.[136–138] Spermatogenesis can be greatly decreased with progestins, high doses of testosterone, or GnRH agonists (discussed earlier in this chapter). Predictable side effects (e.g., increased risk for heart disease with testosterone) and high cost (in the case of GnRH) have thus far precluded these hormone products from being commercially viable. Studies with these drugs nevertheless continue, along with basic research on the biochemistry and pathophysiology of spermatogenesis.

In a multicenter study in seven countries, testosterone enantate injection was found to cause azoospermia in 65% of the men participating in the study.[138] The number of pregnancies per 100 person years was 0.8, comparable with the more effective methods of contraception for women (see Table 23-5). The remaining men did not achieve azoospermia, and thus did not complete the study.

The lack of progress reflects various factors—an earlier concern that contraceptive products would not be used by men, the fact that spermatogenesis is not as cyclic as the menstrual cycle, and a lack of basic information about the biochemistry of the male reproductive system:

> We do not know how to interdict the production of sperm safely and reversibly. . . . We also have no existing products (as models). The result of this dilemma is that we do not know the adequacy of our experimental models to humans. We do not even know, for example, the exact requirements of androgens for maintenance of normal libido. The lack of existing male contraceptive drugs and knowledge about the male reproductive system should not cause pessimism. The same analogous situation existed with female oral contraceptives prior to the time of Pincus, Garcia, Chang, and Rock [in the early 1950s]. . . . [Bartke[139]]
>
> The male reproductive system seems less amenable to interference than does that of the female. . . . First, because the spermatogenic cycle is 74 days, months pass before a drug is effective. Second, because reproductive hormones are generally in a steady state in men, interruption of cyclicity is not an effective contraceptive approach. Third, because the testes are protected by a blood–testis barrier, many agents cannot reach the site of spermatogenesis. . . . [Alexander[140]]
>
> The chief reason. . . is the [earlier] presumption that men would not use a chemical contraceptive through fears of psychological or clinical effects upon libido and masculinity. . . . I have never agreed with this forecast. [Bennett[141]]

Ideally, one would like to have a drug that would inhibit spermatogenesis (without being mutagenic), would not decrease libido, and would not have any other effect on testicular function (e.g., hormone function). Alternatively, drugs that would affect only the spermatozoa after formation would be of great interest (e.g., drugs that could block the fertilizing ability of sperm stored in the epididymis).

Examples of drugs that have some of these properties in animals (and for a few in man) are shown in Fig. 23-22. The potential of copper implants has also received recent attention.[142]

Gossypol and Other Folklore Herbal Contraceptives

One compound that has received considerable attention as a male contraceptive is gossypol. A variety of other compounds have also been used as contraceptives in folk medicine.[143–145]

TABLE 23-5

FAILURE RATE OF CONTRACEPTIVE METHODS

Method	*Pregnancies/100 Woman Years*
Abortion	0
Tubal ligation	0.4
Vasectomy	0.15
Norplant	0.09
Depo-Provera injection	0.3
Combination oral contraceptive	0.1–3
Progestin only minipill (oral)	0.5–3
Progestasert IUD	1.5–2
Copper T IUD	0.6–0.8
Diaphragm	6–18
Condom	5–21
Withdrawal	4–19
Spermicides	6–21
Periodic abstinence (rhythm)	9–20
No contraceptive method	85

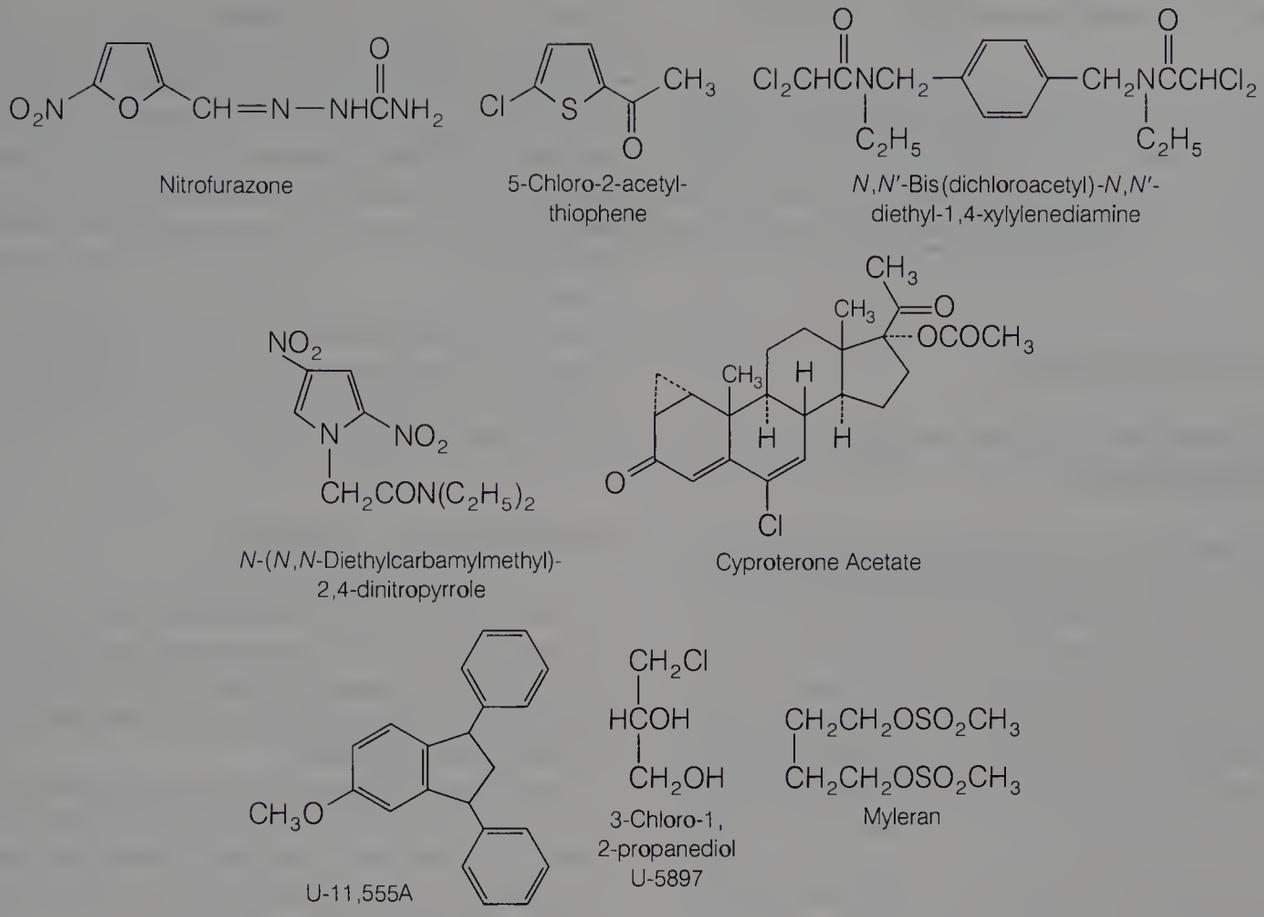
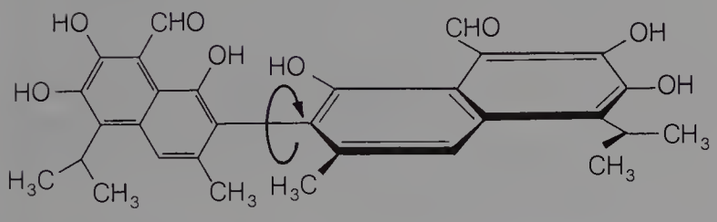


FIG. 23-22. *Examples of chemical contraceptives for males. (Most have been tested only in animals. Some are quite toxic.)*



Gossypol, 1,1′,6,6′,7,7′-hexahydroxy-3,3′-dimethyl-5,5′-bis-(1-methylethyl)-[2,2′-binaphthalene]-8,8′-dicarboxaldehyde, can exist in two different optical isomers. The optical isomers arise because of restricted rotation around the single bond connecting the two naphthalene ring systems. Most research has been done on the racemic mixture, but it appears that the (+) and (−) enantiomers may have significantly different activities. Gossypol is naturally found in cottonseed oil, used for cooking throughout the People's Republic of China.

Gossypol has been found to be a very effective, but toxic, contraceptive agent. Claims of effectiveness have been as high as 99%, with gossypol appearing to act on the spermatid stage of spermatogenesis. Toxicity is also a problem, particularly hypokalemic-induced paralysis. Dietary selenium may significantly enhance gossypol toxicity. Waller and co-authors[145] conclude that:

> Gossypol in its present form is unsuitable and will not be approved by Western drug regulatory agencies as a male contraceptive agent. The therapeutic index . . . is too small. . . . The poor distribution of gossypol to the testes, and high concentrations in organs such as the liver lead to significant exposures of tissues not involved [in the contraceptive action] . . . [However,] gossypol may prove to be a very important compound even if it is never widely used as an antifertility agent.[145]

As a "lead compound" for studying the male reproductive system and for stimulating design of new and less toxic drugs, gossypol has been enormously important. Other herbal contraceptives[144] are also being investigated. Unfortunately, however, recent studies have shown that although gossypol causes a dramatic decrease in spermatogenesis, its actions are often not reversible.[138]

RELATIVE CONTRACEPTIVE EFFECTIVENESS OF VARIOUS METHODS

Some caution is required in interpreting data on the effectiveness of contraceptive methods. Even the "best" method can lead to pregnancy if not used consistently and correctly. Even the generally least effective method is better than no contraceptive at all. Table 23-5 presents some data on numbers of pregnancies per method.

ANDROGENS AND ANABOLIC AGENTS

The commonly used androgenic and anabolic agents are shown in Fig. 23-23. Several excellent reviews on androgens and anabolic agents have been published.[146] The text *Endocrine Physiology* by C. R. Martin[69] is highly recommended for a detailed review of androgen release, biosynthesis, and regulation.

STRUCTURAL CLASSES

Natural Hormones: Testosterone and Dihydrotestosterone

Although produced in small concentrations in females, testosterone and its potent metabolite dihydrotestosterone (Fig. 23-24) are produced in much greater amounts in males. Testosterone has two important activities: androgenic activity (or male sex characteristic-promoting) and anabolic activity (or muscle-building). Compounds that have these two activities are generally called androgens and anabolic agents. Because it would be very useful to have drugs that were anabolic (e.g., to aid the recovery of severely debilitated patients) but not androgenic, many compounds with increased anabolic activity have been synthesized. However, significant levels of androgenic activity have limited the therapeutic uses of all these compounds.

Semisynthetic Analogues

Hundreds of different androgens and anabolic agents have been synthesized and studied.[146] One might suppose that the structure–activity relationships of these drugs have been well delineated. However, the structural requirements for selective anabolic activity are still unclear, and there is even uncertainty about the relationship of structure to androgenic activity. An examination of the compounds in Fig. 23-24 shows that greater planarity and electron density in ring A seem to favor anabolic activity. The discussion of Counsell and Brueggemier[146] is recommended for further reading.

Because bacterial and hepatic oxidation of the 17β-hydroxyl to the 17-one is the primary route of metabolic inactivation, 17α-alkyl groups have been added. Even though 17α-methyltestosterone is only about half as active as testosterone, it can be taken orally. 17α-Ethyltestosterone has greatly reduced activity, as shown in Table 23-6. A disadvantage of the 17α-alkyl testosterones is that hepatic disturbances (and occasionally jaundice) may occur, particularly in the high doses typically used by athletes (see next section).

Table 23-6 illustrates other structure–activity effects of the androgen; for example, the greatly decreased activity of the 17α-ol isomer of testosterone. Many hypotheses have been made in an attempt to summarize the structure–activity relationships of all the known androgens.

Many drugs are available that have improved anabolic/androgenic activity ratios, but none is free of androgenic activity. This has greatly limited their therapeutic utility. Examples of drugs that have marked improvements in anabolic activity are illustrated in Fig. 23-25, but these have not been used clinically because of hepatic toxicity or other side effects. For example, 19-nor steroids are quite anabolic, but their significant progestational activity has generally precluded their use.

As with other compounds we have discussed, hydroxyl groups in the testosterones are often converted to the corresponding esters to prolong activity or to provide some protection from oxidation.

BIOSYNTHETIC SOURCES

As shown in Fig. 23-6, testosterone can be synthesized through progesterone and androstenedione. About 7 mg/day is synthesized by young human adult males. Labeling experiments have also shown that it can be biosynthesized from androst-5-ene-3β,17β-diol.

Testosterone is primarily produced by the interstitial cells of the testes, synthesized largely from cholesterol made in sertoli cells. The ovaries and adrenal cortex also synthesize androstenedione and 5-androsten-17-one-3β-ol (dehydroepiandrosterone), which can be rapidly converted to testosterone in many tissues.

Testosterone levels in the plasma of men are 5 to 100 times higher than the levels in the plasma of women.

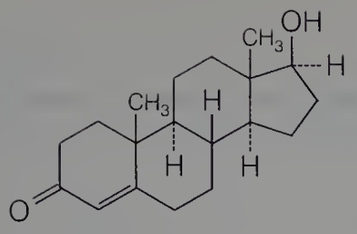
Testosterone is produced in the testes in response to FSH and LH [interstitial cell-stimulating hormone (ICSH)] release by the anterior pituitary, as shown in Fig. 23-10. Testosterone and dihydrotestosterone inhibit the production of LH and FSH by a feedback-inhibition process. This is quite similar to the feedback inhibition by estrogens and progestins in FSH and LH production.

METABOLISM

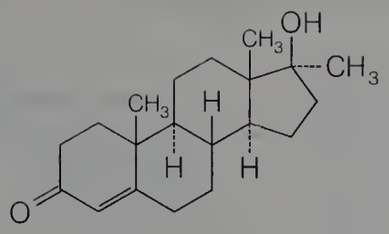
Testosterone is rapidly converted to 5α-dihydrotestosterone in many tissues, and 5α-dihydrotestosterone is also secreted by the testes. In fact, 5α-dihydrotestosterone is the active androgen in many tissues (e.g., in the prostate). The primary route for metabolic inactivation of testosterone and dihydrotestosterone is oxidation to the 17-one. The 3-one group is also reduced to the 3α- and 3β-ols. The metabolites are shown in Fig. 23-24. Others have also been detected.[146]

INHIBITION OF 5α-REDUCTASE

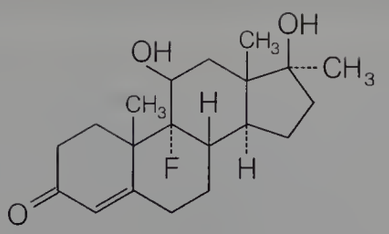
5α-Dihydrotestosterone is important for maintaining prostate function in men. Blocking this enzyme is an important approach for controlling androgen action. The 1994 review



Testosterone
(1 : 1)

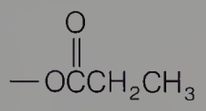


17α-Methyltestosterone
(1 : 1 but 1/2 as potent
as testosterone)

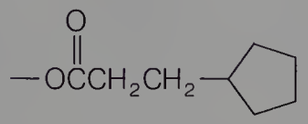


Fluoxymesterone
(1 : 1 to 2 : 1 and 5 to 10 times
more potent than testosterone)

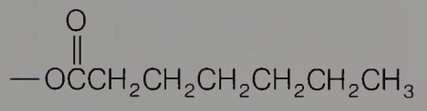
17β-Esters Commercially Available:



Testosterone Propionate

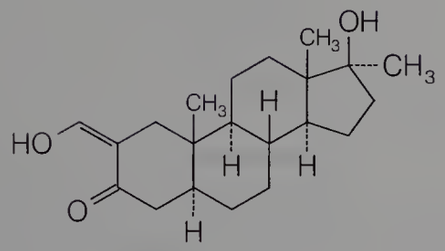


Testosterone Cyclopentylpropionate
(Cypionate)

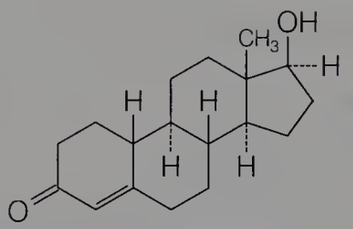


Testosterone Enanthate

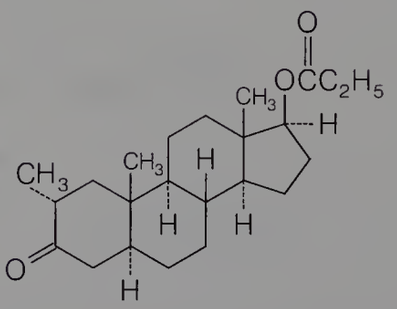
All are IM — some available as implantation pellets



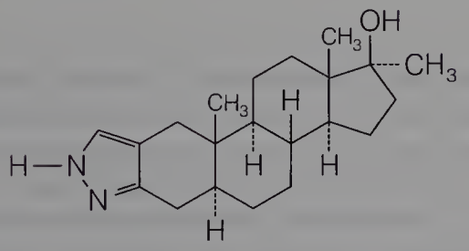
Oxymetholone
(2.5 : 1; 6 : 1 SC)



Nandrolone
(2.5 : 1 to 4 : 1)

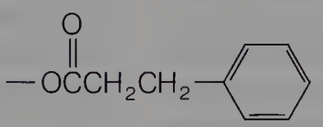


Dromostanolone
(Propionate, 3 : 1 to 4 : 1)

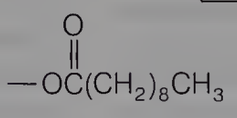


Stanozolol
(3 : 1 to 6 : 1)

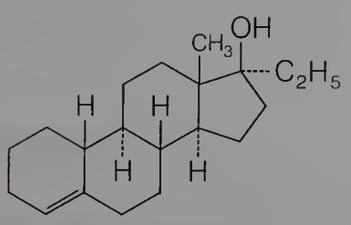
17β-Esters Commercially Available:



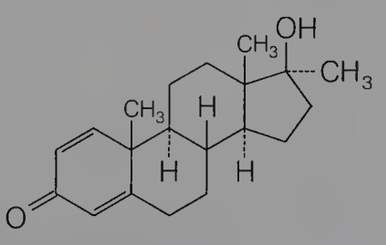
Nandrolone Phenpropionate



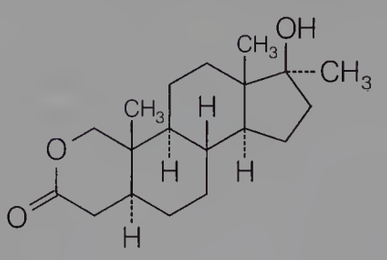
Nandrolone Decanoate



Ethylestrenol
(3 : 1)



Methandrostenolone
(1 : 1)



Oxandrolone

FIG. 23-23. *Androgens and anabolic agents (anabolic/androgenic ratio).*

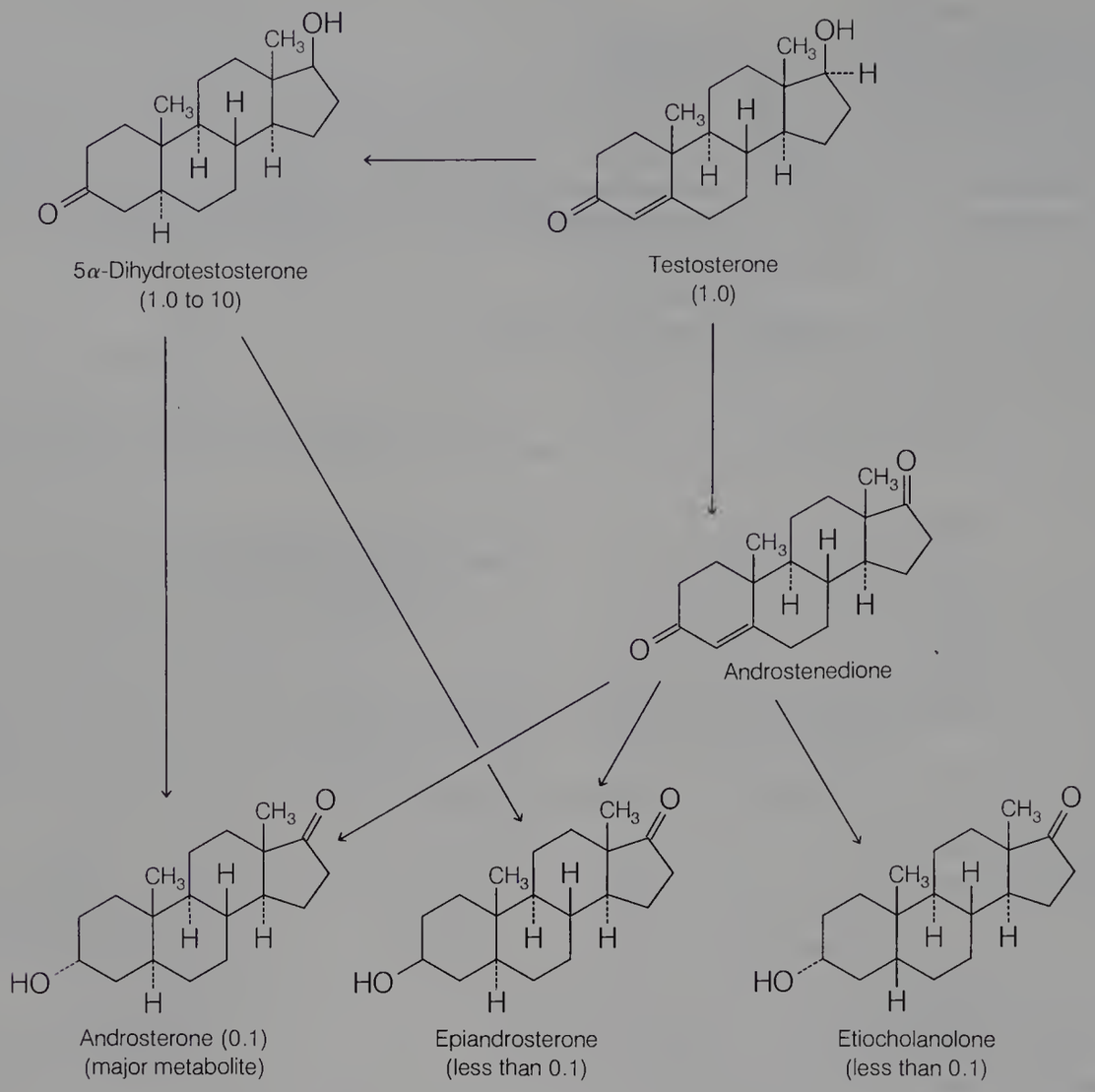


FIG. 23-24. *Metabolism of testosterone and 5α-dihydrotestosterone (relative androgenic activity). Only primary metabolites are shown. (For a full discussion, the review by Martin[69] is recommended.)*

by Rasmussen and Toney[147] is recommended for a detailed overview.

This testosterone metabolite also plays a major role in the pathogenesis of benign prostatic hyperplasia (BPH). Finasteride, (5α,17β)-*N*-(1,1-dimethylethyl)-3-oxo-4-azaandrost-1-ene-17-carboxamide (Proscar), is a potent competitive inhibitor of 5α-reductase,[148] and has been extensively promoted for treatment of BPH. However, it often takes a minimum of 6 months of treatment with finasteride to determine if a patient will respond. In addition, the drug only provides marginal benefit. The Agency for Health Care Policy and Research found that the usual systematic improvement with BHP is only 40% in one year, compared with 51% for patients taking α-adrenergic blockers and 85% for patients who had transurethral resection of the prostate (TURP), and 40% with placebo.[149]

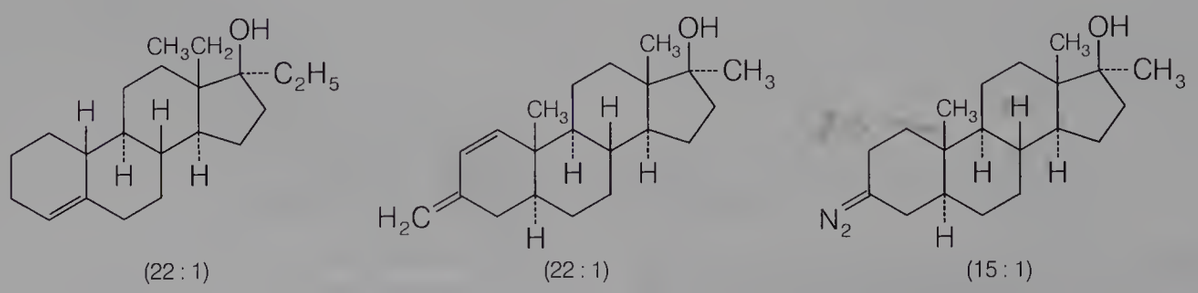
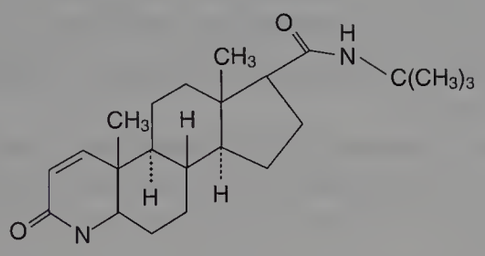


FIG. 23-25. *Experimental compounds with improved anabolic activity (anabolic activity/androgenic activity ratio).*

TABLE 23-6

ANDROGENIC ACTIVITIES OF SOME ANDROGENS[126]

Compound	Micrograms Equivalent to an International Unit
Testosterone (17β-ol)	15
Epitestosterone (17α-ol)	400
17α-Methyltestosterone	25–30
17α-Ethyltestosterone	70–100
17α-Methylandrostane-3α, 17β-diol	35
17α-Methylandrostane-3-one-17β-ol	15
Androsterone	100
Epiandrosterone	700
Androstane-3α, 17β-diol	20–25
Androstane-3α, 17α-diol	350
Androstane-3β, 17β-diol	500
Androstane-17β-ol-3-one	20
Androstane-17α-ol-3-one	300
Δ^5-Androstene-3α, 17β-diol	35
Δ^5-Androstene-3β, 17β-diol	500
Androstanedione-3, 17	120–130
Δ^4-Androstenedione	120



Finasteride

BIOLOGIC ACTIVITIES

Testosterone and dihydrotestosterone cause pronounced masculinizing effects, even in the male fetus. They induce the development of the prostate, penis, and related sexual tissues.

At puberty, the secretion of testosterone by the testes increases greatly, leading to an increase in facial and body hair, a deepening of the voice, an increase in protein anabolic activity and muscle mass, a rapid growth of long bones, and a loss of some subcutaneous fat. Spermatogenesis begins, and the prostate and seminal vesicles increase in activity. Sexual organs increase in size. The skin becomes thicker and sebaceous glands increase in number, leading to acne in many young people. The androgens also play important roles in male psychology and behavior.

THERAPEUTIC USES

The primary use of androgens and anabolic agents is as androgen replacement therapy in men, either at maturity or in adolescence. The cause of testosterone deficiency may be either hypogonadism or hypopituitarism.

The use of the androgens and anabolic agents for their anabolic activity, or for uses other than androgen replacement, has been very limited because of their masculinizing actions. This has greatly limited their use in women and children. Although anabolic activity is often needed clinically, none of the products presently available has been found to be free of significant androgenic side effects.

The masculinizing (androgenic) side effects in females include hirsutism, acne, deepening of the voice, clitoral enlargement, and depression of the menstrual cycle. Furthermore, the androgens and anabolic agents generally alter serum lipid levels and increase the probability of atherosclerosis, characteristically a disease of men and postmenopausal women.

Androgens in low doses are sometimes used in the treatment of dysmenorrhea and postpartum breast enlargement. However, the masculinizing effects of the androgens and anabolic agents, even in small doses, preclude their use in most circumstances. Secondary treatment of advanced or metastatic breast carcinoma in selected patients is generally considered to be the only indication for large-dose, long-term androgen therapy in women.

Androgens and anabolic agents are also used to treat certain anemias, osteoporosis, and to stimulate growth in postpuberal boys. In all cases, use of these agents requires caution.

ANDROGENS AND SPORTS

The use of anabolic steroids by athletes began in the late 1940s[150] and is now widespread. Prior to urine testing requirements, it was estimated that up to 80% of competitive weight lifters and about 75% of professional football players used these drugs, along with a variety of other athletes. One recent review puts the level of use at more than 90% of weight lifters and body builders.[151] During the 1972 Olympic games in Munich, 68% of interviewed track and field athletes (not including long-distance runners) had used anabolic steroids in preparation for the Olympics. Yet, international awareness did not stem the abuse of these drugs in subsequent Olympic games. Canada's Ben Johnson was disqualified as the winner of the 1988 Olympic gold medal in the 100-yard dash for having traces of stanozolol in his urine. The shocking disqualification brought the international misuse of anabolic steroids to headlines worldwide: "The Doped-Up Games" (*Newsweek*, October 10, 1988), "The Drug of Champions" (*Science*, October 14, 1988). The subheadline in *Science* summarized the problem succinctly: "Athletes and body builders support a $100 million black market in steroids, while medical science has been slow to see why."

The understanding by athletes of the rational selection, risks, and risk/benefit ratios of the drugs they take appears to be quite limited.

Many studies have attempted to determine if taking anabolic steroids improves athletic performance.[152–164] However, some failed to use controls (athletes who trained in an

identical manner, but who did not take anabolic steroids). Others failed to use placebos in at least a single-blind research design (neither the treated nor control groups knowing which they were taking).

Of the studies using at least a single-blind protocol, some have reported that anabolic steroids did increase athletic performance, whereas others found they did not. It would be fair to say, therefore, that the benefit of anabolic steroids to athletic performance is uncertain.

The risks of using these drugs appear to outweigh their uncertain benefits.

In late 1987, the FDA issued a special alert about the risks of anabolic steroid use and abuse by athletes,[165] along with a nationwide educational campaign. Other recent reviews on drug abuse and the athlete have shared a similar concern. The shocking book by Robert Goldman, *Death in the Locker Room*,[166] raised public perceptions about the risks of anabolic steroid abuse by athletes.

As summarized by the FDA[165] and recent reviews, the side effects include:

In both sexes

- Increased risk of coronary heart disease, stroke, or obstructed blood vessels
- Increased aggression and antisocial behavior (known as "steroid rage")
- Liver tumors, peliosis hepatis (blood-filled cysts), and jaundice

In men

- Testicular atrophy with consequent sterility or decreased sperm count and abnormal motility and morphology
- Impotence
- Enlarged prostate
- Breast enlargement

In women

- Clitoral enlargement
- Beard growth
- Baldness
- Deepened voice
- Breast diminution

Because of these risks, the International Olympic Committee has banned all anabolic drugs. The top winners in each Olympic event are tested for nontherapeutic drugs of all types. The use of these drugs by athletes has also been criticized and discouraged by coaches,[167] physicians,[168] and other athletic governing bodies.[169]

PRODUCTS

Therapeutic uses of the androgens and anabolic agents have been previously discussed. 17β-Esters and 17α-alkyl products are available for a complete range of therapeutic uses. These drugs are contraindicated in men with prostatic cancer; in men or women with heart disease, kidney disease, or liver disease; and in pregnancy. Diabetics using the androgens and anabolic agents should be carefully monitored. Androgens potentiate the action of oral anticoagulants, causing bleeding in some patients, and they may also interfere with some laboratory tests. Female patients may develop virilization side effects, and doctors should be warned that some of these effects may be irreversible (e.g., voice changes). Virtually all the anabolic agents now commercially available have significant androgenic activity; hence, virilization is a potential problem with all women patients. The 17α-alkyl products may cause cholestatic hepatitis in some patients.

All steroid 4-en-3-ones are light-sensitive and should be kept in light-resistant containers.

Testosterone, USP. 17β-Hydroxyandrost-4-en-3-one is a naturally occurring androgen in men. In women, it serves as a biosynthetic precursor to estradiol. However, it is rapidly metabolized to relatively inactive 17-ones (see Fig. 23-23, so it is not orally active. Testosterone 17β-esters are available in long-acting intramuscular depot preparations, illustrated in Fig. 23-22, including the following:

Testosterone cypionate, USP: Testosterone 17β-cyclopentylpropionate
Testosterone enanthate, USP: Testosterone 17β-heptanoate
Testosterone propionate, USP: Testosterone 17β-propionate
Testosterone is also available in a transdermal delivery system (patch).

Methyltestosterone, USP. 17β-Hydroxy-17-methylandrost-4-en-3-one is only about half as active as testosterone (when compared intramuscularly), but it has the great advantage of being orally active (see Fig. 23-6). (Methyltestosterone given by the buccal route is about twice as active as oral.) Both testosterone and methyltestosterone have high androgenic activity, limiting their usefulness where good anabolic activity–low androgenic activity is desired.

Fluoxymesterone, USP. 9α-Fluoro-11β,17β-dihydroxy-17-methylandrost-4-en-3-one is a highly potent, orally active androgen, about five to ten times more potent than testosterone. It can be used for all the indications discussed previously, but its great androgenic activity has made it useful primarily for treatment of the androgen-deficient male.

Methandrostenolone, USP. 17β-Hydroxy-17-methylandrosta-1,4-dien-3-one is orally active and about equal in potency to testosterone.

Anabolic agents include the commercially available androgens with improved anabolic activity (see Fig. 23-22) and those that are still experimental (examples in Fig. 23-24). It should be emphasized that virtually all the commercial products have significant androgenic properties (ratios given in Fig. 23-22); consequently, virilization in women and children can be expected. Many of the anabolic agents are orally active, as one would predict by noting a 17α-alkyl group in many of them (see Fig. 23-22). Those without the 17α-alkyl (nandrolone and dromostanolone) are active only intramuscularly. The commercially available anabolic agents include

Oxymetholone, USP: 17β-Hydroxy-2-(hydroxymethylene)-17-methylandrostan-3-one

Oxandrolone, USP: 17β-Hydroxy-17-methyl-2-oxaandrostan-3-one

Stanozolol, USP: 17-Methyl-2′*H*-androst-2-eno[3,2,*c*]-pyrazol- 17β-ol

Nandrolone decanoate, USP and nandrolone phenpropionate, USP: 17β-Hydroxyestr-4-en-3-one 17-decanoate and 3′-phenylpropionate

ANTIANDROGENS

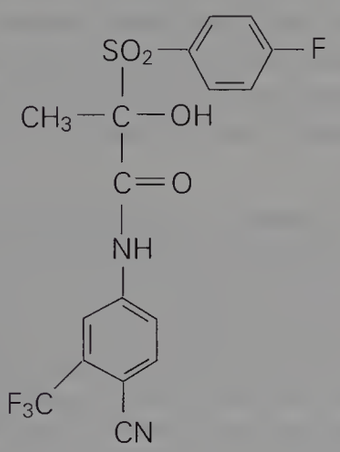
A variety of compounds (see Fig. 23-26) have been intensively studied as androgen antagonists, or antiandrogens. As reviewed by Rasmussen and Toney,[147] a wide range of compounds has been evaluated.

Estrogens have been used as antiandrogens, but their feminizing side effects (e.g., loss of libido) have precluded their extensive use in men. Antiandrogens would be of therapeutic use in treating conditions of hyperandrogenism (e.g., hirsutism, acute acne, and premature baldness) or androgen-stimulated cancers (e.g., prostatic carcinoma). The ideal antiandrogen would be nontoxic, highly active, and devoid of any hormonal activity. Unfortunately, most of the compounds in Fig. 23-26 have not met all these criteria completely.

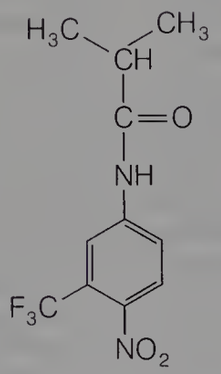
Bicalutamide and Flutamide

Two nonsteroidal antiandrogens are in clinical use in the United States—bicalutamide and flutamide (Fig. 23-26). Bicalutamide, *N*-4-cyano-3-(trifluoromethyl)phenyl-3-[(4-fluorophenyl)sulfonyl]-2-hydroxy-2-methyl-propanamide, Casodex, was approved by the FDA in early 1996 for once a day (50 mg) treatment of advanced prostate cancer. Flutamide, 2-methyl-N-[4-nitro-3(trifluoromethyl)phenyl]propanamide, Eulexin, is given three times daily.

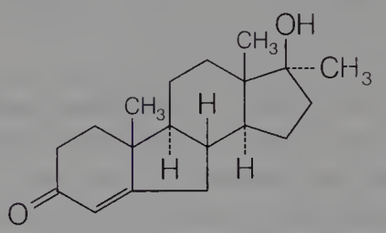
Prostate cancer is strongly androgen sensitive, so by blocking testosterone receptors, the cancer can be inhibited or slowed. However, both flutamide and bicalutamide have a very high incidence of treatment failures (42–53%), and there are no significant differences between them in survival rate, quality of life, subjective response, or time to evidence of disease progression.



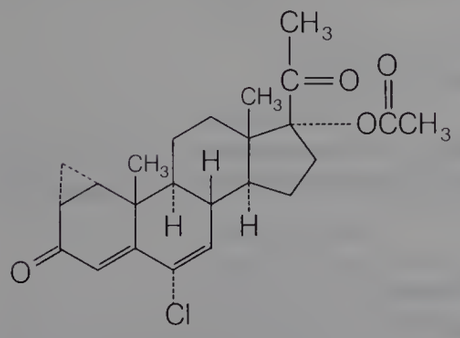
Bicalutamide
(Casodex)
N-4-Cyano-3 (trifluoromethyl) phenyl-3-[(4-fluorophenyl) sulfonyl]-2-hydroxy-2-methylpropanamide



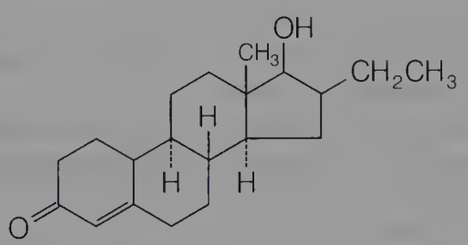
Flutamide
(Eulexin)
(α,α,α-Trifluoro-2-methyl-4′-nitro-*m*-propionotoluidide)



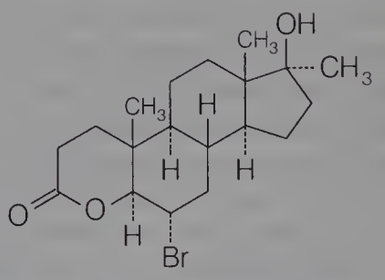
SKF 7690
(benorterone)
(17β-Hydroxy-17-*B* norandrost-4-en-3-one)



Cyproterone Acetate
(6-Chloro-17-hydroxy-1α,2α-methylene-pregna-4,6-diene-3,20-dione 17-acetate)



TSAA-291
(oxendolone)
(16β-Ethyl-19-nortestosterone)



BOMT
(6α-Bromo-17β-hydroxy-17α-methyl-4-oxa-5α-androstane-3-one)

FIG. 23-26. *Antiandrogens.*

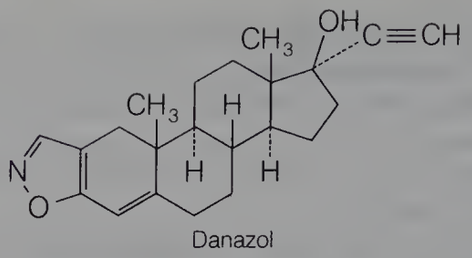
Bicalutamide and flutamide are given in combination with a GnRH agonist such as goserelin or leuprolide. Studies have shown that these drugs completely inhibit the action of testosterone and other androgens by binding to androgen receptors. In clinical trials when given as a single agent for prostate cancer, testosterone and estradiol serum increases have been observed. But when given in combination with a GnRH agonist, bicalutamide and flutamide do not affect testosterone suppression, which is the result of GnRH. As noted earlier in this chapter, GnRH agonists such as leuprolide greatly decrease gonadal function—the medical equivalent of castration in men. Thus, the combination of GnRH with bicalutamide or flutamide blocks the production of testosterone in the testes, and testosterone receptors in the prostate.

Drugs for Endometriosis

Endometriosis is characterized by endometrial tissue growing outside the uterus, especially in the pelvis.[170–174] Constant pain starting 2 to 7 days before menstruation, dyspareunia, and infertility often result. The goal of drug treatment is to preserve the fertility of women wanting future pregnancies, ameliorate symptoms, and simplify future surgery.

The treatment of endometriosis has recently undergone a significant change.[170–174] Most important, other causes of pelvic pain need to be considered before starting treatment. The use of laparoscopy for visualization of endometrial lesions has been a significant enhancement in therapy. A trial of oral contraceptives or medroxyprogesterone acetate is often begun first. It has been found that progestins such as medroxyprogesterone acetate are as effective in suppressing endometriosis as danazol, but without as many side effects. Gonadotropins such as leuprolide are then typically given for up to 6 months followed by laparoscopic excision of the endometriotic lesions. Further controlled studies are needed to clarify the optimal approach to be used for treating endometriosis, and in assisting fertilization in the treatment of endometriosis-associated infertility.

Danazol, 17α-pregna-2,4-dien-20-yno-[2,3-d]isoxazol-17-ol (Danocrine) is a weak androgen that, in spite of the 17α-ethinyl group, has no estrogenic or progestin activity. Danazol has been called a synthetic steroid with diverse biologic effects. Danazol binds to sex hormone binding globulin (SHBG) and decreases the hepatic synthesis of this estradiol and testosterone carrier. Free testosterone thus increases. It also blocks a number of enzymes essential for estradiol, progesterone, testosterone, and glucocorticoid biosynthesis. Danazol inhibits FSH and LH production by the hypothalamus and pituitary. It binds to progesterone receptors, glucocorticoid receptors, testosterone receptors, and estrogen receptors. As a weak androgen, it suppresses endometrium development.



Danazol

ADRENAL CORTEX HORMONES

The adrenal glands (which lie just above the kidneys) secrete over 50 different steroids, including precursors for other steroid hormones. However, the most important hormonal steroids produced by the adrenal cortex are aldosterone and hydrocortisone. Aldosterone is the primary *mineralocorticoid* in humans (i.e., it causes significant salt retention). Hydrocortisone is the primary *glucocorticoid* in humans (i.e., it has its primary effects on intermediary metabolism). The glucocorticoids have become very important in modern medicine, especially for their anti-inflammatory effects.

Sayers and Travis[175] succinctly summarize the importance of the adrenal cortex hormones: ''The adrenal cortex is the organ, par excellence, of homeostasis.'' Aldosterone, and to a lesser extent, other mineralocorticoids maintain a constant electrolyte balance and blood volume.

The glucocorticoids have key roles in controlling carbohydrate, protein, and lipid metabolic. George Chrousos, M.D. (Endocrinology Branch, NIH) has provided an excellent overview:

> Glucocorticoids have an important role in human physiology, and almost every tissue in the human body is affected by them. Glucocorticoids are crucial for the integrity of central nervous system function, and for the maintenance of cardiovascular and metabolic homeostasis. Increased secretion of glucocorticoids during stress is also pivotal in altering central nervous system function, in preventing the inflammatory and immune response systems from over-reacting, and in adjusting energy expenditures, all changes that improve chances for survival.[176]

Medically important adrenal cortex hormones and synthetic mineralocorticoids and glucocorticoids are shown in Fig. 23-27. Because salt-retention activity is usually undesirable, the drugs are classified by their salt-retention activities.

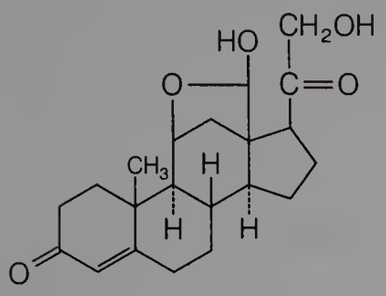
STRUCTURAL CLASSES

As illustrated in Fig. 23-27, the adrenal cortex hormones are classified by their biologic activities into three major groups:

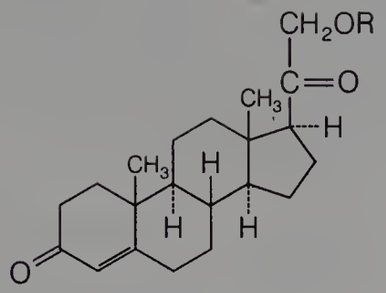
Mineralocorticoids

The mineralocorticoids are adrenal cortex steroids and analogues with high salt-retaining activity. They are used only for treatment of Addison's disease. The naturally occurring

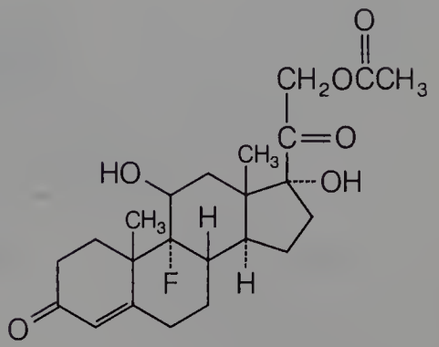
1. Mineralocorticoids (High Salt Retention)



Aldosterone
(not commercially available)



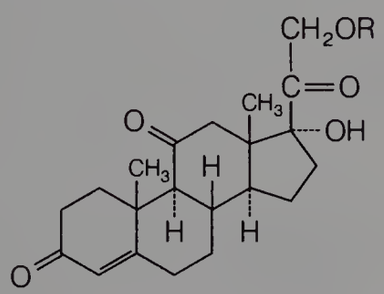
Deoxycorticosterone (R = H)



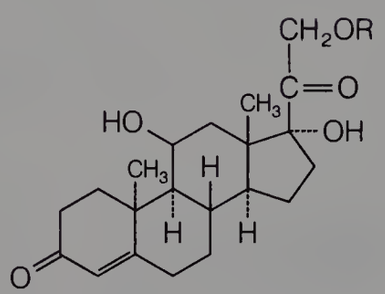
Fludrocortisone Acetate

Esters available:
Deoxycorticosterone Acetate: R = $COCH_3$
Deoxycorticosterone Pivalate: R = $COC(CH_3)_3$

2. Glucocorticoids with Moderate to Low Salt Retention



Cortisone (R = H)

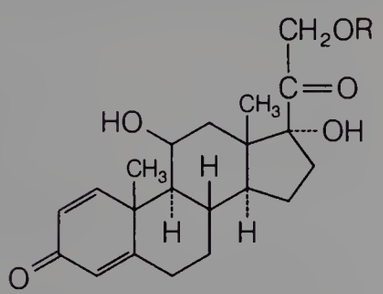


Hydrocortisone (R = H)
(or Cortisol)

Ester available:
Cortisone Acetate: R = $COCH_3$

Esters available:
Hydrocortisone Acetate: R = $COCH_3$
Hydrocortisone Cypionate: R = $COCH_2CH_2$— 

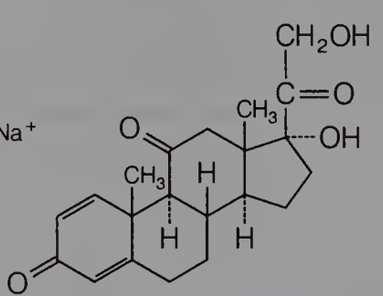
Salts available:
Hydrocortisone Sodium Phosphate: R = $PO_3^{=}(Na^+)_2$
Hydrocortisone Sodium Succinate:
R = $COCH_2CH_2COO^-Na^+$



Prednisolone (R = H)

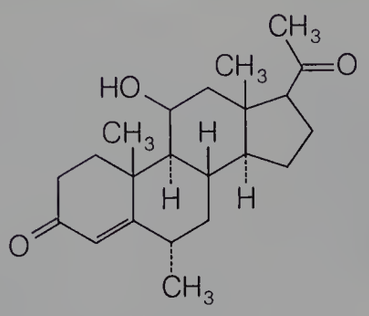
Salts available:
Prednisolone Sodium Phosphate: R = $PO_3^{=}(Na^+)_2$
Prednisolone Sodium Succinate: R = $COCH_2CH_2COO^-Na^+$

Esters available:
Prednisolone Acetate: R = Ac
Prednisolone Succinate: R = $COCH_2CH_2COOH$
Prednisolone Tebutate: R = $COCH_2C(CH_3)_3$

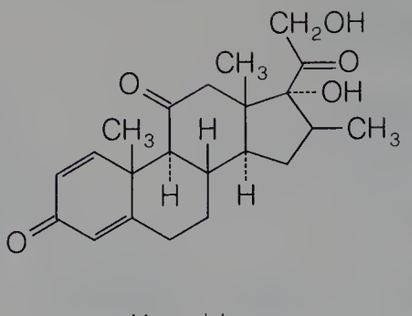


Prednisone

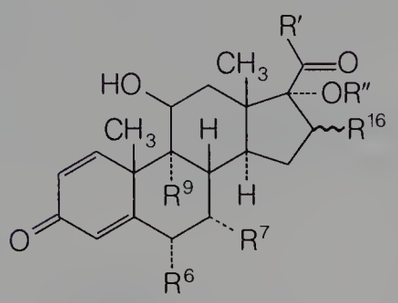
FIG. 23-27. *Natural and semisynthetic adrenal cortex hormones.*

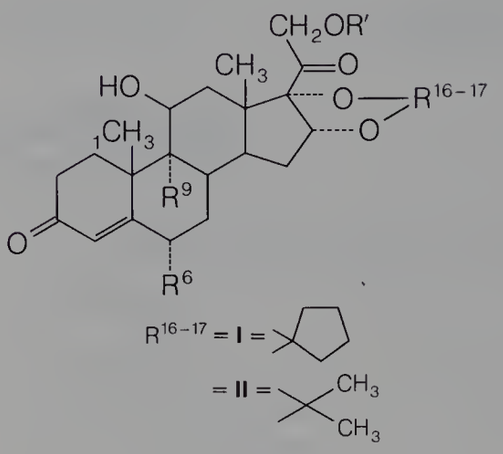


Medrysone



Meprednisone





R^{16-17} = I =

= II =

Alclometasone
R^{16} = ---CH_3
R^7 = Cl
R′ = CH_2OH

Beclomethasone
R^{16} = —CH_3
R^9 = Cl
R′ = CH_2OH
R″ = H

Betamethasone
R^{16} = —CH_3
R^9 = F
R′ = CH_2OH
R″ = H

Dexamethasone
R^{16} = ---CH_3
R^9 = F
R′ = CH_2OH
R″ = H

Diflorasone
R^{16} = —CH_3
R^6 = R^9 = F
R′ = CH_2OH
R″ = H

Flumethasone
R^{16} = ---CH_3
R^6 = R^9 = F
R′ = OH
R″ = H

Methylprednisolone
R^6 = CH_3
R′ = CH_2OH
R″ = H

Paramethasone
R^{16} = —CH_3
R^6 = F
R′ = CH_2OH
R″ = H

Amcinonide
R^{16-17} = I
1-ene
R^9 = F
R′ = $COCCH_3$

Desonide
R^{16-17} = II
1-ene

Flucinolone Acetonide
R^{16-17} = II
1-ene
R^6 = R^9 = F
(The 16,17-diol is flucinonide)

Flunisolide
R^{16-17} = II
1-ene
R^6 = F

Flurandrenolide
R^{16-17} = II
R^6 = F

Triamcinolone Acetonide
R^{16-17} = II
1-ene
R^9 = F
(The 16,17-diol is triamcinolone)

FIG. 23-27. *Continued.*

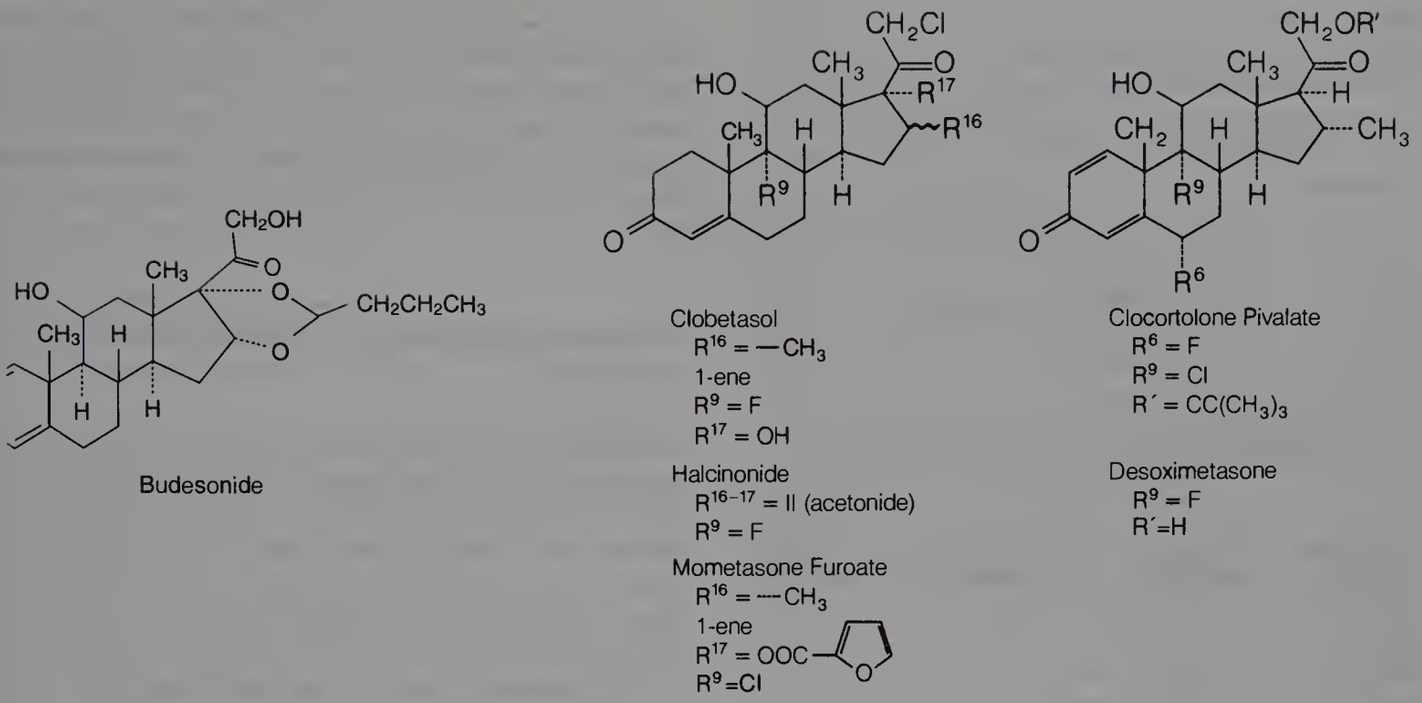


FIG. 23-27. *Continued.*

hormone aldosterone has an 11β-OH and an 18-CHO that naturally bridge to form a hemiacetal (as drawn in Fig. 23-26). Aldosterone is too expensive to produce commercially; therefore other semisynthetic analogues have taken its place for treatment of Addison's disease. The 11-deoxy analogue deoxycorticosterone has significant mineralocorticoid activity, even though it is less than a tenth of aldosterone's (Table 23-7), but still no glucocorticoid or anti-inflammatory activity. Adding a 9α-halogen to hydrocortisone (e.g., to produce fludrocortisone) greatly increases both salt retention and anti-inflammatory activity.

Table 23-8 summarizes the relative effects of various substituents on salt retention and glucocorticoid activity. The salt-retaining actions are approximately additive. For exam-

TABLE 23-7

APPROXIMATE RELATIVE ACTIVITIES OF CORTICOSTEROIDS*

	Biological Half-Life (min)	*Anti-Inflammatory Activity*	*Topical Activity*	*Salt-Retaining Activity*	*Equivalent Dose (mg)*
MINERALOCORTICOIDS					
Aldosterone		0.2	0.2	800	
11-Deoxycorticosterone		0	0	40	
9α-Fluorohydrocortisone (fludrocortisone)		10	5–40	800	2
GLUCOCORTICOIDS					
Hydrocortisone	102	1	1	1	20
Cortisone		0.8	0	0.8	25
Prednisolone	200	4	4	0.6	5
Prednisone		3.5	0	0.6	5
6α-Methylprednisolone (methyprednisolone)		5	5	0	4
16β-Methylprednisolone (meprednisone)		Used in eyes only; comparative data not available			
6α-Fluoroprednisolone (fluprednisolone)		15	7	0	1.5
Triamcinolone acetonide	300	5	5–100	0	4
Triamcinolone	100–200		1–5		
6α-Fluorotriamcinolone acetonide (fluocinolone acetonide)			over 40		
Flurandrenolide (flurandrenolone acetonide)			over 20		
Fluocinolone			over 40		
Fluocinolone 21-acetate (fluocinonide)			over 40–100		
Betamethasone		35	5–100	0	0.6
Dexamethasone (16α-isomer of betamethasone)	200	30	10–35	0	0.75

* The data in this table are only approximate. Blanks indicate that comparative data are not available to the author or that the product is used only for one use (e.g., topically). Data were taken from several sources, and there is an inherent risk in comparing such data. However, the table should serve as a guide of relative activities.

TABLE 23-8

EFFECTS OF SUBSTITUENTS ON GLUCOCORTICOID ACTIVITY

Clinical Antirheumatic Enhancement Factors

Functional Group	Factor	Functional Group	Factor
1-Dehydro	2.8	16α-Methyl	1.6
6-Dehydro	0.9*	6β-Methyl	1.3*
6α-Methyl	0.9*	16α, 17α-Isopropylidenedioxy	0.6*
6α-Fluoro	1.9	17α-Acetoxy	0.3*
9α-Fluoro	4.9	21-Deoxy	0.2*
16α-Hydroxy	0.3	21-Methyl	0.3*

Enhancement Factors for Various Functional Groups of Corticosteroids

Functional Group	Glycogen Deposition	Anti-Inflammatory Activity	Effects on Urinary Sodium†
9α-Fluoro	10	7–10	+ + +
9α-Chloro	3–5	3‡	+ +
9α-Bromo	0.4‡		+
12α-Fluoro	6–8§		+ +
12α-Chloro	4§		
1-Dehydro	3–4	3–4	–
6-Dehydro	0.5–0.7		+
2α-Methyl	3–6	1–4	+ +
6α-Methyl	2–3	1–2	– – –
16α-Hydroxy	0.4–0.5	0.1–0.2	– – – –
17α-Hydroxy	1–2	4	–
21-Hydroxy	4–7	25	+ +
21-Fluoro	2	2	– –

(From Rodig, O. R.: In Burger, A. (ed.). Medicinal Chemistry, Part 2, 3rd ed. New York, Wiley-Interscience, 1970. Used with permission.)

* Two observations or less.

† + = retention; – = excretion.

‡ In 1-dehydrosteroids, this value is 4.

§ In the presence of a 17α-hydroxyl group, this value is <0.01.

ple, a 9α-fluoro group's + + + increase in salt retention can be eliminated by a 6α-methyl's – – –.

Glucocorticoids with Moderate to Low Salt Retention

The glucocorticoids with moderate to low salt retention include cortisone, hydrocortisone, and their 1-enes prednisolone and prednisone.

As shown in Table 23-7, an 11-OH maintains good topical anti-inflammatory activity, but 11-ones have little or none. The 1-ene of prednisolone and prednisone increases anti-inflammatory activity by about a factor of 4 and somewhat decreases salt retention. Duax and co-workers[33,34] have shown that the increase in activity may be due to a change in shape of ring A.

Specifically, it appears that analogues more active than hydrocortisone have their ring A bent underneath the molecule to a much greater extent than in hydrocortisone.

The 11β-OH of hydrocortisone is believed to be of major importance in binding to the receptors. Cortisone may be reduced in vivo to yield hydrocortisone as the active agent. The increase activity of 9α-halo derivatives may be due to the electron-withdrawing inductive effect on the 11β-OH, making it more acidic and, therefore, better able to form noncovalent bonds with the receptor. A 9α-halo substituent also reduces oxidation of the 11β-OH to the less active 11-one.

Glucocorticoids with Very Little or No Salt Retention

Cortisone and hydrocortisone, and even prednisone and prednisolone, have too much salt-retaining activity in the doses needed for some therapeutic purposes. Over the last three decades, a number of substituents have been discovered that greatly decrease salt retention. They include 16α-hydroxy; 16α,17α-ketal; 6α-methyl; 16α- and 16β-methyl. Other substituents have been found to significantly increase both glucocorticoid and mineralocorticoid activities: 1-ene; 2α-methyl; 9α-fluoro; 9α-chloro; and 21-hydroxy.

As a result of the great economic benefit in having a potent anti-inflammatory product on the market, pharmaceutical manufacturers have made every conceivable combination of these various substituents. In every case a 16-hydroxy or methyl (to eliminate salt retention) has been combined with another substituent to increase glucocorticoid or anti-inflammatory activity. The number of permutations and combinations has resulted in a redundant array of analogues with very low salt retention and high anti-inflammatory activity. Few have any significant advantage over any of the others, but prices vary enormously. This group of drugs represents a striking example of "me too" drug development in modern medicine.

A primary goal of these highly anti-inflammatory drugs has been to increase topical potency. As shown in Table 23-7, some are as much as 100 times more active topically than hydrocortisone. Relative potency is as follows:

Very high potency
Clobetasol, 0.05%
Diflorasone (ointment), 0.05%
Halobetasol, 0.05%
High potency
Amcinonide, 0.1%
Betamethasone dipropionate, 0.05%
Desoximetasone, 0.25%
Diflorasone (cream), 0.05%
Fluocinolone, 0.2%
Fluocinonide, 0.05%
Halcinonide, 0.1%
Triamcinolone acetonide, 0.5%
Intermediate potency
Betamethasone benzoate, 0.025%
Betamethasone valerate, 0.1%
Desoximetasone, 0.05%
Fluocinolone acetonide, 0.025%

Triamcinolone acetonide, 0.1%
Low potency
Alclometasone, 0.05%
Betamethasone valerate, 0.01%
Clocortolone, 0.1%
Fluocinolone acetonide, 0.01%
Triamcinolone acetonide, 0.025%
Lowest potency
Dexamethasone, 0.1%
Hydrocortisone, 1.0%
Methylprednisolone acetate, 0.25%

Although, as shown in Table 23-7, cortisone and prednisone are not active topically, most other glucocorticoids are active. Some compounds, such as triamcinolone acetonide, have striking activity topically. Skin absorption is favored by increased lipid solubility of the drug.

Absorption of topical glucocorticoids can also be greatly affected by the extent of skin damage, concentration of the glucocorticoid, cream or ointment base used, and similar factors. One must not assume, therefore, from a study of Table 23-7 that, for example, a 0.25% cream of prednisolone is necessarily exactly equivalent in anti-inflammatory potency to 1% hydrocortisone. Nevertheless, the table can serve as a preliminary guide. Furthermore, particular patients may seem to respond better to one topical anti-inflammatory glucocorticoid than to another, irrespective of the relative potencies shown in Table 23-7.

Risk of Systemic Absorption

Except for fludrocortisone, the topical corticosteroids do not cause absorption effects when used on small areas of intact skin. However, when these compounds are used on large areas of the body, systemic absorption may occur, especially if the skin is damaged or if occlusive dressings are used. Fludrocortisone is more readily absorbed than other topical corticosteroids; hence systemic problems can be expected more frequently with it. (Up to 20% to 40% of hydrocortisone given rectally may also be absorbed.)

BIOSYNTHESIS

As shown in a simplified scheme in Fig. 23-28, aldosterone and hydrocortisone are biosynthesized from pregnenolone through a series of steps involving hydroxylations at C-17, C-11, and C-21 that convert cholesterol to hydrocortisone. Deficiencies in any of the enzymes are the cause of congenital adrenal hyperplasias. Defects in the gene regulation, as well as the enzymes that catalyze the hydroxylation have been intensively studied.[177–184] It has been found that the biosynthesis of steroid hormones (starting with cholesterol) in animal tissue are catalyzed by six forms of cytochrome P-450, and two hydroxysteroid dehydrogenases. Investigators have begun to link defects in particular genes or steroid binding sites to the pathophysiology of patients with the corresponding metabolic diseases.

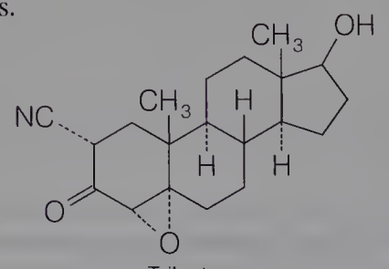
These disorders are usually caused by an inability of the adrenal glands to carry out 11β-, 17α-, or 21-hydroxylations. The most common is a lack of 21-hydroxylase activity, which will result in decreased production of hydrocortisone and a compensatory increase in adrenocorticotropic hormone (ACTH) production. Furthermore, the resultant buildup of 17α-hydroxyprogesterone will lead to an increase of testosterone. When 11β-hydroxylase activity is low, large amounts of 11-deoxycorticosterone will be produced. Because 11-deoxycorticosterone is a potent mineralocorticoid, there will be symptoms of mineralocorticoid excess, including hypertension. When 17α-hydroxylase activity is low, there will be decreased production of testosterone and estrogens as well as hydrocortisone.

Although the details are not completely known, the polypeptide ACTH (corticotropin) produced by the anterior pituitary enhances or is necessary for the conversion of cholesterol to pregnenolone. ACTH also stimulates the synthesis of hydrocortisone. (ACTH is discussed in Chap. 25.) Hydrocortisone then acts by feedback inhibition to suppress the formation of additional ACTH.

The release of the primary mineralocorticoid, aldosterone, is only slightly dependent on ACTH. Aldosterone is an active part of the angiotensin–renin–blood pressure cycle that controls blood volume. A decrease in blood volume stimulates the juxtaglomerular cells of the kidneys to secrete the enzyme renin. Renin, in turn, converts angiotensinogen to angiotensin, then angiotensin stimulates the adrenal cortex to release aldosterone. Aldosterone then causes the kidneys to retain sodium, and blood volume increases. When the blood volume has increased sufficiently, there is a decreased production of renin, until blood volume drops again.

Trilostane–An Inhibitor of Glucocorticoid and Mineralocorticoid Biosynthesis

Trilostane (4,5-epoxy-17-hydroxy-3-oxoandrostane-2-carbonitrile; Modrastane) completely inhibits the conversion of pregnenolone to progesterone, and of 17α-hydroxypregnenolone to 17α-hydroxyprogesterone. It blocks the hydroxylating enzymes (hydroxylases) involved in hydrocortisone and aldosterone biosynthesis (see Fig. 23-28). Thus, trilostane is used for treatment of Cushing's syndrome.[185] However, this drug is teratogenic, and not all patients experience significant decreases in glucocorticoid and mineralocorticoid plasma levels.



Trilostane

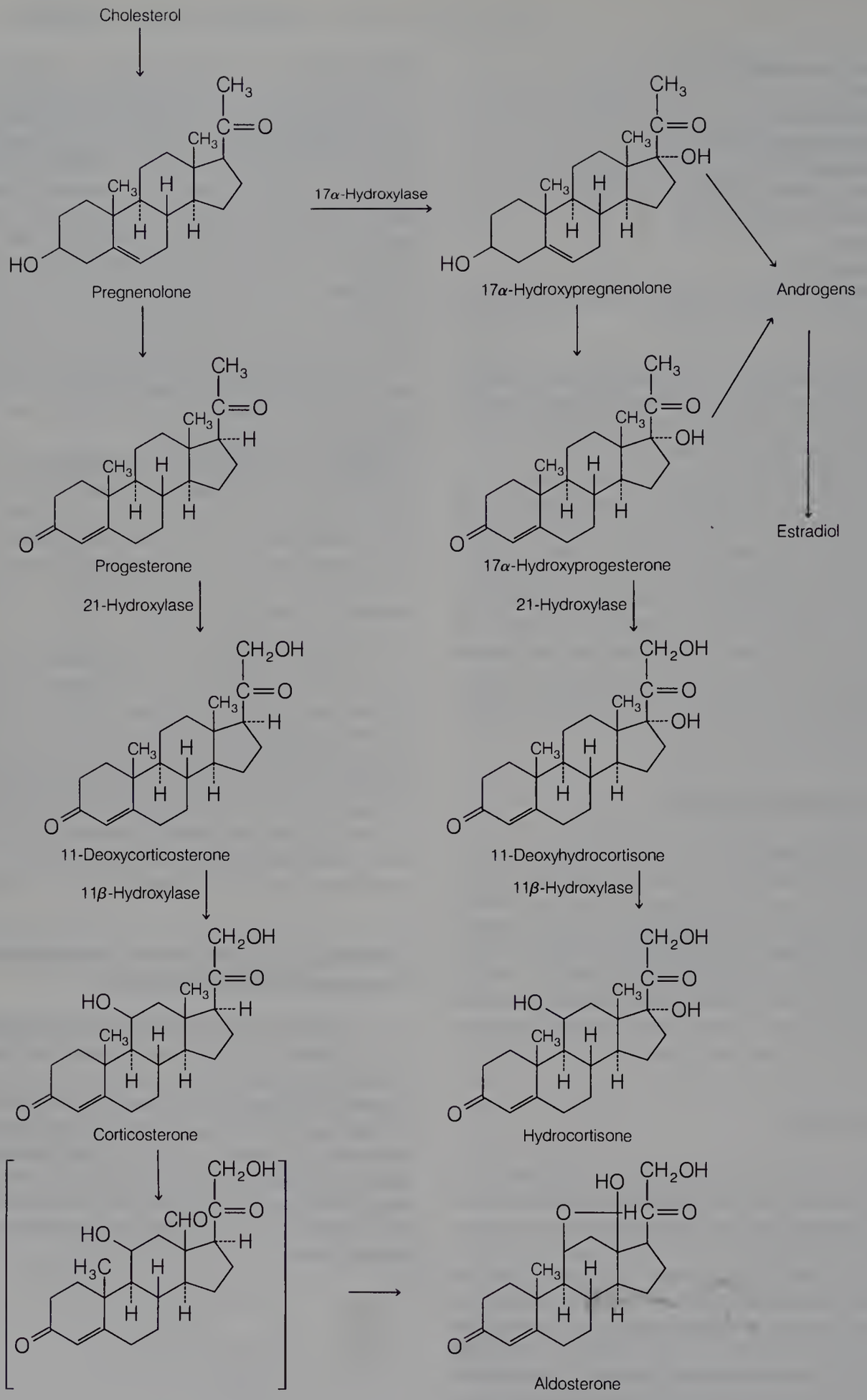


FIG. 23-28. *A simplified scheme of the biosynthesis of hydrocortisone and aldosterone. The biosynthetic pathways are more complex than presented here.*

BIOCHEMICAL ACTIVITIES

The adrenocortical steroids permit the body to adjust to environmental changes, to stress, and to changes in the diet. Aldosterone and, to a lesser extent, other mineralocorticoids maintain a constant electrolyte balance and blood volume, and the glucocorticoids have key roles in controlling carbohydrate, protein, and lipid metabolism.

Aldosterone increases sodium reabsorption in the kidneys. An increase in plasma sodium concentration, in turn, will lead to increased blood volume, because blood volume and urinary excretion of water are directly related to the plasma sodium concentration. Simultaneously, aldosterone increases potassium ion excretion. 11-Deoxycorticosterone also is quite active as a mineralocorticoid. Similar actions are exhibited with hydrocortisone and corticosterone, but to a much smaller degree.

Aldosterone controls the movement of sodium ions in most epithelial structures involved in active sodium transport. Although aldosterone acts primarily on the distal convoluted tubules of the kidneys, it also acts on the proximal covoluted tubules and collecting ducts. Aldosterone also controls the transport of sodium in sweat glands, small intestine, salivary glands, and the colon. In all these tissues, aldosterone enhances the inward flow of sodium ions and promotes the outward flow of potassium ions.

Patients with Addison's disease exhibit muscle weakness and are easily fatigued. This primarily may be due to inadequate blood volume and aldosterone insufficiency, although changes in glucose availability may also be involved.

However, aldosterone (and other steroids with mineral activity) does not cause immediate changes in sodium excretion. There is a latent period after administration of any of the mineralocorticoids. This supports the view that aldosterone acts by stimulation of the synthesis of enzymes that, in turn, are actually responsible for active ion transport. This will be discussed in greater detail with the adrenocortical steroid receptors.

The glucocorticoids have many physiologic and pharmacologic actions. They control or influence carbohydrate, protein, lipid, and purine metabolism. They also affect the cardiovascular and nervous systems and skeletal muscle. They also regulate growth hormone gene expression.

Glucocorticoids stimulate glycogen storage synthesis by inducing the syntheses of glycogen synthase and stimulate gluconeogenesis in the liver. They have a catabolic effect on muscle tissue, stimulating the formation and transamination of amino acids into glucose precursors in the liver. Glucocorticoids induce tyrosine aminotransferase and tryptophan oxidase. The catabolic actions in Cushing's syndrome are demonstrated by a wasting of the tissues, osteoporosis, and reduced muscle mass. Lipid metabolism and synthesis are significantly increased in the presence of glucocorticoids, but the actions usually seem to be dependent on the presence of other hormones or cofactors. Glucocorticoids also protect the body from stress, but the mechanism of this protective effect is unknown. High glucocorticoid production in response to stress can lead to a decrease in the size of the thymus gland by up to 95%. The roles of the thymus in protection against stress (by glucocorticoid stimulation) are, as yet, not fully delineated. Equally fascinating is the glucocorticoid's role in activating some parts of the immune system, but depressing others. A lack of adrenal cortex steroids also causes depression, irritability, and even psychoses, reflecting significant effects on the nervous system. The glucocorticoids also play a role in maintaining bone, cartilage, and skin.

Anti-Inflammatory Actions of Glucocorticoids[186–190]

Glucocorticoid-receptor complexes (see Fig. 23-3) may activate or repress the genes to which they associate. The number of steroid responsive genes per cell is probably between 10 and 100. Repression in particular may have an important role in glucocorticoid anti-inflammatory actions. Glucocorticoids inhibit the transcription of cytokines and other mediators of inflammation. (Cytokines also induce the expression of molecules involved with adhesion of inflammatory cells at the inflammation site, and so the glucocorticoids' inhibition of cytokine transcription helps decrease adhesion as well.)

Glucocorticoids also block the synthesis of some cytokine receptors, and repress target genes for the cytokines themselves. Glucocorticoids inhibit the NK1 receptor involved with many of the inflammatory actions of substance P. Glucocorticoids may also increase the synthesis of lipocortin-1—a protein that inhibits the production of prostaglandins and platelet-activating factor—in some cells.

In addition, glucocorticoids can very effectively inhibit collagenase, an important enzyme involved with inflammation. Although the molecular mechanisms are not delineated, glucocorticoids also appear to inhibit the permeability of capillaries at inflammation sites.

It has also been found that β-adrenergic agonists can reduce glucocorticoid binding to their receptors, which may account for why inhaled β-agonists can interfere with their anti-inflammatory action.

Albert Baldwin[189] and Michael Karin[190] and their coworkers have recently gained new insights on how glucocorticoids suppress immunity. They found that the glucocorticoids stimulate transcription of the gene for synthesis of a protein (IκBα), which binds to nuclear factor kappa B (NF-κB). This nuclear factor is required for the function of many cytokine promotors, as well as responsive elements in interleukin promotion.

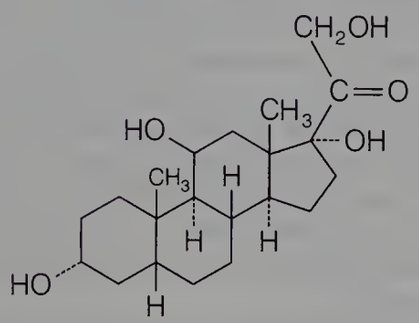
Resistance to Glucocorticoids[186,191]

A few patients with chronic inflammatory illnesses such as asthma and lupus develop resistance to the anti-inflammatory effects of the glucocorticoids. The mechanism is not

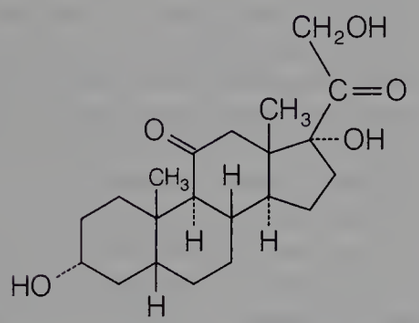
fully understood, but appears to be a decrease in the binding or activation ability of glucocorticoid receptor complexes and their target or "activator" genes.

METABOLISM

Cortisone and hydrocortisone are enzymatically interconvertible, and thus one finds metabolites from both. Most of the metabolic processes occur in the liver, with the metabolites excreted primarily in the urine. Although many metabolites have been isolated, the primary routes of catabolism are (1) reduction of the C-4 double bond to yield 5β-pregnanes; (2) reduction of the 3-one to give 3α-ols; (3) reduction of the 20-one to the corresponding 20α-ol. The two primary metabolites are tetrahydrocortisol and tetrahydrocortisone (shown below) and their conjugates.

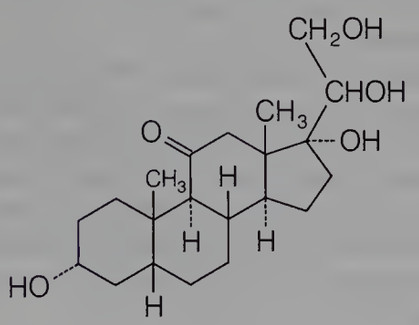


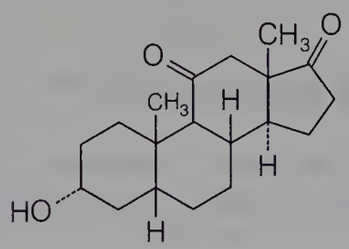
Tetrahydrocortisol



Tetrahydrocortisone

However, other metabolites include 20-ols and derivatives of side chain oxidation and cleavage, as shown below.





The C_{19} metabolites of the latter type are often androgenic.

THERAPEUTIC USES

The adrenocortical steroids are used primarily for their glucocorticoid effects, including immunosuppression, anti-inflammatory activity, and antiallergic activity. The mineralocorticoids are used only for treatment of Addison's disease. Addison's disease is caused by chronic adrenocortical insufficiency and may be due to either adrenal or anterior pituitary failure. The anterior pituitary secretes ACTH, a polypeptide that stimulates the adrenal cortex to synthesize steroids.

The symptoms of Addison's disease illustrate the great importance of the adrenocortical steroids in the body and, especially, the importance of aldosterone. These symptoms include increased loss of body sodium, decreased loss of potassium, hypoglycemia, weight loss, hypotension, weakness, increased sensitivity to insulin, and decreased lipolysis.

Hydrocortisone is also used during postoperative recovery after surgery for Cushing's syndrome—excessive adrenal secretion of glucocorticoids. Cushing's syndrome can be caused by bilateral adrenal hyperplasia or adrenal tumors and is treated by surgical removal of the tumors or resection of hyperplastic adrenal gland(s).

The use of glucocorticoids during recovery from surgery for Cushing's syndrome illustrates a most important principle of glucocorticoid therapy: abrupt withdrawal of glucocorticoids may result in adrenal insufficiency—showing clinical symptoms similar to Addison's disease. For that reason, patients who have been on long-term glucocorticoid therapy must have the dose gradually reduced. Furthermore, prolonged treatment with glucocorticoids can cause adrenal suppression, especially during times of stress. The symptoms are similar to those of Cushing's syndrome, for example, rounding of the face, hypertension, edema, hypokalemia, thinning of the skin, osteoporosis, diabetes, and even subcapsular cataracts. In doses of 45 mg/m^2 of body surface area or more daily, growth retardation occurs in children.

The glucocorticoids are used in the treatment of collagen vascular diseases, including rheumatoid arthritis, disseminated lupus erythematosus, and dermatomyositis.

Although there is usually prompt remission of redness, swelling, and tenderness by the glucocorticoids in rheumatoid arthritis, continued long-term use may lead to serious systemic forms of collagen disease. As a result, the glucocorticoids should be used infrequently in rheumatoid arthritis.

The glucocorticoids are used extensively topically, orally, and parenterally to treat inflammatory conditions. They also usually produce relief from the discomforting symptoms of many allergic conditions—intractable hay fever, exfoliative dermatitis, generalized eczema, and others. The glucocorticoids are also used to treat acute asthmatic symptoms unresponsive to bronchodilators. They have been especially useful in aerosol preparations.

The glucocorticoids' lymphocytopenic actions make them particularly useful for treatment of chronic lymphocytic leukemia in combination with other antineoplastic drugs.

The glucocorticoids are also used in the treatment of congenital adrenal hyperplasias.

The adrenocortical steroids are contraindicated or should be used with great caution in patients having (1) peptic ulcer (in which the steroids may cause hemorrhage), (2) heart disease, (3) infections (the glucocorticoids suppress the body's normal infection-fighting processes), (4) psychoses (since behavioral disturbances may occur during steroid therapy), (5) diabetes (the glucocorticoids increase glucose production, so more insulin may be needed), (6) glaucoma, (7) osteoporosis, and (8) herpes simplex involving the cornea.

When topically administered, the glucocorticoids present relatively infrequent therapeutic problems, but it should be remembered that their anti-inflammatory action can mask symptoms of infection. Many physicians prefer not giving a topical anti-inflammatory steroid until after an infection is controlled with topical antibiotics. The immunosuppressive activity of the topical glucocorticoids can also prevent natural processes from curing the infection. Topical steroids actually may also cause any of several dermatoses in some patients.

Finally, as discussed before with the oral contraceptives, steroid hormones should not be used during pregnancy. If absolutely necessary to use the glucocorticoids topically during pregnancy, they should be limited to small areas of intact skin and used for a limited time.

PRODUCTS

The adrenal corticosteroids used in commercial products are shown in Fig. 23-27. The structures illustrate the usual changes (see Fig. 23-8) made to modify solubility of the products—and, therefore, their therapeutic uses. In particular, the 21-hydroxyl can be converted to an ester to make it less water-soluble to modify absorption, or to a phosphate ester salt or hemisuccinate ester salt to make it more water-soluble and appropriate for intravenous use. The products also reflect the previously discussed structure–activity relationship changes to increase anti-inflammatory activity or potency, or to decrease salt retention.

Again, it must be emphasized that patients who have been on long-term glucocorticoid therapy must have the dose gradually reduced. This "critical rule" and indications have been previously discussed under Therapeutic Uses. Dosage schedules and gradual reduction of dose schedules can be quite complex and specific for each indication. For that reason, specialized indication and dose references such as *Facts and Comparisons* should be consulted before advising physicians on dosages.

Many of the glucocorticoids are available in topical dosage forms, including creams, ointments, aerosols, lotions, and solutions. They are usually applied three to four times a day to well-cleaned areas of affected skin. (The patient should be instructed to apply them with well-washed hands as well.) Ointments are usually prescribed for dry, scaly dermatoses. Lotions are well suited for weeping dermatoses. Creams are of general use for many other dermatoses. When applied to very large areas of skin or to damaged areas of skin, significant systemic absorption can occur. The use of an occlusive dressing can also greatly increase systemic absorption.

Deoxycorticosterone acetate, USP. 21-Acetyloxypregn-4-ene-3,20-dione is a potent mineralocorticoid used only for the treatment of Addison's disease. It has essentially no anti-inflammatory (glucocorticoid) activity but has 100 times the salt-retention (mineralocorticoid) activity of hydrocortisone. (It has only 1/30 the activity of aldosterone.) Hydrocortisone or other glucocorticoids should be given simultaneously for patients with acute adrenal insufficiency. Its great salt-retaining activity can be expressed as edema and pulmonary congestion as toxic doses are reached. It is insoluble in water (as one would predict from Table 23-1). Because Addison's disease is essentially incurable, treatment continues for life. With a serum half-life of only 70 minutes, the drug is sometimes given in the form of subcutaneous pellets administered every 8 to 12 months. The duration of action of deoxycorticosterone pivalate, USP, when given intramuscularly in depot preparations is longer than the acetate, often being administered only once every 4 weeks.

Fluorocortisone acetate, USP. 9α-fluoro-11β,17α,21-trihydroxypregn-4-ene-3,20-dione-21-acetate; 9α-fluorohydrocortisone is used only for the treatment of Addison's disease and for inhibition of endogenous adrenocortical secretions. As shown in Table 23-7, it has up to about 800 times the mineralocorticoid activity of hydrocortisone and about 11 times the glucocorticoid activity. Its potent activity stimulated the synthesis and study of the many fluorinated steroids shown in Fig. 23-27. Although its great salt-retaining activity limits its use to Addison's disease, it has sufficient glucocorticoid activity that, in many cases of the disease, additional glucocorticoids need not be prescribed.

Cortisone acetate, USP. 17α,21-Dihydroxy-4-pregnene,3,11,20-trione 21-acetate is a natural cortical steroid with good anti-inflammatory activity and low to moderate salt-retention activity. It is used for the entire spectrum of uses discussed previously under Therapeutic Uses—collagen diseases, especially rheumatoid arthritis; Addison's disease; severe shock; allergic conditions; chronic lymphatic leukemia; and many other indications. Cortisone acetate is relatively ineffective topically, in part because it must be reduced in vivo to hydrocortisone which is more active. Its plasma half-life is only about 30 minutes, compared to 90 minutes to 3 hours for hydrocortisone.

Hydrocortisone, USP. 11,17,21-Trihydroxy-pregn-4-ene-3,20-dione is the primary natural glucocorticoid in humans. Synthesis of 9α-fluorohydrocortisone during the synthesis of hydrocortisone has led to the array of semisynthetic glucocorticoids shown in Fig. 23-27, many of which have greatly improved anti-inflammatory activity. Nevertheless, hydrocortisone, its esters, and its salts remain a mainstay of modern adrenocortical steroid therapy—and the standard for comparison of all other glucocorticoids and mineralocorticoids (see Table 23-7). It is used for all the indications previ-

ously mentioned. Its esters and salts illustrate the principles of chemical modification to modify pharmacokinetic utility shown in Fig. 23-8. The commercially available salts and esters (see Fig. 23-27) include

Hydrocortisone acetate, USP
Hydrocortisone sodium succinate, USP
Hydrocortisone cypionate, USP
Hydrocortisone sodium phosphate, USP
Hydrocortisone valerate, USP

Prednisolone, USP. Δ^1-Hydrocortisone; 11,17,21-trihydroxypregna-1,4-diene-3,20-dione has less salt-retention activity than hydrocortisone (see Table 23-7), but some patients have more frequently experienced complications such as gastric irritation and peptic ulcers. Because of low mineralocorticoid activity, it cannot be used alone for adrenal insufficiency. Prednisolone is available in a variety of salts and esters to maximize its therapeutic utility (see Fig. 23-8).

Prednisolone acetate, USP
Prednisolone succinate, USP
Prednisolone sodium succinate for injection, USP
Prednisolone sodium phosphate, USP
Prednisolone tebutate, USP

Prednisone, USP. Δ^1-Cortisone; 17,21-dihydroxypregna-1,4-diene-3,11,20-trione has systemic activity very similar to that of prednisolone, and because of its lower salt-retention activity, it is often preferred over cortisone or hydrocortisone.

GLUCOCORTICOIDS WITH LOW SALT RETENTION

Most of the key differences between the many glucocorticoids with low salt retention (see Fig. 23-27) have been summarized in Tables 23-7 and 23-8. The tremendous therapeutic and, therefore, commercial importance of these drugs has been a stimulus to the proliferation of new compounds and their products. Many compounds also are available as salts or esters to give the complete range of therapeutic flexibility illustrated in Fig. 23-8. When additional pertinent information is available, it is given below.

Alclometasone, USP. 7-Chloro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione.

Amcinonide, USP. 21-(Acetyloxy)-16,17-[cyclopentylidenebis(oxy)]-9-fluoro-11-hydroxypregna-1,4-diene-3,20-dione.

Beclomethasone, USP. 9-Chloro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione.

Beclomethasone dipropionate, USP. 9-Chloro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione 17,21-dipropionate is available as a nasal inhaler and is used for seasonal rhinitis poorly responsive to conventional treatment.

Betamethasone, USP. 9-Fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione.

Betamethasone valerate, USP
Betamethasone acetate, USP
Betamethasone sodium phosphate, USP
Betamethasone benzoate, USP
Betamethasone dipropionate, USP

Budesonide, USP. (11β,16α)-16,17-[Butylidenebis(oxy)]-11,21-dihydroxypregna-1,4-diene-3,20-dione.

Clobetasol, USP. 21-Chloro-9-fluoro-11,17-dihydroxy-16-methylpregna-1,4-diene-3,20-dione.

Clocortolone, USP. 9-Chloro-6α-fluoro-11β, 21-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione.

Desonide, USP. 16α-Hydroxyprednisolone-16α,17-acetonide.

Desoximetasone, USP. 9-Fluoro-11,21-dihydroxy-16-methylpregna-1,4-diene-3,20-dione.

Dexamethasone, USP. 9α-Fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione is essentially the 16α-isomer of betamethasone.

Dexamethasone acetate, USP
Dexamethasone sodium phosphate, USP

Diflorasone, USP. 6,9-Difluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione.

Flumethasone pivalate, USP. 6,9-Difluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione 21-pivalate.

Flunisolide, USP. 6α-Fluoro-11β,16α,17,21-tetrahydroxy-pregna-1,4-diene-3,20-dione 16,17-acetonide.

Fluocinolone acetonide, USP. 6α-Fluorotriamcinolone acetonide; 6α,9α-difluoro-11β,16α, 17, 21-tetrahydroxypregna-1,4-diene-3,20-dione 16α, 17α-acetone ketal, the 21-acetonide of fluocinonide. Fluocinonide is about five times more potent than the acetonide in the vasoconstrictor assay.

Flurandrenolide, USP. 6α-Fluoro-11β,21-dihydroxy-16α,17-[(1-methylethylidene)bis(oxy)]pregn-4-ene-3,20-dione has replaced the name *flurandrenolone*. Although a flurandrenolide tape product is available, it can stick to and remove damaged skin, so it should be avoided with vesicular or weeping dermatoses.

Fluticasone propionate, USP. (6α,11β,16α,17α)-6,9-Difluoro-11-hydroxy-16-methyl-3-oxo-17-(1-oxopropoxy)androsta-1,4-diene-17-carbothioic acid S-(fluoromethyl) ester.

Halcinonide. 21-Chloro-9-fluoro-11β,16α,17-trihydroxypregn-4-ene- 3,20-dione 16α,17α-ketal is the first *chloro*-glucocorticoid yet marketed. As with several other glucocorticoids (see Table 23-8), it is used only topically. In one double-blind study with betamethasone valerate cream, halcinonide was superior in the treatment of psoriasis. However, it can be used for the usual range of indications previously described.

Halobetasol propionate, USP. (6, 11, 16)-21-Chloro-6,9-difluoro-11-hydroxy-16-methyl-17-(1-oxopropoxy)-pregna-1,4 -diene-3,20-dione.

Medrysone, USP. 11-Hydroxy-6-methylpregn-4-ene-3,20-dione is unique among the other corticosteroids shown in Fig. 23-26 in that it does not have the usual 17α,21-diol system of the others. Currently, it is used only for treatment of inflammation of the eyes.

Meprednisone, USP. 17,21-Dihydroxy-16β-methylpregna-1,4-diene-3,11,21-trione; 16β-methylprednisolone.

Methylprednisolone, USP. 11,17,21-Trihydroxy-6-methyl-1,4-pregnadiene-3,20-dione

Methylprednisolone acetate, USP
Methylprednisolone hemisuccinate, USP
Methylprednisolone sodium succinate, USP

Mometasone furoate, USP. 9α-21-Dichloro-16α-methylprednisolone 17α-furoate.

Paramethasone acetate, USP. 6α-Fluoro-11β, 17, 21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 21-acetate.

Triamcinolone, USP. 9-Fluoro-11,16,17,21-tetrahydroxypregna-1,4-diene-3,20-dione.

Triamcinolone acetonide, USP. Triamcinolone-16α,17α-acetone ketal; 16α,17α-[(1-methylethylidene)bis(oxy)]triamcinolone.

Triamcinolone hexacetonide, USP. Triamcinolone acetonide 21-[3-(3,3-dimethyl)butyrate].

Triamcinolone diacetate, USP. The hexacetonide is slowly converted to the acetonide in vivo and is given only by intra-articular injection. Only triamcinolone and the diacetate are given orally. When triamcinolone products are given intramuscularly, they are often given deeply into the gluteal region because local atrophy may occur with shallow injections. The acetonide and diacetate may be given by intra-articular or intrasynovial injection, and, additionally, the acetonide may be given by intrabursal or sometimes by intramuscular or subcutaneous injection. A single intramuscular dose of the diacetate or acetonide may last up to 3 or 4 weeks. Plasma levels with intramuscular doses of the acetonide are significantly higher than with triamcinolone itself.

Topically applied triamcinolone acetonide is a potent anti-inflammatory agent (see Table 23-7), about ten times more so than triamcinolone.

CARDIAC STEROIDS AND RELATED INOTROPIC DRUGS

A variety of drugs can increase the force of contraction of the heart. This inotropic action can be particularly useful in treatment of congestive heart failure.[192–196] A failing heart cannot pump sufficient blood to maintain body needs. With more than 2 million patients in the United States alone with congestive heart failure, and millions more throughout the world, inotropic drugs are extremely important. They prolong life, but even with drug treatment, the long-term outlook for these patients is poor. As reviewed by Irene Isu, Ralph Kelly, and Thomas Smith in 1996, mortality rates for heart failure are about 10% one year after diagnosis, and 50% after 5 years.[192,193]

The cardiac glycosides (Fig. 23-29) have been used for centuries, but their narrow therapeutic index has stimulated a wide search for alternatives. The first, amrinone, was reported in 1979.[192]

Primary inotropic drugs (Fig. 23-29) include the following:

- Cardiac steroids, such as digoxin, that act as Na^+, K^+–ATPase inhibitors. These steroids are also widely used to treat atrial fibrillation and flutter.
- Phosphodiesterase inhibitors, such as amrinone and milrinone. Toxicity has limited these drugs' clinical use, although both have been marketed.
- Drugs, such as sulmazole, that increase the Ca^{2+} sensitivity of myocardial contractile proteins. None are marketed in the United States.
- Direct adenylate cyclase stimulants, such as forskolin—not available in the United States.
- Adrenergic agonists (see Chap. 16), such as dopamine, levodopa, dobutamine.
- Indirect adenylate cyclase stimulants (see Chap. 25), such as glucagon and histamine.

The inotropic drugs in Fig. 23-29 all act by affecting the availability of intracellular Ca^{2+} for myocardial contraction or by increasing the sensitivity of myocardial contractile proteins. In normal heart function, Ca^{2+} is essential for heart contraction. During each myocardial action potential, Ca^{2+} enters through slow Ca^{2+} channels, and, in turn, there is a large release of stored Ca^{2+} from sequestration sites on the sarcoplasmic reticulum. The Ca^{2+} binds to troponin-C, and actin and myosin can interact to result in a contraction. (The mechanism involves other myofilament subunits as reviewed by Colucci et al.[193,194])

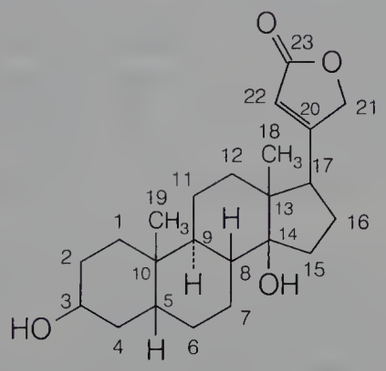
TREATMENT OF HEART FAILURE

Treatment of heart failure is complex (the reviews of Hsu,[195] Kelly and Smith,[196] and Thomas[197] are especially recommended for further study). An overall program includes increasing myocardial contraction, decreasing sodium and water retention, and facilitating ventricular relaxation. Lifestyle modifications (exercise, reduction of dietary sodium, abstaining from alcohol), monitoring weight and symptoms of disease, and compliance with medications all are important.

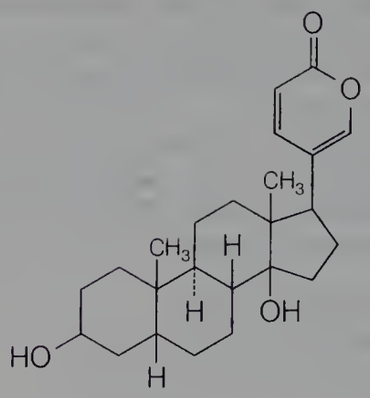
Diuretics reduce peripheral edema for the heart failure patient, as well as pulmonary congestion.

Angiotensin-converting enzyme (ACE) inhibitors taken with diuretics and digoxin have been shown to increase the efficiency of the heart, decrease the progression of the disease, increase the patient's tolerance to exercise, and reduce mortality. The ACE inhibitors have many effects that help reduce preload and afterload in heart failure.

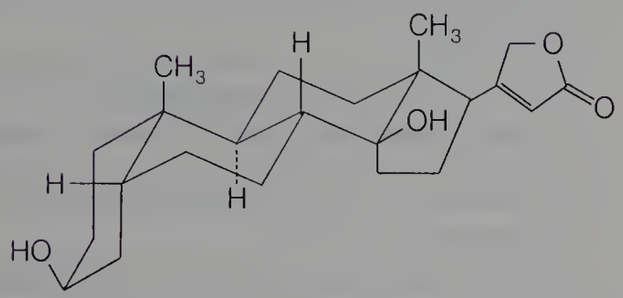
a. The Cardenolides and Bufadienolides (Na^+,K^+– ATPase inhibitors)

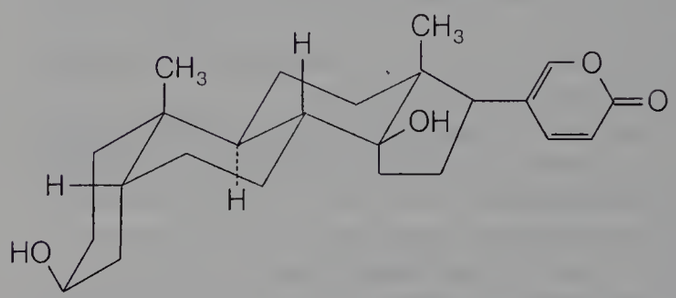


Digitoxigenin
(Cardenolide Prototype)
(Also in commercially
available products)

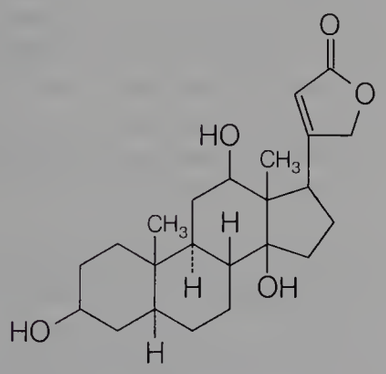


Bufalin
(Bufadienolide Prototype)

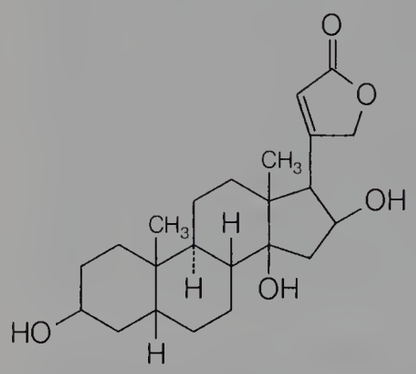




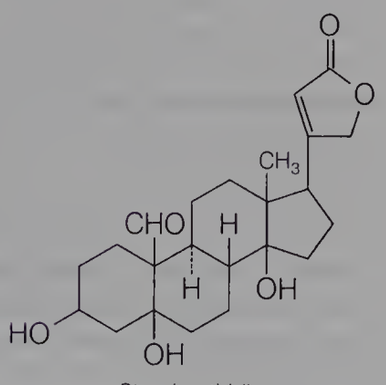
Cardenolide Aglycones
in Commercially Available Products



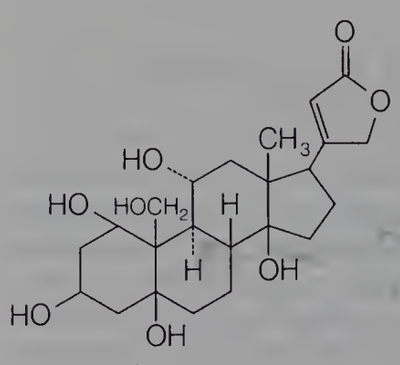
Digoxigenin



Gitoxigenin



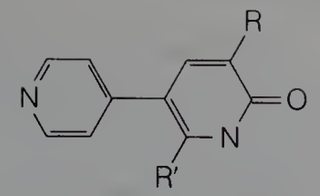
Strophanthidin



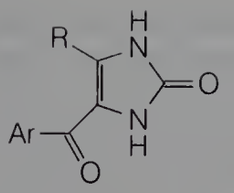
Ouabagenin

FIG. 23-29. *Cardiac steroids and other inotropic drugs. The cardiac steroids are also used to treat atrial fibrillation and flutter (discussed in Chap. 19).*

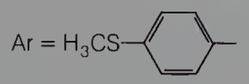
b. Phosphodiesterase inhibitors



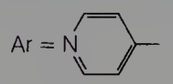
Amrinone R = NH_2, R′ = H
Milrinone R = CN, R′ = CH_3

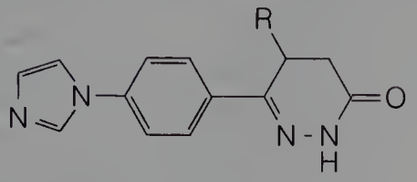


Enoximone R = CH_3



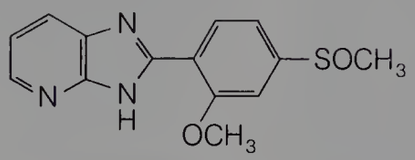
Piroximone R = CH_2CH_3



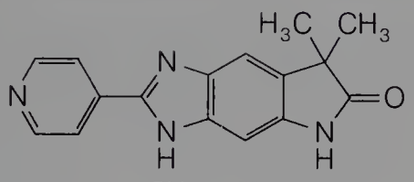


Imazodan R = H
CI-930 R = CH_3

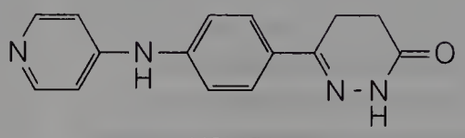
c. Drugs that increase Ca^{2+} sensitivity of myocardial contractile proteins



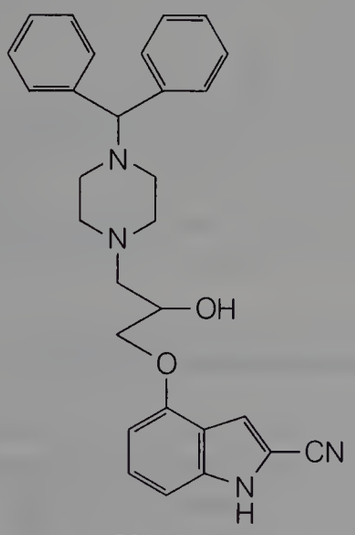
Sulmazole (AR-L 115)



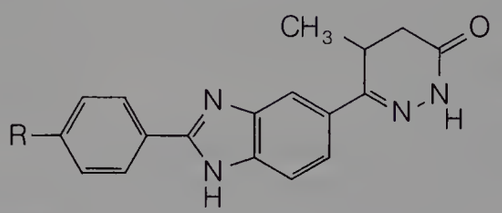
BM 14.478



MCI-154



DPI 201-106



R = OCH_3 Pimobendan
R = OH UD-CG 212

d. Adenylate cyclase stimulants

Forskolin

FIG. 23-29. *Continued.*

Increasing the force of contraction of the heart (inotropic activity), usually with cardiac steroids, is very important for most heart failure patients. (This chapter focuses on these inotropic drugs.) The phosphodiesterase inhibitors (milrinone and amrinone) have been shown to be inotropic, but their toxicity has limited their use to short-term applications. Other inotropic agents include adrenergic agonists (discussed in Chap. 16); although catecholamines have inotropic actions, their pharmacologic effects are too nonspecific and short in duration to be generally useful in treating heart failure.

CARDIAC STEROIDS

Poison and heart tonic—these are the ''split personalities'' of the cardiac steroids that have troubled and fascinated physicians and chemists for several centuries. Plants containing the cardiac steroids have been used as poisons and heart drugs at least since 1500 B.C., with squill appearing in the Ebers Papyrus of ancient Egypt. Throughout history these plants or their extracts have variously been used as arrow poisons, emetics, diuretics, and heart tonics. Toad skins containing cardiac steroids have been used as arrow poisons and even as aids for treating toothaches and as diuretics.

The poison–heart tonic dichotomy continues even today. Cardiac steroids are widely used in the modern treatment of congestive heart failure and for treatment of atrial fibrillation and flutter. Nevertheless, their toxicity remains a serious problem, although advances in biopharmaceutics have reduced the incidence of life-threatening overdoses.

Na^+, K^+–ATPase INHIBITION

The cardiac steroids inhibit Na^+, K^+–ATPase (sodium- and potassium-dependent ATPase), the enzyme that catalyzes the ''sodium pump'' (Fig. 23-30).[40,41,195,197] Its amino acid sequence was determined for the first time in 1985,[28] with the cardiac glycoside-binding site located in 1983,[198] and studied intensively by Repke,[27] Thomas,[197] and co-workers. This enzyme, by essentially being the sodium pump, maintains unequal distribution of Na^+ and K^+ ions across the cell membrane.

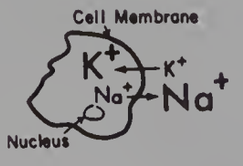


FIG. 23-30. *Movements of ions with the sodium pump. Sodium- and potassium-dependent ATPase catalyzes the pump. The hydrolysis of ATP to ADP provides the needed energy to move the ions against concentration gradients.*

Although the ''pump'' operates in all cells, it performs a critical function in heart contraction. During each contraction, there is an influx of Na^+ and an outflow of K^+. Before the next contraction, Na^+, K^+–ATPase must reestablish the concentration gradient, pumping Na^+ into the cell against a concentration gradient. This process requires energy, and the energy is obtained from hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) by Na^+, K^+–ATPase.

The cardiac steroids inhibit Na^+, K^+–ATPase. The reviews by Smith[40,196] and Thomas[197] are especially recommended for further reading about the cardiac glycosides' actions on Na^+, K^+–ATPase and their clinical uses. Inhibiting all molecules of Na^+, K^+–ATPase in the sarcolemmal membrane would be fatal, because there would be no way for the heart to reestablish the Na^+ and K^+ concentration gradients across the membrane. However, partial inhibition results in a small net increase in intracellular Na^+. Less than a usual amount of Na^+ is pumped out; consequently, a higher than normal amount of Na^+ reìains.

As discussed in Chap. 19, several mechanisms regulate intracellular Ca^{2+}. The blocking of slow Ca^{2+} channels is the basis of the antiarrhythmic and antiangina actions of verapamil and related drugs (Chap. 19). For the cardiac steroids, the sodium–calcium exchanger is most important. When intracellular Na^+ increases in response to partially inhibited sarcolemmal sodium pumps, the sodium–calcium exchanger exhanges three Na^+ for each Ca^{2+}, with a net overall influx of Ca^{2+} across the sarcolemmal membrane. The increased Ca^{2+} triggers the contractile proteins, with a resulting inotropic action.

Inhibition of the sodium pump may result in additional effects that increase the availability of Ca^{2+} for heart contraction. As reviewed by Smith,[40,196] additional Ca^{2+} may enter through slow Ca^{2+} channels. The sodium–hydrogen exchange system may also facilitate additional Na^+ to be transported intracellularly, with increased Ca^{2+} resulting as well.

Phosphodiesterase Inhibitors

In normal heart function, cyclic adenosine monophosphate (cAMP) performs important roles in regulating intracellular Ca^{2+}. Slow Ca^{2+} channels and storage sites for Ca^{2+} on the sarcoplasmic reticulum must be activated by cAMP-dependent protein kinases to result in (1) increased Ca^{2+} influx through the channels and (2) increased release and faster reaccumulation by the sarcoplasmic reticulum. If cAMP is increased by inhibiting its breakdown by phosphodiesterase to AMP, more Ca^{2+} becomes available for cardiac contraction. Drugs such as amrinone and milrinone exert their inotropic actions in this way. Specifically, they inhibit phosphodiesterase F-III.

Unfortunately, amrinone and milrinone increase mortality in patients with heart failure; as a result, these drugs are used only for short-term treatment of patients with especially severe heart failure.

Drugs That Increase the Calcium Ion Sensitivity of Myocardial Contractile Proteins

Following the 1979 report[192] of amrinone's inotropic action, there was an enormous effort made throughout the world to find other nonsteroidal inotropic agents. A number of agents (see Fig. 23-29) were first thought to act pharmacologically as phosphodiesterase inhibitors, but a closer examination has revealed that the inotropic effect requires less drug than for phosphodiesterase inhibition. These drugs appear to increase the effect of existing Ca^{2+} levels, thereby resulting in an inotropic effect. DPI 201-106 has no phosphodiesterase-inhibiting activity at all. It may prolong the opening of Na^+ channels.[199]

Van Meel and co-workers[200] have found, for example, that the (+) and (−) stereoisomers of sulmazole have equivalent vasodilatation and phosphodiesterase-inhibiting potencies. However, the (+) isomer has good inotropic and Ca^{2+} sensitizing activity; the (−) isomer has weak activity for both.

Direct Adenylate Cyclase Stimulants: Forskolin

The diterpene forskolin (see Fig. 23-29) directly stimulates adenylate cyclase or a closely related protein.[194,201–203] The cAMP levels increase in the myocardium, with resulting activation of protein kinases and increases in intracellular Ca^{2+}, as with the phosphodiesterase inhibitors. Vasodilatation also results. In addition, Hoshi and co-workers[204] have reported that forskolin has a direct effect on voltage-dependent K^+ channels, independent of cAMP activation. Wagoner and Pallotta[205] have also reported that some of forskolin's physiologic effects are not mediated by cAMP.

STRUCTURAL CLASSES

As noted in the previous section, cardiac steroids are all Na^+, K^+–ATPase inhibitors. Thus, the classification and discussion that follow use the pharmacologic classification shown in Fig. 23-29.

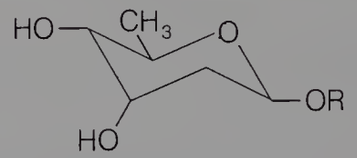
Cardiac Steroids

The cardiac steroids actually include two groups of compounds—the cardenolides and the bufadienolides. The cardenolides, illustrated by digitoxigenin in Fig. 23-29, have an unsaturated butyrolactone ring at C-17, whereas the bufadienolides have an α-pyrone ring. Both have essentially identical pharmacologic profiles and are found in a variety of plant species. The bufadienolides are commonly called "toad poisons" because several are found in the skin secretions of various toad species. Use of the toads as a source of cardiac steroid would quickly result in their extinction; therefore, by far the most historically and commercially important sources of cardiac steroids have been two species of *Digitalis*: *D. purpurea* and *D. lanata*. Whole-leaf digitalis preparations appeared in the *London Pharmacopeia* in the 1500s, but inconsistent results and common fatalities caused their removal. In 1785, William Withering published his classic, *An Account of the Foxglove and Its Medical Uses,* noting that digitalis could be used to treat cardiac insufficiency with its associated dropsy (edema). (The Withering story has been beautifully told by Witzmann[1] and by Skou.[206,207] Nevertheless, it was not until the early 1900s that digitalis and the purified glycosides were commonly used for the treatment of heart disease.

The 5β,14β-stereochemistry of the cardenolides and bufadienolides gives the molecules an interesting shape, caused by the resulting A/B *cis* and C/D *cis* ring junctures. This stereochemistry appears to be an important prerequisite for some, but possibly not all, cardiac steroid activity. This will be discussed later in this section.

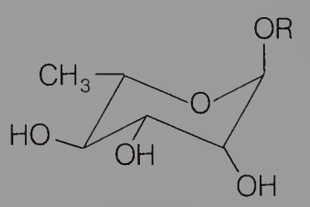
The cardiac steroids are usually found in nature as the corresponding 3β-glycosides. One to four sugars are added to the steroid 3β-hydroxyl to form the glycoside structure. Although hundreds of cardiac steroid glycosides have been found in nature, relatively few different cardenolide or bufadienolide aglycones have been found. For example, as shown in Table 23-9, only three make up the digitalis glycosides. (The substance that forms a glycoside with a sugar is called the aglycone. Thus, the cardenolides and bufadienolides are the *aglycones* or *genins* of the *cardiac glycosides*.) The structure of a representative cardenolide glycoside, lanatoside C, is shown in Fig. 23-31.

The hydrolysis products of other naturally occurring cardiac glycosides are shown in Table 23-9. The two most commonly found glycosides are β-D-digitoxosides (including digoxin, digitoxin, gitoxin) and α-L-rhamnoside (ouabain).



β-D-Digitoxoside

The sugars found as part of the cardiac glycosides are illustrated in Fig. 23-32. As shown in Fig. 23-32, the sugars may



α-L-Rhamnoside
(R = Steroid Genin)

be removed by enzymatic-, acid-, or base-catalyzed hydrolysis. A wide variety of other sugars have been used to synthesize new cardiac glycosides (discussed later in this chapter).

TABLE 23-9

CARDIAC GLYCOSIDES AND HYDROLYSIS PRODUCTS FROM COMMON SOURCES

Structure	*Name*
FROM *DIGITALIS PURPUREA* LEAF	
Glucose-digitoxose$_3$-digitoxigenin	Purpurea glycoside A
Digitoxose$_3$-digitoxigenin	Digitoxin
Glucose-digitoxose$_3$-ditoxigenin	Purpurea glycoside B
Digitoxose$_3$-gitoxigenin	Gitoxin
FROM *DIGITALIS LANATA* LEAF	
Glucose-3-acetyldigitoxose-digitoxose$_2$-digitoxigenin	Lanatoside A
Glucose-digitoxose$_3$-digitoxigenin	Desacetyl lanatoside A (same as purpurea glycoside A)
3-Acetyldigitoxose-digitoxose$_2$-digitoxigenin	Acetyldigitoxin
Digitoxose$_3$-digitoxigenin	Digitoxin
Glucose-3-acetyldigitoxose-digitoxose$_2$-gitoxigenin	Lanatoside B
Glucose-digitoxose$_3$-gitoxigenin	Desacetyl lanatoside B (same as purpurea glycoside B)
3-Acetyldigitoxose-digitoxose$_2$-gitoxigenin	Acetylgitoxin
Digitoxose$_3$-gitoxigenin	Gitoxin
Glucose-3-acetyldigitoxose-digitoxose$_2$-digoxigenin	Lanatoside C
Glucose-digitoxose$_3$-digoxigenin	Desacetyl lanatoside C
3-Acetyldigitoxose-digitoxose$_2$-digoxigenin	Acetyldigoxin
Digitoxose$_3$-digoxigenin	Digoxin
FROM *STROPHANTHUS GRATUS* SEED	
Rhamnose Ouabagenin	Ouabain

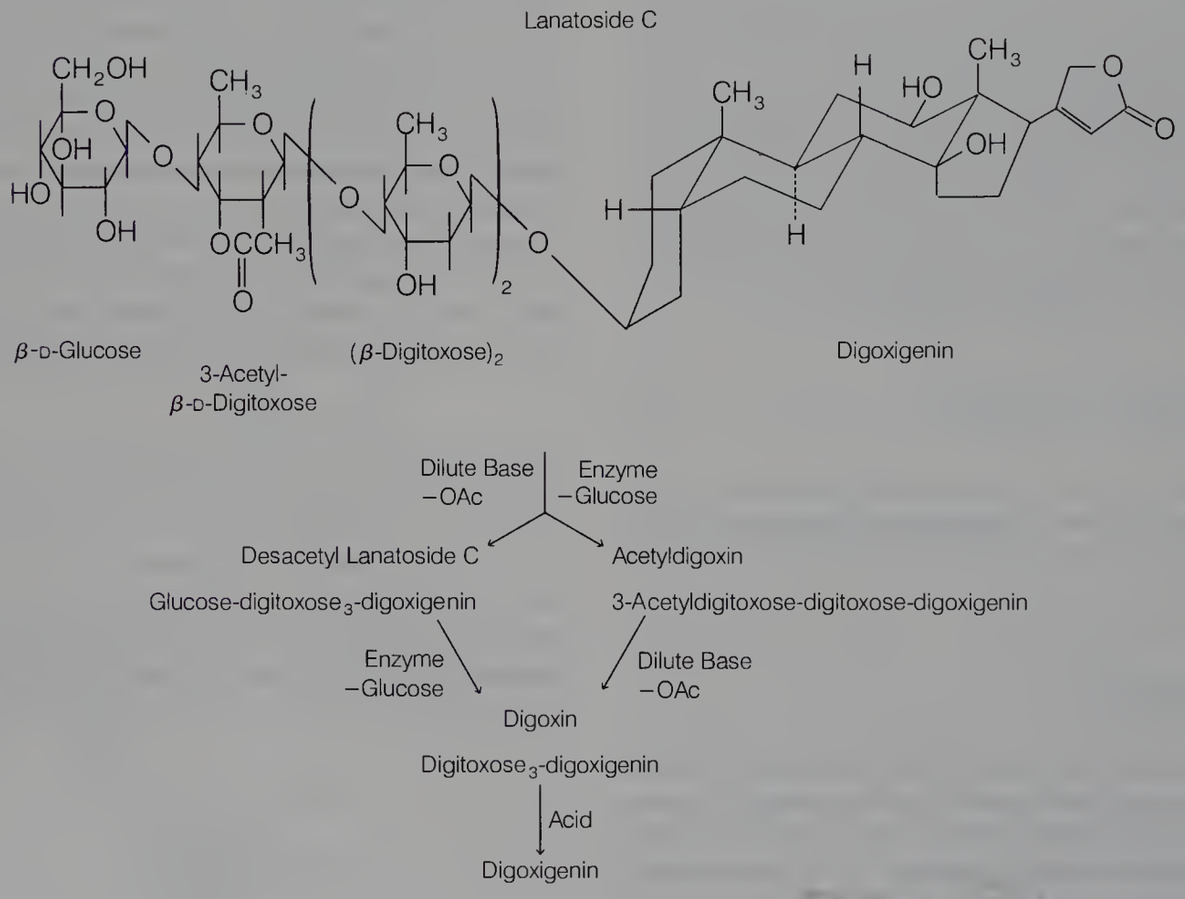


FIG. 23-31. *Selective hydrolysis of naturally occurring cardiac glycosides—a representative example. The sugars are exaggerated in size to clearly show their structure.*

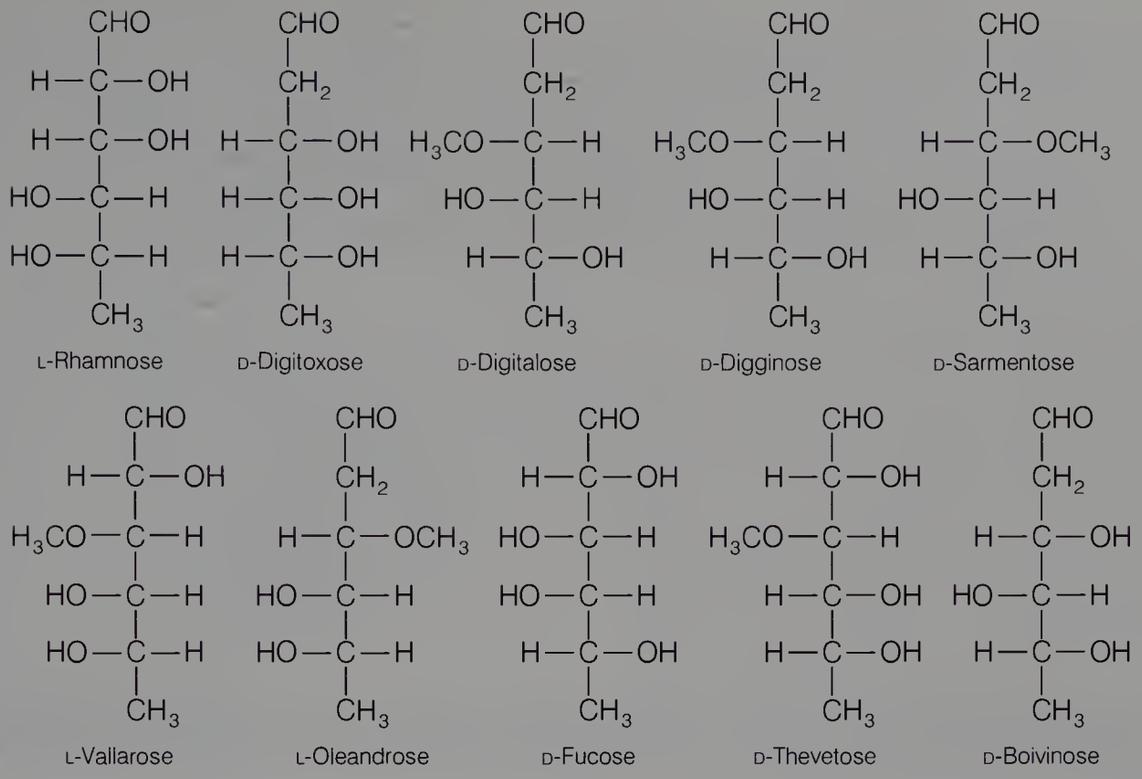


FIG. 23-32. *Sugars found in naturally occurring cardiac glycosides.*

MODELING THE CARDIAC GLYCOSIDE RECEPTOR

The ultimate structure–activity questions to answer with any class of drugs are: (1) How does the drug fit into the receptor? (2) What structures and conformations permit the best fit? and (3) Which parts of the drug molecule are responsible for binding (affinity) and for the pharmacologic response (intrinsic activity)?

Nevertheless, a great amount of research and speculation has appeared in the literature. A combination of drug design, synthesis, conformational energy studies, and computer graphics have been used to indirectly learn about the structural requirements of the cardiac glycoside binding site. This process is called modeling the receptor and is used with many other drugs besides the cardiac steroids. The 1995 and 1996 reviews of Thomas[197] and Repke et al.[27] are recommended for further reading.

Na^+, K^+–ATPase is a dimer made up of two catalytic α subunits and two inert β subunits. The α subunits contain binding sites for cardiac glycosides, ATP, Na^+, K^+, and phosphorylation. The two β subunits are needed for activity, but apparently are not directly catalytic. The β subunits may hold the α subunits in an active conformation, but their exact purpose is as yet unknown. The Na^+, K^+–ATPase enzyme spans through the plasma membrane, with most of the enzyme on the extracellular cytoplasmic surface. Several investigators have proposed how the subunits of the enzyme loop back and forth through the cell membrane.

Electron microscopy and other studies suggest that the α dimer forms a deep cleft in the Na^+, K^+–ATPase. The cleft has been most often proposed as the binding site for cardiac steroid glycosides.

Conformational Flexibility of Cardiac Glycosides

As shown with digoxin in Fig. 23-33, cardiac glycosides are not rigid molecules. With these drugs, therefore, the concept of structure–activity study must include a consideration of conformation. If a newly synthesized analogue is active (or inactive), is the cause of its (in)activity its new structure, or conformations (im)possible at the receptor site?

Two regions of conformational flexibility are the bond connecting the C-17 side group of the steroid ring D, and the two bonds connecting C-3 to the sugar (see Fig. 23-33). The C-17 side group can rotate, but as shown in Fig. 23-33, some conformations are very high in energy. Because cardiac glycosides are uncharged, and the forces that hold them to the receptor are therefore relatively weak, high-energy conformations are very unlikely at the receptor site. In short, low-energy conformations in Fig. 23-34 are the most likely. A similar situation has been found for sugar conformations.[20,22,208,209] Very few combinations of the two bonds that connect the steroid to the glycoside sugar result in conformations that are likely to exist in appreciable quantity at the receptor site.

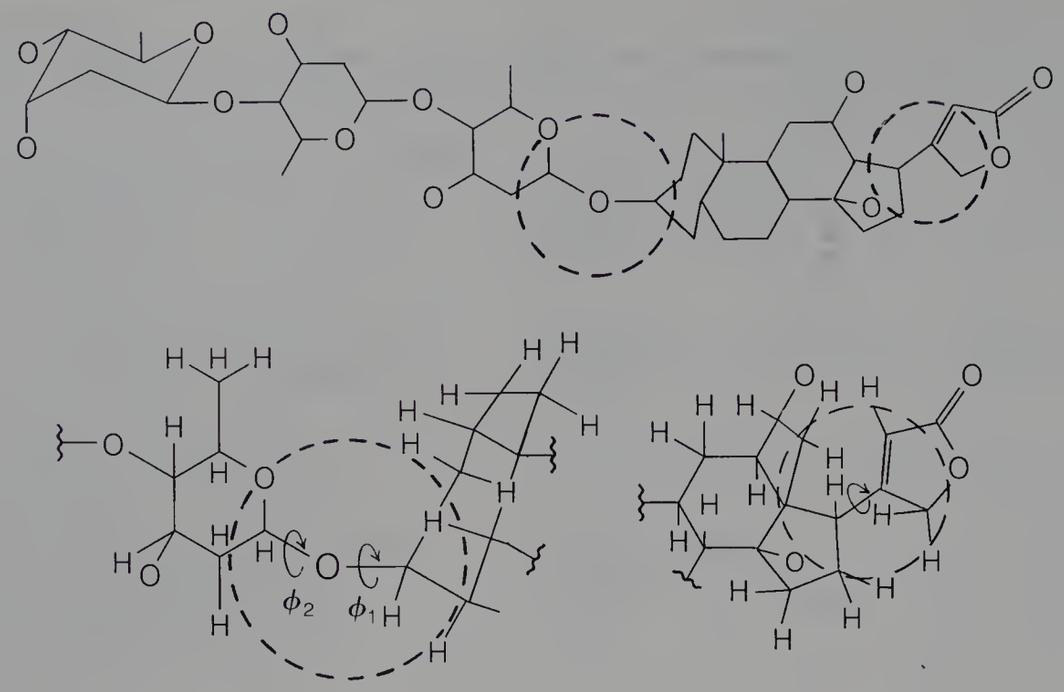


FIG. 23-33. Digoxin. Enlargements show the regions of particular interest in modeling the cardiac glycoside receptor site. The two primary regions of flexibility important to biologic activity are rotation about the two glycosidic bonds (C1′—O3 and O3—C3) and rotation of the C-17 side group. (From Fullerton and co-workers.[20] Used with permission.)

Roles of the Genin and C-17 Side Group

Many often-conflicting models have been proposed over the last two decades to describe structural and geometric features that govern the ability of a particular digitalis genin (cardenolide) or bufalin genin (bufadienolide) to inhibit Na^+, K^+–ATPase (or cause an inotropic response). (The reviews of Thomas,[197] Dittrich et al.,[30] Schonfeld et al.,[210,211] Guntert and Linde,[212] Erdman,[213] and Flasch and Heinz[214] provide a variety of viewpoints.) Since hydrogenation of the side group carbon–carbon double bond was known to decrease activity significantly, an active role in Na^+, K^+–ATPase inhibition was generally envisioned for this double bond. (C-20 also changes from sp^2 to sp^3, greatly moving the side group carbonyl.)

Fullerton, Rohrer, Ahmed, From, and co-workers found several genins that they synthesized and tested did not fit these models.[20,22,215] The most stable conformations (from potential energy diagrams like those in Fig. 23-34) of each of a variety of genins were graphically superimposed with the cardenolide prototype, digitoxigenin. An example is shown in Fig. 23-35 to measure distances between each superimposed analogue and the corresponding atoms in digitoxigenin. Fullerton et al. found that the position of a particular genin's carbonyl oxygen (or nitrile nitrogen) relative to digitoxigenin's was a nearly perfect predictor of its activity (Fig. 23-36).

$$\log I_{50} = 0.44D - 6.36\ r^2 = 0.98$$

where I_{50} = amount of genin required to inhibit 50% of a standard guinea pig brain Na^+, K^+–ATPase preparation in vitro; D equals the distance between carbonyl oxygens of the analogue and digitoxigenin in angstroms. This relationship means that for every 2.2 Å the carbonyl oxygen (or nitrile N, for one compound) moves, activity changes by one order of magnitude (10 or 0.1). The r^2 is the statistical correlation. (Perfect would be 1.00.) As shown in Fig. 23-36, a corre-

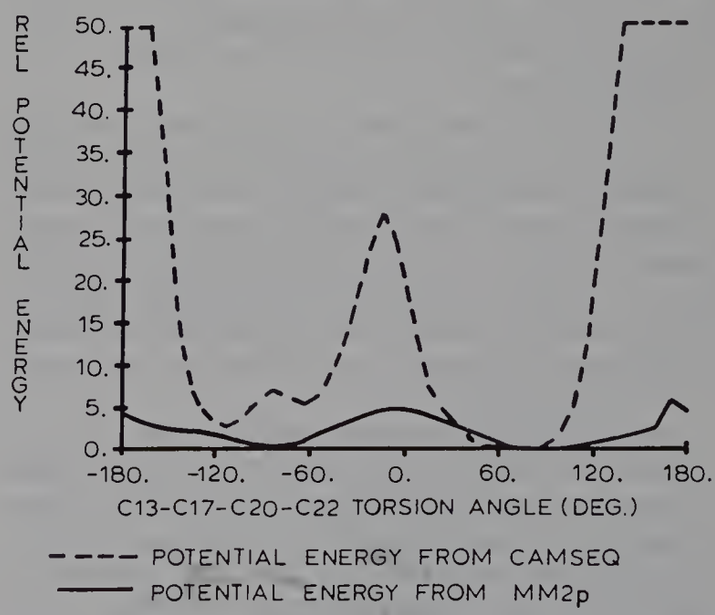


FIG. 23-34. A comparison of the energy calculated at 10° intervals for rotation about the digitoxigenin C17-C20 bond using two different computer programs: a rigid hard sphere model (CAMSEQ; dashed line) and a model in which bond distances and bond angles are allowed to relax at each interval (MM2p; solid line). (From Fullerton and co-workers.[20] Used with permission.)

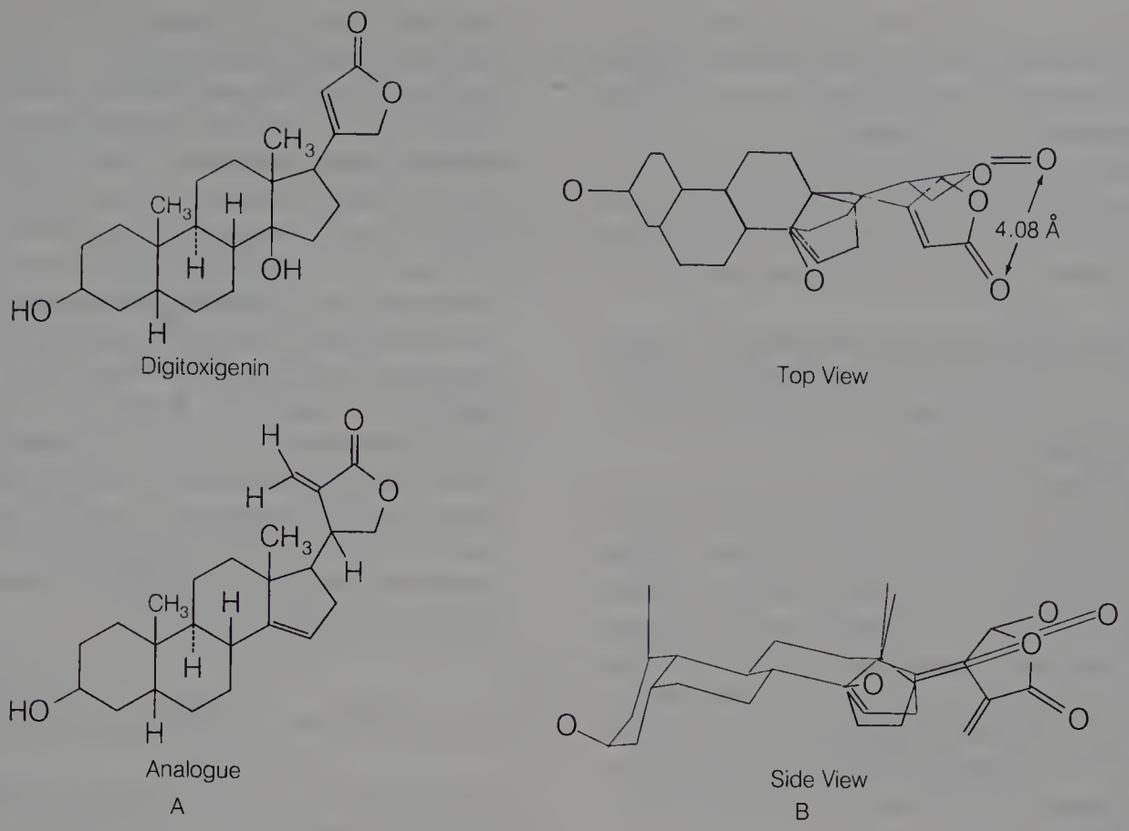


FIG. 23-35. *(A) Digitoxigenin and its 14-ene, 22-methylene analogue. (B) Computer graphics superposition of digitoxigenin and its analogue, both in their most stable C-17 side group conformations, showing distance between carbonyl oxygens; as shown in Fig. 23-36, as the distance between carbonyl oxygens increases, activity (NA^+, K^+-ATPase inhibition) decreases. With the superimposed pair above, it is 4.08 Å. (For additional drawings and discussion, see Fullerton, Rohrer, et al.*[20,22,29]

sponding relationship was found for genins tested in a cat heart Na^+, K^+–ATPase system.

The genins graphed in Fig. 23-36 included bufalin, cardenolide analogues, with and without a 14β-OH, with acyclic side groups such as esters and a nitrile, and also included

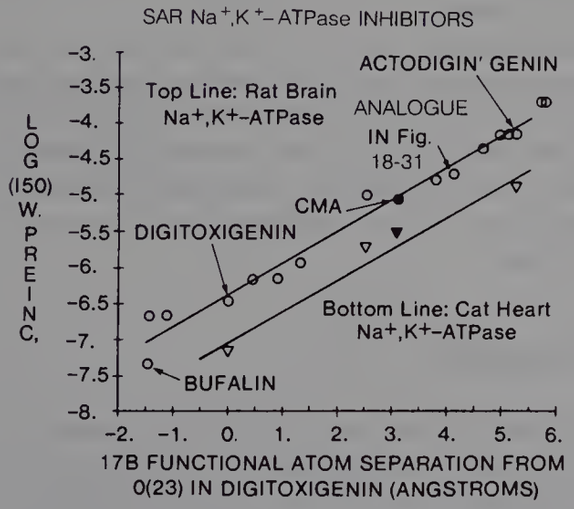


FIG. 23-36. *Relationship of cardiac-steroid-genin-carbonyl-oxygen position to inhibition of NA^+, K^+-ATPase. Circles: genins studied with rat brain NA^+, K^+-ATPase. Triangles: genins studied with cat heart NA^+, K^+-ATPase. Triangles directly under the circles represent the same genin. CMA, chlormadinone acetate. (Data from Fullerton, Ahmed, and co-workers.*[215,216])

the progesterone derivative chlormadinone acetate (CMA). From these studies the following conclusions may be made about digitalis genin–structure–activity relationships.

A-B-C-D Ring System. Both cardenolide and bufadienolide or pregnene ring systems appear to be able to fit the "digitalis" (ouabain) binding site on Na^+, K^+–ATPase. The activity-determining requirement appears to be primarily that the side group carbonyl oxygen (or nitrile *N*) be in the right spot relative to the A-B-C-D rings for maximal activity. It is not yet known what structural feature on Na^+, K^+–ATPase causes this requirement.

14β-Hydroxy. The 14β-OH is not necessary for activity. Some compounds that did not have a 14β-OH have been more active than otherwise identical genins that did. It appears that earlier studies with 14-dehydro analogues may have overlooked the stereochemical change in C-14 changing from sp^3 (with a 14β-OH) to sp^2 (with a double bond).

16β-Hydroxy. *Gitaloxigenin* (gitoxigenin 16β-formate) and its tridigitoxoside *gitaloxin* are naturally occurring cardenolide 16β-formates found in *D. purpurea*.[217] Gitaloxin has even been called the "forgotten" cardiac glycoside of *Digitalis* that may be responsible for most of the plant extract's therapeutic activity.[218] Fullerton, Griffin, and coworkers[219,220] synthesized 17 gitoxigenin 16β-formates, acetates, and methoxycarbonates to learn more about the role of the 16β-OH and 16β-OCHO (formate). A 16β-formate

increased the activity 30 times, and a 16β-acetate 9 to 12 times. The increased activity was not the result of modified carbonyl oxygen position, which suggests the possibility of a separate binding site for C-16 esters.

Lactone Ring. The lactone ring is not necessary, as first shown by Thomas[197] (see, for example, Fig. 23-37). Templeton and co-workers[221] have also identified pregnanes (without C17-lactone rings) with good binding to Na^+, K^+–ATPase.

Side Group Carbon–Carbon Double Bond. It appears that this double bond may just be keeping the side group's carbonyl oxygen in the right place for most efficient Na^+, K^+–ATPase inhibition.

"Lead Structure." Schonfeld and associates[210,211] and Dittrich and co-workers[30] have intensively studied binding energies for cardiac steroids at the Na^+, K^+–ATPase receptor site. They also have studied a variety of other compounds such as cassaine (see Fig. 23-37) that, to varying degrees, appear to fit in the same location on the Na^+, K^+–ATPase. From these studies, these investigators conclude that 5β, 14β-androstane-3β,14β-diol, the steroid nucleus of the naturally occurring cardenolides and bufadienolides, is the minimum structure needed to inhibit Na^+, K^+–ATPase and cause a measurable response.

Roles of the Sugar

The roles of cardiac sugar structure have been intensively studied,[20,29,208,219–229] including the importance of sugar stereochemistry and conformation. Yoda[225,226] was the first to show that there is a separate site for sugar binding on Na^+, K^+–ATPase.

From these many studies, a model of Na^+, K^+–ATPase sugar site-binding requirements has begun to emerge.

1. Sugar site binding is not necessary for good activity. Cardiac genins are usually very active. The I_{50} for digitoxigenin, for example, is about 1.2×10^{-7} M—a very potent drug indeed. Adding a single β-D-digitoxose increases activity about 18 times ($I_{50} = 6.6 \times 10^{-9}$ M). Digitoxin, with three β-D-digitoxose sugars, is about 10 to 12 times more active than digitoxigenin. However, sugars certainly have a significant impact on the pharmacokinetic profiles of cardiac glycosides, affecting both their distribution and speed of elimination (Table 23-10).
2. Stereochemistry of sugar OH groups is important. The stereochemistry of the sugar 4′-OH is especially critical. As shown in Fig. 23-38A, the β-D-glucoside of digitoxigenin is over five times more active than the β-D-galactoside.
3. Changing sugar stereochemistry changes activity. For example, the four possible glycosides of glucose (see Fig. 23-38B) all have different activities. A similar dependence on stereochemistry has been seen with mannosides and other glycosides.[222]
4. With β-L-sugars, an equatorial 4′-OH group is the only sugar OH needed for good activity. As shown in Fig. 23-38C, the α-L-dideoxyrhamnoside of digitoxigenin is as active as the α-L-rhamnoside (with two more sugars), and much more active than the α-L-glucoside and α-L-mannoside (with three more sugars).[227,228]
5. With α-L- and β-D-sugars, an equatorial 4′-OH group is not enough; other OH groups help in sugar site binding. For example, the β-L-dideoxyrhamnoside of digitoxigenin is only three times more active than digitoxigenin; the β-L-rhamnoside is 25 times more active.[227,228]
6. Binding is enhanced by 5′-CH_3 and reduced by 5′-CH_2OH. As shown in Fig. 23-38C, adding an OH group to the 5′-CH_3 of rhamnose (thereby forming mannose) reduces activity significantly. It has been suggested that there is a hydrophobic-binding location for the CH_3 or, alternatively, that the additional OH introduces steric hindrance that diminishes binding.

STRUCTURE AND PARTITION COEFFICIENT

As shown in Table 23-10, commercially available cardiac steroids differ markedly in their degree of absorption, half-life, and time to maximal effect. Usually, this is due to polarity differences caused by the number of sugars at C-3 and the presence of additional hydroxyls on the cardenolide.

Nevertheless, it has always been difficult to visualize how apparently minor structural variations can cause major differences in partition coefficient and absorption. For example, lanatoside C and digoxin differ only by the presence of an additional sugar in lanatoside C (see Table 23-9). One might expect that "one more sugar should not make much difference." However, the $CHCl_3$/16% aqueous MeOH partition coefficients for the compounds are very different indeed: 16.2 for lanatoside C, 81.5 for digoxin, 96.5 for digitoxin, and 10 for gitoxin.

The compounds with increased lipid solubility also are the slowest to be excreted. Additional hydroxyl groups in the more polar compounds provide additional sites for conjugate formation and other metabolic processes. In addition, it is commonly known that more lipid-soluble drugs tend to be excreted more slowly because of increased accumulation in lipid tissues.

METABOLISM

The cardiac glycosides are metabolized to a variety of products. However, with digoxin, a relatively small amount is actually metabolized. Reduction of the C-20(22) double bond results in 20,22-dihydro derivatives with about 1/100 the activity of the original glycosides (because of C-20 changing from sp^2 to sp^3 and thereby moving the carbonyl oxygen). Hydrolysis of digitoxose sugars also occurs, to result in a mixture of bisdigitoxosides and digitoxosides. These

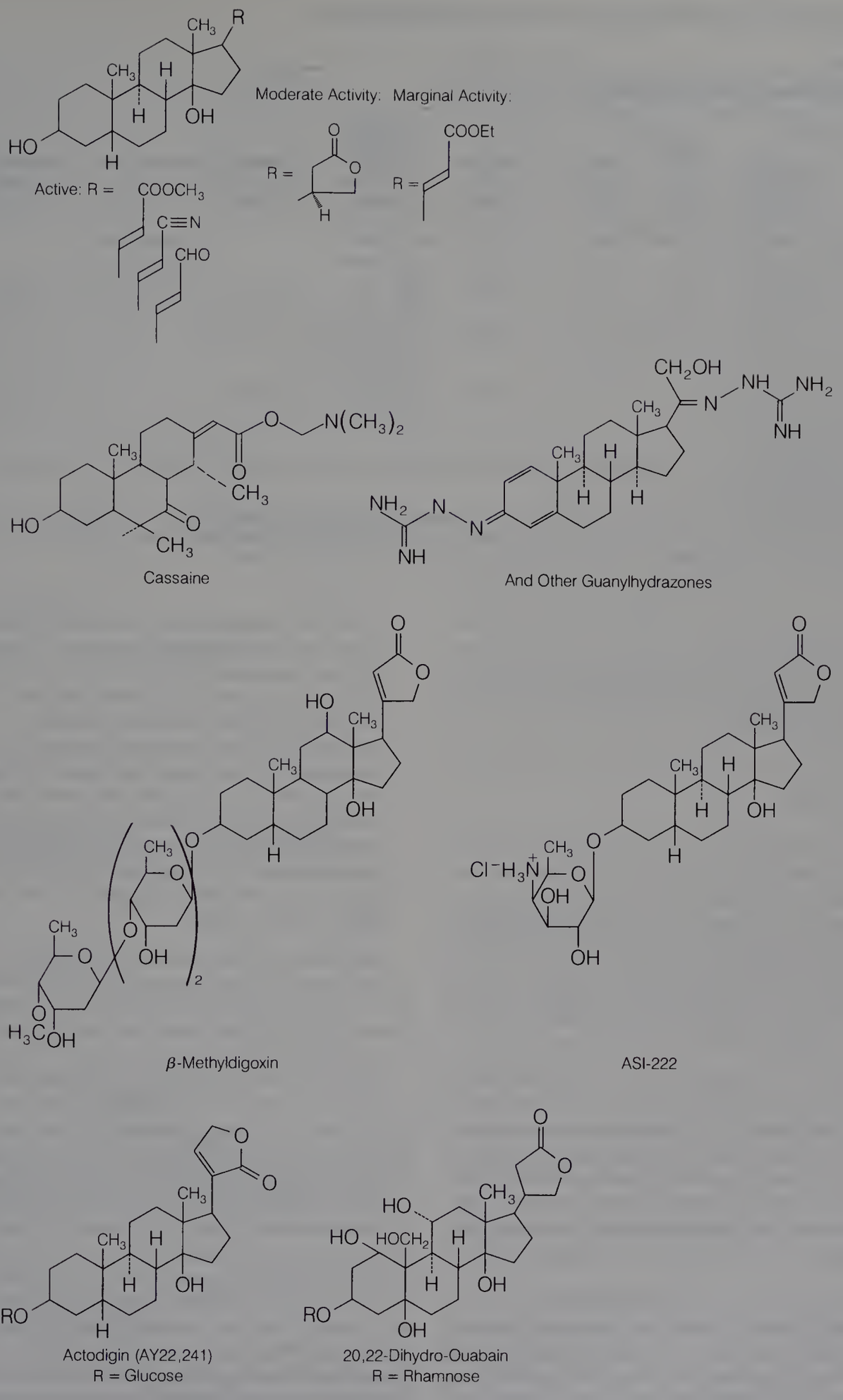


FIG. 23-37. *Cardenolides and other steroids with modified structures that inhibit NA^+, K^+-ATPase.*

TABLE 23-10

CARDIAC GLYCOSIDE PREPARATIONS

Agent	Gastrointestinal Absorption	Onset of Action* (min)	Peak Effect (hr)	Average Half-Life†	Principal Metabolic Route (Excretory) Pathway	Average Digitalizing Dose		Usual Daily Oral Maintenance Dose‖
						Oral‡	IV§	
Ouabain	Unreliable	5–10	0.5–2	21 hr	Renal; some GI excretion		0.3–0.5 mg	
Deslanoside	Unreliable	10–30	1–2	33 hr	Renal		0.8 mg	
Digoxin	55–75%	15–30	1.5–5	36 hr	Renal; some GI excretion	1.25–1.5 mg	0.75–1.0 mg	0.25–0.5 mg
Digitoxin	90–100%	25–120	4–12	4–6 days	Hepatic; renal excretion of metabolites	0.7–1.2 mg	1.0 mg	0.1 mg
Digitalis leaf	About 40%			4–6 days	Similar to digitoxin	0.8–1.2 g		0.1 g

* For intravenous dose.
† For normal subjects (prolonged by renal impairment with digoxin, ouabain, and deslanoside and probably by severe hepatic disease with digitoxin and digitalis leaf).
‡ Divided doses over 12–24 hr at intervals of 6–8 hr.
§ Given in increments for initial subcomplete digitalization; supplement with additional increments p.r.n.
‖ Average for adult patients without renal or hepatic involvement; varies widely among patients and requires close medical supervision.
(Table from Smith, T. W., and Haber, E. N. Engl. J. Med. 289:1063, 1973. Used by permission.)

reactions can also be catalyzed by bacteria in the gastrointestinal tract, one of the primary reasons why absorption can be quite variable among patients. Whereas 60% to 85% of oral tablets are absorbed, liquid-filled gelatin capsules can be 90% or more absorbed.

CLINICAL USE

The cardiac glycosides are used to treat congestive heart failure, atrial fibrillation, and atrial flutter. (Their use with heart arrhythmias has been discussed in Chapter 19.) As reviewed earlier in this chapter, not all patients with congestive heart failure, however, are appropriate candidates for cardiac glycoside (''digitalis'') therapy.

It is important that the pharmacist recognize common symptoms of cardiac glycoside toxicity (Table 23-11) and be familiar with basic principles of cardiac glycoside dosing.

1. A ''loading'' or ''digitalizing'' dose: The potent activity of the digitalis steroids combined with their potential for toxicity makes selection of individual doses more complicated than for almost any other drug. Most importantly, one must carefully consider the renal function of the patient, because much of the dose is excreted in the urine. In patients with normal renal function, the average half-life of digoxin is much shorter than digitoxin (see Table 23-10).
2. A maintenance dose: As with the loading dose (if used at all), the maintenance dose must be carefully tailored to each individual patient. Average doses must not be used without accounting for individual patient variables—kidney function, age, potential drug interactions, presence of heart, thyroid, or hepatic disease.
3. Avoiding or controlling drug interactions: Hypokalemia, such as that brought about by diuretics, or hyperkalemia can cause cardiac arrhythmias—arrhythmias that can also be caused by digitalis. Calcium and digitalis glycosides are synergistic in their actions on the heart.
   Many drugs can affect absorption of digitalis (e.g., cathartics and neomycin). Protein binding can be disturbed by coumarin anticoagulants, phenylbutazone, and some sulfonamides. However, these drug interactions are much less common than those involving potassium or calcium.
4. Prompt treatment of digitalis toxicity, if it occurs, by the physician: Digitalis therapy must be stopped until symptoms are under control.

Most important, digoxin immune Fab (Digibind, digoxin-specific antigen binding fragments) can rapidly reverse life-threatening digitalis glycoside intoxication. This antidote to digoxin toxicity is made from sheep that have been injected with digoxin and a carrier protein.

The antibody fragments' affinity for digoxin is higher than that of Na^+, K^+–ATPase. Since binding to Na^+, K^+–ATPase is reversible, the cardiac glycoside is removed from its binding site on the Na^+, K^+–ATPase and bound to the antibody. Digoxin immune Fab also has high affinity for digitoxin.

Digoxin immune Fab is given by intravenous infusion or by rapid intravenous injection for patients exhibiting severe digitalis-induced arrhythmias or hyperkalemia. The rapid withdrawal of digitalis from Na^+, K^+–ATPase, however, can result in low cardiac output, including congestive heart failure. Although allergic responses have generally not occurred, caution in using any ovine-based product with allergic patients is recommended. Skin testing for allergy to digoxin immune Fab should be considered with high-risk patients.

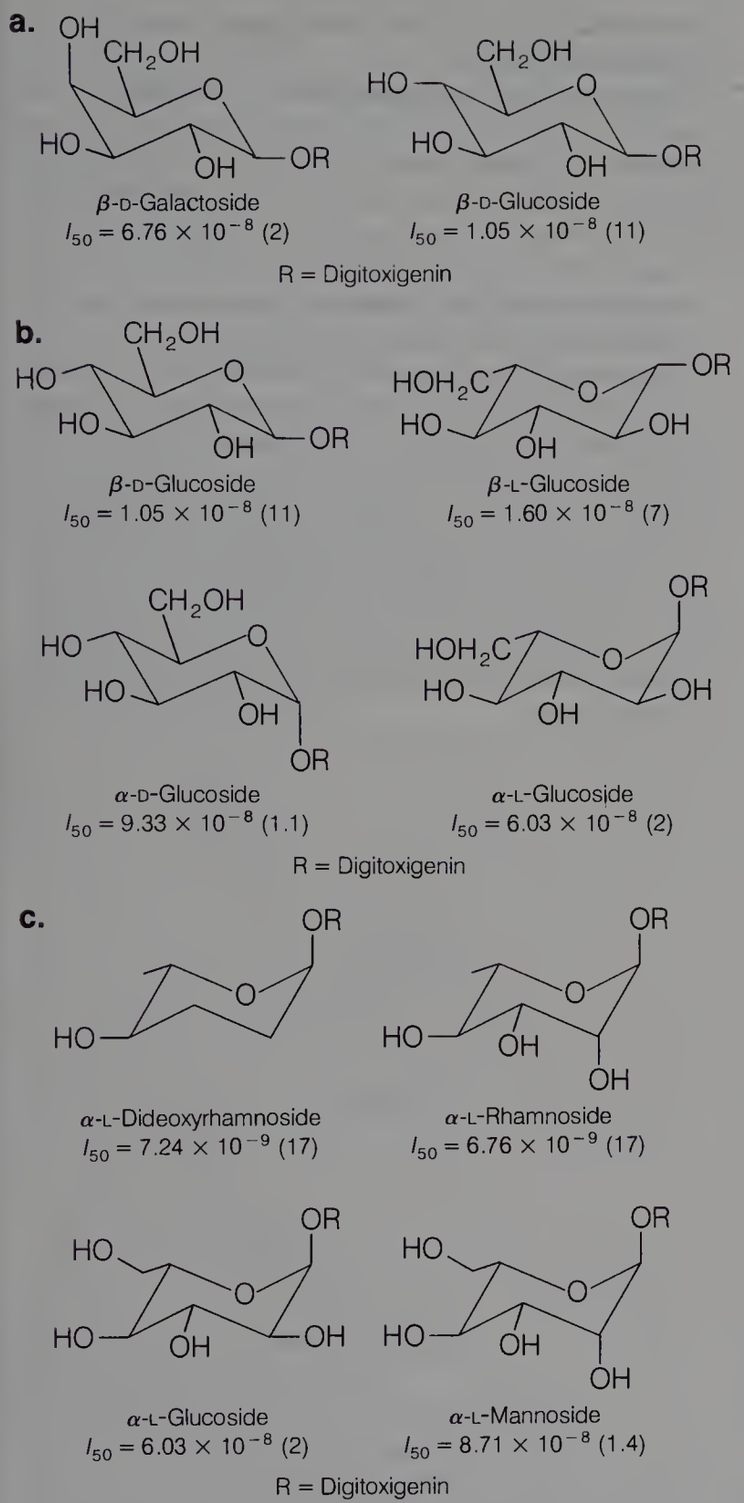
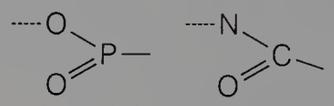


FIG. 23-38. *Na^+, K^+-ATPase inhibitory activities of digitoxigenin glycosides with varying sugars, where R = digitoxigenin. Data in parentheses are activities relative to digitoxigenin. All data from Fullerton, Ahmed, and co-workers.[227,228] Three sets of glycosides* **(a-c)** *illustrate structural relationships discussed in this section.*

MODELING THE PHOSPHODIESTERASE INHIBITOR RECEPTOR

Moos and co-workers[230] have synthesized and studied a variety of cAMP phosphodiesterase III inhibitors using x-ray crystallography, biologic studies, spectroscopy, molecular modeling, and electrostatic potential calculations. They have proposed that these inhibitors mimic the structural and electronic features of cAMP at the active site on phosphodiesterase III (PDE III). It appears that the pyridazone amide of the inhibitors mimics the 5′-phosphate of cAMP,



and that, if the inhibitor molecule is long enough, one of its heterocyclic rings can overlap adenine's 6-NH_2 (Fig. 23-39). Selective inhibition of PDE III depends upon the inhibitor having a relatively flat topography.

TABLE 23-11

NONCARDIAC SYMPTOMS OF DIGITALIS TOXICITY

Symptoms	*Frequency*	*Manifestations*
Gastrointestinal	Most common	Anorexia, nausea, vomiting, diarrhea, abdominal pain, constipation
Neurologic	Common	Headache, fatigue, insomnia, confusion, vertigo
	Uncommon	Neuralgias (especially trigeminal), convulsions, paresthesias, delirium, psychosis
Visual	Common	Color vision, usually green or yellow; colored halos around objects
	Uncommon	Blurring, shimmering vision
	Rare	Scotomata, micropsia, macropsia, amblyopias (temporary or permanent)
Miscellaneous	Rare	Allergic (urticaria, eosinophilia), idiosyncrasy, thrombocytopenia, gastrointestinal hemorrhage, and necrosis

(From Gerbino, P.P., Am. Hosp. Pharm. 30:499. 1973 Copyright 1973, American Society of Hospital Pharmacists, Inc. All rights reserved. Used with permission.)

PRODUCTS

Digitalis products are listed in Table 23-10, and the most important information on the digitalis steroids appears in Table 23-11. Structural differences are shown in Fig. 23-28 and Table 23-9. Any additional pertinent information is given in the following discussion. The previous sections on digitalis toxicity and therapy should be carefully studied as well.

Powdered digitalis, USP, is the dried, powdered leaf of *Digitalis purpurea.* When digitalis is prescribed, powdered digitalis is to be dispensed. One hundred milligrams is equivalent to one USP digitalis unit, used as a relative measure of activity in pigeon assays. Powdered digitalis contains digitoxin, gitoxin, and gitalin, of which digitoxin is usually in

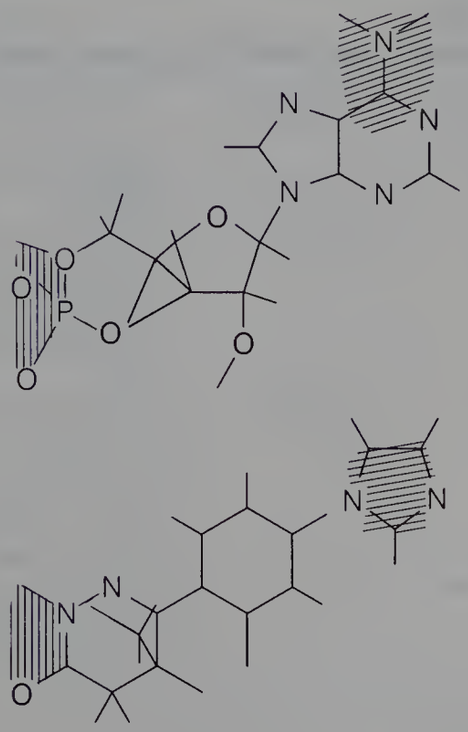


FIG. 23-39. *Pharmacophore model of Moos and co-workers[230] for active conformation of cAMP* (top). *CI-930* (bottom), *as an example, is illustrated in its proposed matching conformation.* Shading on left *shows the mimicking of the pyridazone amide of all the inhibitors for the 5'-phosphate of cAMP. For extended inhibitors, such as imazodan, electron delocalized rings or atoms occupy the corresponding part of the adenine ring* (shading on right).

highest concentration. Because of the sizable presence of digitoxin, powdered digitalis has a slow onset of action and long half-life (see Table 23-10). The long half-life makes toxic symptoms more difficult to treat than with cardiotonic steroids with shorter half-lives.

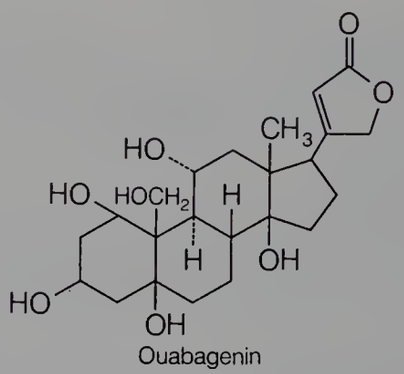
Digoxin, USP, because of its moderately fast onset of action and relatively short half-life (see Table 23-10), has become the most frequently prescribed digitalis steroid. It is a *D. lanata* glycoside of digoxigenin (see Fig. 23-29), 3,12,14-trihydroxycard-20(22)-enolide. Digoxin was first isolated by Smith,[231] in 1930. It may be given orally, intravenously, or intramuscularly (into deep muscle, followed by firm massage).

Digitoxin and digoxin are the most frequently prescribed digitalis steroids. Digoxin is more rapidly excreted and, therefore, also more rapidly accumulates in the presence of impaired renal function. Clinical changes from changing the maintenance dose are quickly observed. Digitoxin is excreted more slowly and accumulates more slowly. Because it is more slowly excreted, its kinetics are less affected by renal function. It has better absorption (bioavailability) than digoxin and, therefore, probably is more reproducible. The rapid excretion of digoxin is certainly useful when toxicity develops. However, if renal function should be impaired during long-term maintenance therapy, the risk of serious toxicity appears to be considerably greater for the patient receiving digoxin rather than digitoxin. Thus, Jelliffe suggests that digitoxin would be preferred with patients with potentially variable renal function.

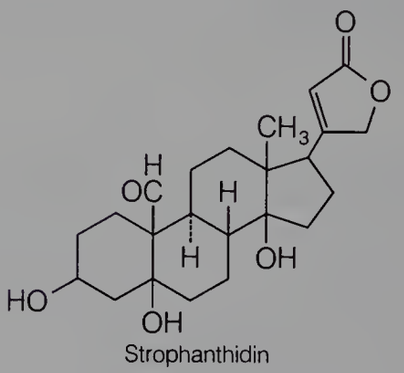
Digitoxin, USP, is obtained from *D. purpurea* and *D. lanata*, as well as several other species of *Digitalis*. It was obtained in crystalline form in 1869 by Nativelle.[232] It is a glycoside of digitoxigenin (see Fig. 23-29), 3,14-dihydroxycard-20(22)-enolide. The properties of digitoxin have been compared with digoxin.

Acetyldigitoxin, USP, is obtained from the enzymatic hydrolysis of lanatoside A (see Table 23-9).

Ouabain, USP, also called G-strophanthin, is a glycoside obtained from the seeds of *Strophanthus gratus* or the wood of *Acokanthera schimperi*. It is too poorly and unreliably absorbed to be used orally, but its extremely fast onset of action (see Table 23-10) makes it useful for rapid digitalization in emergencies (e.g., nodal tachycardia, atrial flutter, or acute congestive heart failure). Its synonym G-strophanthin makes it easily confused with strophanthin (or K-strophanthin), a glycoside obtained from *S. kombe*. The aglycone of ouabain is ouabagenin, whereas the aglycone of strophanthin is strophanthidin.



Ouabagenin



Strophanthidin

Lanatoside C is a digoxigenin glycoside obtained from the leaves of *D. lanata*. It is poorly and irregularly absorbed from the gastrointestinal tract and has a variable metabolic half-life.

Deslanoside, USP, is a digoxigenin glycoside obtained from lanatoside C by alkaline deacetylation (see Table 23-9). It is used only for rapid digitalization in emergency situations and may be given intravenously or intramuscularly.

STEROIDS WITH OTHER ACTIVITIES

As shown in Fig. 23-1, there are a number of important steroids that do not fall into the previous classifications. (Vi-

tamin D precursors are discussed in Chap. 27.) Because these compounds have diverse activities and uses, they will be presented individually in the monographs that follow.

PRODUCTS

Cholesterol, USP, is used as an emulsifying agent. Its biosynthesis and structure are shown in Fig. 23-6, which also illustrates its essential role as a steroid hormone precursor. Cholesterol is the precursor of virtually all other steroid hormones.

It is important to note that significantly more cholesterol is biosynthesized in the body each day (about 1 to 2 g) than is contained in the usual Western diet (about 300 mg). Cholesterol has been implicated in coronary artery disease, but there is increasing evidence that gentic deficiencies in cholesterol metabolism (see section on LDL Receptors), high stress, low exercise, "junk" foods, and smoking are possibly primary causes of heart disease.

Cholesterol is found in most plants and animals. Brain and spinal cord tissues are rich in cholesterol. Gallstones are almost pure cholesterol. In fact, cholesterol was originally isolated from gallstones, by Paulleitier de Lasalle in about 1770. In 1815, Chevreul[232] showed that cholesterol was unsaponifiable and he called it cholesterin (*chole*, bile; *steros*, solid). In 1859, Berthelot established its alcoholic nature, and since then it has been called cholesterol.[233]

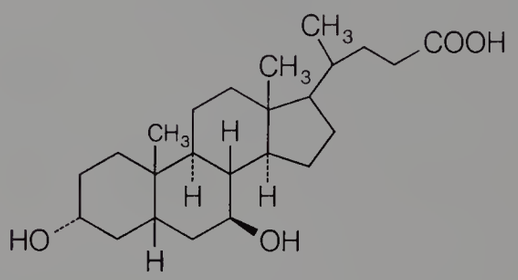
Cholesterol, lanosterol (structure shown in Fig. 23-6), fatty acids and their esters make up *anhydrous lanolin, USP* and *lanolin, USP*. Lanolin (or hydrous wool fat) is the purified, fatlike substance from the wool of sheep, *Ovis aries*, and contains 25% to 30% water. Anhydrous lanolin (or wool fat) contains not more than 0.25% water.

Spironolactone, USP (see Fig. 23-1), 17-Hydroxy-7α-mercapto-3-oxo-17α-pregn-4-ene-3-one- 21-carboxylic acid γ-lactone 7-acetate, is an aldosterone antagonist of great medical importance because of its diuretic activity. Spironolactone is discussed in Chap. 18.

Dehydrocholic acid, USP: 3,7,12-Triketocholanic acid (see Fig. 23-1) is a product obtained by oxidizing bile acids. The bile acids serve as fat emulsifiers during digestion. About 90% of the cholesterol not used for biosynthesis of steroid hormones is degraded to bile acids. All are 5β-steroid-3α-ols, giving rise to the "normal" designation discussed previously in steroid Nomenclature, Stereochemistry, and Numbering. As shown in Fig. 23-40, cholesterol has part of its side chain oxidatively removed in the liver, and two or more hydroxyls are added.[233] The resulting bile acids are then converted to their glycine or taurine conjugate salts, which are secreted in the bile. After entering the large intestine, the conjugate salts are converted to cholic acid, deoxycholic acid, and several other bile acids. Many of the bile acids are then reabsorbed, with cholic acid having a biologic half-life of about 3 days.

The bile acids are anionic detergents that emulsify fats, fat-soluble vitamins, and other lipids so that they may be absorbed. Dehydrocholic acid also stimulates the production of bile (choleretic effect). It is used after surgery on the gallbladder or bile duct to promote drainage and for its lipid-solubilizing effects in certain manifestations of cirrhosis or steatorrhea. A related product, ox bile extract, contains not less than 45% cholic acid and is used for the same purposes as dehydrocholic acid.

Ursodiol, *(ursodeoxycholic acid, Actigall),* is a naturally occurring bile acid recently marketed to dissolve gallbladder stones. Ursodiol decreases cholesterol secretion into bile and may decrease absorption of dietary cholesterol. After absorption and subsequent conjugation by the liver, it is secreted into bile and then reabsorbed to thereby concentrate ursodiol in the circulating bile acids. In clinical trials, about 30% of patients have had their cholesterol gallstones completely dissolved. However, long treatment is required, and when therapy is stopped, the gallstones recur in up to 50% of patients.



Ursodiol

Fusidic acid (see Fig. 23-1) and its sodium salt are used in Europe as antibiotics for gram-positive bacterial infections, particularly with patients who are penicillin-sensitive. It acts by inhibition of G-factor during protein biosynthesis. It is also of interest because it appears that it is formed from an intermediate common to the biosynthesis of lanosterol during squalene epoxide cyclization. Structure–activity studies have been reported by Godtfredsen[234] in 1966, and it was found that just about any minor structural modification of the molecule will result in significantly decreased activity. *Cephalosporin* P_1 and *helvolic acid* are steroids with structures very similar to fusidic acid, and both are antibiotics useful in some gram-positive bacterial infections.

COMMERCIAL PRODUCTION OF STEROIDS

HISTORY

This chapter on steroids would not be complete without brief mention of the fascinating history of the steroid industry. In the 1930s, steroid hormones had to be obtained by extraction of cow, pig, and horse ovaries, adrenal glands, and urine. The extraction process was not only inefficient, it was expensive. Progesterone was valued at over $80 per gram. However, by the late 1940s, progesterone was being sold for less than

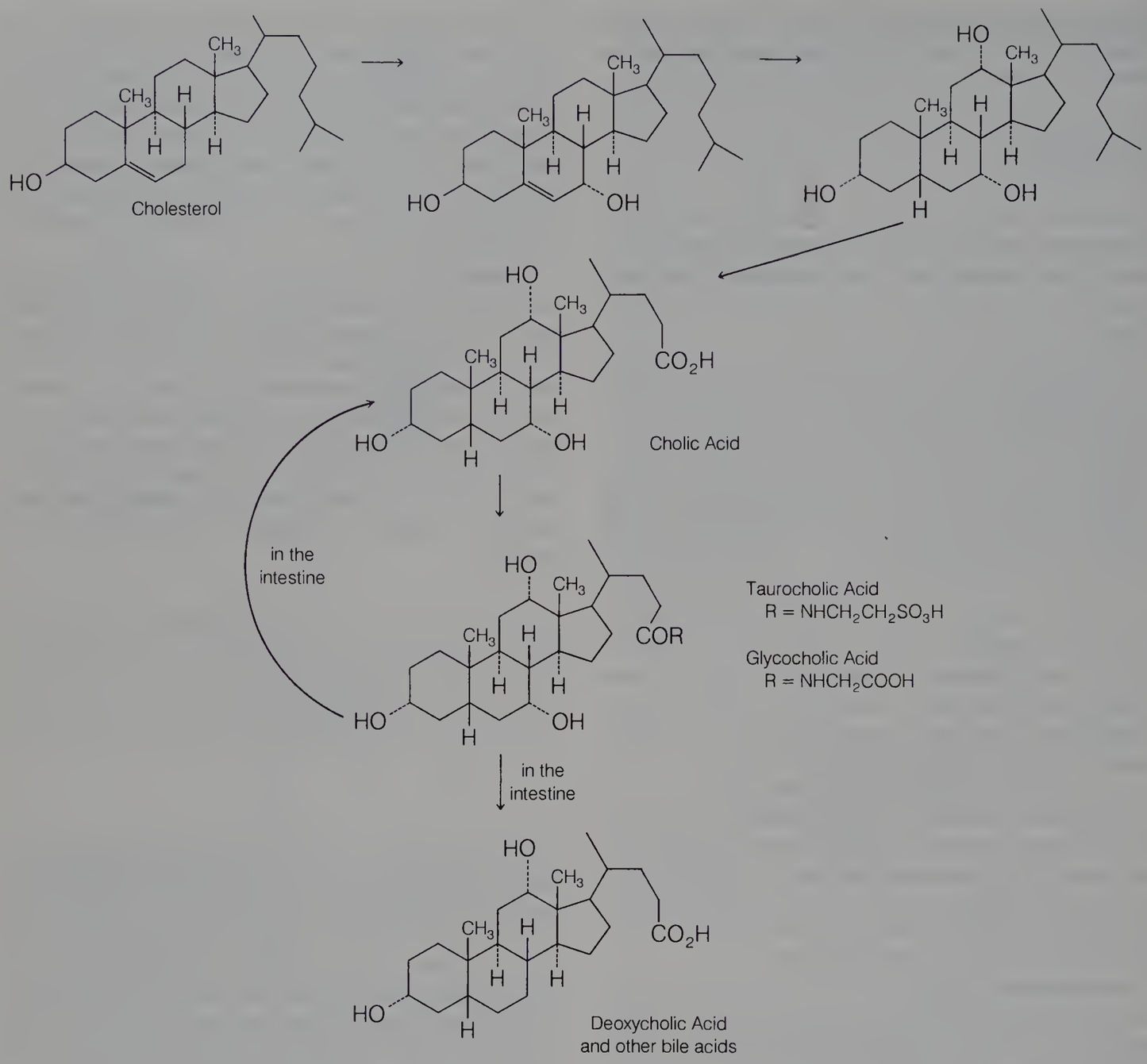


FIG. 23-40. *Metabolism of cholesterol to bile salts.*

50 cents a gram and was available in ton quantities. The man who made steroid hormones cheaply and plentifully available is Russell E. Marker, the "founding father" of the modern steroid industry.[1,235]

After leaving graduate school in 1925, Marker worked in a variety of areas in organic chemistry research. In 1935, he went to the Pennsylvania State University to begin studying steroids, turning his full attention to finding inexpensive starting materials for steroid hormone syntheses. In 1939, he correctly determined the structure of sarsasapogenin, a sapogenin (aglycone of a saponin, i.e., a glycoside that foams in water) whose structure had been incorrectly published by many other chemists a few years earlier.

Marker quickly developed a procedure to degrade the side chain of sarsasapogenin to yield a pregnane. Soon thereafter, he degraded diosgenin (Δ^5-sarsasapogenin) to progesterone (see Fig. 23-41) in excellent yield.

The commercial potential of the process was obvious to Marker. He immediately launched a series of plant-collecting expeditions from 1939 to 1942 to find a high-yield source of diosgenin, isolated previously from a *Dioscorea* species in Japan.[236] Over 400 species were collected (over 40,000 kg of plant material) in Mexico and the American Southwest.

Two particularly high-yielding sources of diosgenin were found in Mexico—*Dioscorea composita* ("barbasco") and *D. macrostachya* ("cabeza de negro")—commonly called "the Mexican yams." Although barbasco had five times the diosgenin content of cabeza, it was in generally inaccessible

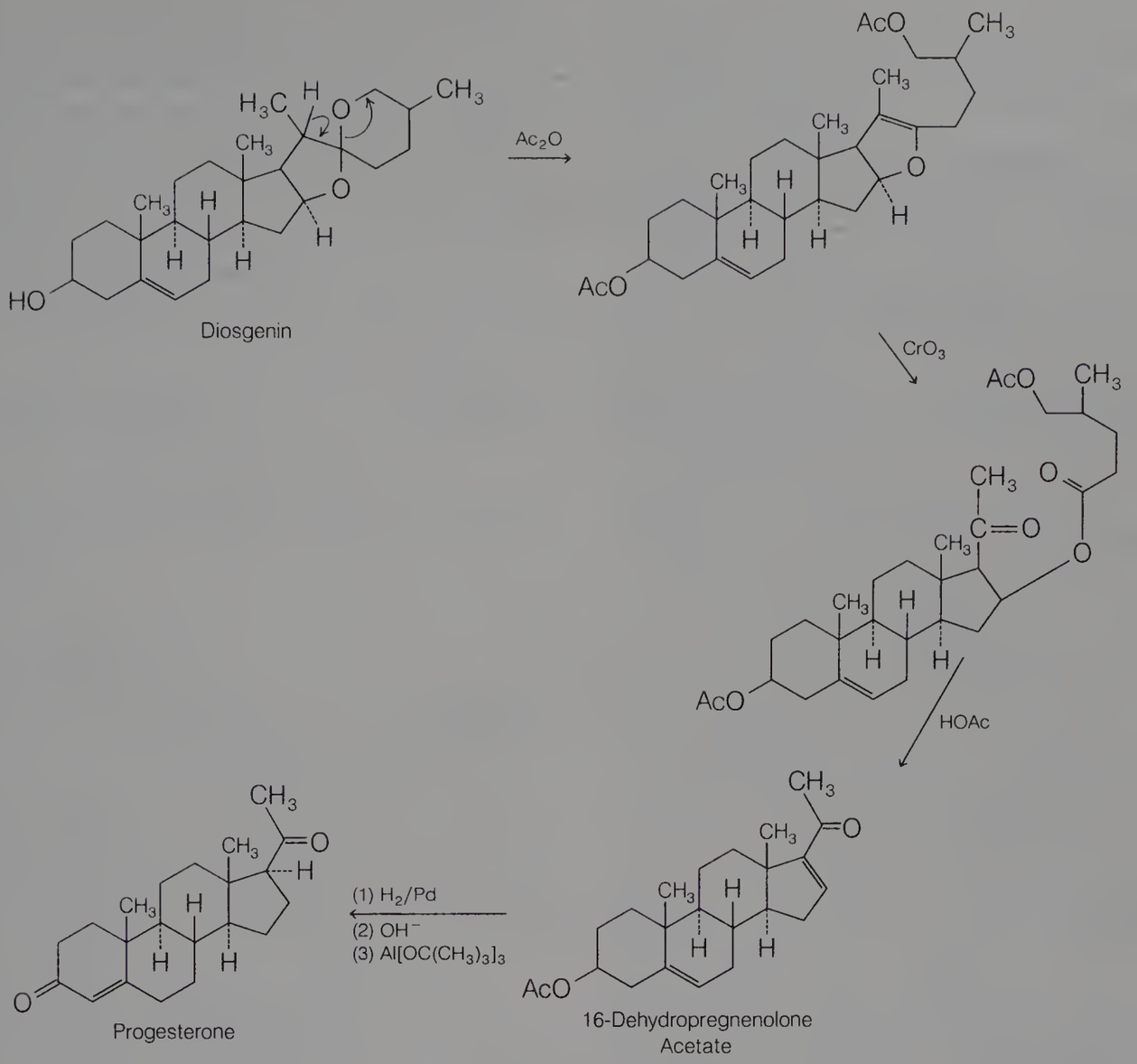


FIG. 23-41. *The Marker synthesis of progesterone from diosgenin.*

areas, and so Marker concentrated on cabeza. He knew he had a high-yield, low-cost course of progesterone, but was unable to interest several American drug companies. In 1943, he returned to Mexico City and promptly made 3 kg of progesterone (valued at $240,000) from cabeza. On January 21, 1944, Marker, Lehmann, and Somlo incorporated Syntex Laboratories, and by 1951 Syntex was taking orders for 10-ton quantities of progesterone. The great demand for progesterone was compounded by it serving as a precursor for cortisone and hydrocortisone (Fig. 23-42) by a microbial process that was patented in 1951 by Upjohn.

However, in 1945, Marker, Somlo, and Lehmann had a general "falling out," and Marker sold his 40% interest in Syntex to the other two partners. Syntex then brought in Rosenkranz, Djerassi, and other chemists to continue the synthesis of hormones from diosgenin. In 1951 and 1953, Frank Colton of G. D. Searle and Co.[237] and Djerassi and Rosenkranz of Syntex Laboratories[238] synthesized norethynodrel and norethindrone, respectively, thus beginning the era of oral contraceptives that continues to this day.

During the 1950s virtually all the steroid hormones had been made from diosgenin by chemists in North America and Europe. The Mexican yams were briefly "nationalized" by Mexico, thus temporarily blocking export. Attempts to grow high-yield barbasco or cabeza in other countries have been generally unsuccessful. Microorganisms have continued to play many key roles in the inexpensive commercial production of steroid drugs.

CURRENT METHODS

Today nearly all steroid hormones are made from diosgenin, stigmasterol (an inexpensive component of soybean oil), or cholesterol (available in ton quantities from wool fat). Microbiological side group-cleaving processes of Sih et al.[239]

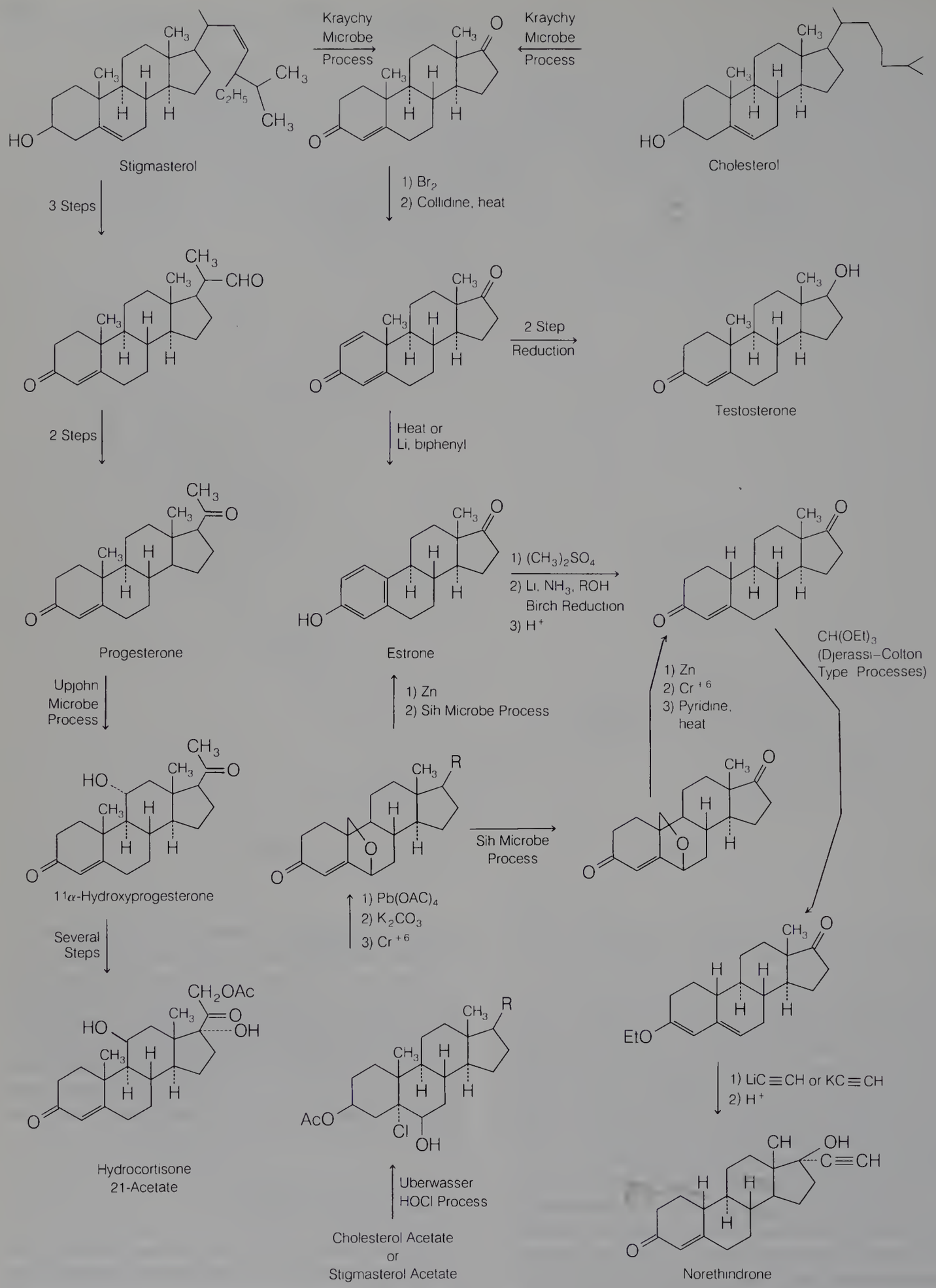


FIG. 23-42. *Commercial production of steroid hormones from cholesterol and stigmasterol.*

and Kraychy et al.[240] are the basis for the routes shown in Fig. 23-42. The 19-methyl group is usually removed by the method of Uberwasser et al.[241] (oxidation to form an oxygen bridge at C-6), but Birch reduction with Li/NH_3, an industrially more difficult process, is also used. The Upjohn microbial process[242,243] for converting progesterone to 11α-hydroxyprogesterone is used to make cortisone and hydrocortisone products. An overview of these processes has been published by Klimstra and Colton.[244] Some total synthetic routes are also used by some companies to make estrone.[245,246]

ACKNOWLEDGMENT

I am grateful for the help of my friend and colleague Deborah E. Atherly, MPH, R.Ph. in writing and revising several parts of this chapter.

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CHAPTER 24

Prostaglandins, Leukotrienes, and Other Eicosanoids

Thomas J. Holmes, Jr.

The prostaglandins (PGA through PGJ) are one group of naturally occurring 20-carbon fatty acid derivatives produced by the oxidative metabolism of 5,8,11,14-eicosatetraenoic acid, which also is called arachidonic acid. Other so-called eicosanoids produced in the complex biologic oxidation scheme called the arachidonic acid cascade (see Figs. 24-1 and 24-2) are thromboxane A_2 (TXA_2), the leukotrienes (LKT A–F), and the highly potent antithrombotic agent prostacyclin (PGI_2). The naming and numbering of these 20-carbon acids is included in Figs. 24-1 to 24-3. Although eicosanoid-derived agents in current human clinical therapy are few, the promise of future contributions from this area is presumed to be very great. This promise stems from the fact that intermediates of arachidonic acid metabolism play an essential modulatory role in many normal and disease-related cellular processes. In fact, much of the pain, fever, swelling, nausea, and vomiting associated with "illness," in general, are probably a result of excessive prostaglandin production in damaged tissues.

HISTORY OF DISCOVERY

Early in this century (1931), it was noted by Kurzrok and Lieb that human seminal fluid could increase or decrease spontaneous muscle contractions of uterine tissue under controlled conditions.[1] This observed effect on uterine musculature was believed to be induced by an acidic vasoactive substance formed in the prostate gland, which was later (1936) termed "prostaglandin" by von Euler.[2] Much later (1950s), it was found that the acidic extract contained not one but several structurally related prostaglandin substances.[3] These materials subsequently were separated, purified, and characterized as the prostaglandins (PGA through PGF), varying somewhat in degree of oxygenation and dehydrogenation and markedly in biologic activity (see Table 24-1). Specific chemical syntheses of the prostaglandins provided access to sufficient quantities of purified materials for wide-scale biologic evaluation and confirmed the structural characterization of these complex substances.[4]

Although a multitude of scientists have contributed to a refined characterization of the eicosanoid biosynthetic pathways and the biologic consequences of this cascade, the profound and persistent pioneering effort of Sune Bergstrom, Bengt Samuellson, and John R. Vane was recognized by the award of a shared Nobel Prize in Medicine in 1982. These scientists not only dedicated themselves to the chemical and biologic characterization of the eicosanoid substances but also were the first to realize the profound significance of the arachidonic acid cascade in disease processes, particularly inflammation. It was these individuals who first proved that the mechanism of the anti-inflammatory action of aspirin and related nonsteroidal anti-inflammatory (NSAI) drugs was directly due to their inhibitory effect on prostaglandin formation. It has been shown subsequently that the analgesic and antipyretic effects of these NSAI agents, as well as their proulcerative and anticoagulant side effects, also result from their effect on eicosanoid metabolism.

A plethora of books have been published describing the role of eicosanoids in the inflammatory process, the immune system, carcinogenesis, the cardiovascular system, reproductive processes, and the central nervous system (see Selected Readings). An annual update of research results in this area has been published since 1975 entitled *Advances in Prostaglandins, Thromboxanes, and Leukotriene Research.* Recent research findings in this area may appear in a variety of biochemical and clinical journals but are the primary concern of two specific journals, *Prostaglandins* and *Prostaglandins, Leukotrienes, and Essential Fatty Acids.*

EICOSANOID BIOSYNTHESIS

Prostaglandins and other eicosanoids are produced by the oxidative metabolism of free arachidonic acid. Under normal circumstances, arachidonic acid is not available for metabo-

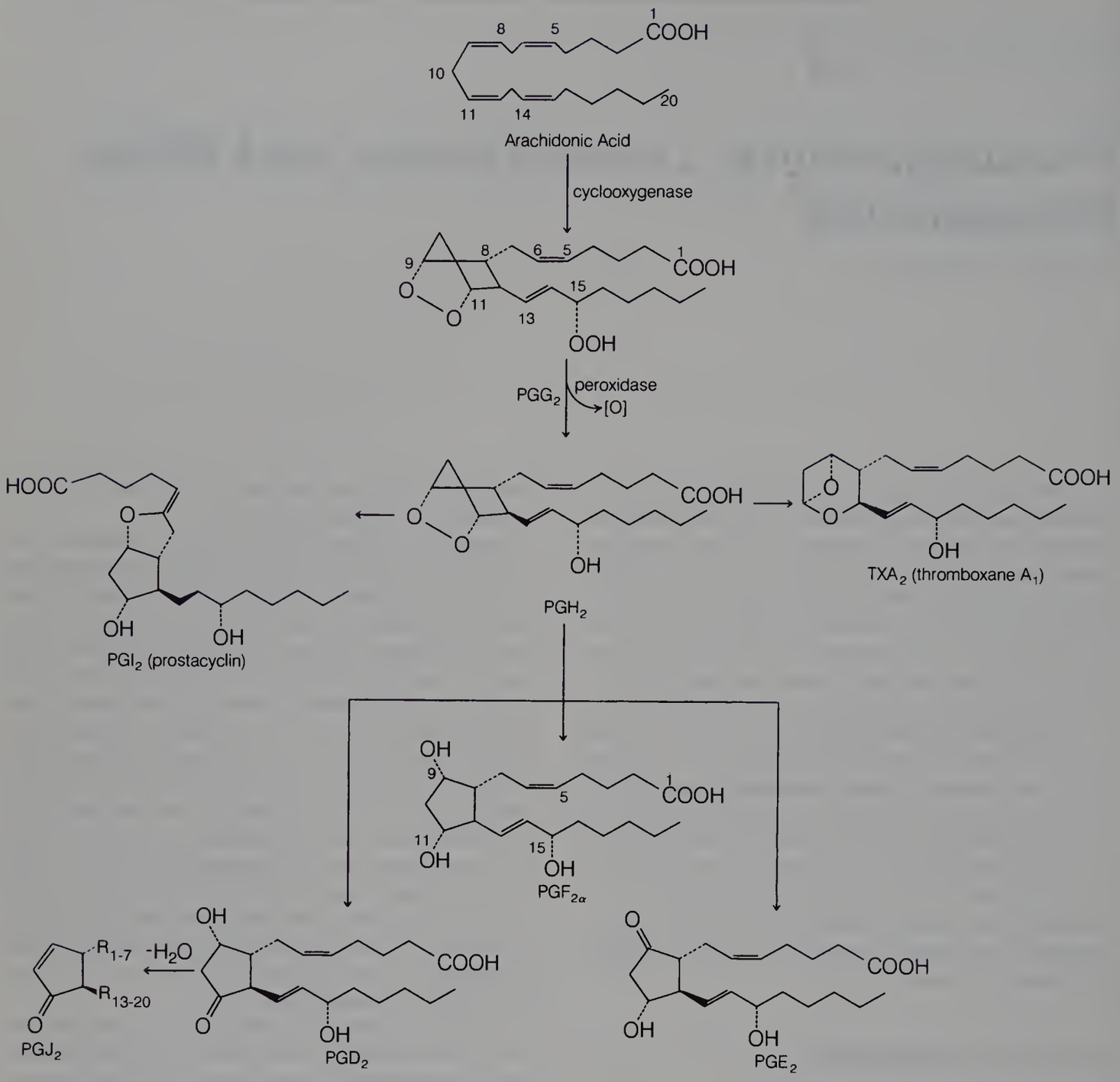


FIG. 24-1. *Cyclooxygenase pathway.*

lism as it is present as a conjugated component of the phospholipid matrix of most cellular membranes. Release of free arachidonic acid, which subsequently may be metabolized, occurs by stimulation of phospholipase (PLA_2) enzyme activity in response to some traumatic event (e.g., tissue damage, toxin exposure, or hormonal stimulation). It is believed that the clinical anti-inflammatory effect of glucocortical steroids (i.e., hydrocortisone) is due to their ability to suppress phospholipase activity via lipocortins and, therefore, prevent the release of free arachidonic acid.[5] Modulation of phospholipase activity by alkali metal ions, toxins, and various therapeutic agents has become a major focus of biologic research because of the changes in eicosanoid production accompanying phospholipase stimulation or suppression. Although initially it was believed that the inflammatory response (swelling, redness, pain) was principally due to PGE_2, recent interest has focused on the interrelationships of PGE-type eicosanoids with prostacyclin and cytokines, such as interleukins-1 and -2, in the modulation of inflammatory reactions.[6]

Two different routes for oxygenation of arachidonic acid have been identified: the cyclooxygenase pathway (Fig. 24-1) and the lipoxygenase pathway (Fig. 24-2). The relative significance of each of these pathways may vary in a particu-

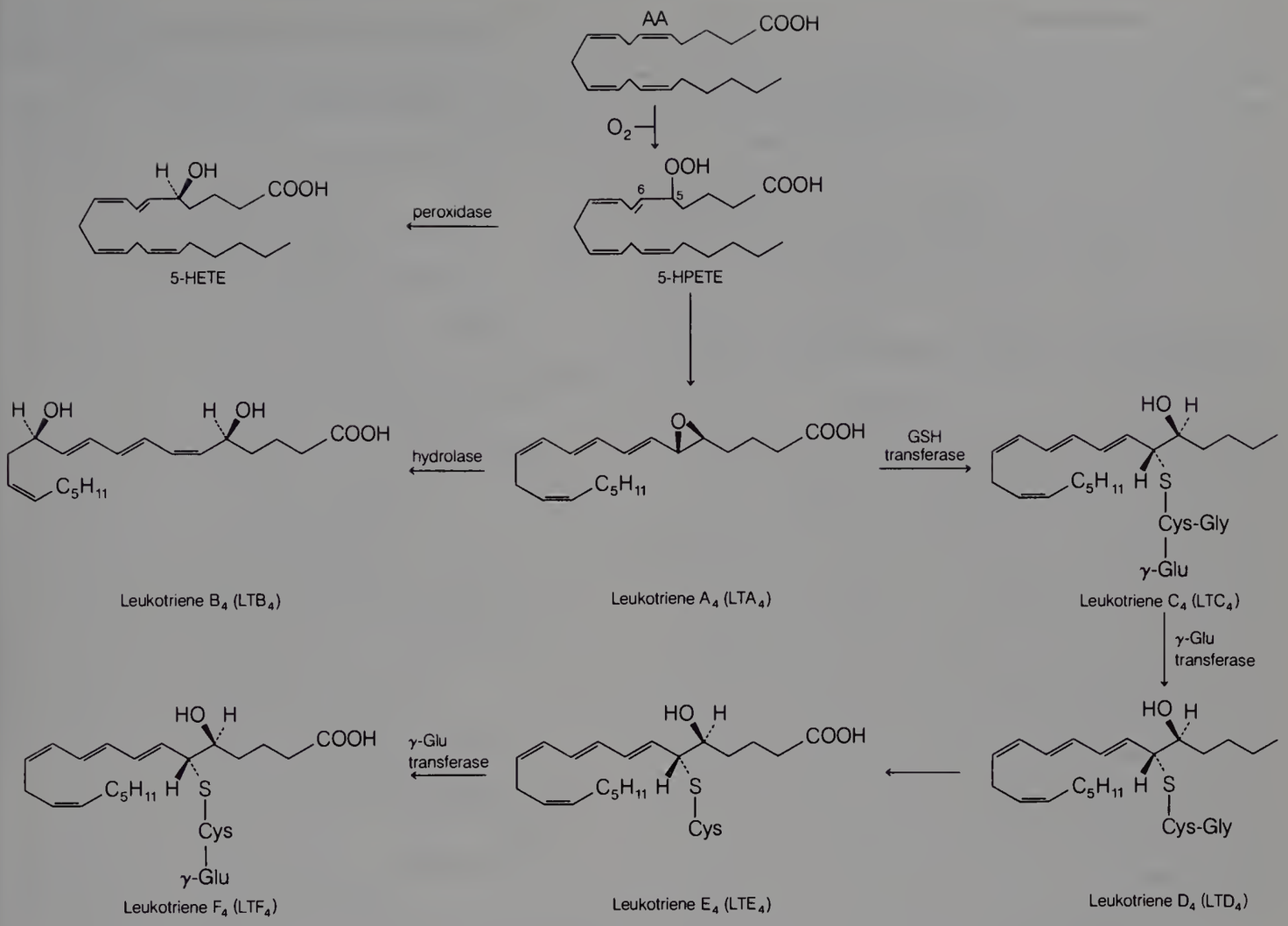


FIG. 24-2. *Lipoxygenase pathway.*

lar tissue or disease state. The cyclooxygenase pathway, so named because of the unusual bicyclic endoperoxide (PGG_2) produced in the first step of the sequence, involves the highly stereospecific addition of two molecules of oxygen to the arachidonic acid substrate, followed by subsequent enzyme-controlled rearrangements to produce an array of oxygenated eicosanoids with diverse biologic activities (see Table 24-1). The first enzyme in this pathway, PGH-synthase, is a hemoprotein that catalyzes both the addition of oxygen (to form PGG_2) and the subsequent reduction (peroxidase activity) of the 15-position hydroperoxide to the 15-(*S*)-configuration alcohol (PGH_2).[7] PGH-synthase (also called cyclooxygenase-1 or PG-synthetase) has been the focus of intense investigation because of its key role as the first enzyme in the arachidonic acid cascade. It is this enzyme in constitutive or inducible form that is susceptible to inhibition by NSAI agents, leading to relief of pain, fever, and inflammation,[6,8] and inhibited by the ω_3-fatty acids (eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) found in certain cold-water fish, leading to beneficial cardiovascular effects.[9] This enzyme will metabolize 20-carbon fatty acids with one more or one less double bond than arachidonic acid, leading to prostaglandins of varied degrees of unsaturation (e.g., PGE_1 or PGE_3, for which the subscript number indicates the number of double bonds in the molecule).

Prostaglandin H_2 serves as a branch-point substrate for specific enzymes, leading to the production of the various prostaglandins, TXA_2, and prostacyclin (PGI_2). Even though most tissues have the capability to produce PGH_2, the relative production of each of these derived eicosanoids is highly tissue-specific and may be subject to secondary modulation by a variety of cofactors. The complete characterization of enzymes involved in branches of the cyclooxygenase pathway is currently under way.

Specific cellular or tissue responses to the eicosanoids are apparently a function of available surface receptor recognition sites.[10] The variety of tissue responses observed upon eicosanoid exposure is outlined in Table 24-1. Nontissue-selective inhibitors of the cyclooxygenase pathway, such as aspirin, thus may exert a diversity of therapeutic effects or side effects (e.g., decreased uterine muscle contraction and platelet aggregation, lowering of elevated body temperature, central and peripheral pain relief, and decreased vascular perfusion) based upon their tissue distribution.

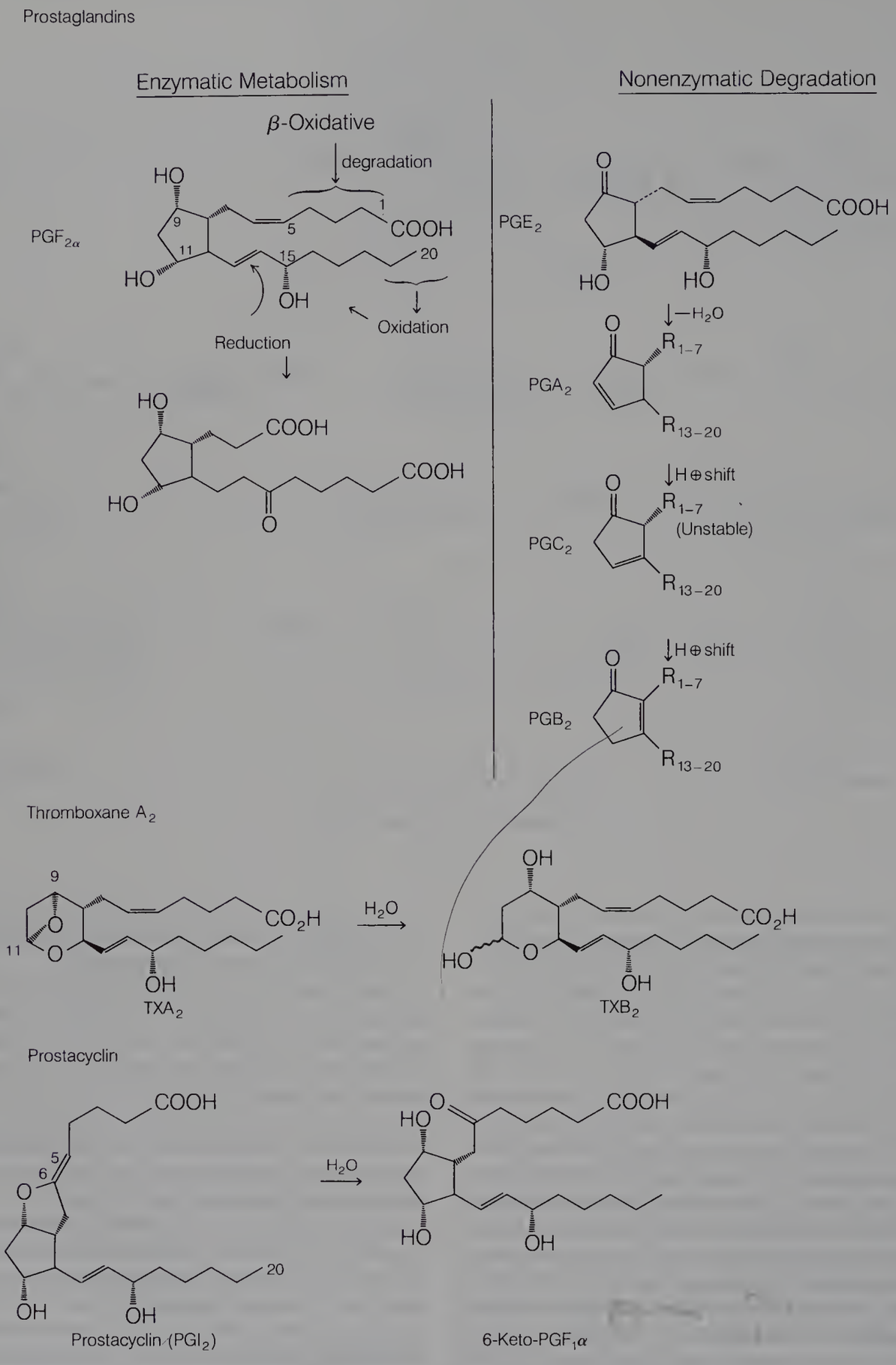


FIG. 24-3. *Eicosanoid degradation.*

TABLE 24-1

BIOLOGIC ACTIVITIES OBSERVED WITH THE EICOSANOIDS

Substance	*Observed Biologic Activity*
PGD_2	Weak inhibitor of platelet aggregation
PGE_1	Vasodilation Inhibitor of lipolysis Inhibitor of platelet aggregation Bronchodilatation Stimulates contraction of gastrointestinal smooth muscle
PGE_2	Stimulates hyperalgesic response Renal vasodilatation Stimulates uterine smooth-muscle contraction Protects gastrointestinal epithelia from acid degradation Reduces secretion of stomach acid Elevates thermoregulatory set-point in anterior hypothalamus
PGF_2	Stimulates breakdown of corpus luteum (luteolysis) in animals Stimulates uterine smooth-muscle contraction
PGI_2	Potent inhibitor of platelet aggregation Potent vasodilator Increases cAMP levels in platelets
PGJ_2	Stimulates osteogenesis Inhibits cell proliferation
TXA_2	Potent inducer of platelet aggregation Potent vasoconstrictor Decreases cAMP levels in platelets Stimulates release of ADP and serotonin from platelets
LTB_4	Increases leukocyte chemotaxis and aggregation
LTC/D_4	Slow-reacting substances of anaphylaxis Potent and prolonged contraction of guinea pig ileum smooth muscle Contracts guinea pig lung parenchymal strips Bronchoconstrictive in humans Increased vascular permeability in guinea pig skin (augmented by PGEs)
5- or 12-HPETE	Vasodilatation of rat and rabbit gastric circulation Inhibits induced platelet aggregation
5- or 12-HETE	Aggregates human leukocytes Promotes leukocyte chemotaxis

The lipoxygenase pathway of arachidonic acid metabolism (Fig. 24-2) produces a variety of acyclic lipid peroxides (hydroperoxyeicosatetraenoic acids [HPETEs]) and derived alcohols (hydroxyeicosatetraenoic acids [HETEs]). Although the specific biologic function of each of these lipoxygenase-derived products is not completely known, they are believed to play a major role as chemotactic factors that promote cellular mobilization toward sites of tissue injury. In addition, the glutathione (GSH) conjugate LKT-D_4 has been characterized as a potent, long-acting bronchoconstrictor, which is released in the lungs during severe hypersensitivity episodes (leading to its initial designation as the "slow-reacting substance of anaphylaxis" [SRSA]). Because of the presumed benefit of preventing formation of LKTs in asthmatic patients, much research effort is being dedicated to the design and discovery of drugs that might selectively inhibit the lipoxygenase pathway of arachidonic acid metabolism, without affecting the cyclooxygenase pathway.[11] It has been proposed that aspirin hypersensitivity in susceptible individuals may result from effectively "shutting down" the cyclooxygenase metabolic route, allowing only the biosynthesis of lipoxygenase pathway intermediates, including the bronchoconstrictive LKTs.[12]

DRUG ACTION MEDIATED BY EICOSANOIDS

The ubiquitous nature of the eicosanoid-producing enzymes implies their significance in a variety of essential cellular processes. Additionally, the sensitivity of these enzymes to structurally varied hydrophobic materials, particularly carboxylic acids and phenolic antioxidants, implies their susceptibility to influence by a variety of exogenously administered drugs. Because most aromatic drug molecules undergo hepatic hydroxylation, phenolic derivatives of administered drugs become readily available in vivo. Even more directly, it has been demonstrated that aromatic molecules upon in vitro incubation with microsomal PGH-synthase will become hydroxylated directly during arachidonic acid metabolism, in a process labeled *cooxidation*.[13] This cooxidative process presumably occurs during the peroxidative conversion of PGG_2 to PGH_2, which effectively makes available a nonspecific oxidizing equivalent. The cooxidation process has been implicated in the activation of polycyclic aromatic hydrocarbons to form proximate carcinogens.[14]

The only group of drugs that has been thoroughly characterized for its effect on arachidonic acid metabolism is the NSAI agents. This large group of acidic, aromatic molecules exerts a diverse spectrum of activities (previously mentioned) by inhibition of the first enzyme in the arachidonic acid cascade, PGH-synthase. Such agents as salicylic acid, phenylbutazone, naproxen, sulindac, and ibuprofen presumably act by a competitive, reversible inhibition of arachidonic acid oxygenation.[15] Aspirin and certain halogenated aromatics (including indomethacin, flurbiprofen, and meclomen) appear to inhibit PGH-synthase in a time-dependent, irreversible manner,[16] although for aspirin this irreversible inhibition appears critical only for its effect on platelet aggregation and, therefore, prolongation of bleeding time.[17]

Interestingly, aspirin's primary competitor in the commercial analgesic marketplace, acetaminophen, has been shown to be a rather weak inhibitor of arachidonic acid oxygenation in vitro.[18] This, in fact, has been observed to be a characteristic of reversible, noncompetitive, phenolic antioxidant inhibitors in general.[19] This determination, in concert with its lack of in vitro anti-inflammatory activity (while maintaining analgesic and antipyretic activity equivalent to the salicylates) has led to the proposal that acetaminophen is more active as an inhibitor of PGH-synthase in the brain, where peroxide levels (which stimulate cyclooxygenase activity) are lower

than in inflamed peripheral joints, where lipid peroxide levels are high.[15] In fact, when in vitro experimental conditions are modified to reduce the so-called peroxide tone, acetaminophen becomes as effective as aspirin in reducing arachidonic acid metabolism.[18]

DESIGN OF EICOSANOID DRUGS

The ability to capitalize successfully on the highly potent biologic effects of the various eicosanoids to develop new therapeutic agents currently seems an unfulfilled promise to medicinal chemists. Although these natural substances are highly potent effectors of various biologic functions, their use as drugs has been hampered by several factors: (1) their chemical complexity and relative instability, which has limited, to some extent, their large-scale production and formulation for clinical testing; (2) their susceptibility to rapid degradation (Fig. 24-3), which limits their effective bioactive half-life; and (3) their ability to affect diverse tissues (particularly the gastrointestinal tract, which may lead to severe nausea and vomiting) if they enter the systemic circulation even in only small amounts.

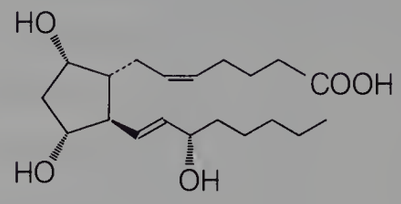
Two approaches have been employed to overcome these difficulties. First, structural analogues of particular eicosanoids have been synthesized, which are more resistant to chemical and metabolic degradation but maintain, to a large extent, a desirable biologic activity. Although commercial production and formulation may be facilitated by this approach, biologic potency usually is reduced by several orders of magnitude. Also, systemic side effects may become troublesome owing to broader distribution as a result of the increased biologic half-life.

Primarily, structural alterations of the eicosanoids have been aimed at reducing or eliminating the very rapid metabolism of these potent substances to relatively inactive metabolites (see Fig. 24-3). Several analogues are presented in Table 24-2 to illustrate approaches that have led to potentially useful eicosanoid drugs. Methylation at the 15- or 16-position will eliminate or reduce oxidation of the essential 15-*(S)*-alcohol moiety. Esterification of the carboxylic acid function may affect formulation or absorption characteristics of the eicosanoid, whereas esterase enzymes in the bloodstream or tissues would be expected to regenerate quickly the active therapeutic agent. Somewhat surprisingly, considering the restrictive configurational requirements at the naturally asymmetric centers, a variety of hydrophobic substituents (including phenyl rings) may replace the alkyl chains with the maintenance of bioactivity.

A second major approach has been aimed at delivering the desired agent, either a natural eicosanoid or a modified analogue, to a localized site of action by using some controlled-delivery method. The exact method of delivery may vary according to the desired site of action (e.g., uterus, stomach, lung) but has included aerosols and locally applied suppository or gel formulations.

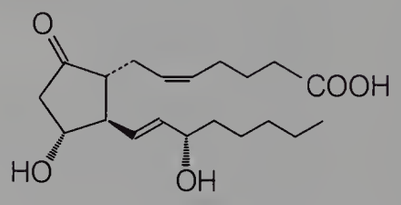
EICOSANOIDS APPROVED FOR HUMAN CLINICAL USE

Prostaglandin $F_{2\alpha}$. Dinoprost (Prostin F2 Alpha). $PGF_{2\alpha}$ is a naturally occurring prostaglandin that is administered intra-amniotically to induce labor or abortion within the first trimester.

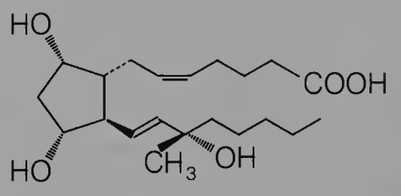


This product is supplied as a solution of the tromethamine salt (5 mg/mL) for direct administration.

Prostaglandin E_2. Dinoprostone (Prostin E2; Cervidil). PGE_2 is a naturally occurring prostaglandin that is administered in a single dose of 20 mg by vaginal suppository to induce labor or abortion.

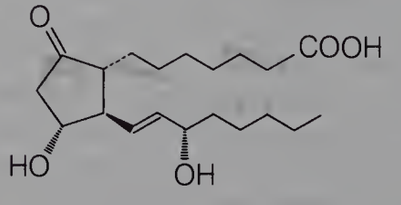


15-(*S*)-Methyl-$PGF_{2\alpha}$. Carboprost tromethamine (Hemabate) is a prostaglandin derivative which has been modified to prevent metabolic oxidation of the 15-position alcohol function.



This derivative is administered in a dose of 250 μg by deep intramuscular injection to induce abortion or to ameliorate severe postpartum hemorrhage.

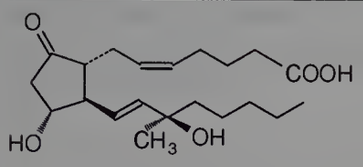
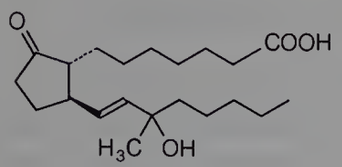
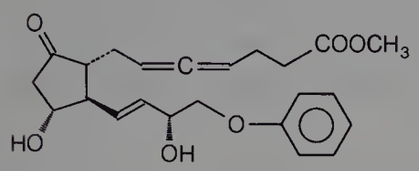
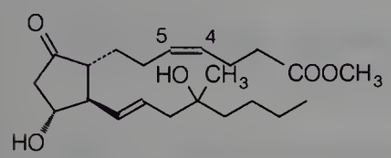
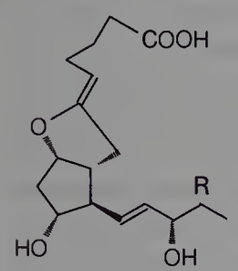
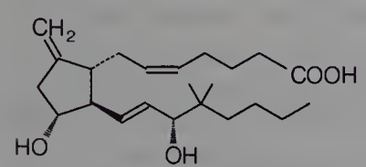
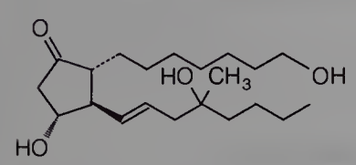
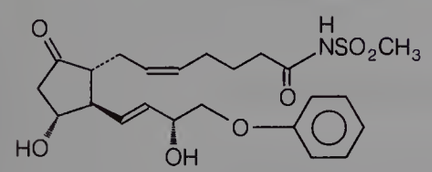
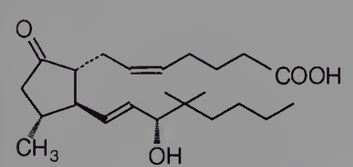
Prostaglandin E_1 USP. Alprostadil (Prostin VR Pediatric). PGE_1 is a naturally occurring prostaglandin that has found particular use in maintaining a patent (opened) ductus arteriosus in infants with congenital defects that restrict pulmonary or systemic blood flow.



Alprostadil must be administered intravenously continually at a rate of approximately 0.1 μg/kg per minute to temporar-

TABLE 24-2

PROSTAGLANDIN ANALOGUES UNDER INVESTIGATION FOR FUTURE CLINICAL USE AND ORPHAN DRUG STATUS

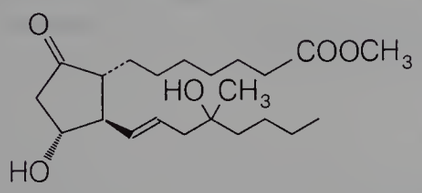
Structure	*Names (Proprietary Name; Manufacturer)*	*Therapeutic Use*
	Arbaprostil (Arbocet; Upjohn); 15-(*R*)-methyl-PGE_2	Gastric antisecretory
	Doxaprost (Ayerst); 15-methyl-11-deoxy-PGE_1	Bronchodilatator
	Enprostil (Syntex); PGE_2-analogue	Antisecretory; antiulcer
	Enisoprost (Searle); 16-(*R,S*)-methyl-16-hydroxy-$\Delta^{4,5}$-PGE_2 methyl ester	Orphan Status: cyclosporine nephrotoxicity
	Epoprostenol (Upjohn and Glaxo-Wellcome); prostacyclin; PGI_2; R= $CH_2\,CH_2\,CH_2\,CH_2\,CH_3$	Orphan Status: pulmonary hypertension: heparin replacement
	Epoprostenol sodium (sodium salt of above) (Cyclo-Prostin; Upjohn / Flolan; Glaxo-Wellcome) Illoprost (Berlex) R = $CH(CH_3)CH_2C{\equiv}CCH_3$	Orphan Status: Raynand's phenomenon
	Meteneprost (Pharmacia and Upjohn); 9-deoxo-9-methylene-16,16-dimethyl-PGE_2	Oxytocic
	Rioprostil (OrthoMiles / Bayer Laboratories); 16-(*R,S*)-methyl-1,16-dihydroxy-PGE_1	Gastric anti-secretory
	Sulprostone (Sulglandin, Pfizer/Schering A. G.); PGE_2 analogue	Oxytocic
	Trimoprostil (Hoffmann-LaRoche); 11-methyl-11-deoxy-16,16-dimethyl-PGE_2	Gastric antisecretory

ily maintain the patency of the ductus arteriosus until corrective surgery can be performed. Up to 80% of circulating alprostadil may be metabolized in a single pass through the lungs. Because apnea has been observed in 10% to 12% of neonates with congenital heart defects, this product should be administered *only* when ventilatory assistance is immediately available. Other commonly observed side effects include decreased arterial blood pressure, which should be monitored during infusion; inhibited platelet aggregation, which might aggravate bleeding tendencies; and diarrhea. Prostin VR Pediatric is provided as a sterile solution in absolute alcohol (0.5 mg/mL) that must be diluted in saline or dextrose solution before intravenous administration.

Alprostadil (Caverject) is also available in glass vials for reconstitution to provide 1.0 mL of solution containing either 10 or 20 μg/mL for intercavernosal penile injection to diagnose or correct erectile dysfunction in certain cases of impotence. An urethral suppository is also available.

Prostaglandin E_1 Cyclodextrin. This polysaccharide complex of PGE_1 (Vasoprost) is available as an orphan drug for the treatment of severe peripheral arterial occlusive disease when grafts or angioplasty are not indicated. Cyclodextrin complexation is used to enhance water solubility and reduce rapid metabolic inactivation.

16-(*R,S*)-Methyl-16-Hydroxy-PGE_1, Methyl Ester. Misoprostol (Cytotec) is a modified prostaglandin analogue which shows potent gastric antisecretory and gastroprotective effects when administered orally.



Misoprostol is administered orally in tablet form in a dose of 100 to 200 μg four times a day to prevent gastric ulceration in susceptible individuals who are taking NSAI drugs. This prostaglandin derivative absolutely should be avoided by pregnant women owing to its potential to induce abortion. In fact, the combined use of intramuscular methotrexate and intravaginal administration of misoprostol has been claimed to be a safe and effective, noninvasive method for the termination of early pregnancy.[20]

VETERINARY USES OF PROSTANOIDS

Since McCracken and co-workers demonstrated that $PGF_{2\alpha}$ acts as a hormone in sheep to induce disintegration of the corpus luteum (luteolysis),[21] salts of this prostaglandin and a variety of analogues have been marketed to induce or synchronize estrus in breed animals (Table 24-3). This procedure allows artificial insemination of many animals during one insemination period.

EICOSANOIDS IN CLINICAL DEVELOPMENT FOR HUMAN TREATMENT

Numerous prostaglandin analogues are under investigation for the treatment of human diseases (see Table 24-2). Efforts are being focused in the areas of gastroprotection as antiulcer therapy, fertility control, the development of thrombolytics (e.g., prostacyclin or thromboxane synthetase inhibitors) to treat cerebrovascular or coronary artery diseases, and the development of antiasthmatics through modulation of the lipoxygenase pathway. However, future application of eicosanoids to the treatment of hypertension or immune system disorders cannot be ruled out. Thus, although progress in this area has been slow, the further use of eicosanoids or eicosanoid analogues as therapeutic agents in the future is almost assured. The recent introduction of the antiasthmatic drugs zafirlukast (Accolate by Zeneca Pharmaceuticals) and

TABLE 24-3

EICOSANOID PRODUCTS FOR VETERINARY USE

Proprietary Name (Manufacturer)	*Chemical Name*	*Therapeutic Use*
Equimate (ICI Corp.)	Fluprostenol; 16-[*m*-(CF_3)-phenoxy]-ω-tetranor-$PGF_{2\alpha}$	Induce estrus; treat equine infertility
Estrumate (ICI Corp.)	Cloprostenol; 16-*m*-chlorophenoxy-ω-tetranor-$PGF_{2\alpha}$	Synchronize estrus in cattle
Fenprostalene (Syntex)	(+)-4,5-Didehydro-16-phenoxy-ω-tetranor-$PGF_{2\alpha}$ methyl ester	Induce abortion in cattle; synchronize estrus in cows
Iliren (Hoechst-Roussel)	Tiaprost; (15*R,S*)-16-(3-thienyloxy)-ω-tetranor-$PGF_{2\alpha}$tromethamine salt	Induce labor; treat pyometra and persistent luteal function in cattle, sheep, pigs, and horses
Lutalyse (Upjohn)	Tromethamine salt of $PGF_{2\alpha}$	Induce and synchronize estrus in mares, cows, and sows
Synchrocept (Syntex)	Prostalene; (+)-4,5-Didehydro-15-methyl-$PGF_{2\alpha}$-methyl ester	Synchronize estrus in mares

montelukast (Singulair by Merck and Co.), competitive leukotriene antagonist, and zilcuton (Zyflo by Abbott Laboratories), a lipoxygenase pathway inhibitor, illustrates how rapidly this promise may be fulfilled.

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CHAPTER 25

Carbohydrates

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Jaime N. Delgado

Carbohydrates represent one of the four major classes of biomolecules that also include proteins, nucleic acids, and lipids. These compounds, usually called "sugars" (e.g., glucose, sucrose, starch, and glycogen), were thought to be represented correctly by the generalized formula $C_x(H_2O)_y$, and thus, the term "carbohydrate" became extensively used. However, many compounds now classified as carbohydrates (2-deoxyribose, digitoxose, glucuronic and gluconic acids, the amino sugars) possess structures that cannot be represented by such a formula. On a functional group basis, carbohydrates are characterized as polyhydroxy aldehydes or polyhydroxy ketones and their derivatives.

Carbohydrates are distributed extensively in both the plant and animal kingdoms. Chlorophyll-containing plant cells produce carbohydrates by photosynthesis, which involves the fixation of CO_2 through reduction by H_2O and requires solar electromagnetic energy. Carbohydrates serve as a source of energy for plants and animals, and, in the form of cellulose and chitin, they function as the supporting structures of plants and insects or crustacea. In plants and microorganisms, carbohydrates are metabolized through various pathways leading to amino acids, purines, pyrimidines, fatty acids, vitamins, and such. Together with other dietary components (such as proteins, lipids, minerals, and vitamins) some carbohydrates are utilized metabolically by animals in many processes, degraded to acetyl coenzyme A (CoA) for the synthesis of lipids, or oxidized to obtain adenosine triphosphate (ATP); and in plants they are used for the synthesis of other organic compounds. Most of the carbohydrate that is utilizable by the human consists of starch, glycogen, sucrose, maltose, or lactose, whereas cellulose, xylans, and pectins cannot be degraded by digestive processes because of the lack of appropriate enzymes.

As the foregoing statements indicate, the biologic importance of carbohydrates is obvious. Various textbooks of biochemistry provide complete discussions of the chemistry and metabolism of carbohydrates.[1–6] Moreover, in medicinal chemistry it is recognized that many pharmaceutical products contain carbohydrates or modified carbohydrates as therapeutic agents or as pharmaceutical necessities. Certain antibiotics are carbohydrates derivatives. The streptomycins, neomycins, paromomycins, gentamicins, and kanamycins are basic carbohydrates that have significant antimicrobial properties.[7] The cardioactive glycosides represent another class of medicinal agents possessing carbohydrate moieties that contribute to their therapeutic efficacy (see Chap. 23).[8] The neuromodulatory actions of adenosine agonists and antagonists and the inhibition of gene expression by oligonucleotides have been reviewed to emphasize their use in rational drug design and biotechnology studies.[9,10]

Some knowledge of the interrelationships of carbohydrates with lipids and proteins in human metabolism is necessary for the study of the medicinal biochemistry of diabetes mellitus and the actions of antidiabetic agents. Accordingly, a brief discussion of these topics will be presented later in this chapter for purposes of emphasizing how some factors affecting carbohydrate metabolism also affect metabolic processes involving lipids and proteins.

CLASSIFICATION

A brief review of elementary characterizations of the more important carbohydrates is fundamental to the understanding of the structural and functional differences among the vast array of natural products that are classified as carbohydrates. The following summary is intended to delineate and exemplify the major classes and types of carbohydrate.

It is conventional to classify carbohydrates as *monosaccharides, oligosaccharides*, and *polysaccharides*, depending on the number of sugar residues present per molecule. Furthermore, monosaccharides containing three carbon atoms are called *trioses*, those containing four carbon atoms are *tetroses*, whereas *pentoses, hexoses*, and *heptoses* contain five, six, and seven carbon atoms, respectively. On a functional group basis, monosaccharides having a potential alde-

hyde group in addition to hydroxyl functions are known as *aldoses* and those bearing a ketone function are *ketoses*. For example, glyceraldehyde is an aldotriose and dihydroxyacetone is a ketotriose, whereas glucose is an aldohexose and fructose is a ketohexose.

Disaccharides, trisaccharides, and tetrasaccharides are oligosaccharides. Sucrose, lactose, maltose, cellobiose, gentiobiose, and melibiose are important disaccharides. Raffinose, melecotose, and gentianose are trisaccharides; stachyose is a tetrasaccharide.

Monosaccharides existing in the form of heterocycles are classified according to the size of the ring system; that is, the six-membered ring structures considered to be related to pyran are called *pyranoses* and the five-membered ring structures related to furan are called *furanoses*. This type of nomenclature can be applied to oligosaccharides and glycoside derivatives. Thus, maltose is 4-D-glucopyranosyl-α-D-glucopyranoside; lactose is 4-D-glucopyranosyl-β-D-galactopyranoside; and sucrose is 1-α-D-glucopyranosyl-β-D-fructofuranoside. (Stereochemical classification of carbohydrates will be considered briefly as the basis for the aforementioned configurational designations.)

Most carbohydrate material in nature exists as high-molecular-weight polysaccharides that on hydrolysis yield monosaccharides or their derivatives. Glucose, mannose, galactose, and arabinose; glucuronic, galacturonic, and mannuronic acids; and some amino sugars occur as structural components of polysaccharides, glucose being the most common component.

Polysaccharides yielding only one variety of monosaccharide are called *homopolysaccharides*, and those yielding a mixture of different monosaccharides are known as *heteropolysaccharides*. Homopolysaccharides of importance include the starches and glycogen, which are mobilizable stores of glucose, whereas cellulose is a structural polymer. All when hydrolyzed yield glucose. Heparin, hyaluronic acid, and the immunochemically specific polysaccharide of type III pneumococcus are representative examples of heteropolysaccharides. Heparin's polymeric structure is composed of α-D-glucuronic acid, α-L-iduronic acid, α-D-glucosamine, and *N*-acetyl-α-D-glucosamine (see the abbreviated structure for heparin in Fig. 25-1).[5] Hyaluronic acid contains glucuronic acid and *N*-acetyl glucosamine units, and the type III pneumococcus polysaccharide on hydrolysis yields glucose and glucuronic acid. These heteropolysaccharides contain two different sugars in each component monomer. Much more complex polysaccharides contain more than two monosaccharides; for example, gums and mucilages upon hydrolysis yield galactose, arabinose, xylose, and glucuronic and galacturonic acids.

Research in the field of structure–activity relationships among polysaccharides continues to increase the understanding of the relationship between their conformations in solution and biologic function. It has been noted that polysaccharides of the pyranose forms of glucose, galactose, mannose, xylose, and arabinose have conformations that are restricted by steric factors. Such polysaccharides have been characterized and classified on the basis of conformation properties: type A, extended and ribbon-like; type B, helical and flexible; type C, rigid and crumpled; and type D, very flexible and extended. Interestingly, most support materials are categorized in type A and most matrix materials belong to type B. Cellulose and chitin form rigid structures; these are the most important structural polysaccharides in nature and possess β-1,4 linkages. Matrix materials form gels, and this property is fundamental to their biologic functions as ground material filling the extracellular spaces of tissue, synovial fluid, or part of the vitreous humor.[5] It has been suggested that some matrix materials produce gels by forming double helices. Hyaluronic acid, which possesses β-1,3 and β-1,4 linkages (see Fig. 25-1), has been studied, and its gelling properties appear to be dependent on double-helix formation.[11] The foregoing summary and particularly the cited reference illustrate the significance of polysaccharides as the fibrous and matrix materials in support structures of plants and animals. However, glycogen and starches, which serve as readily available energy sources for humans, possess mostly α-1,4-glycosidic bonds and some branching through α-1,6-glycosidic bonds. Amylose is the unbranched type of starch and possesses only α-1,4 linkages. Glycogen branching occurs at every eight to ten residues, whereas amylopectin branch points occur at about every 25 to 30 residues to affect their physicochemical properties.[5] The increased branching enhances the solubility of glycogen to increase its mobilizability (see Fig. 25-1).

Many different carbohydrates occur as components of glycoproteins. The term "glycoprotein" is used in a general sense and includes proteins that contain covalently bonded carbohydrates.* Glycoproteins are widely distributed in animal tissues, and some have been found in plants and microorganisms. All plasma proteins (except albumin), proteins of mucous secretions (antibodies, clotting factors), some hormones (e.g., thyroglobulin, chorionic gonadotropin), certain enzymes (e.g., serum cholinesterase, deoxyribonuclease), components of cellular and extracellular membranes (asialoglycoprotein and β-receptors), and constituents of connective tissue are classified as glycoproteins. The bonding of the carbohydrate moiety, oligosaccharide, to the peptide usually involves C-1 of the most internal sugar and a functional group of an amino acid within the peptide chain—for example, the linkage of *N*-acetylglucosamine through a β-glycosidic bond to the amide group of asparagine or by *O*-glycosidic linkage with the side-chain oxygen atom of serine or threonine residues.[6] A variety of oligosaccharides in differ-

* Some textbooks have subcategorized glycoproteins and glycolipids as proteoglycans and peptidoglycans, which are massive aggregates and contain a much greater portion of carbohydrates than proteins or lipids. Hyaluronic acid and heparin have been included in this subcategory.[5]

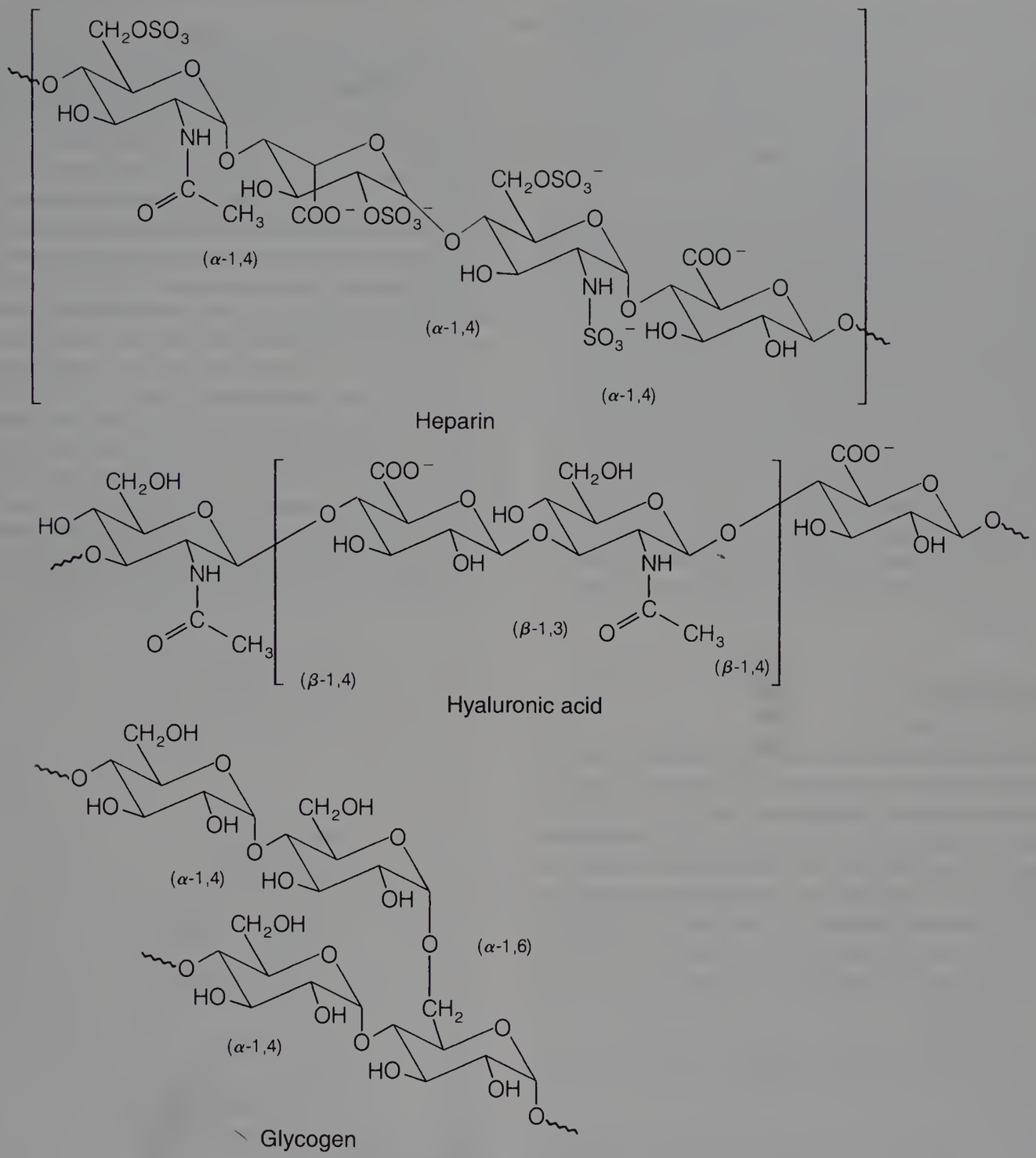


FIG. 25-1. *Structural relationships of three polysaccharides.*

ent patterns have been identified on the outer surface of cell membranes, immunoglobulins, peptide hormones, antibodies, and clotting factors.[6,12,13] The significance of these findings is based on the intricacies of the inherent carbohydrate stereochemistry, different glycosidic linkages, and sequence variations of the basic units. The unique information supplied by each oligosaccharide pattern has initiated studies involving carbohydrates in molecular targeting and cell–cell recognition. A review of cell culture studies implicated the development of antisense oligonucleotide and analogues as potential antiviral or antitumor agents.[10] The synthesis and metabolism of glycoproteins have been studied by numerous investigators, and the major studies have been reviewed.[15–17]

Glycolipids are carbohydrates containing lipids, and some are derivatives of sphingosine. The carbohydrates containing derivatives of ceramides are called *glycosphingolipids.*

Under normal circumstances, there is a steady state of balance between the synthesis and catabolism of glycosphingolipids in all cells. In the absence of any one of the hydrolases necessary for degradation, there is abnormal accumulation of intermediate metabolites, particularly in nervous tissue, which leads to various sphingolipodystrophies. There are three classes of glycosphingolipid: cerebrosides, gangliosides, and ceramide oligosaccharides.

Lipopolysaccharides of gram-negative bacteria have been studied with emphasis on structural elucidation. The peripheral portions of the lipopolysaccharides, called O-antigens, are composed of various carbohydrates arranged as oligosaccharide-repeating units forming high-molecular-weight polysaccharides. Structural details differ with the serotype of the organism. Some somatic O-antigens are highly toxic to animals. The lipopolysaccharide of the Enterobacteriaceae is one of the most complex of all polysaccharides, if not the most complex carbohydrate known. This polysaccharide has a gross structure: the carbohydrate moiety, which is the outermost portion, consists of abequose, mannose, rhamnose, galactose, and *N*-acetylglucosamine units; the lipid fraction includes glucosamine, phosphate, acetates, and β-hydroxymyristic acid.

BIOSYNTHESIS

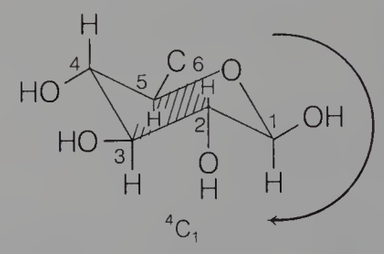
Photosynthesis proceeds in the chlorophyll-containing cells of plants. The photosynthetic process involves the absorption of radiant energy by chlorophyll and the conversion of the absorbed light energy into chemical energy. This chemical energy is necessary for the reduction of CO_2 from the atmosphere to form glucose. The so-formed glucose may be metabolized by the plant cells to form other carbohydrates, degraded to form precursors for the synthesis of other organic compounds, and oxidized as an energy source for the plant's physiology.

In higher plants, sucrose is synthesized through the activated form of glucose, uridine diphosphoglucose (UDPG), and fructose.

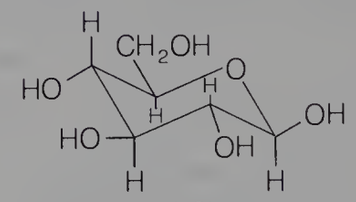
Polysaccharide biosynthesis also requires UDPG. For an illustration of polysaccharide formation, consider glycogenesis in hepatic tissue: a glycogen synthetase enzyme catalyzes the polymerization of glucose units from UDPG. The latter is obtained from the reaction between glucose-1-phosphate and uridine triphosphate (UTP). It is noteworthy that UDPG performs an important role in the formation of the glycosidic linkage fundamental to the structure of oligosaccharides and polysaccharides. Analogous UDP compounds involving other monosaccharides are utilized in the biosynthesis of polysaccharides containing these sugars. The biosynthesis of cellulose is supposed to occur through the guanine-containing analogue of UDPG, guanosine diphosphoglucose (GDPG).

STEREOCHEMICAL CONSIDERATIONS

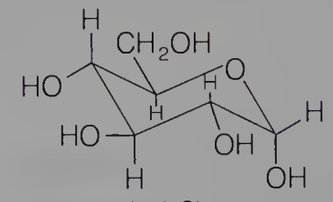
Basic organic chemistry textbooks cover the principles of stereoisomerism relevant to the study of carbohydrates.* The configurational and conformational aspects of carbohydrates have been reviewed by Bentley.[18] The stereochemistry of carbohydrates has presented many challenges to scientists, and there are several books[3,19] that treat this subject comprehensively; hence, here only a brief resumé is presented. Stoddard[19] reviewed stereochemical studies, including nomenclature, on the basis of conformational analysis. In addition to configurational designations (e.g., β-D-glucopyranose), italic letters are used to specify conformation: *C*, chair; *B*, boat; *S*, twist boat; *H*, half-chair; and so on. As an illustration, consider the structure of β-D-glucose: according to this system, conformation is defined by numerals indicating ring atoms lying above or below a defined reference plane; in structure 1 (see diagram) for β-D-(+)-glucose the reference plane contains C-2, C-3, C-5, and O; C-4 is above the plane and C-1 is below; hence, this conformation is designated as $^4C_1$ (compare with α-D-glucose). These symbols were proposed by the British Carbohydrate Nomenclature Committee, and they seem to be receiving general usage.



$^4C_1$



β-D-(+)-Glucose



α-D-(+)-Glucose

Advances in studies of configurational and conformational features of carbohydrate structures have been facilitated by x-ray crystallography, nuclear magnetic resonance

* Symbols *d* and *l* or (+) and (−) are used to designate sign of rotation of plane-polarized light, and the configuration is designated by the symbols in small capitals D and L; monosaccharides are designated as D or L on the basis of the configuration of the highest-numbered asymmetric carbon, the carbonyl being at the top: D if the — OH is on the right and L if the — OH is on the left. (+)-Mannose, (−)-arabinose are assigned to the D-family because of their relation to D-(+)-glucose and D-(+)-glyceraldehyde. Thus, sugars configurationally related to D-glyceraldehyde are said to be members of the D-family, and those related to L-glyceraldehyde belong to the L-series.

spectroscopy, and mass spectrometry combined with gas chromatography.[20,21]

INTERRELATIONSHIPS WITH LIPIDS AND PROTEINS

Various interrelationships of carbohydrates with proteins and lipids have been noted in the foregoing as glycoproteins and glycolipids were characterized. Many other relationships exist among metabolic processes involving carbohydrates, lipids, and proteins. Some of these relationships exemplify how regulation of metabolism is maintained through numerous mechanisms (e.g., feedback regulation and hormonal regulation).

The Krebs citrate cycle requires acetyl-CoA, and this requirement is satisfied by glycolysis and pyruvic acid decarboxylation, by the β-oxidation of fatty acids, by oxidation of glycerol through the glycolytic pathway, and by pyruvic acid from alanine transamination. Even more correlations are found within the steps of the Krebs cycle. Oxaloacetate can be transformed into aspartate or phosphoenolpyruvate and, subsequently, to carbohydrates. The transformation of oxaloacetate into carbohydrates is the metabolic route of gluconeogenesis. Lactate from anaerobic glycolysis is the major starting material for gluconeogenesis; the lactate (from pyruvate and carboxylation of pyruvate) is transformed into the key intermediate phosphoenolpyruvate.

In anabolism, acetyl-CoA, which can originate from carbohydrates, proteins, and lipids, is utilized in the synthesis of important metabolites, such as steroids and fatty acids.

Appreciation of the foregoing correlations between so many important metabolic and anabolic processes facilitates the understanding of how and why factors affecting certain processes directly also affect other processes indirectly.

Glucose must be activated by formation of UDPG before utilization in glycogenesis. The enzyme glycogen synthetase catalyzes the transformation of UDPG into glycogen. Glycogen catabolism to glucose proceeds through the action of phosphorylase *a*, which catalyzes phosphorolysis of glycogen, providing glucose-1-phosphate; the latter then enters glycolysis. At this point, the dynamism of hormonal regulation can be illustrated by referring to the following phenomena. If and when the blood glucose concentration decreases below the normal level, epinephrine (from the adrenal medulla) binds with membrane receptors to activate adenylate cyclase by stimulation of G proteins. Adenylate cyclase then catalyzes the formation of cAMP (cyclic-3′,5′-adenosine monophosphate). Cyclic AMP is a general intracellular mediator of many hormonal actions and stimulates the activation of phosphorylase *b* (inactive), providing phosphorylase *a* (active). Thus, the net effect of phosphorylase *a* action promotes glycogen breakdown, leading to glucose-1-phosphate and an increase in blood glucose concentration. This accounts for epinephrine's hyperglycemic action and explains how epinephrine agonists can affect carbohydrate metabolism, whereas opposite effects can be expected from epinephrine antagonism. Newton and Hornbrook[22] investigated the metabolic effects exerted by adrenergic agonists and antagonists and concluded that the order of potency was isoproterenol > norepinephrine > salbutamol, for stimulation of rat liver adenylate cyclase; a similar order of potency is reported for increased rat liver phosphorylase activity, but epinephrine produced a greater maximal response than isoproterenol. These authors also report that the β-adrenergic antagonist propranolol blocked the effects of isoproterenol or epinephrine on adenylate cyclase, whereas the α-adrenergic blockers ergotamine and phenoxybenzamine produced only partial inhibition. One explanation for this difference is based on the premise that α_1-receptors are associated with the phosphoinositide second-messenger system, whereas β_1- and β_2-receptors are involved in the adenylate cyclase cascade.[6] Therefore, it is noteworthy that the adrenergic metabolic receptor in the liver (rat) reacts to agonists and antagonists in parallel with the responses of those receptors in other tissues that have been designated β-adrenergic receptors. The rationalization behind this observation is based on the relationship between the β-receptor, a 64 kDa protein, and other receptors that are coupled to G proteins involving hormones that utilize cAMP as a second messenger and possess seven transmembrane helices.[23–25]

Abnormally low blood glucose levels also stimulate pancreatic α-cells to release the hormone glucagon, another hyperglycemic hormone. Glucagon, much like epinephrine, activates adenylate cyclase, promoting cAMP formation and leading to enhancement of glycogen catabolism; but glucagon affects liver cells and epinephrine affects both muscle and liver cells.

Adrenocortical hormones (i.e., the glucocorticoids) affect carbohydrate metabolism by promoting gluconeogenesis and glycogen formation. Because gluconeogenesis from amino acids is enhanced and because these hormones also inhibit protein synthesis in nonhepatic tissues, the precursor amino acids are made available for gluconeogenesis in the liver. Glucocorticoids stimulate synthesis of specific proteins in liver while inhibiting protein formation in muscle and other tissues. As protein catabolism continues in these tissues, the ultimate result is a net protein catabolic effect.

Sufficiently high blood glucose concentration stimulates pancreatic β-cells to secrete the hypoglycemic hormone insulin. Insulin exerts numerous biochemical actions,[26] affecting not only carbohydrate metabolism but also lipid and protein metabolism; glycogenesis, lipogenesis, and protein synthesis are enhanced by insulin, whereas ketogenesis from fatty acids, glycogenolysis, and lipolysis are suppressed by insulin. (Insulin deficiency leads to the opposite effects on these processes.) Insulin modifies the reaction rates of many processes in its target cells, and highly specific insulin–receptor interactions have been implicated.[27] Insulin receptors on adipose and liver cells have been characterized, and they appear to have uniform characteristics. Experimental evidence limits insulin action to the plasma membrane of target

cells. Insulin–receptor interactions can lead to modulation of other hormonal actions through mechanisms involving cAMP phosphodiesterase activation and inhibition of adenylate cyclase activation. Cyclic AMP phosphodiesterase is responsible for catalyzing cAMP hydrolysis, which inactivates cAMP; thus, insulin activation of this phosphodiesterase results in reversal of the metabolic effects of hormones that act through cAMP. However, many other compounds are capable of activating phosphodiesterase (e.g., cGMP and nicotinic acid), and even more compounds demonstrate inhibitory activity (e.g., xanthine derivatives, papaverine and related isoquinoline compounds, and some adrenergic amines). The foregoing processes have been reviewed, but a more recent review demonstrated other pharmacologic properties for similar compounds. Adenosine receptor agonists and antagonists, through some overlapping biochemical processes, have produced important central nervous system (CNS) effects. Therefore, the adenosine agonists have been implicated as potential novel neuroleptic agents, whereas the antagonists may become potential CNS stimulants or antidepressant agents.[9,28]

The specific biochemical effects exerted by insulin and glucagon are delineated in more detail in Chap. 26. Suffice it to say here that medicinal agents that promote insulin availability exert actions through insulin and a variety of other effects. Consider that the hypoglycemic sulfonylureas, which stimulate insulin secretion, also might act on phosphodiesterase, thereby inhibiting the inactivation of cAMP; moreover, cAMP has been implicated as a factor promoting insulin release. Reportedly some sulfonylureas also reduce glucagon secretion.

The biguanide phenformin was removed from the pharmaceutical market in 1977 because of the serious side effect, lactic acidosis.[29] The hypoglycemic action of phenformin involves various factors that promote glucose use. Although the exact molecular mechanism of action is unclear, phenformin promotes anaerobic glycolysis and exerts other effects. The action on anaerobic glycolysis is responsible for excessive lactic acid formation (from pyruvic acid reduction) and the development of lactic acidosis.

Another effect of high blood glucose levels is the glycosylation of hemoglobin by small sugar units. Even though the process is not well understood, it has raised interesting questions concerning the diagnosis and treatment evaluation of diabetics. Diabetics appear to have elevated blood levels of glycosylated hemoglobin that return to accepted limits when the concentration of blood glucose is normalized. The symptomatic significance of the elevated levels of glycohemoglobin is not clear, but studies have established that this type of hemoglobin has a lower affinity for oxygen.[5,14]

Feedback regulation of enzyme-catalyzed reactions is another basic mechanism for the regulation of metabolism (i.e., allosteric inhibition of a key enzyme). Phosphofructokinase, the pacemaker enzyme of glycolysis, is inhibited allosterically by ATP, and through such modulation, ATP suppresses carbohydrate catabolism. Of course, there are other cases of feedback regulation. Atkinson's classic article[30] on phenomena associated with biologic feedback control at the molecular level should be consulted to compare negative and positive feedback regulation. In contrast to the effect of ATP, AMP can exert positive regulatory action on phosphofructokinase. The regulatory metabolite acting as modulator modifies the affinity of the enzyme for its substrates, and the terms ''positive'' and ''negative'' are used to indicate whether there is an increase or a decrease, respectively, in affinity.

SUGAR ALCOHOLS

Sorbitol, glucitol, mannitol, galactitol, and dulcitol are natural products that are so closely related to the carbohydrates that it is traditional to classify them as carbohydrate derivatives (i.e., sugar alcohols). These compounds are reduction products of the corresponding aldohexoses—glucose, mannose, and galactose, respectively. Therefore, such sugar alcohols are characterized as hexahydroxy alcohols.

Sorbitol formation has been implicated as a factor contributing to complications of diabetes caused by high glucose concentration in nerve and eye lens cells. Cells that have excessive glucose tend to convert normally minor metabolic pathways to major processes (e.g., glucose reduction to form sorbitol). Sorbitol usually is not metabolized rapidly and cannot be eliminated effectively by the cell; because it accumulates in high concentrations in lens cells of diabetic rodents, osmotic swelling of the lens cells occurs. This swelling has been associated with the development of cataracts as a complication of diabetes. In diabetic rodents, cataract formation can be prevented with agents that inhibit the aldose reductase enzyme that catalyzes glucose reduction to sorbitol.[31] Such aldose reductase inhibitors are, therefore, under investigation as potential medicinal agents.[32] In this connection it should be noted that some hydantoins have demonstrated aldose reductase-inhibitory activity; for example, sorbinil is listed by the *USAN* (United States Adopted Names) *and USP Dictionary of Drug Names*, U.S. Pharmacopeia National Formulary, 1990.

Sorbitol, NF, is very water-soluble and produces sweet and viscous solutions. Hence, it is used in the formulation of some food products, cosmetics, and pharmaceuticals. Sorbitol solution, USP, is a 70% w/w solution that contains at least 64% D-sorbitol, the balance being related sugar alcohols. Upon dehydration it forms tetrahydropyran and tetrahydrofuran derivatives, the fatty acid monoesters of which are the nonionic surface-active agents called Spans. Alternatively, these dehydration products react with ethylene oxide to form the Tweens, which are also useful surfactants.

Mannitol, USP, is a useful medicinal. It acts as an osmotic diuretic and is administered intravenously. After intravenous infusion (in the form of a sterile 25% solution), it is filtered by glomeruli and passes unchanged through the kidneys into the urine; however, while in the proximal tubules, the loops of Henle, the distal tubules, and the collect-

ing ducts, mannitol increases the osmotic gradient against which these structures absorb water and solutes. Because of the foregoing osmotic effect, the urinary water, sodium, and chloride ions are increased. Mannitol also is indicated as an irrigating solution in transurethral prostatic resection.

Mannitol is widely used as an excipient in chewable tablets. In contrast with sorbitol, it is nonhygroscopic. In addition, it has a sweet and cooling taste.

SUGARS

Dextrose, USP. D-(+)-Glucopyranose; grape sugar; D-glucose; glucose (Table 25-1). Dextrose is a sugar usually obtained by the hydrolysis of starch. It can be either α-D-glucopyranose or β-D-glucopyranose or a mixture of the two. A large amount of the dextrose of commerce, whether crystalline or syrupy, usually is obtained by the acid hydrolysis of cornstarch, though other starches can be used.

Although some free glucose occurs in plants and animals, most of it occurs in starches, cellulose, glycogen, and sucrose. It also is found in other polysaccharides, oligosaccharides, and glycosides.

Dextrose occurs as colorless crystals or as a white, crystalline or granular powder. It is odorless and has a sweet taste. One gram of dextrose dissolves in about 1 mL of water and in about 100 mL of alcohol. It is more soluble in boiling water and in boiling alcohol.

TABLE 25-1

SUGAR PRODUCTS

Name	*Preparations*	*Category*	*Application*	*Usual Adult Dose**	*Usual Dose Range**	*Usual Pediatric Dose**
Dextrose USP	Dextrose injection USP	Fluid and nutrient replenisher		IV infusion, 1 L		
	Dextrose and sodium chloride injection USP	Fluid, nutrient, and electrolyte replenisher		IV infusion, 1 L		
	Anticoagulant citrate dextrose solution USP	Anticoagulant for storage of whole blood	For use in the proportion of 75 mL of solution A or 125 mL of solution B for each 500 mL of whole blood			
	Anticoagulant citrate phosphate dextrose solution USP	Anticoagulant for storage of whole blood	For use in the proportion of 70 mL of solution for each 500 mL of whole blood			
Calcium gluconate USP	Calcium gluconate injection USP	Calcium replenisher		IV, 10 mL of a 10% solution at a rate not exceeding 0.5 mL/min at intervals of 1–3 days	1 g/wk to 15 g/day	125 mg/kg of body weight or 3 g/m^2 of body surface, up to qid, diluted and given slowly
	Calcium gluconate tablets USP	Calcium replenisher		1 g 3 or more times daily	1–15 g/day	125 mg/kg of body weight or 3 g/m^2 of body surface, up to qid
Ferrous gluconate USP	Ferrous gluconate capsules USP Ferrous gluconate tablets USP	Iron supplement		300 mg tid	200–600 mg	
Fructose USP	Fructose injection USP	Fluid replenisher and nutrient		IV and SC, as required		
	Fructose and sodium chloride injection USP	Fluid replenisher, nutrient, and electrolyte replenisher		IV and SC, as required		
Lactose NF		Pharmaceutical aid (tablet and capsule diluent)				
Sucrose NF	Compressible sugar NF Confectioner's sugar NF	Pharmaceutical aid (sweetening agent; tablet excipient)				

* See USP DI for complete dosage information.

Aqueous solutions of glucose can be sterilized by autoclaving.

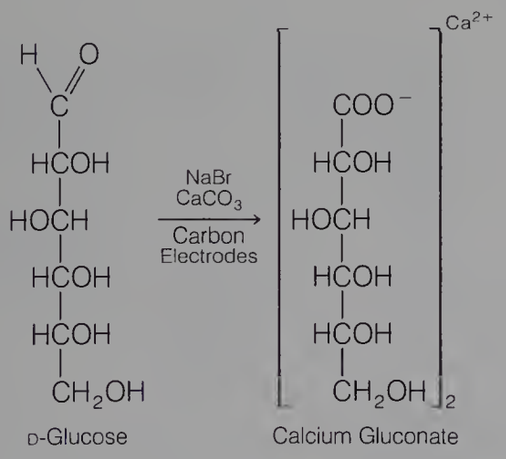
Glucose can be used as a ready source of energy in various forms of starvation. It is the sugar found in the blood of animals and in the reserve polysaccharide glycogen present in the liver and muscle. It can be used in solution intravenously to supply fluid and to sustain the blood volume temporarily. It has been used in the management of the shock that may follow the administration of insulin used in the treatment of schizophrenia because a "hypoglycemia" results from the use of insulin in this type of therapy and the "hypoglycemic" state can be reversed by the use of dextrose intravenously. When dextrose is used intravenously, its solutions (5% to 50%) usually are made with physiologic salt solution or Ringer's solution. The dextrose used for intravenous injection must conform to the *USP* requirements for dextrose.

Liquid glucose, NF, is a product obtained by the incomplete hydrolysis of starch. It consists chiefly of dextrose (D-glucose, $C_6H_{12}O_6$), with dextrins, maltose, and water. This glucose usually is prepared by the partial acid hydrolysis of cornstarch; hence, the common name "corn syrup" and other trade names refer to a product similar to liquid glucose. The official product contains not more than 21% of water.

Liquid glucose is a colorless or yellowish, thick, syrupy liquid. It is odorless, or nearly so, and sweet. Liquid glucose is very soluble in water but sparingly soluble in alcohol.

Liquid glucose is used extensively as a food (sweetening agent) for both infants and adults. It is used in the massing of pills, in the preparation of pilular extracts, and for other similar uses. It is not to be used intravenously.

Calcium Gluconate, USP. The gluconic acid used in the preparation of calcium gluconate can be prepared by the electrolytic oxidation of glucose as follows:



D-Glucose — Calcium Gluconate

Gluconic acid is produced on a commercial scale by the action of a number of fungi, bacteria, and molds upon 25% to 40% solutions of glucose. The fermentation is best carried out in the presence of calcium carbonate and oxygen to give almost quantitative yields of gluconic acid. Several organisms can be used, for example, *Acetobacter oxydans, A. aceti, A. rancens, B. gluconicum, A. xylinum, A. roseus,* and *Penicillium chrysogenum.* The fermentation is complete in 8 to 18 days.

Calcium gluconate occurs as a white, crystalline or granular powder without odor or taste. It is stable in air. Its solutions are neutral to litmus paper. One gram of calcium gluconate dissolves slowly in about 30 mL of water and in 5 mL of boiling water. Each milliliter of 10% solution represents 9.3 mg of calcium. It is insoluble in alcohol and in many other organic solvents.

Calcium gluconate will be decomposed by the mineral acids and other acids that are stronger than gluconic acid. It is incompatible with soluble sulfates, carbonates, bicarbonates, citrates, tartrates, salicylates, and benzoates.

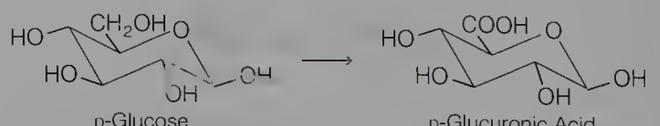
Calcium gluconate fills the need for a soluble, nontoxic, well-tolerated form of calcium that can be employed orally, intramuscularly, or intravenously. Calcium therapy is indicated in conditions such as parathyroid deficiency (tetany) and general calcium deficiency and when calcium is the limiting factor in increased clotting time of the blood. It can be used both orally and intravenously.

Calcium Gluceptate, USP. Calcium glucoheptonate is a sterile, aqueous, approximately neutral solution of the calcium salt of glucoheptonic acid, a homologue of gluconic acid. Each milliliter of calcium gluceptate injection, USP, represents 18 mg of calcium. Its uses and actions are the same as those of calcium gluconate.

Ferrous Gluconate, USP. Iron (2+) gluconate (Fergon) occurs as a fine yellowish gray or pale greenish yellow powder with a slight odor like that of burnt sugar. One gram of this salt is soluble in 10 mL of water; however, it is nearly insoluble in alcohol. A 5% aqueous solution is acid to litmus.

Ferrous gluconate can be administered orally or by injection for the utilization of its iron content.

Glucuronic acid occurs naturally as a component of many gums, mucilages and hemicelluloses and in the mucopolysaccharide portion of a number of glycoproteins. It is used by animals and humans to detoxify such substances as camphor, menthol, phenol, salicylates, and chloral hydrate. None of the foregoing can be used to prepare glucuronic acid for commercial purposes. It is prepared by oxidizing the terminal primary alcohol group of glucose or a suitable derivative thereof, such as 1,2-isopropylidine-D-glucose. It is a white, crystalline solid that is water-soluble and stable. It exhibits both aldehydic and acidic properties. It also may exist in a lactone form and, as such, is marketed under the name Glucurone, an abbreviation of glucuronolactone.

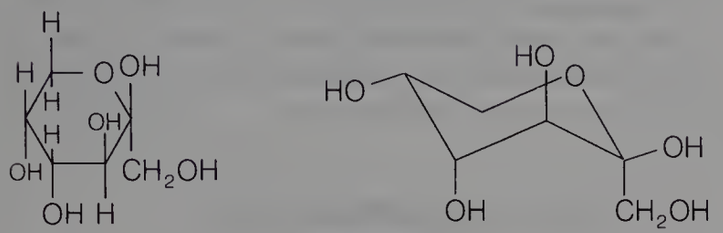


D-Glucose — D-Glucuronic Acid

An average of 60% effectiveness was obtained in the relief of certain arthritic conditions by the use of glucuronic acid. A possible rationale for the effectiveness of glucuronic acid in the treatment of arthritic conditions is based upon the fact that it is an important component of cartilage, nerve sheath,

joint capsule tendon, synovial fluid, and intercellular cement substances. The dose is 500 mg to 1.0 g orally four times a day or 3 to 5 mL of a 10% buffered solution given intramuscularly.

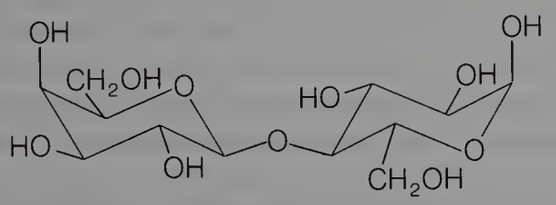
Fructose, USP. D-(−)-Fructose; levulose; β-D-(−)-fructopyranose. Fructose is a sugar usually obtained by hydrolysis of aqueous solutions of sucrose and subsequent separation of fructose from glucose.* It occurs as colorless crystals or as a white or granular powder that is odorless and sweet. It is soluble 1:15 in alcohol and freely soluble in water. Fructose is considerably more sensitive to heat and decomposition than glucose, and this is especially true in the presence of bases.



β-D-Fructopyranose

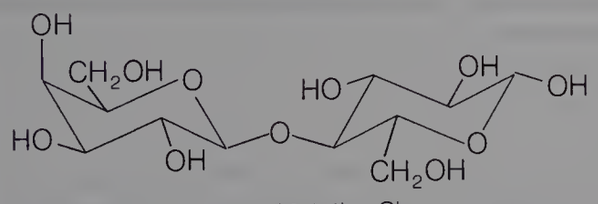
Fructose (a 2-ketohexose) can be utilized to a greater extent than glucose by diabetics and patients who must be fed by the intravenous route.

Lactose, NF. Saccharum lactis; milk sugar. This is a sugar obtained from milk. Lactose is a by-product of *whey*, the portion of milk that is left after the fat and the casein have been removed for the production of butter and cheese. Cow milk is 2.5% to 3% lactose, whereas that of other mammals contains 3% to 5%. Although common lactose is a mixture of the α- and β-forms, the pure β-form is sweeter than the slightly sweet-tasting mixture.



Galactose (β-1,4) Glucose

α-Lactose



Galactose (β-1,4) Glucose

β-Lactose

Lactose occurs as white, hard, crystalline masses or as a white powder. It is odorless and faintly sweet. It is stable in air but readily absorbs odors. Its solutions are neutral to litmus paper. One gram of lactose dissolves in 5 mL of water and in 2.6 mL of boiling water. Lactose is very slightly soluble in alcohol and insoluble in chloroform and ether.

Lactose is hydrolyzed readily in acid solutions to yield one molecule each of D-glucose and D-galactose. It reduces Fehling solution.

Lactose is used as a diluent in tablets and powders and as a nutrient for infants.

β-Lactose when applied locally to the vagina brings about a desirable lower *p*H. The lactose probably is fermented, with the production of lactic acid.

Maltose. Malt sugar; 4-D-glucopyranosyl-α-D-glucopyranoside is an end product of the enzymatic hydrolysis of starch by the enzyme diastase. It is a reducing disaccharide that is fermentable and is hydrolyzed by acids or the enzyme maltose to yield two molecules of glucose.

Maltose is a constituent of malt extract and is used for its nutritional value for infants and adult invalids.

Malt extract is a product obtained by extracting malt, the partially and artificially germinated grain of one or more varieties of *Hordeum vulgare* Linné (Gramineae). Malt extract contains maltose, dextrins, a small amount of glucose, and amylolytic enzymes.

Malt extract is used in the brewing industry because of its enzyme content, which converts starches to fermentable sugars. It also is used in infant feeding for its nutritive value and laxative effect.

The usual dose is 15 g.

Dextrins are obtained by the enzymatic (diastase) degradation of starch. These degradation products vary in molecular weight in the following decreasing order: amylodextrin, erythrodextrin, and achroodextrin. Lack of homogeneity precludes the assignment of definite molecular weights. With the decrease in molecular weight, the color produced with iodine changes from blue to red to colorless.

Dextrin occurs as a white, amorphous powder that is incompletely soluble in cold water but freely soluble in hot water.

Dextrins are used extensively as a source of readily digestible carbohydrate for infants and adult invalids. They often are combined with maltose or other sugars.

Sucrose, NF. Saccharum; sugar; cane sugar; beet sugar. Sucrose is a sugar obtained from *Saccharum officinarum* Linné (Gramineae), *Beta vulgaris* Linné (Chenopodiaceae), and other sources. Sugar cane (15% to 20% sucrose) is expressed, and the juice is treated with lime to neutralize the plant acids. The water-soluble proteins are coagulated by heat and removed by skimming. The resultant liquid is decolorized with charcoal and concentrated. Upon cooling, the sucrose crystallizes out. The mother liquor, upon concentration, yields more sucrose, brown sugar, and molasses.

Sucrose occurs as colorless or white, crystalline masses or blocks or as a white, crystalline powder. It is odorless, sweet, and stable in air. Its solutions are neutral to litmus.

* The crystalline form of fructose is the β-anomer, having a six-membered ring; when dissolved in water, it is converted not only to the α-form but to the β-form also. The fructofuranose forms were called "gamma"

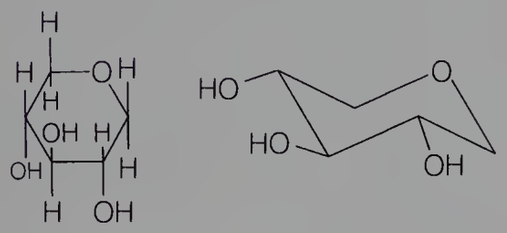
One gram of sucrose dissolves in 0.5 mL of water and in 170 mL of alcohol.

Sucrose does not respond to tests for reducing sugars (i.e., reduction of Fehling solution and others). It is hydrolyzed readily, even in the cold, by acid solutions to give one molecule each of D-glucose and D-fructose. This hydrolysis also can be effected by the enzyme invertase. Sucrose caramelizes at about 210°C.

Sucrose is used in the preparation of syrups and as a diluent and sweetening agent in several pharmaceutic products (e.g., troches, lozenges, and powdered extracts). In a concentration of 800 mg/mL, sucrose is used as a sclerosing agent.

Invert sugar (Travert) is a hydrolyzed product of sucrose (invert sugar) prepared for intravenous use.

Xylose, USP, is used as a diagnostic aid in testing for intestinal absorptive capacity in the diagnosis of celiac disease.



α-D-Xylose

STARCH AND DERIVATIVES

Starch, NF. Amylum. Cornstarch consists of the granules separated from the grain of *Zea mays* Linné (Gramineae). Corn, which contains about 75% dry weight of starch, is first steeped with sulfurous acid and then milled to remove the germ and the seed coats. It then is milled with cold water, and the starch is collected and washed by screens and flotation. Starch is a high-molecular-weight carbohydrate composed of 10% to 20% of a hot water-soluble "amylose" and 80% to 90% of a hot water-insoluble "amylopectin." Amylose is hydrolyzed completely to maltose by the enzyme β-amylase, whereas amylopectin is hydrolyzed only incompletely (60%) to maltose. The glucose residues are in the form of branched chains in the amylopectin molecule. The chief linkages of the glucose units in starch are α-1,4, because β-amylase hydrolyzes only α-linkages and maltose is 4-D-glucopyranosyl-α-D-glucopyranoside.

Starch occurs as irregular, angular, white masses or as a fine powder and consists chiefly of polygonal, rounded, or spheroidal grains from 3 to 35 μm in diameter, usually with a circular or several-rayed central cleft. It is odorless and has a slight characteristic taste. Starch is insoluble in cold water and in alcohol.

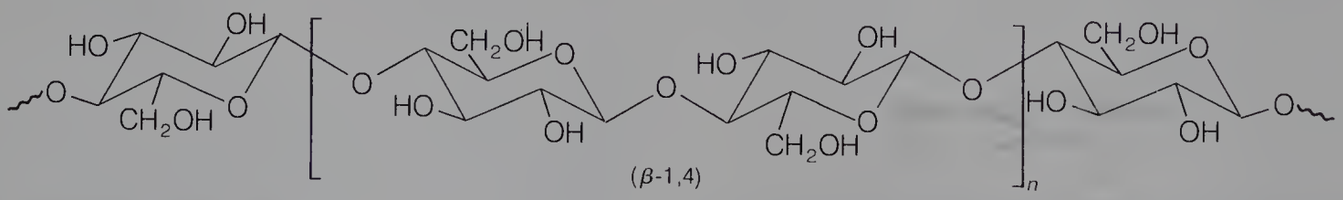
Amylose gives a blue color on treatment with iodine, and amylopectin gives a violet to red-violet color.

Starch is used as an absorbent in starch pastes, as an emollient in the form of a glycerite, and in tablets and powders.

Pregelatinized Starch, NF. This is starch that has been modified to make it suitable for use as a tablet excipient. It has been processed in the presence of water to rupture most of the starch granules and then dried.

CELLULOSE AND DERIVATIVES

"Cellulose" is the name generally given to a group of very closely allied substances, rather than to a single entity (Table 25-2). The celluloses are anhydrides of β-glucose, possibly existing as long chains that are not branched, consisting of 100 to 200 β-glucose residues. These chains may be cross-linked by residual valences (hydrogen bonds) to produce the supporting structures of the cell walls of plants. The cell walls found in cotton, pappi on certain fruits, and other sources are the purest forms of cellulose; however, because they are cell walls, they enclose varying amounts of substances that are proteinaceous, waxy, or fatty. These must be removed by proper treatment to obtain pure cellulose. Cellulose from almost all other sources is combined by ester linkages, glycoside linkages, and other combining forms with encrusting substances, such as lignin, hemicelluloses, and pectins. These can be removed by steam under pressure, weak acid or alkali solutions, and sodium bisulfite and sulfurous acid. Plant celluloses, especially those found in wood, can be resolved into β-cellulose, which is soluble in 17.5% sodium hydroxide, and alkali-insoluble α-cellulose. The cellulose molecule can be depicted in part as shown in the diagram below.



(β-1,4) (β-1,4) (β-1,4)

Cellulose

TABLE 25-2

PHARMACEUTICALLY IMPORTANT CELLULOSE PRODUCTS

Name Proprietary Name	*Preparations*	*Category*	*Usual Adult Dose**	*Usual Dose Range**
Purified cotton USP		Surgical aid		
Purified rayon USP		Surgical aid		
Microcrystalline cellulose NF		Pharmaceutical aid (tablet diluent)		
Powdered cellulose NF		Pharmaceutical aid (tablet diluent; adsorbant; suspending agent)		
Methylcellulose USP		Pharmaceutical aid (suspending agent; tablet excipient; viscosity-increasing agent)		
	Methylcellulose ophthalmic solution USP	Topical protectant (ophthalmic)		
	Methylcellulose tablets USP	Cathartic	1–1.5 g bid to qid	1–6 g/day
Ethylcellulose NF		Pharmaceutical aid (tablet binder)		
Hydroxypropyl methylcellulose USP		Pharmaceutical aid (suspending agent; tablet excipient; viscosity-increasing agent)	Topically to the conjunctiva, 0.05–0.1 mL of a 0.5–2.5% solution tid or qid, or as needed, as artificial tears or contact lens solution	
	Hydroxypropyl methylcellulose ophthalmic solution USP	Topical protectant (ophthalmic)		
Oxidized cellulose USP *Oxycel*		Local hemostatic	Topically as necessary to control hemorrhage	
Carboxymethylcellulose sodium USP		Pharmaceutical aid (suspending agent; tablet excipient; viscosity-increasing agent)		
	Carboxymethylcellulose sodium tablets USP	Cathartic	1.5 g tid	
Pyroxylin USP		Pharmaceutical necessity for Collodion USP		
Cellulose acetate phthalate NF		Pharmaceutical aid (tablet-coating agent)		

* See USP DI for complete dosage information.

Purified cotton, USP, is the hair of the seed of cultivated varieties of *Gossypium hirsutum* Linné or of other species of *Gossypium* (Malvaceae), freed from adhering impurities, deprived of fatty matter, bleached, and sterilized.

Microcrystalline cellulose, NF, is purified, partially depolymerized cellulose prepared by treating α-cellulose, obtained as a pulp from fibrous plant material, with mineral acids.

It occurs as a fine, white, odorless, crystalline powder that is insoluble in water, in dilute alkalies, and in most organic solvents.

Methylcellulose, USP, (Syncelose, Cellothyl, Methocel) is a methyl ether of cellulose, the methoxyl content of which varies between 26% and 33%. A 2% solution has a centipoise (cp) range of not less than 80% and not more than 120% of the labeled amount when cp is 100 or less and not less than 75% or more than 140% of the labeled amount for viscosity types higher than 100 cp.

Methyl- and ethylcellulose ethers (Ethocel) can be prepared by the action of methyl and ethyl chlorides or methyl and ethyl sulfates, respectively, on cellulose that has been treated with alkali. Purification is accomplished by washing the reaction product with hot water. The degree of methylation or ethylation can be controlled to yield products that vary in their viscosities when they are in solution. Seven viscosity types of methylcellulose are produced commercially and have the following centipoise values: 10, 15, 25, 100, 400, 1500, and 4000, respectively. Other intermediate viscosities can be obtained by the use of a blending chart. The ethyl celluloses have similar properties.

Methylated celluloses of a lower methoxy content are soluble in cold water, but, in contrast with the naturally occurring gums, they are insoluble in hot water and are precipitated out of solution at or near the boiling point. Solutions of powdered methylcellulose can be prepared most readily by first mixing the powder thoroughly with one-fifth to one-third of the required water as hot water (80° to 90°C) and

allowing it to macerate for 20 to 30 minutes. The remaining water is then added as cold water. With the increase in methoxy content, the solubility in water decreases until complete water insolubility is reached.

Methylcellulose resembles cotton in appearance and is neutral, odorless, tasteless, and inert. It swells in water and produces a clear to opalescent, viscous, colloidal solution. Methylcellulose is insoluble in most of the common organic solvents. However, aqueous solutions of methylcellulose can be diluted with ethanol.

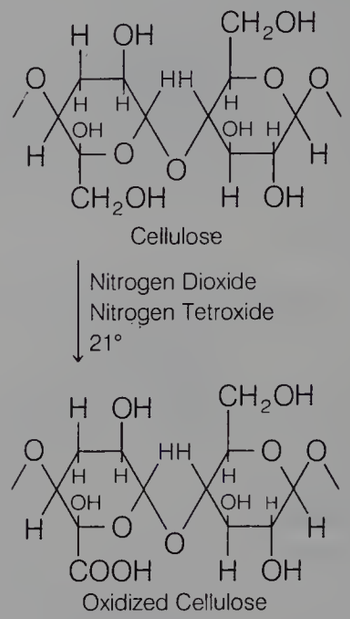
Methylcellulose solutions are stable over a wide range of *p*H (2 to 12) with no apparent change in viscosity. The solutions do not ferment and will carry large quantities of univalent ions, such as iodides, bromides, chlorides, and thiocyanates. However, smaller amounts of polyvalent ions, such as sulfates, phosphates, carbonates, and tannic acid or sodium formaldehyde sulfoxylate, will cause precipitation or coagulation.

The methylcelluloses are used as substitutes for the natural gums and mucilages, such as gum tragacanth, gum karaya, chrondrus, or quince seed mucilage. They can be used as bulk laxatives and in nose drops, ophthalmic preparations, burn preparations, ointments, and like preparations. Although methylcellulose when used as a bulk laxative takes up water quite uniformly, tablets of methylcellulose have caused fecal impaction and intestinal obstruction. Commercial products include Hydrolose syrup, Anatex, Cologel liquid, Premocel tablets, and Valo-call. In general, methylcellulose of the 1500 or 4000 cp viscosity type is the most useful as a thickening agent when used in 2% to 4% concentrations. For example, a 2.5% concentration of a 4000 cp-type methylcellulose will produce a solution with a viscosity obtained by 1.25% to 1.75% of tragacanth.

Ethylcellulose, NF, is an ethyl ether of cellulose containing not less than 45% and not more than 50% of ethoxy groups, prepared from ethyl chloride and cellulose. It occurs as a free-flowing, stable white powder that is insoluble in water, glycerin, and propylene glycol but freely soluble in alcohol, ethyl acetate, or chloroform. Aqueous suspensions are neutral to litmus. Films prepared from organic solvents are stable, clear, continuous, flammable, and tough.

Hydroxypropyl Methylcellulose 2208, USP. Propylene glycol ether of methyl cellulose contains a degree of substitution of no less than 19% or more than 24% as methoxyl groups (OCH_3) and no less than 4% or more than 12% as hydroxypropyl groups (OC_3H_6OH). It occurs as a white, fibrous or granular powder that swells in water to produce a clear to opalescent, viscous, colloidal solution.

Oxidized cellulose, USP, (Oxycel) when thoroughly dry contains no less than 16% or more than 24% of carboxyl groups. Oxidized cellulose is cellulose in which some of the terminal primary alcohol groups of the glucose residues have been converted to carboxyl groups. Therefore, the product is possibly a synthetic polyanhydrocellobiuronide. Although the *USP* accepts carboxyl contents as high as 24%, it is reported that products that contain 25% carboxyl groups are too brittle (friable) and too readily soluble to be of use. Those products that have lower carboxyl contents are the most desirable. Oxidized cellulose is slightly off-white, is acid to the taste, and possesses a slight, charred odor. It is prepared by the action of nitrogen dioxide or a mixture of nitrogen dioxide and nitrogen tetroxide upon cellulose fabrics at ordinary temperatures. Because cellulose is a high-molecular-weight carbohydrate composed of glucose residues joined 1,4- to each other in their β-forms, the reaction must be as diagrammed on the cellulose molecule in part.



The oxidized cellulose fabric, such as gauze or cotton, resembles the parent substance. It is insoluble in water and acids but soluble in dilute alkalies. In weakly alkaline solutions, it swells and becomes translucent and gelatinous. When wet with blood, it becomes slightly sticky and swells, forming a dark brown gelatinous mass. Oxidized cellulose cannot be sterilized by autoclaving. Special methods are needed to render it sterile.

Oxidized cellulose has noteworthy hemostatic properties. However, when it is used in conjunction with thrombin, it should be neutralized previously with a solution of sodium bicarbonate. It is used in various surgical procedures in much the same way as gauze or cotton, by direct application to the oozing surface. Except when used for hemostasis, it is not recommended as a surface dressing for open wounds. Oxidized cellulose implants in connective tissue, muscle, bone, serous and synovial cavities, brain, thyroid, liver, kidney, and spleen were absorbed completely in varying lengths of time, depending on the amount of material introduced, the extent of operative trauma, and the amount of blood present.

Carboxymethylcellulose Sodium, USP. CMC, sodium cellulose glycolate, is the sodium salt of a polycarboxymethyl ether of cellulose, containing, when dried, 6.5% to 9.5% of sodium. It is prepared by treating alkali cellulose with sodium chloroacetate. This procedure permits control

of the number of $—OCH_2COO^- Na^+$ groups that are to be introduced. The number of $—OCH_2COO^- Na^+$ groups introduced is related to the viscosity of aqueous solutions of these products. CMC is available in various viscosities, 5 to 2000 cp in 1% solutions. Therefore, high-molecular-weight polysaccharides containing carboxyl groups have been prepared whose properties in part resemble those of the naturally occurring polysaccharides, whose carboxyl groups contribute to their pharmaceutic and medicinal usefulness.

Carboxymethylcellulose sodium occurs as a hygroscopic white powder or granules. Aqueous solutions may have a *p*H between 6.5 and 8. It is easily dispersed in cold or hot water to form colloidal solutions that are stable to metal salts and *p*H conditions from 2 to 10. It is insoluble in alcohol and organic solvents.

It can be used as an antacid but is more adaptable for use as a nontoxic, nondigestible, unabsorbable, hydrophilic gel used as an emollient-type bulk laxative. Its bulk-forming properties are not as great as those of methylcellulose; however, its lubricating properties are superior, with little tendency to produce intestinal blockage.

Pyroxylin, USP. Soluble guncotton is a product obtained by the action of nitric and sulfuric acids on cotton and consists chiefly of cellulose tetranitrate $[C_{12}H_{16}O_6(NO_3)_4]$. The glucose residues in the cellulose molecule contain three free hydroxyl groups that can be esterified. Two of these three hydroxyl groups are esterified to give the official pyroxylin, and therefore, it is really a dinitrocellulose or cellulose dinitrate which conforms to the official nitrate content.

Pyroxylin occurs as a light yellow, matted mass of filaments, resembling raw cotton in appearance but harsh to the touch. It is exceedingly flammable and decomposes when exposed to light, with the evolution of nitrous vapors and a carbonaceous residue. Pyroxylin dissolves slowly but completely in 25 parts of a mixture of 3 volumes of ether and 1 volume of alcohol.

In the form of collodion and flexible collodion, it is used for coating purposes or in conjunction with certain medicinal agents.

Cellulose acetate phthalate, NF, is a partial acetate ester of cellulose that has been reacted with phthalic anhydride. One carboxyl of the phthalic acid is esterified with the cellulose acetate. The finished product contains about 20% acetyl groups and about 35% phthalyl groups. In the acid form, it is soluble in organic solvents and insoluble in water. The salt form is readily soluble in water. This combination of properties makes it useful in enteric coating of tablets because it is resistant to the acid condition of the stomach but soluble in the more alkaline environment of the intestinal tract.

HEPARIN

Heparin is a mucopolysaccharide composed of α-D-glucuronic acid, α-L-iduronic acid, α-D-glucosamine, and *N*-acetyl-α-D-glucosamine; these monosaccharide units are partially sulfated and linked in the polymeric form through 1 → 4 linkages, as indicated by the structure shown in Fig. 25-1. Heparin is present in animal tissue of practically all types, but mainly in lung and liver tissue.[33]

The chemistry and pharmacology of heparin have been reviewed by Ehrlich and Stivala.[34] This review comprehensively covers most topics pertinent to medicinal chemistry. Heparin is included among the *AMA Drug Evaluations* (1983) and USP DI (1995) anticoagulants.[35,36] Its greatest use has been in the prevention and arrest of thrombosis (see Chap. 26 on the biochemical functions performed by thrombin, fibrinogen, and fibrin in normal blood coagulation).

The mechanism of anticoagulant action exerted by heparin has been investigated from various standpoints, and now it is recognized that the mechanism involves the plasma protein inhibitor of serine proteases, *antithrombin III*. This naturally occurring inhibitor inactivates various critical clotting factors, which are enzymes designated as IXa, Xa, XIa, thrombin, and perhaps XIIa that have a serine residue within the reactive center. Antithrombin III interacts with and inhibits these factors irreversibly. Heparin interacts with antithrombin III and induces conformational changes that complement the interaction between antithrombin III and the aforementioned factors.[37]

Jaques[38] summarized studies that have shown that heparin is a biochemical representative of a class of compounds characterized as linear anionic polyelectrolytes. Such compounds demonstrate interesting specific reactions with biologically active proteins, forming stable complexes that change the bioactivity of these proteins. These complexations increase the negative charge of cell surfaces, including those of the blood vessel walls. The increase in the negative charge of the vessel wall is considered to be a factor that contributes to the prevention of thrombosis by heparin and similar compounds.

Heparin also affects fibrinolysis. It seems to reduce the inhibition of antifibrinolysin and, thereby, enhances fibrinolysis. Heparin's effects on platelets have been studied, and it was shown to prevent conversion of degenerated platelets in solution from forming a gel, to inhibit platelet adhesion to intercellular cement, and to prevent platelet disintegration and release of phospholipids. Another major effect is on blood lipids. It stimulates the release of lipoprotein lipase, an enzyme that catalyzes the hydrolysis of triglycerides associated with chylomicrons and, through this action, promotes the clearing of lipemic plasma. Research has included the possible effect of heparin on tumor growth and metastasis. Some studies show that heparin is a miotic inhibitor in Ehrlich's ascites tumor. Other investigations have produced negative data, and, hence, the question remains unanswered.[34]

Protamine has been characterized as a heparin antagonist, but it has the characteristic of prolonging clotting time on its own. Protamine (discussed also in Chap. 26) is basic enough to interact with heparin (which is acidic owing to its *O*-sulfate and *N*-sulfate groups). When protamine and heparin interact, they neutralize the action of each other.

It appears that the reticuloendothelial system may be involved in the disposition of heparin; that is, heparin may leave the plasma by uptake into the reticuloendothelial system. Data from kinetic studies of heparin removal from circulation of the minipig are consistent with this suggestion.[39]

Heparin is metabolized primarily in the liver by partial cleavage of the sulfate groups to form uroheparin and excreted by the kidneys, primarily as a partially sulfated product. Up to 50% may be excreted unchanged when high doses are given. The partially desulfated product excreted in the urine has been shown to be one-half as active as heparin in anticoagulant properties.

Heparan sulfate is the polysaccharide found as a by-product in the preparation of heparin from lung and liver tissue. Heparan sulfate has a lower sulfate content, and its glucosamine residues are partially acetylated and *N*-sulfated. Heparan sulfate isolated from the aorta has negligible antithrombin activity.

Heparin Calcium, USP; Heparin Sodium, USP. Heparin may be prepared commercially from lung and liver, employing the procedure of Kuizenga and Spaulding[33] combined with suitable methods for purifying the isolated heparin. The calcium salt has been prepared from porcine intestinal mucosa and the sodium salt from either porcine intestinal mucosa or bovine lung tissue. The sodium and calcium salts are white, amorphous, hygroscopic powders that are soluble (1 : 20) in water but poorly soluble in alcohol. A 1% aqueous solution has an adjusted *p*H of 5 to 7.5. It is relatively stable to heat, and solutions may be sterilized by atuoclaving but should never be frozen.

For full-dose therapy, heparin is administered intravenously in two ways: (1) the intermittent injection method and (2) the continuous infusion method or by deep subcutaneous (intrafat) injection. The fixed-combination preparation containing dihydroergotamine mesylate and heparin sodium is administered *only* by deep subcutaneous injection. More importantly, heparin should not be administered intramuscularly because of the frequency of irritation, pain, and hematoma at the injection site. The continuous infusion method is preferred because it provides a more constant anticoagulating activity and lower incidence of bleeding complications. A constant-rate infusion pump also is recommended.[40]

The therapeutic use of subcutaneous heparin in low doses was investigated extensively, and some reports favorably evaluated this mode of administration. Therefore, a fixed low-dose therapy also is utilized, which involves the administration of heparin calcium or heparin sodium by deep subcutaneous injection.[41–43]

A common side effect with heparin can be hemorrhage, but this can be minimized with the low-dose regimen.[44]

Category: anticoagulant

Usual full-dose: parenteral, the following amounts, as indicated by prothrombin-time determinations: IV, 10,000 USP heparin units initially, then 5000 to 10,000 U every 4 to 6 hours; infusion: 20,000 to 40,000 U/L at a rate of 1000 U/hour over a 24-hour period; subcutaneous: 10,000 to 20,000 U initially, then 8000 to 10,000 U every 8 hours or 15,000 to 20,000 U every 12 hours.

Usual pediatric dose: IV injection: 50 U/kg of body weight initially, then 50 to 100 U/kg of body weight every 4 hours; infusion: 50 U/kg of body weight initially, followed by 100 U/kg, added and absorbed every 4 hours.

Occurrence: Heparin sodium injection USP; Heparin calcium injection USP

LOW-MOLECULAR-WEIGHT HEPARINS[45,46]

Low-molecular-weight (LMW) heparins are fragments or fractions of standard (unfractionated) commercial grade heparin produced by either enzymatic or chemical depolymerization of standard heparin. LMW heparins may contain up to 25 different molecular fragments, each having a molecular weight between 4000 and 9000 and the average molecular weight is between 4000 and 5000.[47] Each fragment may consist of two to 50 monosaccharide units and exhibit 9 varying degrée of antithrombotic activity.[48] Hydrolysis with nitrous acid, enzymatic cleavage with heparinase, and hydrolytic degradation with hydrogen peroxide are the three most commonly used methods to depolymerize standard heparin. The LMW heparin fragments resulting from any of these methods of depolymerization contain the unique pentassaccharide required for specific binding with antithrombin III but in lower proportions than found in standard heparin preparations.[45]

Two major differences in the therapeutic profiles of LMW heparins and standard heparin have been noted. First, the LMW heparins were observed to progressively (with decreasing size) lose their ability to prolong the activated thromboplastin time (APTT) while maintaining their ability to inhibit activated factor X (factor Xa). Secondly, for an equivalent antithrombotic effect, the LMW heparins produce significantly less bleeding in experimental studies compared to standard heparin. In general, the LMW heparins possess greater anti-factor Xa activity relative to their antithrombin activity compared to standard heparin. The antithrombin activity of LMW heparins is directly dependent on the size of the fragments, with the larger fragments (more than 18 saccharide units) exhibiting higher antithrombin activity. The pharmacologic profile of LMW heparins relative to their molecular weights and size is found in Table 25-3.[45]

The LMW heparins exhibit lower binding affinity for several plasma proteins compared to standard heparin and minimal, if any, binding to endothelial cell-surface or macrophage receptors. The more predictable and higher bioavailability of LMW heparins following subcutaneous administration is believed to be due to lower intracellular degradation, resulting from lower binding of LMW heparins to cell-surface receptors. Following subcutaneous adminis-

TABLE 25-3

ACTIVITY OF LOW-MOLECULAR-WEIGHT HEPARINS[45]

Product	Ratio Antifactor Xa to Antithrombin Activity	Average Molecular Weight	Number of Saccharide Units	Half-Life (min)
Standard heparin	1:1	15,000	40–50	30–150
Enoxaparin	2.7:1	4,500	10–27	129–180
Dalteparin	2.0:1	5,000	7–30	119–139
Nadroparin	3.2:1	4,500	7–27	132–162
Ardeparin	2.0:1	6,000	7–50	200
Tinzaparin	1.9:1	4,500	10–20	111

tration, the LMW heparins have substantially longer half-lives than standard heparin. The lower risk of bleeding associated with LMW heparins also may be due to the lack of affinity for endothelial cell-surface receptors. LMW heparins cause less interference with platelet adhesion than standard heparin because of their lower affinity for binding to the von Willebrand factor.[45,46]

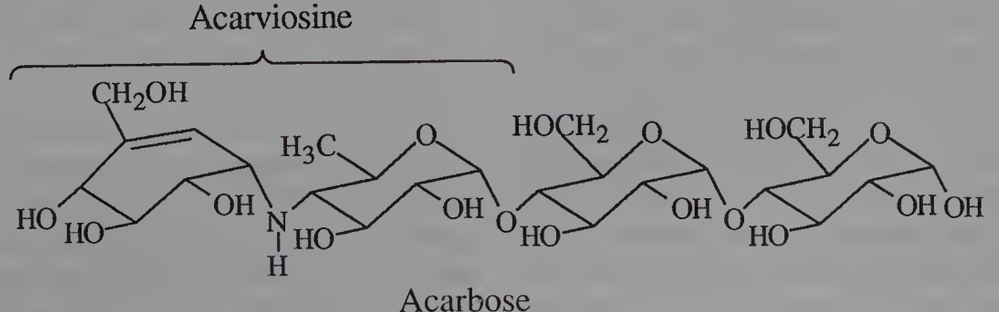
LMW heparins have higher anticoagulant potency in whole blood than standard heparin. It is postulated that during coagulation platelet factor 4, a potent inhibitor of standard heparin, is released. This factor has no inhibitory effect on the LMW heparins. Also, it has been observed that factor Xa bound to the platelet membrane in the prothrombinase complex is resistant to inactivation by standard heparin but is susceptible to inactivation by LMW heparins.[45]

Enoxaparin[49] (Lovenox) was the first LMW heparin approved in the United States, in 1993. Refer to Table 25-3 for comparative data on activity and structural differences between LMW heparins and standard heparin. It is indicated for the prophylaxis of deep venous thrombosis following total hip replacement surgery. It also is indicated for the prevention of thromboembolism in patients undergoing orthopedic or gynecologic surgical procedures. Absorption of enoxaparin following subcutaneous administration is almost complete. Enoxaparin has the longer duration of action, with respect to anti-factor Xa activity, compared to dalteparin or nodraparin. Its half-life following an intravenous dose of 40 mg is approximately 4.6 hours. Its onset of action, following subcutaneous injection, is approximately 3 hours. The normal dosage is 30 mg subcutaneously, twice daily. The primary route of elimination is renal. The most common adverse effect of enoxaparin is hemorrhagic complications.

Dalteparin[49] (Fragmin) is an LMW heparin, approved in the United States in 1994. Refer to Table 25-3 for comparative data on activity and structural differences between LMW heparins and standard heparin. Its indications are similar to enoxaparin, and it offers no advantages over the latter. Following subcutaneous administration, dalteparin exhibits peak anti-factor Xa activity in 2 to 4 hours, and its plasma half-life is 3 to 4 hours. Its normal dosage is 2500 IU (16 mg), administered subcutaneously 1 to 2 hours prior to surgery and then once daily for 5 to 10 days postoperatively. Hemorrhage is the primary adverse effect of dalteparin.

ALPHA-GLUCOSIDASE INHIBITOR

Acarbose (Precose) is a complex oligosaccharide initially isolated from a culture of actinomycetes. It competitively inhibits intestinal brush-border alpha-glucosidases including glucoamylase, sucrase, maltase, and isomaltase. The degree of inhibition depends upon the specific enzyme involved. The rank order of inhibition (most to least inhibited) is glucoamylase > sucrase > maltase > isomaltase. Acarbose has no inhibitory effect on lactase. The alpha-glucosidases are oligosaccharidases found in the brush-border of the intestinal mucosa and are responsible for the digestion of di-, tri-, and oligosaccharides (dextrins). Inhibition of alpha-glucosidases by acarbose causes a delay in the digestion of sucrose and other polysaccharides, thus retarding the rate of absorption of glucose and fructose. In clinical trials, acarbose was observed to significantly lower postprandial blood glucose levels and, therefore, may be employed as an adjunct to conventional treatment of diabetes mellitus involving oral hypoglycemic agents, insulin, and dietary measures.[50,51]



Acarbose

Structure–activity relationship studies have revealed that all inhibitors of alpha-glucosidases possess a common structural moiety known as acarviosine, which includes a substituted cyclohexene ring and a 4,6-dideoxy-4-amino-D-glucose moiety. Furthermore, it has been postulated that the secondary amino group of acarviosine may be involved in preventing an essential carboxyl group on the alpha-glucosidase enzyme from protonating the glucosidic oxygen of the substrate. The specificity of inhibitory action against various alpha-glucosidases appears to be determined by the number of glucose moieties linearly linked to acarviosine.[50,52]

Acarbose is indicated primarily in controlling hyperglycemia in patients with diabetes mellitus. The recommended dosage of acarbose for initiation of therapy is 25 mg three times daily at the beginning of each meal. The maintenance dose is 50 to 100 mg three times daily. In the treatment of diabetes, 50 to 200 mg of acarbose may be administered three times daily by the oral route. Systemic absorption of acarbose is less than 2%. Peak reduction of blood and serum glucose occurs at 69 minutes after the administration of an oral dose.[53] The most common adverse effects of acarbose are related to the impaired absorption of carbohydrates. These effects include diarrhea, flatulence, abdominal distention or pain, and meteorism.

GLYCOSIDES

Because several plant constituents yielded glucose and an organic hydroxide upon hydrolysis, ''glucoside'' was introduced as a generic term for these substances. The fact that many plant constituents yielded sugars other than glucose led to the suggestion of the less specific term ''glycoside.'' When the nature of the sugar residue is known, more specific terms can be used when desired, such as ''glucoside,'' ''fructoside,'' ''rhamnoside,'' and others, respectively. The nonsugar portion of the glycoside generally is referred to as the *aglycon* or *genin.*

Two general types of glycoside are known: the nitrogen glycosides and the conventional glycoside. The conventional glycoside has an acetal structure and can be illustrated by the simplest type, in which methyl alcohol is the aglycon or organic hydroxide. Two forms of this, as well as of all other glycosides, are possible: namely, α and β because of the asymmetry centering about C-1 of the sugar residue that contains the acetal structure. It is thought that all naturally occurring glycosides are of the β variety because the enzyme emulsin, which cannot hydrolyze synthetic α-glycosides, hydrolyzes naturally occurring glycosides. Some of the β-glycosides also are hydrolyzed by amygdalase, cellobiase, gentiobiase, and the phenol glycosidases. The α-glycosides are hydrolyzed by maltase, mannosidase, and trehalase.

Glycosides usually are hydrolyzed by acids and are relatively stable toward alkalies. Some glycosides are much more resistant to hydrolysis than others. For example, those glycosides that contain a 2-desoxy sugar are cleaved easily by weak acids, even at room temperature. However, most of the glycosides containing the normal-type sugars are quite resistant to hydrolysis, and of these, some may require rather drastic hydrolytic measures. The drastic treatment required for the hydrolysis of some glycosides causes chemical changes to take place in the aglycon portion of the molecule; these changes present problems in the elucidation of their structures. Conversely, those glycosides that are hydrolyzed very easily present problems in isolation and storage. Examples of the latter are the cardiac glycosides.

Although most glycosides are stable to hydrolysis by bases, the structure of the aglycon may determine its base sensitivity (e.g., picrocrocin has a half-life of 3 hours in 0.007 N KOH at 30°C).

The sugar component of glycosides may be a mono-, di-, tri-, or tetrasaccharide. A wide variety of sugars are found in the naturally occurring glycosides. Most of the unusual and rare sugars found in nature are components of glycosides.

The aglycons, or nonsugar portions of glycosides, are represented by a wide variety of organic compounds, as illustrated by the cardiac glycosides, the saponins, and others (see Chap. 23).

Because of the complexity of the structures of the naturally occurring glycosides, no generalizations are possible about their stabilities if the stabilities of the glycosidic linkages are excluded. It also follows that considerable deviations are met within their solubility properties. Many glycosides are soluble in water or hydroalcoholic solutions because the solubility properties of the sugar residues exert a considerable effect. Some glycosides, such as the cardiac glycosides, are slightly soluble or insoluble in water. For these, the steroid aglycon is markedly insoluble in water and offsets the solubility properties of the sugar residues. Most glycosides are insoluble in ether. Some glycosides are soluble in ethyl acetate, chloroform, or acetone.

Glycosides are widely distributed in nature. They are found in varying amounts in seeds, fruits, roots, bark, and leaves. Occasionally, two or more glycosides are found in the same plant (e.g., cardiac glycosides and saponins). Glycosides often are accompanied by enzymes that are capable of synthesizing or hydrolyzing them. This phenomenon introduces problems in the isolation of glycosides because the disintegration of plant tissues, with no precautions to inhibit enzymatic activity, may lead to partial or complete hydrolysis of the glycosides.

Most glycosides are bitter, although there are many that are not. Glycosides per se or their hydrolytic products furnish a number of drugs, some of which are very valuable. Some plants that contain the cyanogenetic-type glycoside present an agricultural problem. Cattle have been poisoned by eating plants that are rich in the cyanogenetic-type glycosides.

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CHAPTER 26

Amino Acids, Proteins, Enzymes, and Peptide Hormones

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Vilas A. Prabhu

Proteins are essential components of all living matter. As cellular components, proteins perform numerous functions. The chemical reactions fundamental to the life of the cell are catalyzed by proteins called enzymes. Other proteins are structural constituents of protoplasm and cell membranes. Some hormones are characterized as proteins or protein-like compounds because of their polypeptide structural features.

Protein chemistry is essential not only to the study of molecular biology in understanding how cellular components participate in the physiologic processes of organisms, but also to medicinal chemistry. An understanding of the nature of proteins is necessary for the study of those medicinal agents that are proteins or protein-like compounds and their physicochemical and biochemical properties relating to mechanisms of action. Also, in medicinal chemistry, drug–receptor interactions are implicated in the rationalization of structure–activity relationships and in the science of rational drug design. Drug receptors are considered to be macromolecules, some of which seem to be proteins or protein-like.

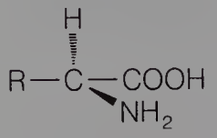
Recombinant DNA technology[1] has had a dramatic impact on our ability to produce complex proteins and polypeptides, structurally identical with those found endogenously. Many of these endogenous proteins or polypeptides have exhibited neurotransmitter and hormonal properties that regulate a variety of physiologic processes. Recombinant DNA-derived technology products, which are currently being used, are discussed later in this chapter.

This chapter reviews the medicinal chemistry of proteins and includes some discussion of those amino acids that are products of protein hydrolysis. Some amino acids (e.g., dopa) are useful therapeutic agents, and their mode of action relates to amino acid metabolism. Some medicinals are amino acid antagonists, and their biochemical effects relate to their therapeutic uses; hence, brief mention of some representative cases of amino acid antagonism will be made in the appropriate context. Moreover, the hormones with protein-like structure also are discussed, with emphasis on their biochemical effects.

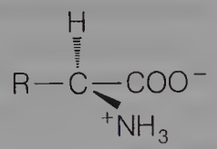
A study of medicinal chemistry cannot be made without including some enzymology, not only because many drugs affect enzyme systems, and vice versa, but also because fundamental lessons of enzymology have been applied to the study of drug–receptor interactions. Accordingly, this chapter includes a section on enzymes.

AMINO ACIDS

Proteins are biosynthesized from α-amino acids, and when proteins are hydrolyzed, amino acids are obtained. Some very complex (conjugated) proteins yield other hydrolysis products in addition to amino acids. α-Amino acids are commonly characterized with the generalized structure*:



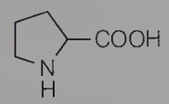
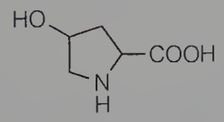
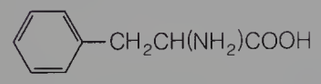
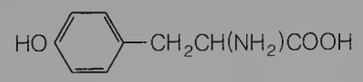
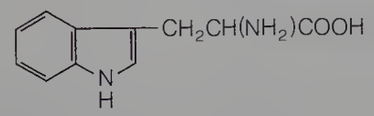
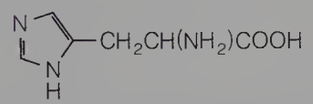
The most important amino acids are described in Table 26-1. Although the foregoing structure for amino acids is widely used, physical, chemical, and some biochemical properties of these compounds are more consistent with a dipolar ion structure.



* All α-amino acids, except glycine, are optically active because the *R* for the generalized structure represents some moiety other than hydrogen: the amino acids of proteins have the same absolute configuration as L-alanine, which is related to L-glyceraldehyde. (The D and L designations refer to configuration rather than to optical rotation.)

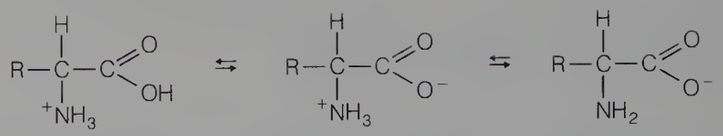
TABLE 26-1

NATURALLY OCCURRING AMINO ACIDS

Name	Symbol	Formula
Glycine	Gly	H_2NCH_2COOH
Alanine	Ala	$CH_3CH(NH_2)COOH$
Valine	Val	$(CH_3)_2CHCH(NH_2)COOH$
Leucine	Leu	$(CH_3)_2CHCH_2CH(NH_2)COOH$
Isoleucine	Ile	$CH_3CH_2CH(CH_3)CH(NH_2)COOH$
Serine	Ser	$HOCH_2CH(NH_2)COOH$
Threonine	Thr	$CH_3CH(OH)CH(NH_2)COOH$
Cysteine	Cys	$HSCH_2CH(NH_2)COOH$
Cystine	Cys	$(—SCH_2CH(NH_2)COOH)_2$
Methionine	Met	$CH_3SCH_2CH_2CH(NH_2)COOH$
Proline	Pro	
Hydroxyproline	Hyp	
Phenylalanine	Phe	
Tyrosine	Tyr	
Tryptophan	Trp	
Aspartic acid	Asp	$HOOCCH_2CH(NH_2)COOH$
Glutamic acid	Glu	$HOOCCH_2CH_2CH(NH_2)COOH$
Lysine	Lys	$H_2NCH_2CH_2CH_2CH_2CH(NH_2)COOH$
Arginine	Arg	$H_2NC(=NH)NH_2CH_2CH_2CH_2CH(NH_2)COOH$
Histidine	His	

The relatively high melting point, solubility behavior, and acid–base properties characteristic of amino acids can be accounted for on the basis of the dipolar ion structure (commonly called *zwitterion*). Amino acids in the dry solid state are dipolar ions (inner salts).

Amino acids, when dissolved in water, can exist as dipolar ions and, in this form, would make no contribution to migration in an electric field. The concentration of the dipolar ion will vary depending on the pK_a value of the amino acids and the hydronium ion concentration of the aqueous solution according to the following equilibrium:



The hydronium ion concentration of the solution can be adjusted, and, if expressed in terms of *p*H, the *p*H at which the concentration of the dipolar form is maximal has been called the *isoelectric point* for the amino acid. (Because proteins are polymers of amino acids, they also have zwitterion character and isoelectric points.)

Glycine has $pK_{a_1} = 2.34$ for the carboxyl group and $pK_{a_2} = 9.6$ for the protonated amino group. The R groups of other amino acids change the pK_a values slightly. The positive charge of I tends to repel a proton from the carboxyl group so that I is more strongly acidic than acetic acid ($pK_a = 4.76$). The pK_{a_2} value for III is less than methylamine because of the electron-withdrawing effect of the carboxyl group (see structure).

Table 26-1 demonstrates that most amino acids have complex side chains and that some amino acids have other functions (in addition to the α-carboxyl and α-amino groups) such as —OH, $—NH_2$, $—CO_2H$, —SH, phenolic —OH, guanidine, etc. These functions contribute to the physicochemical and biochemical properties of the respective amino acids or to their derivatives, including the proteins in which they are present. It has been customary to designate those amino acids that cannot be synthesized in the organism (animal) at a rate adequate to meet metabolic requisites as *essential amino acids.* Nutritionally essential amino acids (for humans) are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine.[2] At this point, it is important to note that some of these essential amino acids participate in the biosynthesis of other important metabolites; for example, histamine (from histidine), catecholamines and the thyroid hormones (from phenylalanine through tyrosine), serotonin from tryptophan, and others.

Amino acid antagonists have received the attention of many medicinal chemists. As antimetabolites these compounds interfere with certain metabolic processes and thereby exert, in some cases, therapeutically useful pharmacologic actions (e.g., α-methyldopa as a dopa decarboxylase inhibitor). Table 26-2 lists some other amino acid antagonists. The study of such antimetabolites as potential chemotherapeutic agents continues. Research in cancer chemotherapy has involved experimentation with many antagonists of amino acids. The glutamine antagonists azaserine and 6-diazo-5-oxonorleucine (DON), interfere with the metabolic processes that require glutamine and, thereby, disrupt nucleic acid synthesis (glutamine is required for nucleic acid formation; glutamine is derived from glutamic acid). The phenomenon *lethal synthesis* involves incorporation of the

TABLE 26-2

SELECTED AMINO ACID ANTAGONISTS

Amino Acid Antagonist	*Amino Acid Antagonized*	*Other Inhibitory Effects*
D-Alanine	L-Alanine	Carboxypeptidase
D-Phenylalanine	L-Phenylalanine	D-Amino acid oxidase
α-Methyl-L-methionine	L-Methionine	D-Amino acid oxidase
α-Methyl-L-glutaric acid	L-Glutaric acid	Glutamic decarboxylase
Ethionine	Methionine	
α-Methyldopa	Dopa	Dopa decarboxylase
Allyl glycine	Methionine	Growth of *E. coli*
Propargylglycine	Methionine	Growth of *E. coli*
2-Amino-5-heptenoic acid	Methionine	Growth of *E. coli*
2-Thienylalanine	Phenylalanine	Growth of yeast
p-Fluorophenylalanine	Phenylalanine	Incorporation of phenylalanine into protein molecules
L-*O*-Methylthreonine	Isoleucine	Competitive incorporation of leucine into proteins
4-Oxalysine	Lysine	Growth of *E. coli, L. casei*, etc.
6-Methyltryptophan	Tryptophan	
5,5,5-Trifluoronorvaline	Leucine, methionine	Growth of *E. coli*, etc.
3-Cyclohexene-l-glycine	Isoleucine	Inhibits *E. coli*
O-Carbamyl-L serine	L-Glutamine	Inhibits *E. coli, S. lactis*

antimetabolite into the protein structure or into the structure of some other macromolecule, and this unnatural macromolecule alters metabolic processes dependent on it. *O*-Methylthreonine competes with isoleucine for incorporation into protein molecules, whereas *O*-ethylthreonine is incorporated into tRNA in *Escherichia coli.*

Although all of the naturally occurring amino acids have been synthesized and several of them are available by the synthetic route, others are available more economically by isolation from hydrolyzed proteins. The latter are leucine, lysine, cystine, cysteine, glutamic acid, arginine, tyrosine, the prolines, and tryptophan.

PRODUCTS

Some pharmaceutically important amino acids are listed in Table 26-3.

Aminoacetic Acid, USP. Glycine (Glycocoll) contains not less than 98.5% and not more than 101.5% $C_2H_5NO_2$. It occurs as a white, odorless, crystalline powder, having a sweetish taste. It is insoluble in alcohol but soluble in water (1:4) to make a solution that is acid to litmus paper.

A 1.5% solution is preferred over the 2.1% isotonic solution for use as an irrigating solution during transurethral resection of the prostate gland. From 10 to 15 L of the solution may be used during the surgical operation.

Methionine, USP. DL-2-amino-4-(methylthio)-butyric acid (Amurex) occurs as white, crystalline platelets or a powder with a slight, characteristic odor; it is soluble in water (1:30), and a 1% solution has a *p*H of 5.6 to 6.1. It is insoluble in alcohol. The racemic compound has been produced in ever-increasing quantities and at considerably reduced cost. The human body needs proteins that furnish methionine to prevent pathologic accumulation of fat in the liver, a condition that can be counteracted by administration of the acid or proteins that provide it. Methionine also has a function in the synthesis of choline, cystine, lecithin, and, probably, creatine. Deficiency not only limits growth in rats, but also inhibits progression of tumors.

In therapy, methionine has been employed in the treatment of liver injuries caused by poisons such as carbon tetrachloride, chloroform, arsenic, and trinitrotoluene. Although many physicians are enthusiastic about its value under such circumstances, this action has not been established satisfactorily.

Another use for methionine is as a urinary acidifier to help control the odor and dermatitis caused by ammoniacal urine in incontinent patients. It has been reported to be effective in both short- and long-term use. Treatment must be continued for 3 or 4 days before the ammoniacal odor is eliminated.

Dihydroxyaluminum Aminoacetate, USP. Basic aluminum glycinate (Hyperacid) may be represented by the formula $H_2NCH_2COOAl(OH)_2$. It is a white, odorless, water-insoluble powder that is faintly sweet and is employed as a gastric antacid in the same way as aluminum hydroxide gel. Over the latter, it is claimed to have the advantages of more prompt, greater, and more lasting buffering action. Also, it is said to have less astringent and constipative effects because of its smaller content of aluminum. However, all medical authorities are not yet satisfied that any of these claims are justified. The compound is furnished in powder, magma, or tablets containing 500 mg.

Aminocaproic Acid, USP. 6-Aminohexanoic acid (Amicar) occurs as a fine, white, crystalline powder that is freely soluble in water, slightly soluble in alcohol, and practically insoluble in chloroform.

Aminocaproic acid is a competitive inhibitor of plasminogen activators, such as streptokinase and urokinase. It is effective because it is an analogue of lysine, the position of which in proteins is attacked by plasmin. To a smaller degree, it also inhibits plasmin (fibrinolysin). Lowered plasmin levels lead to more favorable amounts of fibrinogen, fibrin, and other important clotting components.

Aminocaproic acid has been used in the control of hemorrhage in certain surgical procedures. It is of no value in controlling hemorrhage caused by thrombocytopenia or other coagulation defects or vascular disruption (e.g., bleeding ulcers, functional uterine bleeding, post-tonsillectomy bleeding). Because it inhibits the dissolution of clots, it may interfere with normal mechanisms for maintaining the patency of blood vessels.

Aminocaproic acid is absorbed well orally. Plasma peaks occur in about 2 hours. It is excreted rapidly, largely unchanged.

TABLE 26-3

PHARMACEUTICALLY IMPORTANT AMINO ACIDS

Name Proprietary Name	*Preparations*	*Category*	*Application*	*Usual Adult Dose**	*Usual Dose Range**
Aminoacetic acid USP	Aminoacetic acid irrigation USP	Irrigating solution	Topically to the body cavities, as a 1.5% solution		
Methionine USP *Amurex, Odor-Scrip, Oradash, Uranap*	Methionine capsules USP Methionine tablets USP	Acidifier (urinary)		400–600 mg/day	
Dihydroxyaluminum aminoacetate USP *Hyperacid*	Dihydroxyaluminum aminoacetate magma USP Dihydroxyaluminum aminoacetate tablets USP	Antacid		500 mg–1 g qid	500 mg–2 g
Aminocaproic acid USP *Amicar*	Aminocaproic acid injection USP Aminocaproic acid syrup USP Aminocaproic acid tablets USP	Hemostatic		Oral and IV, initial, 5 g followed by 1–1.25 g every hour to maintain a plasma level of 13 mg/100 mL. No more than 30 g/24-hr period is recommended	
Acetylcysteine USP *Mucomyst*	Acetylcysteine solution USP	Mucolytic agent			By inhalation of nebulized solution, 3–5 mL of a 20% solution or 4–10 mL of a 10% solution tid or qid; by direct instillation, 1–2 mL of a 10 or 20% solution every 1–4 hr
Levodopa USP *Larodopa, Levopa, Dopar*	Levodopa capsules USP Levodopa tablets USP	Antiparkinsonian		Initial, 250 mg bid to qid, gradually increasing the total daily dose in increments of 100–750 mg every 3–7 days as tolerated	500 mg–8 g/day
Glutamic acid hydrochloride *Acidulin*	Glutamic acid hydrochloride capsules	Acidifier (gastric)			Oral, 340 mg–1 g tid before meals

*See USP DI for complete dosage information.

Acetylcysteine, USP (Mucomyst) is the *N*-acetyl derivative of L-cysteine. It is used primarily to reduce the viscosity of the abnormally viscid pulmonary secretions in patients with cystic fibrosis of the pancreas (mucoviscidosis) or various tracheobronchial and bronchopulmonary diseases.

Acetylcysteine is more active than cysteine, and its mode of action in reducing the viscosity of mucoprotein solutions, including sputum, may be by opening the disulfide bonds in the native protein.

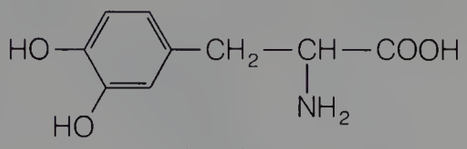
Acetylcysteine is most effective in 10% to 20% solutions with a *p*H of 7 to 9. It is used by direct instillation or by aerosol nebulization. It is available as a 20% solution of the sodium salt in 10 and 30 mL containers. An opened vial of acetylcysteine must be covered, stored in a refrigerator, and used within 48 hours.

Glutamic Acid Hydrochloride (Acidulin) is essentially a pure compound that occurs as a white, crystalline powder soluble 1:3 in water and insoluble in alcohol. It has been used in place of glycine in the treatment of muscular dystrophies, with rather unpromising results. It also is combined (8 to 20 g/day) with anticonvulsants for the petit mal attacks of epilepsy, a use that appears to depend on a change in the *p*H of urine.

The hydrochloride, which releases the acid readily, has been recommended under a variety of names for furnishing acid to the stomach in the achlorhydria of pernicious anemia and other conditions. The usual dosage range is 600 mg to 1.8 g taken during meals.

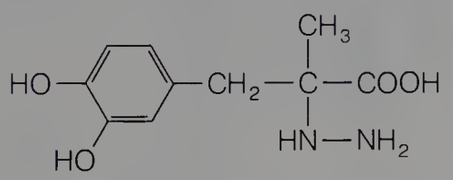
Levodopa, USP (−)-3-(3,4-Dihydroxyphenyl)-L-alanine (Larodopa, Dopar, Levopa), occurs as a colorless, crystalline material. It is slightly soluble in water and insoluble in alcohol. Levodopa is a precursor of dopamine and is of value in the treatment of Parkinson's disease. Dopamine does not cross the blood–brain barrier and, therefore, is inef-

fective. Levodopa does cross the blood–brain barrier and presumably is converted metabolically to dopamine in the basal ganglia. The dose must be determined carefully for each patient.



Levodopa

Carbidopa, USP. The drug Sinemet is a combination of carbidopa and levodopa. The former is the hydrazine analogue of α-methyldopa, and it is an inhibitor of aromatic acid decarboxylation. Accordingly, when carbidopa and levodopa are administered in combination, carbidopa inhibits decarboxylation of peripheral levodopa but does not cross the blood–brain barrier and, hence, does not affect the metabolism of levodopa in the central nervous system. Because carbidopa's decarboxylase-inhibiting activity is limited to extracerebral tissues, it makes more levodopa available for transport to the brain. Thus, carbidopa reduces the amount of levodopa required by approximately 75%.



Carbidopa

Sinemet is supplied as tablets in two strengths: Sinemet-10/100, containing 10 mg of carbidopa and 100 mg of levodopa, and Sinemet-25/250, containing 25 mg of carbidopa and 250 mg of levodopa.

Management of acute overdosage with Sinemet is fundamentally the same as management of acute overdosage with levodopa; however, pyridoxine is not effective in reversing the actions of Sinemet.

PROTEIN HYDROLYSATES

In therapeutics, agents affecting volume and composition of body fluids include various classes of parenteral products. Idealistically, it would be desirable to have parenteral fluids available that would provide adequate calories and important proteins and lipids to mimic as closely as possible an appropriate diet. However, this is not so. Usually, sufficient carbohydrate is administered intravenously to prevent ketosis, and in some cases, it is necessary to give further sources of carbohydrate by vein to reduce the wasting of protein. Sources of protein are made available in the form of protein hydrolysates, and these can be administered to influence favorably the balance.

Protein deficiencies in human nutrition sometimes are treated with protein hydrolysates. The lack of adequate protein may result from several conditions, but the problem is not always easy to diagnose. The deficiency may be due to insufficient dietary intake; temporarily increased demands, as in pregnancy; impaired digestion or absorption; liver malfunction; increased catabolism; or loss of proteins and amino acids, as in fevers, leukemia, hemorrhage, surgery, burns, fractures, or shock.

PRODUCTS

Protein Hydrolysate Injection, USP, protein hydrolysates (intravenous) (Aminogen, Travamin). Protein hydrolysate injection is a sterile solution of amino acids and short-chain peptides that represent the approximate nutritive equivalent of the casein, lactalbumin, plasma, fibrin, or other suitable protein from which it is derived by acid, enzymatic, or another method of hydrolysis. It may be modified by partial removal and restoration or addition of one or more amino acids. It may contain dextrose or another carbohydrate suitable for intravenous infusion. Not less than 50% of the total nitrogen present is in the form of α-amino nitrogen. It is a yellowish to red-amber transparent liquid that has a *p*H of 4 to 7.

Parenteral preparations are employed for the maintenance of a positive nitrogen balance in patients for whom there is interference with ingestion, digestion, or absorption of food. In such patients, the material to be injected must be nonantigenic and must not contain pyrogens or peptides of high molecular weight. Injection may result in untoward effects, such as nausea, vomiting, fever, vasodilatation, abdominal pain, twitching and convulsions, edema at the site of injection, phlebitis, and thrombosis. Sometimes these reactions are due to inadequate care in cleanliness or too rapid administration.

Category: fluid and nutrient replenisher

Usual dose: IV infusion, 2 to 3 L of a 5% solution once daily at a rate of 1.5 to 2 mL/min initially, then increased gradually as tolerated to 3 to 6 mL/min

Usual dose range: 2 to 8 L/day

Usual pediatric dose: infants, IV infusion, 2 to 3 g of protein per kg body weight in a 4% to 7% solution once daily at a rate not exceeding 0.2 mL/min initially, then increased gradually as tolerated to 0.2 to 0.6 mL/min. Children, IV infusion, 1 to 2 g of protein per kg in a 4% to 7% solution once daily at a rate not exceeding 0.2 mL/min initially, then increased gradually as tolerated to 1 to 3 mL/min.

AMINO ACID SOLUTIONS

These solutions contain a mixture of essential and nonessential crystalline amino acids with or without electrolytes (e.g., Aminosyn, Freeamine III, Procalamine, Trowasol, Novamine). Protein hydrolysates are being replaced by crystalline amino acid solutions for parenteral administration be-

cause the free amino acids are utilized more efficiently than the peptides produced by the enzymatic cleavage of protein hydrolysates.[3]

PROTEINS AND PROTEIN-LIKE COMPOUNDS

The chemistry of proteins is complex, and some of the most complex facets remain to be clearly understood. Protein structure usually is studied in basic organic chemistry and, to a greater extent, in biochemistry, but for the purposes of this chapter some of the more important topics will be summarized, with emphasis on relationships to medicinal chemistry. Much progress has been made in the understanding of the more sophisticated features of protein structure[4] and its correlation with physicochemical and biologic properties. With the total synthesis of ribonuclease in 1969, new approaches to the study of structure–activity relationships among proteins have involved the synthesis of modified proteins.

Many types of compound important in medicinal chemistry are classified structurally as proteins. Enzymes, antigens, and antibodies are proteins.* Numerous hormones are low relative molecular mass proteins; hence, relative to the foregoing they are called *simple proteins*. Fundamentally, all proteins are composed of one or more polypeptide chains; that is, the primary organizational level of protein structure is the polypeptide (polyamide) chain composed of naturally occurring amino acids bonded to one another by amide linkages. An extended polypeptide chain can be visualized with the aid of Fig. 26-1. The specific physicochemical and biologic properties of proteins depend not only on the nature of the specific amino acids and their sequence within the polypeptide chain, but also on conformational characteristics.

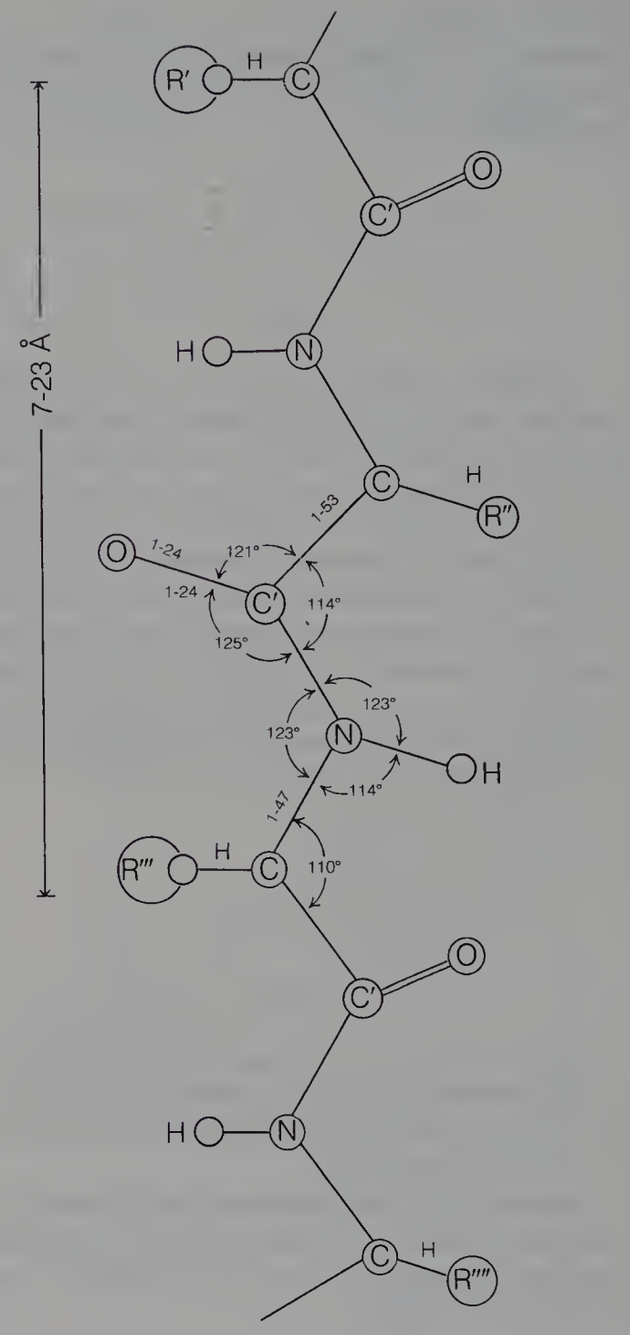


FIG. 26-1. *A diagrammatic representation of a fully extended polypeptide chain with the bond lengths and the bond angles derived from crystal structures and other experimental evidence. (From Corey, R. B., and Pauling, L.: Proc. R. Soc. Lond. Ser. B 141:10, 1953.)*

CONFORMATIONAL FEATURES OF PROTEIN STRUCTURE

As just indicated, the polypeptide chain is considered to be the primary level of protein structure, and the folding of the polypeptide chains into a specific coiled structure is maintained through hydrogen-bonding interactions (intramolecular) (Fig. 26-2). The folding pattern is called the secondary level of protein structure. The intramolecular hydrogen bonds involve the partially negative oxygens of amide carbonyl groups and the partially positive hydrogens of the amide —NH. Additional factors such as ionic bonding between positively charged and negatively charged groups and disulfide bonds contribute to the stabilization of such folded structures.

The arrangement and interfolding of the coiled chains into layers determine the tertiary and higher levels of protein structure. Such final conformational character is determined by various types of interaction, primarily hydrophobic forces and, to some extent, hydrogen bonding and ion pairing.[4,5] Hy-

* The term "interferon" is applied generally to the antiviral proteins naturally produced by various cells. Since the characterization of interferon as an antiviral protein in 1957, much has been learned about the purification and characterization of human leukocyte interferon (e.g., purification by high-performance liquid chromatography), antiviral properties of interferon from various species, cloning and expression of human interferons in bacteria, and monoclonal antibodies of human leukocyte interferon. The interested reader should refer to S. Pestka et al.: Annu. Rep. Med. Chem. 16: 229, 1981.

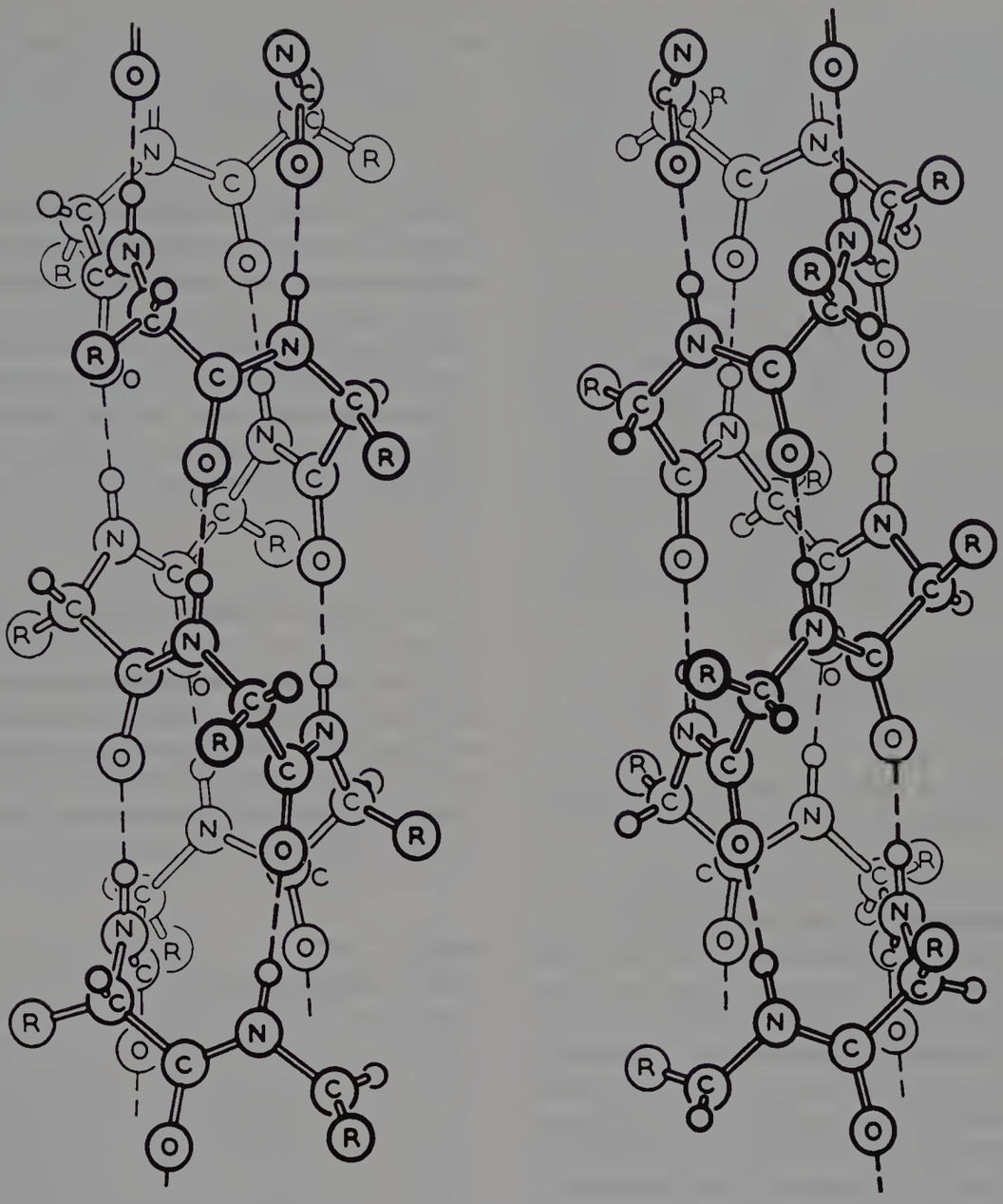


FIG. 26-2. *Left-handed and right-handed α-helices. The R and H groups on the α-carbon atom are in the correct position corresponding to the known configuration of the L-amino acids in proteins. (L. Pauling and R. B. Corey, unpublished drawings.)*

drophobic forces are implicated in many biologic phenomena associated with protein structure and interactions.[6] The side chains (R groups) of various amino acids have hydrocarbon moieties that are hydrophobic, and they have minimal tendency to associate with water molecules, whereas water molecules are strongly associated through hydrogen bonding. Such hydrophobic R groups tend to get close to one another, with exclusion of water molecules, to form "bonds" between different segments of the chain or between different chains. These often are termed "hydrophobic bonds," "hydrophobic forces," or "hydrophobic interactions."

The study of protein structure has required several physicochemical methods of analysis.[4] Ultraviolet spectrophotometry has been applied to the assessment of conformational changes that proteins undergo. Conformational changes can be investigated by the direct plotting of the difference in absorption between the protein under various sets of conditions. X-ray analysis has been most useful in the elucidation of the structures of several proteins (e.g., myoglobulin and lysozyme). Absolute determinations of conformation and helical content can be made by x-ray diffraction analysis. Optical rotation of proteins also has been studied fruitfully. It is interesting that the specific rotations of proteins are always negative and that extreme changes in *p*H (when the protein is in solution) and conditions that promote denaturation (urea solutions, increased temperatures) tend to augment the negative optical rotation. Accordingly, it is rationalized that the changes in rotation are due to conformational changes (i.e., changes in protein structure at the secondary and higher levels of organization). Optical

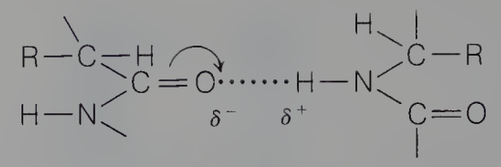
rotatory dispersion also has been experimented with in the study of conformation alterations and conformational differences among globular proteins. Additionally, circular dichroism methodology has been involved in structural studies. The shape and magnitude of rotatory dispersion curves and circular dichroism spectra are very sensitive to conformational alterations; thus, the effects of enzyme inhibitors on conformation can be analyzed. Structural studies have included the investigation of the tertiary structures of proteins in high-frequency nuclear magnetic resonance (NMR).[7] NMR spectroscopy has been of some use in the study of interactions between drug molecules and proteins such as enzymes, proteolipids, and others. NMR has been applied to the study of binding of atropine analogues to acetylcholinesterase[8] and of interactions involving cholinergic ligands to housefly brain and torpedo electroplax.[9] Kato[10,11] has investigated the binding of inhibitors (e.g., physostigmine) to acetylcholinesterase utilizing NMR spectroscopy.*

FACTORS AFFECTING PROTEIN STRUCTURE

Conditions that promote the hydrolysis of amide linkages affect protein structure, as noted above under Protein Hydrolysates.

The highly ordered conformation of a protein can be disorganized (without hydrolysis of the amide linkages), and, in the process, the protein's biologic activity is obliterated. This process is customarily called *denaturation*, and it involves unfolding of the polypeptide chains, loss of the native conformation of the protein, and disorganization of the uniquely ordered structure, without the cleavage of covalent bonds. The rupture of native disulfide bonds usually is considered to be a more extensive and drastic change than denaturation. Criteria for the detection of denaturation involve detection of previously masked —SH, imidazole, and —NH_2 groups; decreased solubility; increased susceptibility to the action of proteolytic enzymes; decreased diffusion constant and increased viscosity of protein solution; loss of enzymatic activity if the protein is an enzyme; and modification of antigenic properties.

Eyring and co-workers[12,13] have carried out studies of factors affecting protein structure and, therefore, biochemical processes. Another study,[14] involving interactions between general anesthetic molecules and proteins, is fundamental to medicinal chemistry and pharmacology. The interested reader should consult the references cited; however, herein brief mention must be made to exemplify the importance of hydrophobic phenomena in mechanisms of drug action involving proteins or other macromolecules.



Eyring et al. propose that anesthetics affect the action of proteins necessary for central nervous system function. It is emphasized that there are certain proteins needed for the maintenance of consciousness. To function normally, the protein must have a particular conformation. Anesthetic molecules are implicated as interacting with the hydrophobic regions of the protein, thus disrupting (unfolding) the conformation. These conformational changes in essential proteins affect their activities and function; hence, it is believed that these effects lead to blockade of synapses.[14]

PURIFICATION AND CLASSIFICATION

It may be said that it is old-fashioned to classify proteins according to the following system as so much progress has been made in the understanding of protein structure. Nevertheless, an outline of this system of classification is given because the terms used are still found in the pharmaceutical and medical literature. Table 26-4 includes the classification

TABLE 26-4

SIMPLE (TRUE) PROTEINS

Class	*Characteristics*	*Occurrence*
Albumins	Soluble in water, coagulable by heat and reagents	Egg albumin, lactalbumin, serum albumin, leucosin of wheat, legumelin of legumes
Globulins	Insoluble in water, soluble in dilute salt solution, coagulable	Edestin of plants, vitelline of egg, serum globulin, lactoglobulin, amandin of almonds, myosin of muscles
Prolamines	Insoluble in water or alcohol, soluble in 60–80% alcohol, not coagulable	Found only in plants (e.g., gliadin of wheat, hordein of barley, zein of corn, and secalin of rye)
Glutelins	Soluble only in dilute acids or bases, coagulable	Found only in plants (e.g., glutenin of wheat and oryzenin of rice)
Protamines	Soluble in water or ammonia, strongly alkaline, not coagulable	Found only in the sperm of fish (e.g., salmine from salmon)
Histones	Soluble in water, but not in ammonia, predominantly basic, not coagulable	Globin of hemoglobin, nucleohistone from nucleoprotein
Albuminoids	Insoluble in all solvents	In keratin of hair, nails, and feathers; collagen of connective tissue; chondrin of cartilage; fibroin of silk; and spongin of sponges

* C. M. Deber et al. have reviewed some modern approaches to the deduction of peptide conformation in solution: ^{13}CNMR, conformational energy calculations, and circular dichroism (Deber, C. M., et al.: Science 9:106, 1976).

and characterization of simple proteins. Before classification it must be ensured that the protein material is purified to the extent practically possible, and this is a very challenging task. Several criteria are used to determine homomolecularity; for example, crystallinity, constant solubility at a given temperature, osmotic pressure in different solvents, diffusion rate, electrophoretic mobility, dielectric constant, chemical assay, spectrophotometry, and quantification of antigenicity. The methodology of purification is complex; procedures can involve various techniques of chromatography (column), electrophoresis, ultracentrifugation, and others. In some cases, high-performance liquid chromatography (HPLC) has been applied to the separation of peptides; for example, Folkers et al.[15] have reported the purification of some hypothalamic peptides by a combination of chromatographic methods including HPLC.

Conjugated proteins contain a nonprotein structural component in addition to the protein moiety, whereas *simple proteins* contain only the polypeptide chain of amino acid units. *Nucleoproteins* are conjugated proteins containing nucleic acids as structural components. *Glycoproteins* are carbohydrate-containing conjugated proteins (e.g., thyroglobulin). *Phosphoproteins* contain phosphate moieties (e.g., casein). *Lipoproteins* are lipid-bearing. *Metalloproteins* have some bound metal. *Chromoproteins*, such as hemoglobin or cytochrome, have some chromophoric moiety.

PROPERTIES OF PROTEINS

The classification delineated in Table 26-4 is based on solubility properties. Fibrous proteins are water-insoluble and highly resistant to hydrolysis by proteolytic enzymes; the collagens, elastins, and keratins are in this class. Globular proteins (albumins, globulins, histones, and protamines) are relatively water-soluble; they are also soluble in aqueous solutions containing salts, acids, bases, or ethanol. Enzymes, oxygen-carrying proteins, and protein hormones are globular proteins.

Another important characteristic of proteins is the amphoteric behavior. In solution, proteins migrate in an electric field, and the direction and rate of migration are a function of the net electrical charge of the protein molecule, which in turn depends on the *p*H of the solution. The isoelectric point is the *p*H value at which a given protein does not migrate in an electric field, and it is a constant for any given protein and can be used as an index of characterization. Proteins differ in rate of migration and in their isoelectric points. Electrophoretic analysis is used to determine purity and for quantitative estimation because proteins differ in electrophoretic mobility at any given *p*H.[4]

Because they are ionic in solution, proteins bind with cations and anions depending on the *p*H of the environment. Sometimes, complex salts are formed and precipitation takes place (e.g., trichloracetic acid is a precipitating agent for proteins and is used for deproteinizing solutions).

Proteins possess chemical properties characteristic of their component functional groups, but in the native state, some of these groups are "buried" within the tertiary protein structure and may not readily react. Certain denaturation procedures can expose these functions and allow them to respond to the usual chemical reagents (e.g., an exposed $—NH_2$ group can be acetylated by ketene, $—CO_2H$ can be esterified with diazomethane).

COLOR TESTS, MISCELLANEOUS SEPARATION AND IDENTIFICATION METHODS

Proteins respond to the following color tests: (1) biuret, pink to purple with an excess of alkali and a small amount of copper sulfate; (2) ninhydrin, a blue color when boiled with ninhydrin (triketohydrindene hydrate) that is intensified by the presence of pyridine; (3) Millon's test for tyrosine, a brick-red color or precipitate when boiled with mercuric nitrate in an excess of nitric acid; (4) Hopkins-Cole test for tryptophan, a violet zone with a salt of glyoxylic acid and stratified over sulfuric acid; and (5) xanthoproteic test, a brilliant orange zone when a solution in concentrated nitric acid is stratified under ammonia.

Almost all so-called alkaloidal reagents will precipitate proteins in slightly acid solution.

The qualitative identification of the amino acids found in proteins and other substances has been simplified greatly by the application of paper chromatographic techniques to the proper hydrolysate of proteins and related substances. End-member degradation techniques for the detection of the sequential arrangements of the amino acid residues in polypeptides (proteins, hormones, enzymes, and such) have been developed to such a high degree with the aid of paper chromatography that very small samples of the polypeptides can be utilized. These techniques, together with statistical methods, have led to the elucidation of the sequential arrangements of the amino acid residues in oxytocin, vasopressin, insulin, hypertensin, glucagon, corticotropins, and others.

Ion-exchange chromatography has been applied to protein analysis and to the separation of amino acids. The principles of ion-exchange chromatography can be applied to the design of automatic amino acid analyzers with appropriate recording instrumentation.[4] One- or two-dimensional thin-layer chromatography also has been used to accomplish separations not possible with paper chromatography. Another method for separating amino acids and proteins involves a two-dimensional analytical procedure that uses electrophoresis in one dimension and partition chromatography in the other. The applicability of HPLC was noted earlier.[15]

PRODUCTS

Gelatin, NF, is a protein obtained by the partial hydrolysis of collagen, an albuminoid found in bones, skins, tendons,

TABLE 26-5

PHARMACEUTICALLY IMPORTANT PROTEIN PRODUCTS

Name Proprietary Name	*Category*
Gelatin, NF	Pharmaceutical acid (encapsulating agent; suspending agent; tablet binder and coating agent)
Gelatin film, absorbable, USP *Gelfilm*	Local hemostatic
Gelatin sponge, absorbable, USP *Gelfoam*	Local hemostatic

cartilage, hoofs, and other animal tissues. The products seem to be of great variety, and from a technical standpoint, the raw material must be selected according to the purpose intended (Table 26-5). This is because collagen usually is accompanied in nature by elastin and, especially, mucoids, such as chondromucoid, which enter into the product in a small amount. The raw materials for official gelatin, and that used generally for food, are skins of calf or swine and bones. First, the bones are treated with hydrochloric acid to remove the calcium compounds and, second, are digested with lime for a prolonged period, which converts most other impurities to a soluble form. The fairly pure collagen is extracted with hot water at a *p*H of about 5.5, and the aqueous solution of gelatin is concentrated, filtered, and cooled to a stiff gel. Calf skins are treated in about the same way, but those from hogs are not given any lime treatment. The product derived from an acid-treated precursor is known as type A and exhibits an isoelectric point between *p*H 7 and 9, whereas that for which alkali is used is known as type B and exhibits an isoelectric point between *p*H 4.7 and 5. The minimum gel strength officially is that a 1% solution, kept at 0°C for 6 hours, must show no perceptible flow when the container is inverted.

Gelatin occurs in sheets, shreds, flakes, or coarse powder. It is white or yellowish, has a slight but characteristic odor and taste, and is stable in dry air but subject to microbial decomposition if moist or in solution. It is insoluble in cold water but swells and softens when immersed and gradually absorbs five to ten times its own weight of water. It dissolves in hot water to form a colloidal solution; it also dissolves in acetic acid and in hot dilute glycerin. Gelatin commonly is bleached with sulfur dioxide, but that used medicinally must have not over 40 parts per million of sulfur dioxide. However, a proviso is made that for the manufacture of capsules or pills it may have certified colors added, may contain as much as 0.15% sulfur dioxide, and may have a lower gel strength.

Gelatin is used in the preparation of capsules and the coating of tablets and, with glycerin, as a vehicle for suppositories. It also has been employed as a vehicle for other drugs when slow absorption is required. When dissolved in water, the solution becomes somewhat viscous, and such solutions are used to replace the loss in blood volume in cases of shock. This is accomplished more efficiently now with blood plasma, which is safer to use. In hemorrhagic conditions, it sometimes is administered intravenously to increase the clotting of blood or is applied locally for the treatment of wounds.

The most important value in therapy is as an easily digested and adjuvant food. It fails to provide any tryptophan at all and is lacking notably in adequate amounts of other essential amino acids; approximately 60% of the total amino acids consist of glycine and the prolines. Nevertheless, when supplemented, it is very useful in various forms of malnutrition, gastric hyperacidity or ulcer, convalescence, and general diets of the sick. It is especially recommended in the preparation of modified milk formulas for feeding infants.

Gelatin Film, Absorbable, USP (Gelfilm), is a sterile, nonantigenic, absorbable, water-insoluble gelatin film. The gelatin films are prepared from a solution of specially prepared gelatin–formaldehyde combination, by spreading on plates and drying under controlled humidity and temperature. The film is available as light yellow, transparent, brittle sheets 0.076 mm to 0.228 mm thick. Although insoluble in water, they become rubbery after being in water for a few minutes.

Gelatin Sponge, Absorbable, USP (Gelfoam), is a sterile, absorbable, water-insoluble, gelatin-based sponge that is a light, nearly white, nonelastic, tough, porous matrix. It is stable to dry heat at 150°C for 4 hours. It absorbs 50 times its own weight of water or 45 times oxalated whole blood.

It is absorbed in 4 to 6 weeks when used as a surgical sponge. When applied topically to control capillary bleeding, it should be moistened with sterile isotonic sodium chloride solution or thrombin solution.

Nonspecific Proteins. The intravenous injection of foreign protein is followed by fever, muscle and joint pain, sweating, and decrease and then increase in leukocytes; it even can result in serious collapse. The results have been used in the treatment of various infections, originally the chronic form. The method is presumed to be of value in acute and chronic arthritis, peptic ulcer, certain infections of the skin and eye, some vascular diseases, cerebrospinal syphilis, especially dementia paralytica, and other diseases. Because a fever is necessary in this system, the original program has developed into the use of natural fevers, such as malaria, of external heat, and of similar devices. However, the slightly purified proteins of milk still are recommended for some diseases; they are available commercially as Activin, Caside, Clarilac, Bu-Ma-Lac, Lactoprotein, Mangalac, Nat-i-lac, Neolacmanese, and Proteolac. Muscosol is a purified beef peptone, and Omniadin is a similar purified bacterial protein. Synodal contains nonspecific protein with lipoids, animal fats, and emetine hydrochloride and is designed for the treatment of peptic ulcer. One of the favorite agents of this class has been typhoid vaccine.

Venoms. Cobra (*Naja*) venom solution, from which the hemotoxic and proteolytic principles have been removed, has been credited with virtues owing to toxins and has been injected intramuscularly as a nonnarcotic analgesic in doses of 1 mL/day. Snake venom solution of the water moccasin is employed subcutaneously in doses of 0.4 to 1.0 mL as a hemostatic in recurrent epistaxis and thrombocytopenic purpura and as a prophylactic before tooth extraction and minor surgical procedures. Stypven, from the Russell viper, is used topically as a hemostatic and as a thromboplastic agent in Quick's modified clotting-time test. Ven-Apis, the purified and standardized venom from bees, is furnished in graduated strengths of 32, 50, and 100 bee-sting units. It is administered topically in acute and chronic arthritis, myositis, and neuritis.

Nucleoproteins. The nucleoproteins previously mentioned are found in the nuclei of all cells and in the cytoplasm. They can be deproteinized by several methods. Those compounds that occur in yeast usually are treated by grinding with a very dilute solution of potassium hydroxide, adding picric acid in excess, and precipitating the nucleic acids with hydrochloric acid, leaving the protein in solution. The nucleic acids are purified by dissolving in dilute potassium hydroxide, filtering, acidifying with acetic acid, and finally precipitating with a large excess of ethanol.

The nucleoproteins found in the nucleus of eukaryotic cells include a variety of enzymes, such as DNA and RNA polymerases (involved in nucleic acid synthesis), nucleases (involved in the hydrolytic cleavage of nucleotide bonds), isomerases, and others. The nucleus of eukaryotic cells also contains specialized proteins, such as tubulin (involved in the formation of mitotic spindle before mitosis) and histones. *Histones* are proteins rich in the basic amino acids arginine and lysine, which together make up one-fourth of the amino acid residues. Histones combine with negatively charged double-helical DNA to form complexes that are held together by electrostatic interactions. Histones package and order the DNA into structural units called "nucleosomes."

ENZYMES

Those proteins that have catalytic properties are called *enzymes* (i.e., enzymes are biologic catalysts of protein nature).* Some enzymes have full catalytic reactivity per se; these are considered to be simple proteins because they do not have a nonprotein moiety. Other enzymes are conjugated proteins, and the nonprotein structural components are necessary for reactivity. Occasionally, enzymes require metallic ions. Because enzymes are proteins or conjugated proteins, the general review of protein structural studies presented earlier in this chapter (e.g., protein conformation and denaturation) is fundamental to the following topics. Conditions that affect denaturation of proteins usually have adverse effects on the activity of the enzyme.

General enzymology is discussed effectively in numerous standard treatises, and one of the most concise discussions appears in the classic work by Ferdinand,[16] who includes reviews of enzyme structure and function, bioenergetics, and kinetics and appropriate illustrations with a total of 37 enzymes selected from the six major classes. Accordingly, for additional basic studies of enzymology, the reader should refer to this classic monograph and to a comprehensive review of this topic.[17]

RELATION OF STRUCTURE AND FUNCTION

Koshland[18] has reviewed concepts concerning correlations of protein conformation and conformational flexibility of enzymes with enzyme catalysis. Enzymes do not exist initially in a conformation complementary to that of the substrate. The substrate induces the enzyme to assume a complementary conformation. This is the so-called induced-fit theory. There is proof that proteins do possess conformational flexibility and undergo conformational changes under the influence of small molecules. It is emphasized that this does not mean that all proteins must be flexible; nor does it mean that conformationally flexible enzymes must undergo conformational changes when interacting with all compounds. Furthermore, a regulatory compound that is not directly involved in the reaction can exert control on the reactivity of the enzyme by inducing conformational changes (i.e., by inducing the enzyme to assume the specific conformation complementary to the substrate). (Conceivably, hormones as regulators function according to the foregoing mechanism of affecting protein structure.) So-called flexible enzymes can be distorted conformationally by molecules classically called "inhibitors." Such inhibitors can induce the protein to undergo conformational changes, disrupting the catalytic functions or the binding function of the enzyme. In this connection, it is interesting to note how the work of Belleau[18a] and the molecular perturbation theory of drug action relate to Koshland's studies (see Chap. 2).

Evidence continues to support the explanation of enzyme catalysis on the basis of the *active site* (reactive center) of amino acid residues, which is considered to be that relatively small region of the enzyme's macromolecular surface involved in catalysis. Within this site, the enzyme has strategically positioned functional groups (from the side chains of amino acid units) that participate cooperatively in the catalytic action.[19]

Some enzymes have absolute specificity for a single substrate, but others catalyze a particular type of reaction that various compounds undergo. In the latter, the enzyme is said to have relative specificity. Nevertheless, when compared with other catalysts, enzymes are outstanding in their specificity for certain substrates.[20] The physical, chemical, conformational, and configurational properties of the substrate

* Important factors limiting rates of enzyme-catalyzed reactions have been evaluated critically by W. W. Cleland (Acc. Chem. Res. 8:145, 1975).

determine its complementarity to the enzyme's reactive center. These factors, therefore, determine whether a given compound satisfies the specificity of a particular enzyme. Enzyme specificity must be a function of the nature, including conformational and chemical reactivity, of the reactive center, but when the enzyme is a conjugated protein with a coenzyme moiety, the nature of the coenzyme also contributes to specificity characteristics.

It appears that in some instances the active center of the enzyme is complementary to the substrate molecule in a strained configuration, corresponding to the "activated" complex for the reaction catalyzed by the enzyme. The substrate molecule is attracted to the enzyme and is caused by the forces of attraction to assume the strained state, with conformational changes that favor the chemical reaction; that is, the activation energy requirement of the reaction is decreased by the enzyme to such an extent that the reaction is caused to proceed at an appreciably greater rate than it would in the absence of the enzyme. If the enzymes were always completely complementary in structure to the substrates, then no other molecule would be expected to compete successfully with the substrate in combination with the enzyme, which in this respect would be similar in behavior to antibodies. However, occasionally, an enzyme complementary to a strained substrate molecule might attract more strongly to itself a molecule resembling the strained substrate molecule itself; for example, the hydrolysis of benzoyl-L-tyrosylglycineamide was practically inhibited by an equal amount of benzoyl-D-tyrosylglycineamide. This example illustrates a type of antimetabolite activity.

Several types of interaction contribute to the formation of enzyme–substrate complexes: attractions between charged (ionic) groups on the protein and the substrate, hydrogen bonding, hydrophobic forces (the tendency of hydrocarbon moieties of side chains of amino acid residues to associate with the nonpolar groups of the substrate in a water environment), and London forces (induced dipole interactions).

Many studies of enzyme specificity have been made on proteolytic enzymes (proteases). Configurational specificity can be exemplified by the aminopeptidase that cleaves L-leucylglycylglycine, but does not affect D-leucylglycylglycine. D-Alanylglycylglycine is cleaved slowly by this enzyme. These phenomena illustrate the significance of steric factors; at the active center of aminopeptidase, a critical factor is a matter of closeness of approach that affects the kinetics of the reaction.

One can easily imagine how difficult it is to study the reactivity of enzymes on a functional group basis because the mechanism of enzyme action is so complex.[18] Nevertheless, it can be said that the —SH group probably is found in more enzymes as a functional group than are the other polar groups. It should be noted that in some enzymes (e.g., urease), the less readily available SH groups are necessary for biologic activity and cannot be detected by the nitroprusside test, which is used to detect the freely reactive SH groups.

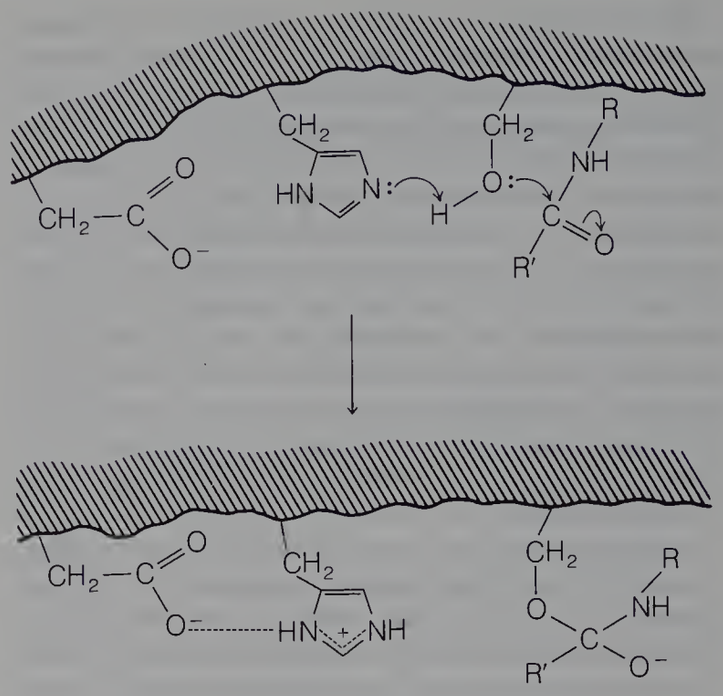


FIG. 26-3. *Generalized mechanism of protease catalysis. (Adapted from Chem. Eng. News, Apr. 16, 1979, p. 23.)*

A free —OH group of the tyrosyl residue is necessary for the activity of pepsin. Both the —OH of serine and the imidazole portion of histidine appear to be necessary parts of the active center of certain hydrolytic enzymes, such as trypsin and chymotrypsin, and furnish the electrostatic forces involved in a proposed mechanism (Fig. 26-3), in which E denotes enzyme, the other symbols being self-evident.*

These two groups (i.e., —OH and =NH) could be located on separate peptide chains in the enzyme as long as the specific three-dimensional structure formed during activation of the zymogen brought them near enough to form a hydrogen bond. The polarization of the resulting structure would cause the serine oxygen to be the nucleophilic agent that attacks the carbonyl function of the substrate. The complex is stabilized by the simultaneous "exchange" of the hydrogen bond from the serine oxygen to the carbonyl oxygen of the substrate.

The intermediate acylated enzyme is written with the proton on the imidazole nitrogen. The deacylation reaction involves the loss of this positive charge simultaneously with the attack of the nucleophilic reagent (abbreviated Nu:H).

Roberts[21] effectively used nitrogen-15 (^{15}N) NMR to study the mechanism of protease catalysis. A schematic summary of the generalized mechanism is represented in Figure 26-4. It is concluded that the tertiary N-1 nitrogen of the histidine unit within the reactive center of the enzyme deprotonates the hydroxyl of the neighboring serine unit and si-

* Alternative mechanisms have been proposed;[17] esterification and hydrolysis have been studied extensively by M. L. Bender (J. Am. Chem. Soc. 79:1258, 1957; 80:5338, 1958; 82:1900, 1960; 86:3704, 5330, 1964). D. M. Blow has reviewed studies concerning the structure and mechanism of chymotrypsin (Acc. Chem. Res. 9:145, 1976).

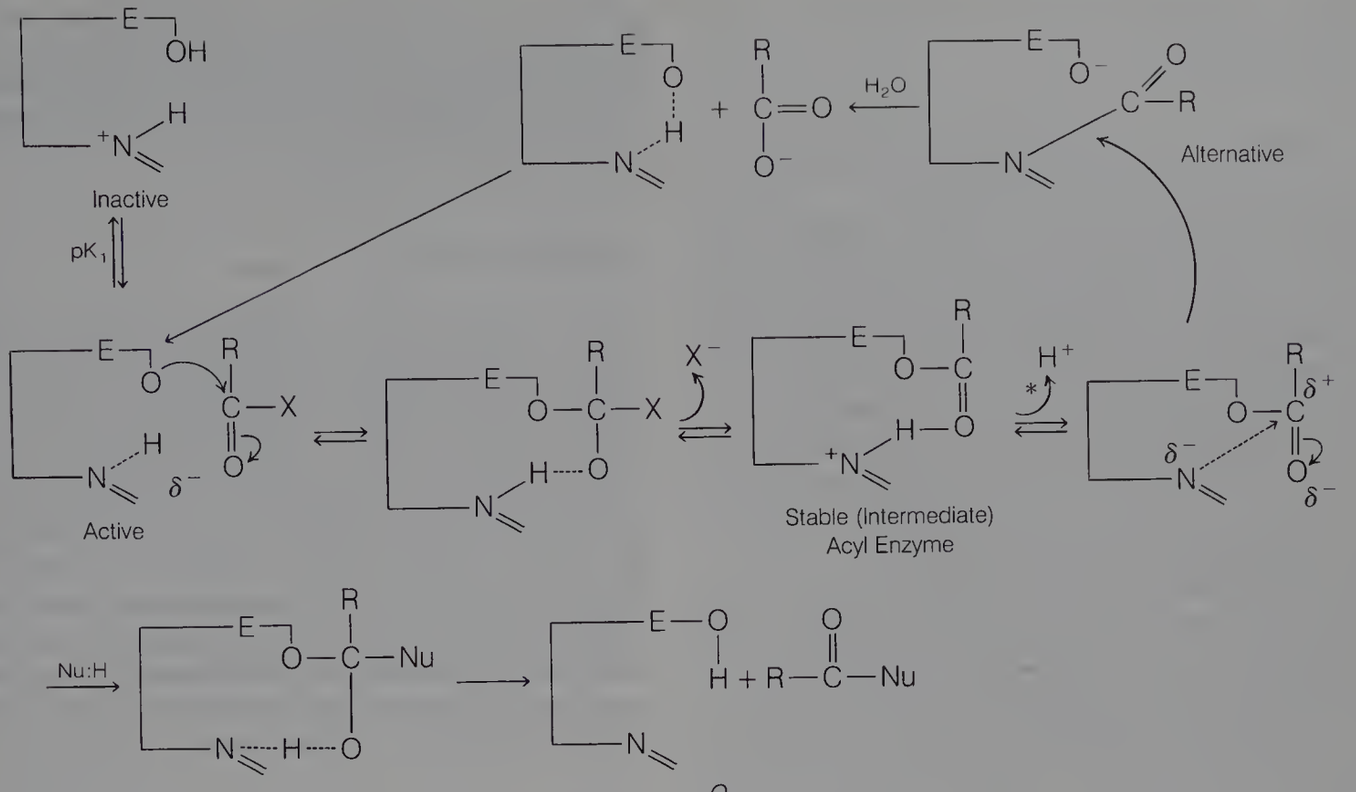


FIG. 26-4. *Enzyme-catalyzed hydrolysis of* $R-\overset{O}{\overset{\|}{C}}-X$*: A proposed generalized mechanism.*

multaneously the hydroxyl oxygen exerts nucleophilic attack on the carbonyl carbon of the amide substrate, as depicted in the scheme. A tetrahedral intermediate is implicated, and the carboxylate group of the aspartate unit (the third functional group within the reactive center) stabilizes the developing imidazolium ion by a hydrogen bonding to the N-3 hydrogen. Finally, decomposition of the anionic tetrahedral intermediate toward product formation (amine and acylated serine) is promoted by prior protonation of the amide nitrogen by the imidazolium group.

A possible alternative route to deacylation would involve the nucleophilic attack of the imidazole nitrogen on the newly formed ester linkage of the postulated acyl intermediate, leading to the formation of the acyl imidazole. The latter is unstable in water, hydrolyzing rapidly to give the product and regenerated active enzyme.

The reaction of an alkyl phosphate in such a scheme may be written in an entirely analogous fashion, except that the resulting phosphorylated enzyme would be less susceptible to deacylation through nucleophilic attack. The following diagrammatic scheme (Fig. 26-5) has been proposed to explain the function of the active thiol ester site of papain. This ester site is formed and maintained by the folding energy of the enzyme (protein) molecule.

ZYMOGENS (PROENZYMES)

Zymogens, also called "proenzymes," are enzyme precursors. These proenzymes are said to be activated when they are transformed to the enzyme. This activation usually involves catalytic action by some proteolytic enzyme. Occasionally, the activators merely effect a reorganization of the tertiary structure (conformation) of the protein so that the

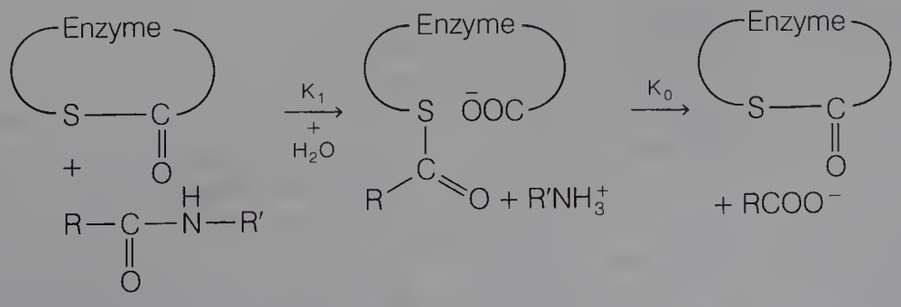


FIG. 26-5. *The action of papain: A proposed scheme.*

groups involved within the reactive center become functional (i.e., unmasked).

SYNTHESIS AND SECRETION OF ENZYMES

Exportable proteins (enzymes), such as amylase, ribonuclease, chymotrypsin(ogen), trypsin(ogen), insulin, and such, are synthesized on the ribosomes. They pass across the membrane of the endoplasmic reticulum into the cisternae and directly into a smooth vesicular structure, which effects further transportation. They are stored finally in highly concentrated form within membrane-bound granules. These are called "zymogen granules," the exportable protein content of which may reach a value of 40% of the total protein of the gland cell. In the foregoing enzyme sequences, the newly synthesized exportable protein (enzymes) is not free in the cell sap. The stored exportable proteins are released into the extracellular milieu for the digestive enzymes and into adjacent blood capillaries for hormones. Release of these proteins is initiated by specific inducers: for example, cholinergic agents (but not epinephrine) and Ca^{2+} effect a discharge of amylase, lipase, or other into the medium; increase in glucose levels stimulates the secretion of insulin and so on. This release of the reserve enzymes and hormones is completely independent of the synthetic process, as long as the stores in the granules are not depleted. Energy oxidative phosphorylation does not play an important role in these releases. Electron microscope studies indicate a fusion of the zymogen granule membrane with the cell membrane such that a direct opening of the granule into the extracellular lumen of the gland is formed.

CLASSIFICATION

There are various systems for the classification of enzymes (e.g., the International Union of Biochemistry system). This system includes some of the terminology used in the literature of medicinal chemistry, and in many instances the terms are self-explanatory: for example, oxidoreductases, transferases (catalyze transfer of a group, such as methyltransferase), hydrolases (catalyze hydrolysis reactions, such as esterases and amidases), lyases (catalyze nonhydrolytic removal of groups leaving double bonds), isomerases, and ligases. Other systems sometimes are used to classify and characterize enzymes, and the following terms are frequently encountered: lipases, peptidases, proteases, phosphatases, kinases, synthetases, dehydrogenases, oxidases, reductases.

PRODUCTS

Pharmaceutically important enzyme products are listed in Table 26-6.

TABLE 26-6

PHARMACEUTICALLY IMPORTANT ENZYME PRODUCTS

Name Proprietary Name	*Preparations*	*Category*	*Application*	*Usual Adult Dose* *	*Usual Dose Range* *
Pancreatin USP *Panteric*	Pancreatin capsules USP Pancreatin tablets USP	Digestive aid		325 mg–1 g	
Trypsin crystallized USP	Trypsin crystallized for aerosol USP	Proteolytic enzyme		Aerosol, 125,000 USP units in 3 mL of saline daily	
Pancrelipase USP *Cotazym*	Pancrelipase capsules USP Pancrelipase tablets USP	Digestive aid			An amount of pancrelipase equivalent to 8,000–24,000 USP units of lipolytic activity before each meal or snack, or to be determined by the practitioner according to the needs of the patient
Chymotrypsin USP *Chymar*	Chymotrypsin for ophthalmic solution USP	Proteolytic enzyme (for zonule lysis)	1–2 mL by irrigation to the posterior chamber of the eye, under the iris, as a solution containing 75–150 U/mL		
Hyaluronidase for injection USP *Alidase, Wydase*	Hyaluronidase injection USP	Spreading agent		Hypodermoclysis, 150 USP hyaluronidase units	
Sutilains USP *Travase*	Sutilains ointment USP	Proteolytic enzyme	Topical, ointment, bid to qid		

*See USP DI for complete dosage information.

Pancreatin, USP (Panteric), is a substance obtained from the fresh pancreas of the hog or of the ox and contains a mixture of enzymes, principally pancreatic amylase (amylopsin), protease, and pancreatic lipase (steapsin). It converts not less than 25 times its weight of USP Potato Starch Reference Standard into soluble carbohydrates and not less than 25 times its weight of casein into proteoses. Pancreatin of a higher digestive power may be brought to this standard by admixture with lactose, sucrose containing not more than 3.25% of starch, or pancreatin of lower digestive power. Pancreatin is a cream-colored, amorphous powder having a faint, characteristic, but not offensive, odor. It dissolves slowly but incompletely in water and is insoluble in alcohol. It acts best in neutral or faintly alkaline media, and excessive acid or alkali renders it inert. Pancreatin can be prepared by extracting the fresh gland with 25% alcohol or with water and subsequently precipitating with alcohol. Besides the enzymes mentioned, it contains some trypsinogen, which can be activated by enterokinase of the intestines; chymotrypsinogen, which is converted by trypsin to chymotrypsin; and carboxypeptidase.

Pancreatin is used largely for the predigestion of food and for the preparation of hydrolysates. The value of its enzymes orally must be very small because they are digested by pepsin and acid in the stomach, although some of them may escape into the intestines without change. Even if they are protected by enteric coatings, it is doubtful if they could be of great assistance in digestion.

Trypsin Crystallized, USP, is a proteolytic enzyme crystallized from an extract of the pancreas gland of the ox, *Bos taurus*. It occurs as a white to yellowish white, odorless, crystalline or amorphous powder, and 500,000 USP trypsin units are soluble in 10 mL of water or saline TS.

Trypsin has been used for several conditions in which its proteolytic activities relieve certain inflammatory states, liquefy tenacious sputum, and such; however, the many side reactions encountered, particularly when it is used parenterally, mitigate against its use.

Pancrelipase, USP (Cotazym). This preparation has a greater lipolytic action than other pancreatic enzyme preparations. Hence, it is used to help control steatorrhea and in other conditions in which pancreatic insufficiency impairs the digestion of fats in the diet.

Chymotrypsin, USP (Chymar). This enzyme is extracted from mammalian pancreas and is used in cataract surgery. A dilute solution is used to irrigate the posterior chamber of the eye to dissolve the fine filaments that hold the lens.

Hyaluronidase for Injection, USP (Alidase, Wydase), is a sterile, dry, soluble enzyme product prepared from mammalian testes and capable of hydrolyzing the mucopolysaccharide hyaluronic acid. It contains not more than 0.25 μg of tyrosine for each USP hyaluronidase unit. Hyaluronidase in solution must be stored in a refrigerator. Hyaluronic acid, an essential component of tissues, limits the spread of fluids and other extracellular material, and, because the enzyme destroys this acid, injected fluids and other substances tend to spread farther and faster than normal when administered with this enzyme. Hyaluronidase may be used to increase the spread and consequent absorption of hypodermoclytic solutions; to diffuse local anesthetics, especially in nerve blocking; and to increase diffusion and absorption of other injected materials, such as penicillin. It also enhances local anesthesia in surgery of the eye and is useful in glaucoma because it causes a temporary drop in intraocular pressure.

Hyaluronidase is practically nontoxic, but caution must be exercised in the presence of infection because the enzyme may cause a local infection to spread, through the same mechanism; it never should be injected in an infected area. Sensitivity to the drug is rare.

The activity of hyaluronidase is determined by measuring the reduction of turbidity that it produces on a substrate of native hyaluronidate and certain proteins or by measuring the reduction in viscosity that it produces on a buffered solution of sodium or potassium hyaluronidate. Each manufacturer defines its product in turbidity or viscosity units, but values are not the same because they measure different properties of the enzyme.

Sutilains, USP (Travase), is a proteolytic enzyme obtained from cultures of *Bacillus subtilis* and is used to dissolve necrotic tissue occurring in second- and third-degree burns, as well as in bed sores and ulcerated wounds.

Many substances are contraindicated during the topical use of sutilains. These include detergents and anti-infectives, which have a denaturing action on the enzyme preparation. The antibiotics penicillin, streptomycin, and neomycin do not inactivate sutilains. Mafenide acetate is also compatible with the enzyme.

Streptokinase (Kabikinase, Streptase) is a catabolic product secreted by group C β-hemolytic streptococci. It is a protein with no known enzymatic activity. Streptokinase activates plasminogen to plasmin, a proteolytic enzyme that hydrolyzes fibrin and promotes the dissolution of thrombi.[22] Plasminogen is activated when streptokinase forms a 1:1 stoichiometric complex with it. Allergic reactions to streptokinase are a common occurrence because of antibody formation in individuals treated with it. Furthermore, the antibodies inactivate streptokinase and reduce its ability to prolong thrombin time. Streptokinase is indicated for acute myocardial infarction, for local perfusion of an occluded vessel, and before angiography, by intravenous, intra-arterial and intracoronary administration, respectively.

Urokinase (Abbokinase) is a glycosylated serine protease consisting of two polypeptide chains connected by a single disulfide bond. It is isolated from human urine or tissue culture of human kidneys. The only known substrate of urokinase is plasminogen, which is activated to plasmin, a fibrinolytic enzyme.[22] Unlike streptokinase, urokinase is a direct activator of plasminogen. Urokinase is nonantigenic

because it is an endogenous enzyme and, therefore, may be used when streptokinase use is impossible because of antibody formation. It is administered intravenously or by the intracoronary route. Its indications are similar to those of streptokinase.

Alteplase (Activase) is a tissue plasminogen activator (t-PA) produced by recombinant DNA technology. It is a single-chain glycoprotein protease consisting of 527 amino acid residues. Native t-PA is isolated from a melanoma cell line. The single-chain molecule is susceptible to enzymatic digestion to a two-chain molecule, in which the two chains remain linked with a disulfide bond. Both forms of the native t-PA are equipotent in fibrinolytic (and plasminogen-activating) properties.[22] It is an extrinsic plasminogen activator associated with vascular endothelial tissue, which preferentially activates plasminogen bound to fibrin. The fibrinolytic action of alteplase (t-PA) is confined to thrombi, with minimal systemic activation of plasminogen. It is produced commercially by recombinant DNA methods by inserting the alteplase gene (acquired from human melanoma cells) into ovarian cells of the Chinese hamster, serving as host cells. The melanoma-derived alteplase is immunologically and chemically identical with the uterine form.[22] Alteplase is indicated for the intravenous management of acute myocardial infarction.

Papain, USP (Papase), the dried and purified latex of the fruit of *Carica papava* L. (Caricaceae), has the power of digesting protein in either acid or alkaline media; it is best at a *p*H of from 4 to 7 and at 65° to 90°C. It occurs as light brownish gray to weakly reddish brown granules or as a yellowish gray to weakly yellow powder. It has a characteristic odor and taste and is incompletely soluble in water to form an opalescent solution. The commercial material is prepared by evaporating the juice, but the pure enzyme also has been prepared and crystallized. In medicine, it has been used locally in various conditions similar to those for which pepsin is employed. It has the advantage of activity over a wider range of conditions, but it is often much less reliable. Intraperitoneal instillation of a weak solution has been recommended to counteract a tendency to adhesions after abdominal surgery, and several enthusiastic reports have been made about its value under these conditions. Papain has been reported to cause allergies in persons who handle it, especially those who are exposed to inhalation of the powder.

Bromelains (Ananase) is a mixture of proteolytic enzymes obtained from the pineapple plant. It is proposed for use in the treatment of soft-tissue inflammation and edema associated with traumatic injury, localized inflammations, and postoperative tissue reactions. The swelling that accompanies inflammation may be caused by occlusion of the tissue spaces with fibrin. If this is true, sufficient amounts of Ananase would have to be absorbed and reach the target area after oral administration to act selectively on the fibrin. This is yet to be established, and its efficacy as an anti-inflammatory agent is inconclusive. However, an apparent inhibition of inflammation has been demonstrated with irritants such as turpentine and croton oil (granuloma pouch technique).

Ananase is available in 50,000-unit tablets for oral use.

Diastase (Taka-Diastase) is derived from the action of a fungus, *Aspergillus oryzae* Cohn (*Eurotium O.* Ahlburg), on rice hulls or wheat bran. It is a yellow, hygroscopic, almost tasteless powder that is freely soluble in water and can solubilize 300 times its weight of starch in 10 minutes. It is employed in doses of 0.3 to 1.0 g in the same conditions as malt diastase. Taka-Diastase is combined with alkalies as an antacid in Takazyme, with vitamins in Taka-Combex, and in other preparations.

HORMONES

The hormones discussed in this chapter may be classified structurally as polypeptides, proteins, or glycoproteins. These hormones include metabolites elaborated by the hypothalamus, pituitary gland, pancreas, gastrointestinal tract, parathyroid gland, liver, and kidneys. A comprehensive review of the biochemistry of these polypeptides and other related hormones is beyond the scope of this chapter. For a detailed discussion, the reader should refer to the review by Wallis et al.[23] and to other literature cited throughout this chapter.

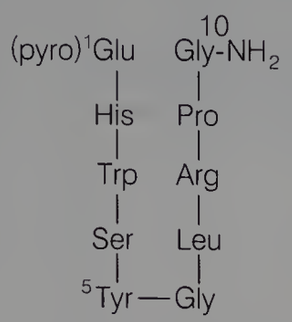
HORMONES FROM THE HYPOTHALAMUS

The physiologic and clinical aspects of hypothalamic-releasing hormones have been reviewed.[24] Through these hormones, the central nervous system regulates other essential endocrine systems, including the pituitary, which in turn controls other systems (e.g., the thyroid).

Thyroliberin (thyrotropin-releasing hormone; TRH) is the hypothalamic hormone responsible for the release of the pituitary's thyrotropin. Thyrotropin stimulates the production of thyroxine and liothyronine by the thyroid. The latter thyroid hormones, by feedback regulation, inhibit the action of TRH on the pituitary. Thyroliberin is a relatively simple tripeptide that has been characterized as pyroglutamyl-histidyl-prolinamide. TRH possesses interesting biologic properties. In addition to stimulating the release of thyrotropin, it promotes the release of prolactin. It also has some central nervous system effects that have been evaluated for antidepressant therapeutic potential, but, as yet, the results of clinical studies are not considered to be conclusive.[24,25]

Gonadoliberin, as the name implies, is the gonadotropin-releasing hormone (Gn-RH), also known as luteinizing hormone-releasing hormone (LH-RH). This hypothalamic decapeptide stimulates the release of luteinizing hormone (LH)

and follicle-stimulating hormone (FSH) by the pituitary. LH-RH is considered to be of potential therapeutic importance in the treatment of hypogonadotropic infertility in both males and females.[26]



Luteinizing Hormone-Releasing Hormone (LH-RH)

A hypothalamic growth-releasing factor (GRF), also called somatoliberin, continues to be under intensive investigation. Its identification and biologic characterization remain to be completed, but physiologic and clinical data support the existence of hypothalamic control of pituitary release of somatotropin.

Somatostatin is another very interesting hypothalamic hormone.[24] It is a tetradecapeptide possessing a disulfide bond linking two cysteine residues, 3-14, in the form of a 38-member ring. Somatostatin suppresses several endocrine systems. It inhibits the release of somatotropin and thyrotropin by the pituitary. It also inhibits the secretion of insulin and glucagon by the pancreas. Gastrin, pepsin, and secretin are intestinal hormones that are likewise affected by somatostatin. The therapeutic potential of somatostatin will be discussed later in relation to the role of glucagon in the pathology of human diabetes.

Other hypothalamic hormones include the luteinizing hormone release-inhibiting factor (LHRIF), prolactin-releasing factor (PRF), corticotropin-releasing factor (CRF), melanocyte-stimulating hormone-releasing factor (MRF), and melanocyte-stimulating hormone release-inhibiting factor (MIF).

As the foregoing discussion illustrates, the hypothalamic endocrine system performs many essential functions affecting other endocrine systems.[27] In turn, the thalamus and cortex exert control on the secretion of these (hypothalamic) factors. A complete review of this field is beyond the scope of this chapter; hence, the interested reader should refer to the literature cited.[23–27]

PITUITARY HORMONES

The pituitary gland, or the hypophysis, is located at the base of the skull and is attached to the hypothalamus by a stalk. The pituitary gland plays a major role[23] in regulating activity of the endocrine organs, including the adrenal cortex, the gonads, and the thyroid. The neurohypophysis (posterior pituitary), which originates from the brain, and the adenohypophysis (anterior pituitary), which is derived from epithelial tissue, are the two embryologically and functionally different parts of the pituitary gland. The adenohypophysis is under the control of hypothalamic regulatory hormones, and it secretes adrenocorticotropic hormone (ACTH), growth hormone (GH), LH, FSH, prolactin, and others. The neurohypophysis is responsible for the storage and secretion of the hormones vasopressin and oxytocin, controlled by nerve impulses traveling from the hypothalamus.

Adrenocorticotropic Hormone

ACTH (adrenocorticotropin, corticotropin) is a medicinal agent that has been the center of much research. In the late 1950s, its structure was elucidated, and the total synthesis was accomplished in the 1960s. Related peptides also have been synthesized, and some of these possess similar physiologic action. Human ACTH has 39 amino acid units within the polypeptide chain.

Structure–activity relationship studies of ACTH[28] showed that the COOH-terminal sequence is not particularly important for biologic activity. Removal of the NH_2-terminal amino acid results in complete loss of steroidogenic activity. Full activity has been reported for synthetic peptides containing the first 20 amino acids. A peptide containing 24 amino acids has full steroidogenic activity, without allergenic reactions. This is of practical importance because natural ACTH preparations sometimes produce clinically dangerous allergic reactions.

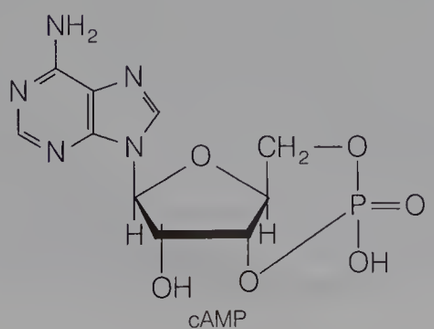
Corticotropin exerts its major action on the adrenal cortex, promoting steroid synthesis by stimulating the formation of pregnenolone from cholesterol. An interaction between ACTH and specific receptors is implicated in the mechanism leading to stimulation of adenylate cyclase and acceleration of steroid production. The rate-limiting step in the biosynthesis of steroids from cholesterol is the oxidative cleavage of the side chain of cholesterol, which results in the formation of pregnenolone. This rate-limiting is step-regulated by cyclic adenosine monophosphate (cAMP).[29] Corticotropin, through cAMP, stimulates the biosynthesis of steroids from cholesterol by increasing the availability of free cholesterol. This involves activation of cholesterol esterase by phosphorylation. Corticotropin also stimulates the uptake of cholesterol from plasma lipoproteins. Other biochemical effects exerted by ACTH include stimulation of phosphorylase and hydroxylase activities. Glycolysis also is increased by this hormone. Enzyme systems that catalyze processes involving the production of reduced nicotinamide adenine dinucleotide phosphate (NADPH) also are stimulated. (It is noteworthy that NADPH is required by the steroid hydroxylations that take place in the overall transformation of cholesterol to hydrocortisone, the major glucocorticoid hormone.) Pharmaceutically important ACTH products are listed in Table 26-7.

TABLE 26-7

PHARMACEUTICALLY IMPORTANT ACTH PRODUCTS

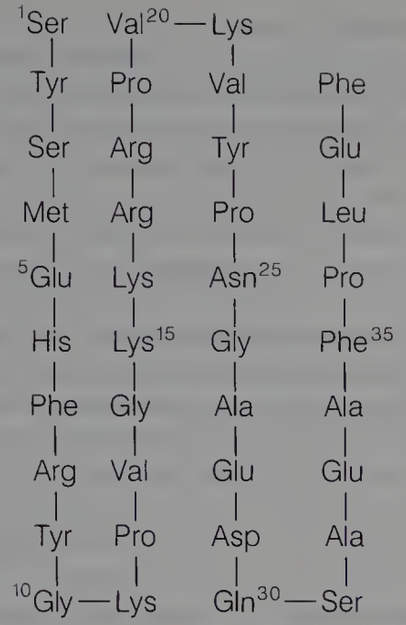
Preparation Proprietary Name	*Category*	*Usual Adult Dose**	*Usual Dose Range**	*Usual Pediatric Dose**
Corticotropin injection USP Corticotropin for injection USP *Acthar*	Adrenocorticotropic hormone; adrenocortical steroid (anti-inflammatory); diagnostic aid (adrenocortical insufficiency)	Adrenocorticotropic hormone: parenteral, 20 USP units, qid. Adrenocortical steroid (anti-inflammatory): parenteral, 20 USP units qid. Diagnostic aid (adrenocortical insufficiency): rapid test — IM or IV, 25 USP units, with blood sampling in 1 hr; adrenocortical steroid output — IV infusion, 25 U in 500–1,000 mL of 5% dextrose injection over a period of 8 hr on each of 2 successive days, with 24-hr urine collection done on each day	Adrenocorticotropic hormone: 40–80 U/day; adrenocortical steroid (anti-inflammatory): 40–80 U/day	Parenteral, 0.4 U/kg of body weight or 12.5 U/m^2 of body surface, qid
Repository corticotropin injection USP *Acthar Gel, Cortrophin Gel*	Adrenocorticotropic hormone; adrenocortical steroid (anti-inflammatory); diagnostic aid (adrenocortical insufficiency)	Adrenocorticotropic hormone: IM or SC, 40–80 U every 24–72 hr; IV infusion, 40–80 U in 500 mL of 5% dextrose injection given over an 8-hr period, qd. Adrenocortical steroid (anti-inflammatory): IM or SC, 40–80 U every 24–72 hr; IV infusion, 40–80 U in 500 mL of 5% dextrose injection given over an 8-hr period, qd. Diagnostic aid (adrenocortical insufficiency): IM, 40 U bid on each of 2 successive days, with 24-hr urine collection done each day		Adrenocorticotropic hormone: parenteral, 0.8 U/kg of body weight or 25 U/m^2 of body surface per dose
Sterile corticotropin zinc hydroxide suspension USP *Cortrophin-Zinc*	Adrenocorticotropic hormone; adrenocortical steroid (anti-inflammatory); diagnostic aid (adrenocortical insufficiency)	Adrenocorticotropic hormone: IM, initial, 40–60 U/day, increasing interval to 48, then 72 hr; reduce dose per injection thereafter; maintenance, 20 U/day to twice weekly. Adrenocortical steroid (anti-inflammatory): IM, initial, 40–60 U/day, increasing interval to 48, then 72 hr; reduce dose per injection thereafter; maintenance, 20 U/day to twice weekly. Diagnostic aid (adrenocortical insufficiency): IM, 40 U on each of 2 successive 24-hr periods		
Cosyntropin *Cortrosyn*	Diagnostic aid (adrenocortical insufficiency)	IM or IV, 250 μg		Children 2 yr of age or less, 0.125 mg

*See USP DI for complete dosage information.



cAMP

Corticotropin Injection, USP (ACTH injection). Adrenocorticotropin injection is a sterile preparation of the principle or principles derived from the anterior lobe of the pituitary of mammals used for food by humans. It occurs as a colorless or light straw-colored liquid or a soluble, amorphous solid by drying such liquid from the frozen state. It exerts a tropic influence on the adrenal cortex. The solution has a *p*H range of 3.0 to 7.0 and is used for its adrenocorticotropic activity.



Corticotropin

Repository Corticotropin Injection, USP (corticotropin gel, purified corticotropin). ACTH purified is corticotropin in a solution of partially hydrolyzed gelatin to be used intramuscularly for a more uniform and prolonged maintenance of activity.

Sterile Corticotropin Zinc Hydroxide Suspension, USP, is a sterile suspension of corticotropin, adsorbed on zinc hydroxide, which contains no less than 45 and no more than 55 μg of zinc for each 20 USP corticotropin units. Because of its prolonged activity owing to slow release of corticotropin, an initial dose of 40 USP units can be administered intramuscularly, followed by a maintenance dose of 20 units, two or three times a week.

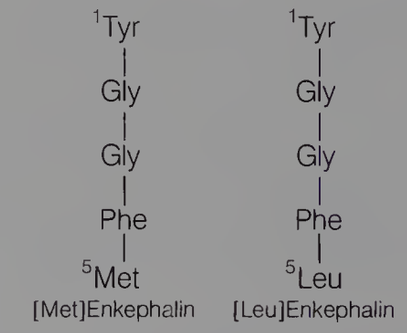
Cosyntropin (Cortrosyn) is a synthetic peptide containing the first 24 amino acids of natural corticotropin. Cosyntropin is used as a diagnostic agent to test for adrenal cortical deficiency. Plasma hydrocortisone concentration is determined before and 30 minutes after the administration of 250 μg of cosyntropin. Most normal responses result in an approximate doubling of the basal hydrocortisone concentration in 30 to 60 minutes. If the response is not normal, adrenal insufficiency is indicated. Such adrenal insufficiency could be due to either adrenal or pituitary malfunction, and further testing is required to distinguish between the two. Cosyntropin (250 μg infused within 4 to 8 hours) or corticotropin (80 to 120 U/day for 3 to 4 days) is administered. Patients with functional adrenal tissue should respond to this dosage. Patients who respond accordingly are suspected of hypopituitarism, and the diagnosis can be confirmed by other tests for pituitary function. However, little or no response is shown by patients who have Addison's disease.

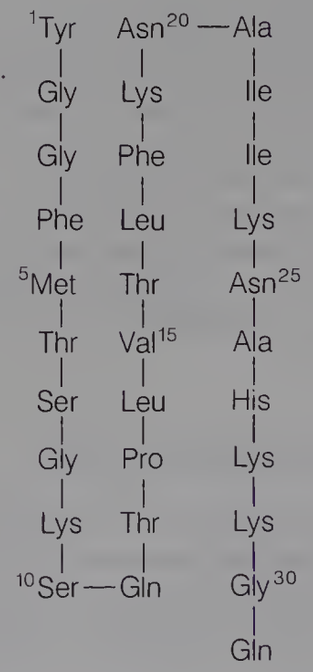
Melanotropins (Melanocyte-Stimulating Hormone)

Melanocyte-stimulating hormone (MSH) is elaborated by the intermediate lobe of the pituitary gland and regulates pigmentation of skin in fish, amphibians, and, to a lesser extent, humans. Altered secretion of MSH has been implicated in causing changes in skin pigmentation during the menstrual cycle and pregnancy. The two major types of melanotropin, α-MSH and β-MSH, are derived from ACTH and β-lipotropin, respectively. α-MSH contains the same amino acid sequence as the first 13 amino acids of ACTH; β-MSH has 18 amino acid residues. A third melanotropin, γ-melanotropin, is derived from a larger peptide precursor, proopiomelanocortin (POMC). Some important endocrinologic correlations of interest include inhibitory actions of hydrocortisone on the secretion of MSH and the inhibitory effects of epinephrine and norepinephrine on MSH action.

Lipotropins (Enkephalins and Endorphins)

Opiates, such as opium and morphine, have been known to humans for centuries as substances that relieve pain and suffering. Neuropharmacologists have theorized that opiates interact with receptors in the brain that are affected by endogenous substances that function as regulators of pain perception. The important breakthrough came in 1975, with the isolation of two peptides with opiate-like activity[30] from pig brains. These related pentapeptides, called methionine-enkephalin and leucine-enkephalin, are abundant in certain nerve terminals and have been shown to occur in the pituitary gland.





β-Endorphin (sheep)

An examination of the structures of enkephalins revealed that the amino acid sequence of met-enkephalin was identical with the sequence of residues 61–65 of β-lipotropin (β-LPH), a larger peptide found in the pituitary gland. This discovery suggested that β-LPH may be a precursor for other larger peptides containing the met-enkephalin sequence. Soon after the structural relationship between β-LPH and met-enkephalin was established, longer peptides, called endorphins, were isolated from the intermediate lobe of the pituitary gland. The endorphins (α, β, and γ) contained the met-enkephalin amino acid sequence and possessed morphine-like activity.[31] The longest of these peptides, β-endorphin, a 31-residue peptide (residues 61–91 of β-LPH), is about 20 to 50 times more potent than morphine as an analgesic and has a considerably longer duration of action than that of enkephalins. Numerous enkephalin analogues and derivatives have been prepared and their biologic activity evaluated. Like morphine, β-endorphin and the enkephalins can induce tolerance and dependence.

In addition to the enkephalins and endorphins, several other opioid peptides have been extracted from pituitary, adrenal, and nervous tissue, including dynorphins and neoendorphins. It is now clear that β-LPH, ACTH, and γ-MSH are derived from the same precursor, POMC. Krieger and Liotta[31a] have summarized and critically reviewed the available data concerning synthesis, distribution, regulation, and function of these hormones, and they conclude that pituitary hormones originating in the brain may be involved in central coordination of responses independently of those affected by peripheral secretion of such pituitary hormones. However, those hormones that appear to enter the brain by possible retrograde portal blood flow may participate in short-loop feedback regulation of anterior pituitary function. Another peptide, proenkephalin, is the primary precursor for met- and leu-enkephalins. The neoendorphins and dynorphins are derived from the same peptide precursor, prodynorphin.[31]

The endorphins and enkephalins have a wide range of biologic effects, and most of their actions are in the central nervous system. Their actions include inhibition of release of dopamine in the brain tissue and inhibition of release of acetylcholine from neuromuscular junctions. The role of endorphins and enkephalins as inhibitory neurotransmitters agrees well with the observed biologic effects of these peptides in lowering response to pain and other stimuli. The role of endorphins and enkephalins as neurotransmitters and neuromodulators, with emphasis on receptor interactions, has been reviewed.[32]

Growth Hormone (Somatotropin)

GH is a 191-residue polypeptide elaborated by the anterior pituitary. The amino acid sequence of GH has been determined, and comparison with growth hormones of different species has revealed a considerable amount of structural variation.[33]

The major biologic action of GH is to promote overall somatic growth. Deficiency in the secretion of this hormone can cause dwarfism, and an overproduction of this hormone can cause acromegaly and giantism. Secretion of this hormone is stimulated by growth hormone-releasing hormone (GH-RH), a 44-residue polypeptide secreted by the hypothalamus. Secretion of GH is inhibited by somatostatin.

GH stimulates protein synthesis, both in the skeletal muscles and in the liver. In the liver, GH stimulates uptake of amino acids and promotes the synthesis of all forms of RNA. It stimulates glucagon secretion by the pancreas, increases synthesis of glycogen in muscles, augments the release of fatty acids from adipose tissue, and increases osteogenesis. It also causes acute hypoglycemia followed by elevated blood glucose concentration and, perhaps, glycosuria.

GH has been recognized as an effective replacement therapy for GH-deficient children. The supply of GH, however, was very limited because its source was the pituitary glands of human cadavers. There were also several reports of deaths in children with Creutzfeldt-Jakob disease (caused by viral contamination of GH), which halted the distribution of GH in 1977. Both of these problems were solved with the application of recombinant DNA technology in the commercial production of somatrem and somatropin.

Somatrem (systemic) (Protropin) is a biosynthetic form of human GH that differs from the pituitary-derived GH and recombinant somatotropin by addition of an extra amino acid, methionine. Because of its structural difference from the natural GH, patients receiving somatrem may develop antibodies, which may result in a decreased response to it. Somatrem is administered intramuscularly or subcutaneously, and the therapy is continued as long as the patient is responsive, until the patient reaches a mature adult height, or until the epiphyses close. The dosage range is 0.05 to 0.1 IU.

Somatropin (rDNA origin) for injection (Humatrope) is a natural-sequence human GH of recombinant DNA origin. Its composition and sequence of amino acids are identical with those of human GH of pituitary origin. It is administered intramuscularly or subcutaneously. The dosage range is from 0.05 to 0.1 IU.

Prolactin

Prolactin (PRL), a hormone secreted by the anterior pituitary, was discovered in 1928. It is a 198-residue polypeptide with general structural features similar to those of GH.[34]

PRL stimulates lactation of parturition.

GONADOTROPIC HORMONES

The two principal gonadotropins elaborated by the adenohypophysis are FSH and LH. LH is also known as interstitial cell-stimulating hormone. The gonadotropins along with thyrotropin form the glycoprotein group of hormones. FSH

and LH may be produced by a single cell, the gonadotroph. The secretion of FSH and LH is controlled by the hypothalamus, which produces LH-RH. LH-RH stimulates the secretion of both FSH and LH, although its effects on the secretion of LH are more pronounced.

Follicle-Stimulating Hormone

FSH promotes the development of ovarian follicles to maturity as well as spermatogenesis in testicular tissue. It is a glycoprotein, the carbohydrate component of which is considered to be associated with its activity.

Luteinizing Hormone

LH is another glycoprotein. It acts after the maturing action of FSH on ovarian follicles, stimulates production of estrogens, and transforms the follicles into corpora lutea. LH also acts in the male of the species by stimulating the Leydig cells that produce testosterone.

Menotropins

Pituitary hormones, prepared from the urine of postmenopausal women whose ovarian tissue does not respond to gonadotropin, are available for medicinal use in the form of the product menotropins (Pergonal). The latter has FSH and LH gonadotropin activity in a 1 : 1 ratio. Menotropins is useful in the treatment of anovular women whose ovaries are responsive to pituitary gonadotropins but who have a gonadotropin deficiency caused by either pituitary or hypothalamus malfunction. Usually, menotropins is administered intramuscularly: initial dose of 75 IU of FSH and 75 IU of LH daily for 9 to 12 days, followed by 10,000 IU of chorionic gonadotropin 1 day after the last dose of menotropins.

THYROTROPIN

The thyrotropic hormone, also called thyrotropin and thyroid-stimulating hormone (TSH), is a glycoprotein consisting of two polypeptide chains. This hormone promotes production of thyroid hormones by affecting the kinetics of the mechanism whereby the thyroid concentrates iodide ions from the bloodstream, thereby promoting incorporation of the halogen into the thyroid hormones and release of hormones by the thyroid.

TSH (Thytropar) appears to be a glycoprotein (M_r 26,000 to 30,000) containing glucosamine, galactosamine, mannose, and fucose, the homogeneity of which is yet to be established. It is produced by the basophil cells of the anterior lobe of the pituitary gland. TSH enters the circulation from the pituitary, presumably traversing cell membranes in the process. After exogenous administration, it is widely distributed and disappears very rapidly from circulation. Some evidence suggests that the thyroid may directly inactivate some of the TSH by an oxidation mechanism that may involve iodine. TSH thus inactivated can be reactivated by certain reducing agents. TSH regulates the production by the thyroid gland of thyroxine, which stimulates the metabolic rate. Thyroxine feedback mechanisms regulate the production of TSH by the pituitary gland.

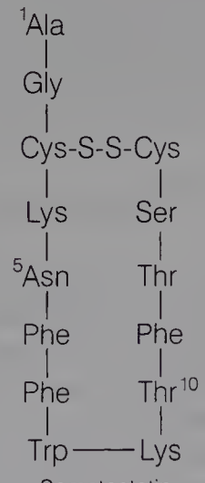
The decreased secretion of TSH from the pituitary is a part of a generalized hypopituitarism that leads to hypothyroidism. This type of hypothyroidism can be distinguished from primary hypothyroidism by the administration of TSH in doses sufficient to increase the uptake of radioiodine or to elevate the blood or plasma protein-bound iodine (PBI) as a consequence of enhanced secretion of hormonal iodine (thyroxine). Interestingly, massive doses of vitamin A inhibit the secretion of TSH. Thyrotropin is used as a diagnostic agent to differentiate between primary and secondary hypothyroidism. Its use in hypothyroidism caused by pituitary deficiency has limited application; other forms of treatment are preferable.

Dose, intramuscular or subcutaneous, 10 IU.

SOMATOSTATIN

Somatostatin was discovered in the hypothalamus. It is now established that it is elaborated by the δ-cells of the pancreas and elsewhere in the body. Somatostatin is an oligopeptide (14 amino acid residues) and is referred to as somatotropin release-inhibiting factor (SRIF).[35]

Its primary action is inhibiting the release of GH from the pituitary gland. Somatostatin also suppresses the release of both insulin and glucagon. It causes a decrease in both cAMP levels and adenylate cyclase activity. It also was found to inhibit calcium ion influx into the pituitary cells and to suppress glucose-induced pancreatic insulin secretion by activating and deactivating potassium ion and calcium ion permeability, respectively. The chemistry, structure–activity relationships, and potential clinical applications have been reviewed.[24,36]



Somatostatin

A powerful new synthetic peptide that mimics the action of somatostatin, octreotide acetate (Sandostatin), has received approval from the FDA for the treatment of certain rare forms of intestinal endocrine cancers, such as malignant carcinoid tumors and vasoactive intestinal peptide-secreting tumors (VIPomas).

PLACENTAL HORMONES

Human Chorionic Gonadotropin

Human chorionic gonadotropin (hCG) is a glycoprotein synthesized by the placenta. Estrogens stimulate the anterior pituitary to produce placentotropin, which in turn stimulates hCG synthesis and secretion. hCG is produced primarily during the first trimester of pregnancy. It exerts effects that are similar to those of pituitary LH.

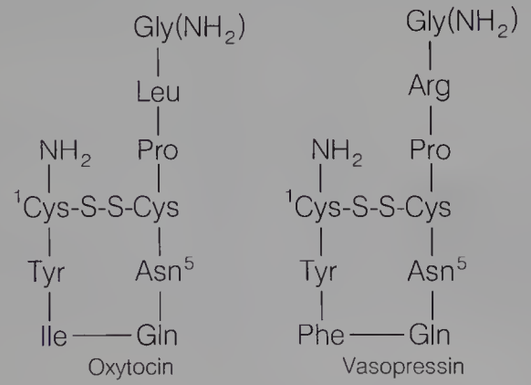
hCG is used therapeutically in the management of cryptorchidism in prepubertal boys. It also is used in women in conjunction with menotropins to induce ovulation when the endogenous availability of gonadotropin is not normal.

Human Placental Lactogen

Human placental lactogen (hPL) also is called human chorionic mammotropin and chorionic growth-hormone prolactin. This hormone exerts numerous actions. In addition to mammotropic and lactotropic effects, it exerts somatotropic and luteotropic actions. It has been identified as a protein composed of 191 amino acid units in a single-peptide chain with two disulfide bridges.[26] hPL resembles human somatotropin.

NEUROHYPOPHYSEAL HORMONES (OXYTOCIN, VASOPRESSIN)

The posterior pituitary (neurohypophysis) is the source of vasopressin, oxytocin, α- and β-MSH, and coherin. The synthesis, transport, and release of these hormones have been reviewed by Brownstein.[37] Vasopressin and oxytocin are synthesized and released by neurons of the hypothalamic–neurohypophyseal system. These peptide hormones, and their respective neurophysin carrier proteins, are synthesized as structural components of separate precursor proteins, and these proteins appear to be partially degraded into smaller bioactive peptides in the course of transport along the axon.



The structures of vasopressin and oxytocin have been elucidated, and these peptides have been synthesized. Actually, three closely related nonapeptides have been isolated from mammalian posterior pituitary: oxytocin and arginine vasopressin from most mammals and lysine vasopressin from pigs. The vasopressins differ from one another in the nature of the eighth amino acid residue: arginine and lysine, respectively. Oxytocin has leucine at position 8 and its third amino acid is isoleucine instead of phenylalanine. Several analogues of vasopressin have been synthesized and their antidiuretic activity evaluated. Desmopressin, 1-desamino-8-arginine-vasopressin, is a synthetic derivative of vasopressin. It is a longer-acting and more potent antidiuretic than vasopressin, with much less pressor activity. Desmopressin is much more resistant to the actions of peptidases because of the deamination at position 1, which accounts for its longer duration of action. The substitution of D- for L-arginine in position 8 accounts for its sharply lower vasoconstrictive effects.[38]

Vasopressin also is known as the pituitary antidiuretic hormone (ADH). This hormone can effect graded changes in the permeability of the distal portion of the mammalian nephron to water, resulting in either conservation or excretion of water; thus, it modulates the renal tubular reabsorption of water. ADH has been shown to increase cAMP production in several tissues. Theophylline, which promotes cAMP by inhibiting the enzyme (phosphodiesterase) that catalyzes its hydrolysis, causes permeability changes similar to those caused by ADH. Cyclic AMP also effects similar permeability changes; hence, it is suggested that cAMP is involved in the mechanism of action of ADH.

The nonrenal actions of vasopressin include its vasoconstrictor effects and neurotransmitter actions in the central nervous system, such as regulation of ACTH secretion, circulation, and body temperature.[39]

ADH is therapeutically useful in the treatment of diabetes insipidus of pituitary origin. It also has been used to relieve intestinal paresis and distention.

Oxytocin is appropriately named on the basis of its oxytocic action. Oxytocin exerts stimulant effects on the smooth muscle of the uterus and mammary gland and has a relaxing effect on vascular smooth muscle when administered in high doses. It is considered to be the drug of choice to induce labor, particularly in cases of intrapartum hypotonic inertia. Oxytocin also is used in inevitable or incomplete abortion after the 20th week of gestation. It also may be used to prevent or control hemorrhage and to correct uterine hypotonicity. In some cases, oxytocin is used to promote milk ejection; it acts by contracting the myoepithelium of the mammary glands. Oxytocin is usually administered parenterally by intravenous infusion, intravenous injection, or intramuscular injection. Oxytocin citrate buccal tablets are also available, but the rate of absorption is unpredictable and buccal administration is less precise. Topical administration (nasal spray) 2 or 3 minutes before nursing to promote milk ejection sometimes is recommended.[40] Refer to Table 26-8 for product listing.

TABLE 26-8

NEUROHYPOPHYSEAL HORMONES: PHARMACEUTICAL PRODUCTS

Preparation Proprietary Name	*Category*	*Usual Adult Dose* *	*Usual Pediatric Dose* *
Oxytocin injection USP *Pitocin, Syntocinon*	Oxytocic	IM, 3–10 U after delivery of placenta; IV, initially no more than 1–2 mU/min, increased every 15–30 min in increments of 1–2 mU	
Oxytocin nasal solution, USP *Syntocinon*	Oxytocic	1 spray or 3 drops in 1 or both nostrils 2–3 min before nursing or pumping of breasts	
Vasopressin injection USP *Pitressin*	Antidiuretic posterior pituitary hormone	IM or SC, 2.5–10 U tid or qid as necessary	IM or SC, 2.5–10 U tid or qid as necessary
Sterile vasopressin tannate oil suspension *Pitressin*	Antidiuretic posterior pituitary hormone	IM, 1.5–5 U every 1–3 days	IM, 1.25–2.5 U every 1–3 days
Desmopressin acetate nasal solution *DDAVP*	Antidiuretic posterior pituitary hormone	Maintenance: Intranasal, 2–4 μg/day, as a single dose or in 2–3 divided doses	Maintenance: Intranasal, 2–4 μg/kg of body weight per day or 5–30 mg/day or in 2–3 divided doses
Desmopressin acetate injection *DDAVP, Stimate*	Antidiuretic posterior pituitary hormone	IV or SC, 2–4 μg/day usually in 2 divided doses in the morning or evening	IV, 3 μg/kg of body weight diluted in 0.9% sodium chloride injection USP

*See USP DI for complete dosage information.

Oxytocin Injection, USP, is a sterile solution in water for injection of oxytocic principle prepared by synthesis or obtained from the posterior lobe of the pituitary of healthy, domestic animals used for food by humans. The *p*H is 2.5 to 4.5; expiration date, 3 years.

Oxytocin preparations are widely used with or without amniotomy to induce and stimulate labor. Although injection is the usual route of administration, the sublingual route is extremely effective. Sublingual and intranasal spray (Oxytocin Nasal Solution, USP) routes of administration also will stimulate milk let-down.

Vasopressin Injection, USP (Pitressin), is a sterile solution of the water-soluble pressor principle of the posterior lobe of the pituitary of healthy, domestic animals used for food by humans; it also may be prepared by synthesis. Each milliliter possesses a pressor activity equal to 20 USP posterior pituitary units; expiration date, 3 years.

Vasopressin Tannate (Pitressin Tannate) is a water-insoluble tannate of vasopressin administered intramuscularly (1.5 to 5 pressor units daily) for its prolonged duration of action by the slow release of vasopressin. It is particularly useful for patients who have diabetes insipidus, but it never should be used intravenously.

Felypressin. 2-L-Phenylalanine-8-L-lysine vasopressin has relatively low antidiuretic activity and little oxytocic activity. It has considerable pressor (i.e., vasoconstrictor) activity, which, however, differs from that of epinephrine (i.e., following capillary constriction in the intestine it lowers the pressure in the vena portae, whereas epinephrine raises the portal pressure). Felypressin also causes an increased renal blood flow in the cat, whereas epinephrine brings about a fall in renal blood flow. Felypressin is five times more effective as a vasopressor than is lysine vasopressin and is recommended in surgery to minimize blood flow, especially in obstetrics and gynecology.

Lypressin is synthetic 8-L-lysine vasopressin, a polypeptide similar to ADH. The lysine analogue is considered to be more stable, and it is absorbed rapidly from the nasal mucosa. Lypressin (Diapid) is pharmaceutically available as a topical solution, spray, 50 pressor units (185 μg)/mL in 5 mL containers. Usual dosage, topical (intranasal), one or more sprays applied to one or both nostrils one or more times daily.[29]

Desmopressin Acetate (DDAVP, Stimate) is synthetic 1-desamino-8-D-arginine vasopressin. Its efficacy, ease of administration (intranasal), long duration of action, and lack of side effects make it the drug of choice for the treatment of central diabetes insipidus. It also may be administered intramuscularly or intravenously. It is preferred to vasopressin injection and oral antidiuretics for use in children. It is indicated in the management of temporary polydipsia and polyuria associated with trauma to, or surgery in, the pituitary region.

PANCREATIC HORMONES

Relationships between lipid and glucose levels in the blood and the general disorders of lipid metabolism found in diabetic subjects have received the attention of many chemists and clinicians. To understand diabetes mellitus, its complications, and its treatment, one has to begin at the level of the basic biochemistry of the pancreas and the ways carbohydrates are correlated with lipid and protein metabolism (see Chap. 25). The pancreas produces insulin, as well as gluca-

gon; β-cells secrete insulin and α-cells secrete glucagon. Insulin will be considered first.

Insulin

One of the major triumphs of this century occurred in 1922, when Banting and Best extracted insulin from dog pancreas.[41] Advances[41a] in the biochemistry of insulin have been reviewed with emphasis on proinsulin biosynthesis, conversion of proinsulin to insulin, insulin secretion, insulin receptors, metabolism, effects by sulfonylureas, and so on.[42–44]

Insulin is synthesized by the islet β-cells from a single-chain, 86-amino-acid polypeptide precursor, proinsulin.[45] Proinsulin itself is synthesized in the polyribosomes of the rough endoplasmic reticulum of the β-cells from an even larger polypeptide precursor, preproinsulin. The B-chain of preproinsulin is extended at the NH_2-terminus by at least 23 amino acids. Proinsulin then traverses the Golgi apparatus and enters the storage granules, in which the conversion to insulin occurs.

The subsequent proteolytic conversion of proinsulin to insulin is accomplished by the removal of Arg-Arg residue at positions 31 and 32 and Arg-Lys residue at positions 64 and 65 by an endopeptidase that resembles trypsin in its specificity and a thiol-activated carboxypeptidase B-like enzyme.[46]

The actions of these proteolytic enzymes on proinsulin result in the formation of equimolar quantities of insulin and the connecting C-peptide. The resulting insulin molecule consists of chains A and B, having 21 and 31 amino acid residues, respectively. The chains are connected by two disulfide linkages, with an additional disulfide linkage within chain A (Fig. 26-6).

The three-dimensional structure of insulin has been determined by x-ray analysis of single crystals. These studies have demonstrated that the high bioactivity of insulin depends on the integrity of the overall conformation. The biologically active form of the hormone is thought to be the

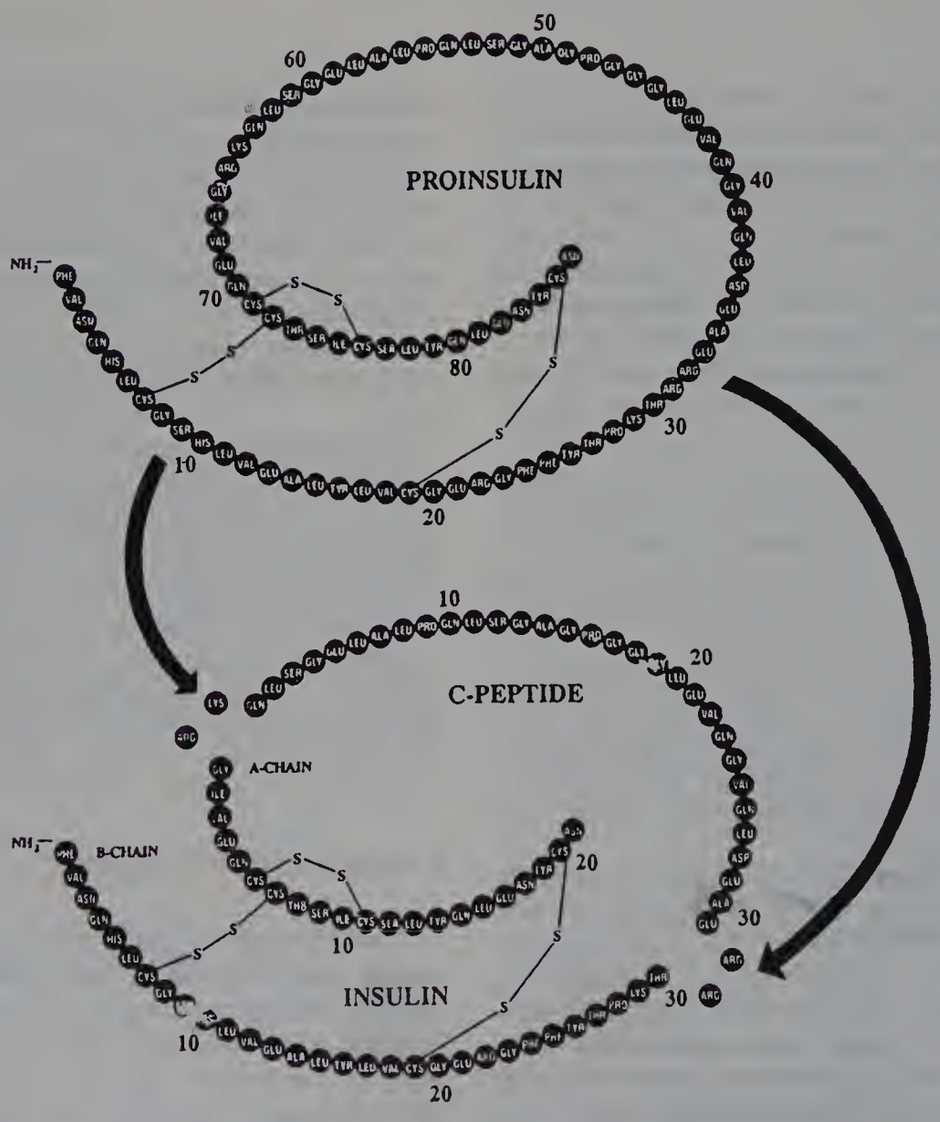


FIG. 26-6. Conversion of proinsulin to insulin.

TABLE 26-9†

SOME SEQUENCE DIFFERENCES IN INSULINS OF VARIOUS SPECIES

	A-chain					B-chain		
Species	1	8	9	10	−1	1	29	30
Human	Gly.	Thr.	Ser.	Ile.		Phe.	Lys.	Thr.
Pork	Gly.	Thr.	Ser.	Ile.		Phe.	Lys.	Ala.
Beef	Gly.	Ala.	Ser.	Val.		Phe.	Lys.	Ala.
Sheep	Gly.	Ala.	Gly.	Val.		Phe.	Lys.	Ala.
Horse	Gly.	Thr.	Gly.	Ile.		Phe.	Lys.	Ala.
Rabbit	Gly.	Thr.	Ser.	Ile.		Phe.	Lys.	Ser.
Chicken	Gly.	His.	Asn.	Thr.		Ala.	Lys.	Ala.
Cod	Gly.	His.	Arg.	Pro.	Met.	Ala.	Lys.	—
Rat I*	Gly.	Thr.	Ser.	Ile.		Phe.	Lys.	Ser.
Rat II*	Gly.	Thr.	Ser.	Ile.		Phe.	Met.	Ser.

*Asp substitution for Glu at position 4 on A-chain.
†See Reference 44 for details.

monomer. The receptor-binding region, consisting of A-1 Gly, A-4 Glu, A-5 Gln, A-19 Tyr, A-21 Asn, B-12 Val, B-16 Tyr, B-24 Phe, and B-26 Tyr, has been identified. The three-dimensional crystal structure appears to be conserved in solution and during its receptor interaction.[26]

The amino acid sequence of insulins from various animal species have been examined.[44] Details of these are shown in Table 26-9. It is apparent from the analysis that frequent changes in sequence occur within the interchain disulfide ring (positions 8, 9, and 10). The hormonal sequence for porcine insulin is the closest to that of humans, differing only by the substitution of an alanine residue at the COOH-terminus of the B-chain. Porcine insulin, therefore, is a good starting material for the synthesis of human insulin.

Insulin composes 1% of pancreatic tissue, and secretory protein granules contain about 10% insulin. These granules fuse with the cell membrane with simultaneous liberation of equimolar amounts of insulin and the C-peptide. Insulin enters the portal vein, and about 50% is removed in its first passage through the liver. The plasma half-life of insulin is approximately 4 minutes, compared with 30 minutes for the C-peptide.

Usually, exogenous insulin is weakly antigenic. Insulin antibodies have been observed to neutralize the hypoglycemic effect of injected insulin. The antibody-binding sites on insulin are quite different from those involved in binding of insulin with its receptors.[48]

Regulation of insulin secretion is affected by numerous factors, such as food, hormonal and neuronal stimuli, and ionic mechanisms.[49] In humans, the principal substrate that stimulates the release of insulin from the islet β-cells is glucose. In addition to glucose, other substrates (e.g., amino acids, free fatty acids, and ketone bodies) also can directly stimulate insulin secretion. Secretin and ACTH can directly stimulate the secretion of insulin. Glucagon and other related peptides can cause an increase in the secretion of insulin, whereas somatostatin inhibits its secretion.

Autonomic neuronal mechanisms also play an important role in regulating insulin release. In the sympathetic nervous system, α-adrenergic agonists inhibit insulin release, whereas β-adrenergic agonists stimulate the release of insulin. In the parasympathetic nervous system, cholinomimetic drugs stimulate the release of insulin.

"Clinical" insulin that has been crystallized five times and then subjected to countercurrent distribution (2-butanol: 1% dichloroacetic acid in water) yields about 90% insulin A, with varying amounts of insulin B together with other minor components. A and B differ by an amide group and have the same activity. End-member analysis, sedimentation, and diffusion studies indicate an M_r of about 6000. The value of 12,000 M_r for insulin containing trace amounts of zinc (obtained by physical methods) is probably a bimolecular association product through the aid of zinc. Insulin was the first protein for which a complete amino acid sequence was determined. The extensive studies of Sanger[50] and others have elucidated the amino acid sequence and structure of insulin. Katsoyannis[51] and others followed with the synthesis of A- and B-chains of human, bovine, and sheep insulin. The A- and B-chains were combined to form insulin in 60% to 80% yields, with a specific activity comparable with that of the natural hormone.[35]

The total synthesis of human insulin has been reported by Rittel et al.[51a] These workers were able to synthesize selectively the final molecule appropriately cross-linked by disulfide (—S—S—) groups in yields ranging between 40% and 50%, whereas earlier synthetic methods involved random combination of separately prepared A- and B-chains of the molecule.

Recombinant DNA technology has been applied successfully in the production of human insulin on a commercial scale. Human insulin is produced in genetically engineered *E. coli*.[52] Eli Lilly and Co., in cooperation with Genentech, began marketing of recombinant DNA-derived human insulin (Humulin) in 1982. There are two available methods of applying recombinant DNA technology in the production of human insulin. The earlier method involved insertion of genes, for production of either the A- or the B-chain of the insulin molecule, into a special strain of *E. coli* (KI2) and

subsequently combining the two chains chemically to produce an insulin that is structurally and chemically identical with pancreatic human insulin. The second, and most recent, method involves the insertion of genes for the entire proinsulin molecule into special *E. coli* bacteria, which are then grown in fermentation process. The connecting C-peptide is then enzymatically cleaved from proinsulin to produce human insulin.[53] Human insulin produced by recombinant DNA technology is less antigenic than that from animal sources.

Although insulin is readily available from natural sources (e.g., porcine and bovine pancreatic tissue), partial syntheses and molecular modifications have been developed as the basis for structure–activity relationship (SAR) studies. Such studies have shown that amino acid units cannot be removed from the insulin peptide chain A without significant loss of hormonal activity. However, several amino acids of chain B are not considered to be essential for activity. Up to the first six and the last three amino acid units can be removed without significant decrease in activity.[26]

Two insulin analogues, which differ from the parent hormone in that the NH_2-terminus of chain A (A^1) glycine has been replaced by L- and D-alanine, respectively, have been synthesized by Cosmatos et al.[54] for SAR studies. The relative potencies of the L- and D-analogues reveal interesting SARs. The L- and D-alanine analogues are 9.4% and 95%, respectively, as potent as insulin in glucose oxidation. The relative binding affinity to isolated fat cells is reported to be approximately 10% for the L- and 100% for the D-analogue. Apparently, substitution on the α-carbon of A^1 glycine of insulin with a methyl in a particular configuration interferes with the binding; hence, the resulting analogue (that of L-alanine) is much less active. Methyl substitution in the opposite configuration affects neither the binding nor the bioactivity.

It appears that molecular modifications of insulin on the amino groups lead to reduction of bioactivity, but modifications of the ϵ-amino group of lysine number 29 on chain B (B-29) may yield active analogues. Accordingly, May et al.[55] synthesized *N*-ϵ-(+)-biotinyl insulin, which was demonstrated to be equipotent with natural insulin. Complexes of this biotinyl–insulin derivative with avidin also were prepared and evaluated biologically; these complexes showed a potency decrease to 5% of that of insulin. Such complexes conjugated with ferritin are expected to be useful in the development of electron microscope stains of insulin receptors.

Alteration in the tertiary structure of insulin appears to drastically reduce biologic activity as well as receptor binding. The three-dimensional structure provided by x-ray crystallography of the insulin monomer has revealed an exposed hydrophobic face that is thought to be involved directly in interacting with the receptor.[56] Thus, loss of biologic activity in insulin derivatives, produced by chemical modification, can be interpreted in terms of adversely affecting this hydrophobic region. Also, species variation in this hydrophobic region is very unusual.

Insulin is inactivated in vivo by (1) an immunochemical system in the blood of insulin-treated patients, (2) reduction of disulfide bonds (probably by glutathione), and (3) insulinase (a proteolytic enzyme) that occurs in liver. Pepsin and chymotrypsin will hydrolyze some peptide bonds that lead to inactivation. Insulin is inactivated by reducing agents such as sodium bisulfite, sulfurous acid, and hydrogen.

Advances in the area of insulin's molecular mechanisms have been reviewed[56a] with emphasis on receptor interactions, effect on membrane structure and functions, effects on enzymes, and the role of second messengers.[36] The insulin receptor is believed to be a glycoprotein complex with a high relative molecular mass (M_r). The receptor is thought to consist of four subunits: two identical α-units with an M_r of about 130,000 Da and two identical β-units with an M_r of 95,000 Da, joined together by disulfide bonds. The α-subunits are primarily responsible for binding insulin to its receptor, and the β-subunits are thought to possess intrinsic protein kinase activity that is stimulated by insulin. The primary effect of insulin may be a kinase stimulation leading to phosphorylation of the receptor as well as other intracellular proteins.[57,58] Additionally, insulin binding to its receptors may result in the generation of a soluble intracellular second messenger (possibly a peptide) that may mediate some insulin activity relating to activation of enzymes such as pyruvate dehydrogenase and glycogen synthetase.[59] The insulin–receptor complex becomes internalized and may serve as a vehicle for translocating insulin to the lysosomes, in which it may be broken down and recycled back to the plasma membrane. The half-life of insulin is about 10 hours.

The binding of insulin to its target tissue is determined by several factors. The number of receptors in the target tissue and their affinity for insulin are two important determinants. These factors vary substantially from tissue to tissue. Another important consideration is the concentration of insulin itself. Elevated levels of circulating insulin decrease the number of insulin receptors on target cell surfaces and vice versa. Other factors that affect insulin binding to its receptors include *p*H, temperature, membrane lipid composition, and ionic strength.[58,60] It is conceivable, therefore, that conditions associated with insulin resistance, such as obesity and type I and type II diabetes mellitus, could be caused by altered receptor kinase activity or impaired generation of second messengers (low relative molecular mass peptides), increased degradation of the messenger, or decreased substrates (enzymes involved in metabolic activity) for the messenger or receptor kinase.[61]

Metabolic Effects of Insulin. Insulin has pronounced effects on the metabolism of carbohydrates, lipids, and proteins.[62] The major tissues affected by insulin are muscle (cardiac and skeletal), adipose tissue, and liver. The kidney is much less responsive, whereas others, such as brain tissue and red blood cells, do not respond at all. The actions of insulin are highly complex and diverse. Because many of the actions of insulin are mediated by second messengers, it is difficult to distinguish between its primary and secondary actions.

In muscle and adipose tissue, insulin promotes transport of glucose and other monosaccharides across cell membranes; it also facilitates transport of amino acids, potassium ions, nucleosides, and ionic phosphate. Insulin also activates certain enzymes—kinases and glycogen synthetase in muscle and adipose tissue. In adipose tissue, insulin decreases the release of fatty acids induced by epinephrine or glucagon. Cyclic AMP promotes fatty acid release from adipose tissue; therefore, it is possible that insulin decreases fatty acid release by reducing tissue levels of cAMP. Insulin also facilitates the incorporation of intracellular amino acids into protein.

Insulin is believed to influence protein synthesis at the ribosomal level in various tissues.[63] In skeletal muscles, insulin predominantly stimulates translation by increasing the rate of initiation of protein synthesis and the number of ribosomes. In the liver, the predominant effect is on transcription. In cardiac muscles, insulin is believed to decrease the rate of degradation of proteins.

In the liver, there is no barrier to the transport of glucose into cells; nevertheless, insulin influences liver metabolism, decreasing glucose output, decreasing urea production, lowering cAMP, and increasing potassium and phosphate uptake. The lowering of cAMP results in decreased activity of glycogen phosphorylase, leading to diminished glycogen breakdown and increased activity of glycogen synthetase. It appears that insulin exerts induction of specific hepatic enzymes involved in glycolysis, while inhibiting gluconeogenic enzymes. Thus, insulin promotes glucose utilization through glycolysis by increasing the synthesis of glucokinase, phosphofructokinase, and pyruvate kinase. Insulin decreases the availability of glucose from gluconeogenesis by suppressing pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1,6-diphosphatase, and glucose-6-phosphatase.

Insulin's effects on lipid metabolism also are important. In adipose tissue, it has an antilipolytic action (i.e., an effect opposing the breakdown of fatty acid triglycerides). It also decreases the supply of glycerol to the liver. Thus, at these two sites, insulin decreases the availability of precursors for the formation of triglycerides. Insulin is necessary for the activation and synthesis of lipoprotein lipases, enzymes responsible for lowering very low-density lipoprotein (VLDL) and chylomicrons in peripheral tissue. Other effects include stimulation of the synthesis of fatty acids (lipogenesis) in the liver.[64]

Diabetes mellitus is a systemic disease caused by a decrease in the secretion of insulin or in a reduced sensitivity or responsiveness to insulin by target tissue (insulin receptor activity). The disease is characterized by hyperglycemia, hyperlipidemia, and hyperaminoacidemia. Diabetes mellitus frequently is associated with the development of micro- and macrovascular diseases, neuropathy, and atherosclerosis. Various types of diabetes have been recognized and classified and their pathophysiology discussed.[65]

The two major types of diabetes are type I, insulin-dependent diabetes mellitus (IDDM), and type II, non-insulin-dependent diabetes mellitus (NIDDM). Type I diabetes (also known as juvenile-onset diabetes) is characterized by a destruction of pancreatic β-cells, resulting in a deficiency of insulin secretion. Autoimmune complexes and viruses have been mentioned as two possible causes of β-cell destruction. Generally, in type I diabetes, receptor sensitivity to insulin is not decreased. Type II diabetes, also known as adult-onset diabetes, is characterized primarily by insulin receptor defects or postinsulin receptor defects. There is no destruction of β-cells, and insulin secretion is relatively normal. In reality, however, the two types of diabetes show a considerable overlap of clinical features.[65]

Diabetes mellitus is associated with both *microangiopathy* (damage to smaller vessels, e.g., the eyes and kidney) and *macroangiopathy* (damage to larger vessels, e.g., atherosclerosis). Hyperlipidemia (characterized by an increase in the concentration of lipoproteins such as VLDL, intermediate density lipoprotein [IDL], and LDL) has been implicated in the development of atherosclerosis and is known to occur in diabetes. Severe hyperlipidemia may lead to life-threatening attacks of acute pancreatitis. It also seems that severe hyperlipidemia causes xanthoma. Researchers also are investigating the relationship between diabetes and endogenous hyperlipidemia (hypertriglyceridemia).[64] Considering the effects of insulin on lipid metabolism, as summarized earlier, one can rationalize that in type II diabetes, in which the patient may actually have an absolute excess of insulin, in spite of the evidence of glucose tolerance tests, the effect of the excessive insulin on lipogenesis in the liver may be enough to increase the levels of circulating triglycerides and VLDL. In type I diabetes, with a deficiency of insulin, the circulating level of lipids may rise because too much precursor is available, with fatty acids and carbohydrates going to the liver.

The relationship between the carbohydrate metabolic manifestations of diabetes and the development of micro- and macrovascular diseases has been studied extensively.[66,67] It is becoming increasingly clear that hyperglycemia plays a major role in the development of vascular complications of diabetes, including intercapillary glomerulosclerosis, premature atherosclerosis, retinopathy with its specific microaneurysms and retinitis proliferans, leg ulcers, and limb gangrene. First, hyperglycemia causes an increase in the activity of lysine hydroxylase and galactosyl transferase, two important enzymes involved in glycoprotein synthesis. An increase in the glycoprotein synthesis in the collagen of kidney basement membrane may lead to the development of diabetic glomerulosclerosis. Second, an increase in the uptake of glucose by non-insulin-sensitive tissues (such as nerve Schwann cells and ocular lens cells) occurs during hyperglycemia. Intracellular glucose is converted enzymatically first to sorbitol and then to fructose. The buildup of these sugars inside the cells increases the osmotic pressure in ocular lens cells and Schwann cells, resulting in an increase in water uptake and impairing cell functions. Some forms of diabetic cataracts and diabetic neu-

ropathy are believed to be caused by this pathway. Third, hyperglycemia may precipitate nonenzymatic glycosylation of a variety of proteins in the body, including hemoglobin, serum albumin, lipoprotein, fibrinogen, and basement membrane protein. Glycosylation is believed to alter the tertiary structures of proteins and possibly their rate of metabolism. The rate of glycosylation is a function of plasma glucose concentration and the duration of hyperglycemia. Needless to say, this mechanism might play an important role in both macro- and microvascular lesions. Finally, hyperglycemia increases the rate of aggregation and agglutinization of circulating platelets. Platelets play an important role in promoting atherogenesis. The increase in the rate of platelet aggregation and agglutinization leads to the development of microemboli, which can cause transient cerebral ischemic attacks, strokes, and heart attacks.[65]

Modern concepts on the therapeutics of diabetes mellitus have been reviewed by Maurer.[68] This review emphasizes that insulin therapy does not always prevent serious complications. Even diabetics who are considered to be well under insulin therapeutic control experience wide fluctuations in blood glucose concentration, and it is hypothesized that these fluctuations eventually cause the serious complications of diabetes (e.g., kidney damage, retinal degeneration, premature atherosclerosis, cataracts, neurologic dysfunction, and a predisposition to gangrene).

Insulin Preparations. The various commercially available insulin preparations are listed in Table 26-10. Amorphous insulin was the first form made available for clinical use. Further purification afforded crystalline insulin, which is now commonly called "regular insulin." Insulin injection, USP, is made from zinc insulin crystals. For some time, regular insulin solutions have been prepared at a *p*H of 2.8 to 3.5; if the *p*H were increased above the acidic range, particles would be formed. However, more highly purified insulin can be maintained in solution over a wider *p*H range even when unbuffered. Neutral insulin solutions have greater stability than acidic solutions; neutral insulin solutions maintain nearly full potency when stored up to 18 months at 5° and 25°C. As noted in Table 26-10, the various preparations differ in onset and duration of action. A major disadvantage of regular insulin is its short duration of action (5 to 7 hours), which necessitates its administration several times daily.

Many attempts have been made to prolong the duration of action of insulin, for example, the development of insulin forms possessing less water solubility than the highly soluble (in body fluids) regular insulin. Protamine insulin preparations proved to be less soluble and less readily absorbed from body tissue. Protamine zinc insulin (PZI) suspensions were even longer-acting (36 hours) than protamine insulin; these are prepared by mixing insulin, protamine, and zinc chloride with a buffered solution. The regular insulin/PZI ratios in clinically useful preparations range from 2:1 to 4:1.

Isophane insulin suspension incorporates some of the qualities of regular insulin injection and is usually sufficiently long-acting (although not as much as PZI) to protect the patient from one day to the next (the term "isophane" is derived from the Greek *iso* and *phane*, meaning equal and appearance, respectively). Isophane insulin is prepared by the careful control of the protamine/insulin ratio and the formation of a crystalline entity containing stoichiometric amounts of insulin and protamine. (Isophane insulin also is known as NPH; the *N* indicates neutral *p*H, the *P* stands for protamine, and the *H* for Hegedorn, the developer of the product.) NPH insulin has a quicker onset and a shorter duration of action (28 hours) than PZI. NPH is given in single morning doses and normally exhibits greater activity during the day than at night. NPH and regular insulin can be combined conveniently and effectively for many patients with diabetes.

The posology of various insulin preparations is summarized in Table 26-10.

TABLE 26-10

INSULIN PREPARATIONS

Name	Particle Size (μm)	Action	Composition	pH	Duration (hr)
Insulin injection* USP		Prompt	Insulin + $ZnCl_2$	2.5–3.5	5–7
Prompt insulin zinc suspension* USP	2‡	Rapid	Insulin + $ZnCl_2$ + buffer	7.2–7.5	12
Insulin zinc suspension* USP	10–40 (70%) 2 (30%)‡	Intermediate	Insulin + $ZnCl_2$ + buffer	7.2–7.5	18–24
Extended insulin zinc suspension* USP	10–40	Long-acting	Insulin + $ZnCl_2$ + buffer	7.2–7.5	24–36
Globin zinc insulin injection* USP		Intermediate	Globin§ + $ZnCl_2$ + insulin	3.4–3.8	12–18
Protamine zinc insulin suspension† USP		Long-acting	Protamine‖ + insulin + Zn	7.1–7.4	24–36
Isophane insulin suspension* USP	30	Intermediate	Protamine# $ZnCl_2$ insulin buffer	7.1–7.4	18–24

*Clear or almost clear.
†Turbid.
‡Amorphous.
§Globin (3.6–4.0 mg/100 USP units of insulin) prepared from beef blood.
‖Protamine (1.0–1.5 mg/100 USP units of insulin) from the sperm or the mature testes of fish belonging to the genus *Oncorhynchus* or *Salmo*.
#Protamine (0.3–0.6 mg/100 USP units of insulin).

TABLE 26-11

DOSAGE AND SOURCE OF INSULIN PREPARATIONS

USP Insulin Type	*Strengths and Sources*	*Usual Adult Dose**
Insulin injection (regular insulin, crystalline zinc insulin)	U-40 mixed, U-100 mixed: purified beef, pork; purified pork; biosynthetic human; semisynthetic human U-500: purified pork	Diabetic hyperglycemia: SC, as directed by physician 15–30 min before meals up to tid or qid
Isophane insulin suspension (NPH insulin)	U-40 mixed, U-400 mixed: beef; purified beef, pork; purified pork; biosynthetic human; semisynthetic human	SC, as directed by physician, qd 30–60 min before breakfast. An additional dose before breakfast may be necessary for some patients about 30 min before a meal or at bedtime
Isophane insulin suspension (70%) and insulin injection (30%)	U-100: purified pork; semisynthetic human	SC, as directed by physician, qd, 15–30 min before breakfast, or as directed
Insulin zinc suspension (Lente insulin)	U-40 mixed, U-100 mixed: beef; purified beef; purified pork; biosynthetic human; semisynthetic human	SC, as directed by physician, qd 30–60 min before breakfast. An additional dose may be necessary for some patients about 30 min before a meal or at bedtime
Extended insulin zinc suspension (Ultralente insulin)	U-40 mixed, U-100 mixed: beef; purified beef	SC, as directed by physician, qd 30–60 min before breakfast
Prompt insulin zinc suspension (Semilente insulin)	U-40 mixed, U-100 mixed: beef; purified pork	SC, as directed by physician, qd 30–60 min before breakfast. An additional dose may be necessary for some patients about 30 min before a meal or at bedtime
Protamine zinc insulin suspension (PZI Insulin)	U-40 mixed, U-100 mixed: purified pork	SC, as directed by physician, qd 30–60 min before breakfast

*See USP DI for complete dosage information.

A major concern for PZI and NPH insulins is the potential antigenicity of protamine (obtained from fish). This concern led to the development of Lente insulins. By varying the amounts of excess zinc, by using an acetate buffer (instead of phosphate), and by adjusting the *p*H, two types of Lente insulin were prepared. At high concentrations of zinc, a microcrystalline form precipitates and is called Ultralente. Ultralente insulin is relatively insoluble and has a slower onset and a longer duration of action than PZI. At a relatively low zinc concentration, an amorphous form precipitates and is called Semilente insulin. The latter is more soluble and has a quicker onset and a shorter duration of action than regular insulins. A third type of insulin suspension, Lente insulin, is a 70:30 mixture of Ultralente and Semilente insulins. Lente insulin has a rapid onset and an intermediate duration of action (comparable with that of NPH insulin). Lente insulins are chemically incompatible with the PZI and NPH insulins because of the different buffer system used in the preparation of these insulins (an acetate buffer is used in Lente insulins and a phosphate buffer is used in PZI and NPH insulins). Dosage and sources are summarized in Table 26-11.

Additionally, regular insulin will remain fast acting when combined with NPH but not when added to Lente. The rapid action of regular insulin is neutralized by the excess zinc present in Lente insulin.[61] Similar products[68a] containing recombinant DNA-derived human insulin (instead of the bovine- and porcine-derived insulin) are available.

Advances made in the area of insulin production and delivery techniques have been reviewed by Chick[69] and Salzman.[70] Progress in alternative routes of delivery of insulin have been prompted by problems associated with conventional insulin therapy, mentioned earlier. First, various types of electromechanical device (infusion pumps) have been developed with the aim of reducing fluctuations in blood glucose levels associated with conventional insulin therapy (subcutaneous injections). These continuous-infusion pumps are either close-loop or open-loop systems. The ultimate goal of research in this area is to develop a reliable implantable (miniature) device for long-term use that would eliminate the need for daily administration and monitoring of blood glucose levels. The second area of research is to study alternative routes of administration such as oral, nasal, and rectal. Preliminary results indicate that absorption of insulin at these sites is not uniform and is unpredictable. The third approach to correcting the problems of conventional insulin therapy is to supplement the defective pancreas by transplantation

with a normally functioning pancreas from an appropriate donor. The major problem with this approach is the rejection of the donor pancreas by the recipient, as well as problems associated with the draining of exocrine enzymes. A modified procedure is to transplant only viable pancreatic islet cells or fetal or neonatal pancreas. The possibility remains, however, that in type I diabetes the newly transplanted pancreatic β-cells could be destroyed by the same autoimmune process that caused the disease in the first place.

Glucagon

Glucagon, USP. The hyperglycemic–glycogenolytic hormone elaborated by the α-cells of the pancreas is known as glucagon. It contains 29 amino acid residues in the sequence shown. Glucagon has been isolated from the amorphous fraction of a commercial insulin sample (4% glucagon).

Glucagon

Attention has been focused on glucagon as a factor in the pathology of human diabetes. According to Unger et al.,[71] the following observations support this implication of glucagon: an elevation in glucagon blood levels (hyperglucagonemia) has been observed in association with every type of hyperglycemia; when secretion of both glucagon and insulin are suppressed, hyperglycemia is not observed unless the glucagon levels are restored to normal by the administration of glucagon; the somatostatin-induced suppression of glucagon release in diabetic animals and humans restores blood sugar levels to normal and alleviates certain other symptoms of diabetes.

Unger et al.[71] propose that although the major role of insulin is regulation of the transfer of glucose from the blood to storage in insulin-responsive tissues (e.g., liver, fat, and muscle), the role of glucagon is regulation of the liver-mediated mobilization of stored glucose. The principal consequence of high concentrations of glucagon is liver-mediated release into the blood of abnormally high concentrations of glucose, thereby causing persistent hyperglycemia. Therefore, it is indicated that the presence of a relative excess of glucagon is an essential factor in the development of diabetes.[71]

Glucagon's solubility is 50 μg/mL in most buffers between *p*H 3.5 and 8.5. It is soluble, 1 to 10 mg/mL, in the *p*H ranges 2.5 to 3.0 and 9.0 to 9.5. Solutions of 200 μg/mL at *p*H 2.5 to 3.0 are stable for at least several months at 4°C if sterile. Loss of activity by fibril formation occurs readily at high concentrations of glucagon at room temperature or above at *p*H 2.5. The isoelectric point appears to be at *p*H 7.5 to 8.5. Because it has been isolated from commercial insulin, its stability properties should be comparable with those of insulin.

As with insulin and some of the other polypeptide hormones, glucagon-sensitive receptor sites in target cells bind glucagon. This hormone–receptor interaction leads to activation of membrane adenylate cyclase, which catalyzes cAMP formation. Thus, intracellular cAMP is elevated. The mode of action of glucagon in glycogenolysis is basically the same as the mechanism of epinephrine (i.e., by stimulation of adenylate cyclase). Subsequently, the increase in cAMP results in activating the protein kinase that catalyzes phosphorylation of phosphorylase kinase → phosphophosphorylase kinase. The latter is necessary for the activation of phosphorylase to form phosphorylase *a*. Finally, phosphorylase *a* catalyzes glycogenolysis, and this is the basis for the hyperglycemic action of glucagon. Although both glucagon and epinephrine exert hyperglycemic action through cAMP, glucagon affects liver cells and epinephrine affects both muscle and liver cells.

Fain[72] reviewed the many phenomena associated with hormones, membranes, and cyclic nucleotides, including several factors activating glycogen phosphorylase in rat liver. These factors involve not only glucagon but also vasopressin and the catecholamines. Glucagon and β-catecholamines mediate their effects on glycogen phosphorylase through cAMP but may involve other factors as well.

Glucagon exerts other biochemical effects. Gluconeogenesis in the liver is stimulated by glucagon, and this is accompanied by enhanced urea formation. Glucagon inhibits the incorporation of amino acids into liver proteins. Fatty acid synthesis is decreased by glucagon. Cholesterol formation also is reduced. However, glucagon activates liver lipases and stimulates ketogenesis. Ultimately, the availability of fatty acids from liver triglycerides is elevated, fatty acid oxidation increases acetyl-CoA and other acyl-CoAs, and ketogenesis is promoted. As glucagon effects elevation of cAMP levels, release of glycerol and free fatty acids from adipose tissue also is increased.

Glucagon's regulatory effects on carbohydrates and fatty acid metabolism have been reviewed,[36] with particular emphasis on the enzyme systems involved.

Glucagon is therapeutically important. It is recommended for the treatment of severe hypoglycemic reactions caused by the administration of insulin to diabetic or psychiatric patients. Of course, this treatment is effective only when hepatic glycogen is available. Nausea and vomiting are the most frequently encountered reactions to glucagon.

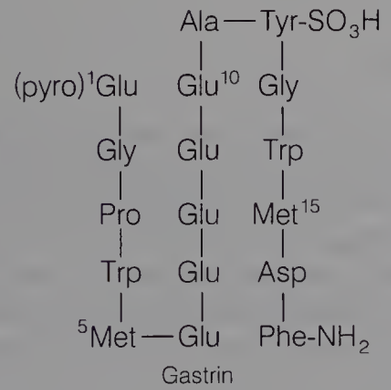
Usual dose: parenteral, adults, 500 μg to 1 mg (0.5 to 1 unit), repeated in 20 minutes if necessary; pediatric, 25 μg/kg of body weight, repeated in 20 minutes if necessary.

GASTROINTESTINAL HORMONES

There is a formidable array of polypeptide hormones of the gastrointestinal tract that includes secretin, pancreozymin–cholecystokinin, gastrin, motilin, neurotensin, vasoactive intestinal peptide, somatostatin, and others. The biosynthesis, chemistry, secretion, and actions of these hormones have been reviewed.[73]

Gastrin

Gastrin is a 17-residue polypeptide isolated from the antral mucosa. It was isolated originally in two different forms. In one of the forms, the tyrosine residue in position 12 is sulfated. Both forms are biologically active. Cholinergic response to the presence of food in the gastrointestinal tract provides the stimulus for gastrin secretion. The lowering of *p*H in the stomach inhibits the secretion of gastrin. The effects of structural modification of gastrin on gastric acid secretion have been reviewed.[74] These studies have revealed that the four residues at the COOH-terminal retain significant biologic activity and that the aspartate residue is the most critical for activity. The most important action of gastrin is to stimulate the secretion of gastric acid and pepsin. Other actions of gastrin include increase in the secretion of pancreatic enzymes; contraction of smooth muscles; water and electrolyte secretion by the stomach and pancreas; water and electrolyte absorption by the small intestine; and secretion of insulin, glucagon, and somatostatin. A synthetic pentapeptide derivative, pentagastrin, is currently employed as a gastric acid secretagogue.



Gastrin

Pentagastrin (Peptavlon), a physiologic gastric acid secretagogue, is the synthetic pentapeptide derivative *N*-*t*-butyloxycarbonyl-β-alanyl-L-tryptophyl- L-methionyl-L-aspartyl-L-phenylalanyl amide. It contains the COOH-terminal tetrapeptide amide (H · Try · Met · Asp · Phe · NH_2), which is considered to be the active center of the natural gastrins. Accordingly, pentagastrin appears to have the physiologic and pharmacologic properties of the gastrins, including stimulation of gastric secretion, pepsin secretion, gastric motility, pancreatic secretion of water and bicarbonate, pancreatic enzyme secretion, biliary flow and bicarbonate output, intrinsic factor secretion, and contraction of the gallbladder.

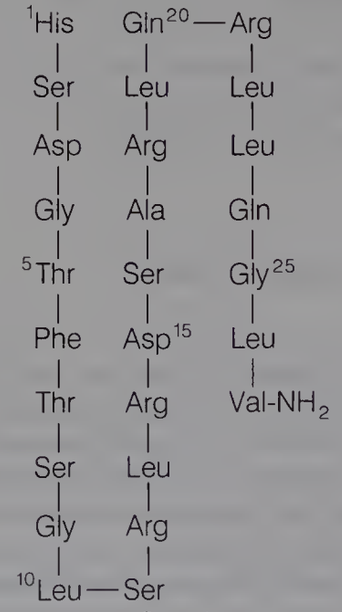
Pentagastrin is indicated as a diagnostic agent to evaluate gastric acid secretory function, and it is useful in testing for anacidity in patients with suspected pernicious anemia, atrophic gastritis or gastric carcinoma, hypersecretion in suspected duodenal ulcer or postoperative stomal ulcers, and Zollinger-Ellison tumor.

Pentagastrin usually is administered subcutaneously; the optimal dose is 6 μg/kg. Gastric acid secretion begins approximately 10 minutes after administration and peak responses usually occur within 20 to 30 minutes. The usual duration of action is from 60 to 80 minutes. Pentagastrin has a relatively short plasma half-life, perhaps under 10 minutes. The available data from metabolic studies indicate that pentagastrin is inactivated by the liver, kidney, and tissues of the upper intestine.

Contraindications include hypersensitivity or idiosyncrasy to pentagastrin. It should be used with caution in patients with pancreatic, hepatic, or biliary disease.

Secretin

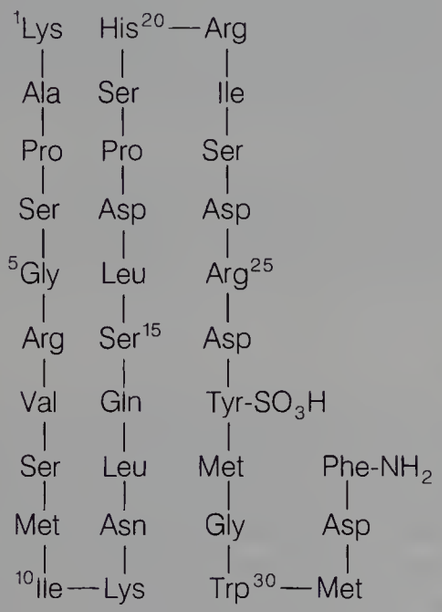
Secretin is a 27-amino-acid polypeptide that is structurally similar to glucagon. The presence of acid in the small intestine is the most important physiologic stimulus for the secretion of secretin. The primary action of secretin is on pancreatic acinar cells that regulate the secretion of water and bicarbonate. Secretin also promotes the secretion of pancreatic enzymes, to a lesser extent. Secretin inhibits the release of gastrin and, therefore, gastric acid. It also increases stomach-emptying time by reducing the contraction of the pyloric sphincter.[73]



Secretin

Cholecystokinin–Pancreozymin

It was thought originally that cholecystokinin and pancreozymin were two different hormones. Cholecystokinin was thought to be responsible for contraction of the gallbladder, whereas pancreozymin was believed to induce secretion of pancreatic enzymes. It is now clear that both actions are caused by a single 33-residue polypeptide, referred to as cholecystokinin–pancreozymin (CCK-PZ). CCK-PZ is secreted in the blood in response to the presence of food in the duodenum, especially long-chain fatty acids. The five COOH-terminal amino acid residues are identical with those in gastrin. The COOH-terminal octapeptide retains full activity of



Cholecystokinin

the parent hormone. The octapeptide is found in the gut as well as the central nervous system. SARs of cholecystokinin have been reviewed.[73] The COOH-terminal octapeptide is present in significant concentrations in the central nervous system. Its possible actions here, the therapeutic implications in the treatment of Parkinson's disease and schizophrenia, and its SAR have been reviewed.[74]

Vasoactive Intestinal Peptide

Vasoactive intestinal peptide (VIP) is widely distributed in the body and is believed to occur throughout the gastrointestinal tract. It is a 28-residue polypeptide and has structural similarities to secretin and glucagon. It causes vasodilatation and increases cardiac contractibility. VIP stimulates bicarbonate secretion, relaxes gastrointestinal and other smooth muscles, stimulates glycogenesis, inhibits gastric acid secretion, and stimulates insulin secretion. Its hormonal and neurotransmitter role has been investigated.[75]

Vasoactive Intestinal Peptide

Gastric Inhibitory Peptide

Gastric inhibitory peptide (GIP) is a 43-amino-acid polypeptide isolated from the duodenum. Secretion of GIP into the blood is stimulated by food. The primary action of GIP is inhibition of gastric acid secretion. Other actions include stimulation of insulin and glucagon secretion and stimulation of intestinal secretion.[73]

Motilin

Motilin is a 22-residue polypeptide isolated from the duodenum. Its secretion is stimulated by the presence of acid in the duodenum. Motilin inhibits gastric motor activity and delays gastric emptying.

Neurotensin

Neurotensin is a 13-amino-acid peptide, first isolated from bovine hypothalamus. It has now been identified in the intestinal tract. The ileal mucosa contains 90% of the total neurotensin of the body. It is implicated as a releasing factor for several adenohypophyseal hormones. It causes vasodilatation, increases vascular permeability, and increases gastrin secretion. It decreases secretion of gastric acid and secretin.

PARATHYROID HORMONE

This hormone is a linear polypeptide containing 84 amino acid residues. SAR studies[76] of bovine parathyroid hormone have revealed that the biologic activity is retained by an NH_2-terminal fragment consisting of 34 amino acid residues.

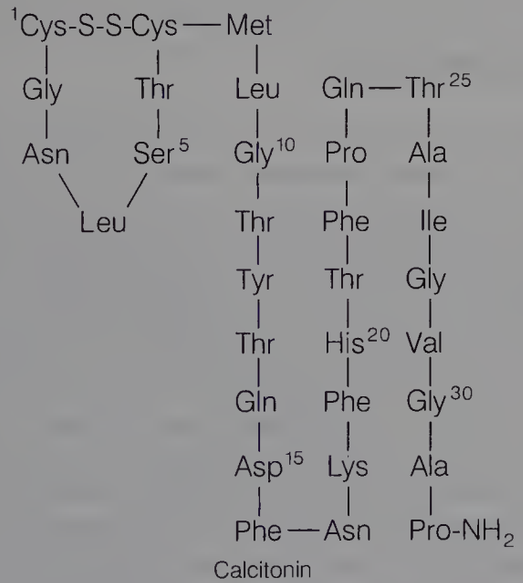
It regulates the concentration of calcium ion in the plasma within the normal range, in spite of variations in calcium intake, excretion, and anabolism into bone.[77] Also, for this hormone, cAMP is implicated as a second messenger. Parathyroid hormone activates adenylate cyclase in renal and skeletal cells, and this effect promotes formation of cAMP from ATP. The cAMP increases the synthesis and release of the lysosomal enzymes necessary for the mobilization of calcium from bone.

Parathyroid Injection, USP, has been employed therapeutically as an antihypocalcemic agent for the temporary control of tetany in acute hypoparathyroidism.

Calcitonin

Calcitonin (thyrocalcitonin) is a 32-amino-acid polypeptide hormone secreted by parafollicular cells of the thyroid glands in response to hypocalcemia. The entire 32-residue peptide appears to be required for activity because smaller fragments are totally inactive. Common structural features of calcitonin isolated from different species are a COOH-terminal prolinamide, a disulfide bond between residues 1 and 7 at the NH_2-terminus, and a chain length of 32 residues.[78] Calcitonin inhibits calcium resorption from bone, causing hypocalcemia, with parallel changes in plasma phosphate concentration. In general, calcitonin negates the osteolytic effects of parathyroid hormone.

The potential therapeutic uses of calcitonin are in the treatment of hyperparathyroidism, osteoporosis and other bone disorders, hypercalcemia of malignancy, and idiopathic hypercalcemia.



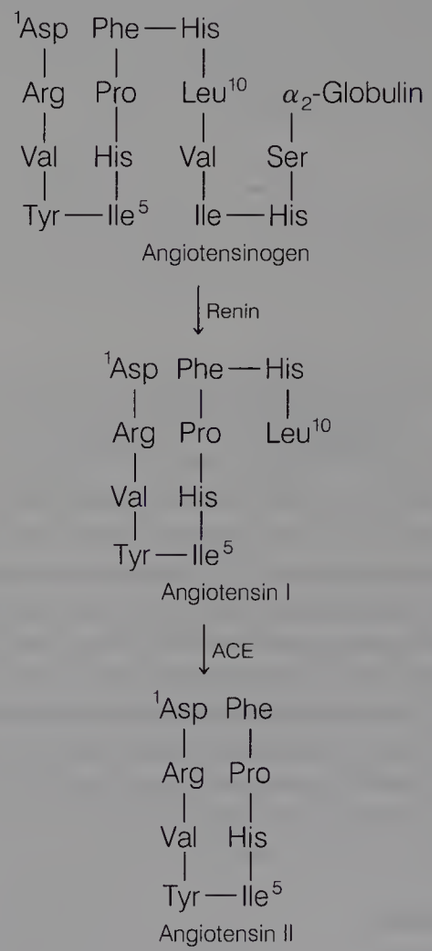
Calcitonin

ANGIOTENSINS

The synthesis of angiotensins in the plasma is initiated by the catalytic action of *renin* (a peptidase elaborated by the kidneys) on *angiotensinogen,* an α-globulin produced by the liver and found in the plasma. The hydrolytic action of renin on angiotensinogen yields *angiotensin I,* a decapeptide consisting of the first ten residues of the NH_2-terminal segment of angiotensinogen. Angiotensin I has weak pharmacologic activity. It is converted to angiotensin II, an octapeptide, by the catalytic actions of angiotensin-converting enzyme (ACE). Angiotensin II is a highly active peptide and is hydrolyzed to angiotensin III, a heptapeptide, by an aminopeptidase. Angiotensin III retains most of the pharmacologic activity of its precursor. Further degradation of angiotensin III leads to pharmacologically inactive peptide fragments.

Angiotensin II is the most active form; hence, it is the most investigated angiotensin for pharmacologic action and SARs. The two primary actions of angiotensin II are vasoconstriction and stimulation of synthesis and secretion of aldosterone by the adrenal cortex. Both of these actions lead to hypertension.

Mechanisms and sites of action of angiotensin agonists and antagonists in terms of biologic activity



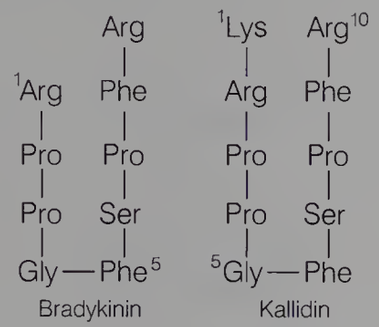
and receptor interactions have been reviewed.[79] Additionally, compounds that inhibit ACE have found therapeutic use as antihypertensive agents (e.g., captopril). The synthesis and biologic activity of several ACE inhibitors has been reviewed.[80]

Angiotensin Amide (Hypertensin) is a synthetic polypeptide (1-L-aspariginyl-5-L-valine angiotensin octapeptide)

and has twice the pressor activity of angiotensin II. It is pharmaceutically available as a lyophilized powder for injection (0.5 to 2.5 mg diluted in 500 mL of sodium chloride injection or 5% dextrose for injection) to be administered by continuous infusion. The pressor effect of angiotensin is due to an increase in peripheral resistance; it constricts resistance vessels but has little or no stimulating action on the heart and little effect on the capacitance vessels. Angiotensin has been utilized as an adjunct in various hypotensive states. It is mainly useful in controlling acute hypotension during administration of general anesthetics that sensitize the heart to the effects of catecholamines.

PLASMAKININS

Bradykinin and kallidin are potent vasodilators and hypotensive agents that have different peptide structures: bradykinin is a nonapeptide, whereas kallidin is a decapeptide. Kallidin is lysylbradykinin; that is, it has an additional lysine at the NH_2-terminus of the chain. These two compounds are made available from kininogen, a blood globulin, upon hydrolysis. Trypsin, plasmin, or the proteases of certain snake venoms can catalyze the hydrolysis of kininogen.

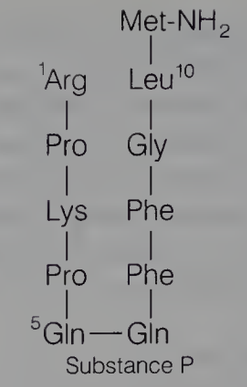


Bradykinin is one of the most powerful vasodilators known; 0.05 to 0.5 μg/kg intravenously can produce a decrease in blood pressure in all mammals investigated so far.

Although the kinins per se are not used as medicinals, kallikrein enzyme preparations that release bradykinin from the inactive precursor have been used in the treatment of Raynaud's disease, claudication, and circulatory diseases of the eyegrounds. (''Kallikreins'' is the term used to designate the group of proteolytic enzymes that catalyze the hydrolysis of kininogen, forming bradykinin.)

Substance P

Substance P is a polypeptide consisting of 11 amino acid residues. It has been implicated in the transmission of ''painful'' sensory information through the spinal cord to higher centers in the central nervous system.[74] Substance P is localized in the primary afferent sensory fibers. Other pharmacologic effects are vasodilatation, stimulation of smooth muscles, stimulation of salivary secretion, and diuresis.



Atrial Natriuretic Factors

Atrial natriuretic factors (ANFs) are peptides elaborated by the secretory granules in the atria of mammalian hearts. Two distinct natriuretic peptides have been identified in humans and are believed to be obtained from a common precursor, atriopeptigen, a 151-amino-acid peptide. A 28-amino-acid peptide is the most common ANF identified. These endogenous peptides promote natriuresis and diuresis.[82]

THYROGLOBULIN

Thyroglobulin, a glycoprotein, is composed of several peptide chains; it also contains 0.5% to 1% iodine and 8% to 10% carbohydrate in the form of two types of polysaccharide. The formation of thyroglobulin is regulated by TSH. Thyroglobulin has no hormonal properties. It must be hydrolyzed to release the hormonal iodothyronines thyroxine and liothyronine (see Chap. 19).

BLOOD PROTEINS

The blood is the transport system of the organism and thus performs important distribution functions. Considering the multitude of materials transported by the blood (e.g., nutrients, oxygen, carbon dioxide, waste products of metabolism, buffer systems, antibodies, enzymes, and hormones), its chemistry is very complex. Grossly, approximately 45% consists of the formed elements that can be separated by centrifugation, and of these, only 0.2% are other than erythrocytes. The 55% of removed plasma contains approximately 8% solids, of which a small portion (less than 1%) can be removed by clotting to produce defibrinated plasma, which is called serum. Serum contains inorganic and organic compounds, but the total solids are chiefly protein, mostly albumin, and the rest nearly all globulin. The plasma contains the protein fibrinogen, which is converted by coagulation to insoluble fibrin. The separated serum has an excess of the clotting agent thrombin.

Serum globulins can be separated by electrophoresis into α-, β-, and γ-globulins, which contain most of the antibod-

ies. The immunologic importance of globulins is well known. Many classes and groups of immunoglobulins are produced in response to antigens or even to a single antigen. The specificity of antibodies has been studied from various points of view, and Richards et al.[83] have suggested that even though immune serums appear to be highly specific for antigen binding, individual immunoglobulins may not only interact with several structurally diverse determinants, but may bind such diverse determinants to different sites within the combining region.

The importance of the blood coagulation process has been obvious for a long time. Coagulation mechanisms are covered in several biochemistry texts;[84,85] hence, herein a brief summary suffices. The required time for blood clotting is normally 5 minutes, and any prolongation beyond 10 minutes is considered abnormal. Thrombin, the enzyme responsible for the catalysis of fibrin formation, originates from the inactive zymogen prothrombin; the prothrombin → thrombin transformation is dependent on calcium ions and thromboplastin. The fibrinogen → fibrin reaction catalyzed by thrombin involves proteolytic cleavage (partial hydrolysis), polymerization of the fibrin monomers from the preceding step, and actual clotting (hard clot formation). The final process forming the hard clot occurs in the presence of calcium ions and the enzyme fibrinase.

Thrombin, USP, is a sterile protein substance prepared from prothrombin of bovine origin. It is used as a topical hemostatic because of its capability of clotting blood, plasma, or a solution of fibrinogen without adding other substances. Thrombin also may initiate clotting when combined with gelatin sponge or fibrin foam.

For external use: topically to the wound, as a solution containing 100 to 2000 NIH units/mL in sodium chloride irrigation or sterile water for injection or as a dry powder.

HEMOGLOBIN

Erythrocytes contain 32% to 55% hemoglobin, about 60% water, and the rest as stroma. The last can be obtained, after hemolysis of the corpuscles by dilution, through the process of centrifuging and consists of lecithin, cholesterol, inorganic salts, and a protein, stromatin. Hemolysis of the corpuscles, or ''laking'' as it sometimes is called, may be brought about by hypotonic solution, by fat solvents, by bile salts that dissolve the lecithin, by soaps or alkalies, by saponins, by immune hemolysins, and by hemolytic serums, such as those from snake venom and numerous bacterial products.

Hemoglobin (Hb) is a conjugated protein, the prosthetic group being heme (hematin) and the protein (globin), which is composed of four polypeptide chains, usually in identical pairs. The total relative molecular mass is about 66,000, including four heme molecules. The molecule has an axis of symmetry and, therefore, is composed of identical halves with an overall ellipsoid shape of the dimensions 55 × 55 × 70 Å.[85]

Iron in the heme of hemoglobin (ferrohemoglobin), is in the ferrous state and can combine reversibly with oxygen to function as a transporter of oxygen.

$$\text{Hemoglobin} + \text{Oxygen } (O_2) \rightleftharpoons \text{Oxyhemoglobin}$$

In this process, the formation of a stable oxygen complex, the iron remains in the ferrous form because the heme moiety lies within a cover of hydrophobic groups of the globin. Both Hb and O_2 are magnetic, whereas HbO_2 is dimagnetic because the unpaired electrons in both molecules have become paired. When oxidized to the ferric state (methemoglobin or ferrihemoglobin), this function is lost. Carbon monoxide will combine with hemoglobin to form carboxyhemoglobin (carbonmonoxyhemoglobin) to inactivate it.

The stereochemistry of the oxygenation of hemoglobin is very complex, and it has been investigated to some extent. Some evidence from x-ray crystallographic studies reveals that the conformations of the α- and β-chains are altered when their heme moieties complex with oxygen, thus promoting the complexation with oxygen. It is assumed that hemoglobin can exist in two forms, the relative position of the subunits in each form being different. In the deoxy form, α- and β-subunits are bound to each other by ionic bonds in a compact structure that is less reactive toward oxygen than is the oxy form. Some ionic bonds are cleaved in the oxy form, relaxing the conformation. The latter conformation is more reactive to oxygen.[85,86]

THE IMPACT OF BIOTECHNOLOGY ON THE DEVELOPMENT AND COMMERCIAL PRODUCTION OF PROTEINS AND PEPTIDES AS PHARMACEUTICAL PRODUCTS

Over the past decade and a half, far-reaching and revolutionary breakthroughs in molecular biology, especially research involving gene manipulations, that is, genetic engineering, have led the way in the development of new biotechnology-derived products for the treatment of diseases. The term ''biotherapy'' has been coined to describe the clinical and diagnostic use of biotechnology-derived products. Generally, these products are proteins, peptides, or nucleic acids which are structurally and/or functionally similar to naturally occurring biomolecules. The large-scale production of these complex biomolecules was beyond the capabilities of traditional pharmaceutical technologies. According to the 1995 survey[87] conducted by the Pharmaceutical Research and Manufacturers of America, there are currently more than 230 biotechnology-derived products in various stages of development and 24 approved biotechnology-derived products available in the market. The currently approved biotechnology products are listed in Table 26-12. There are 14 approval applications pending at the FDA and 49 in the third and final

TABLE 26-12

APPROVED BIOTECHNOLOGY OF DRUGS AND VACCINES*

Product Name	*Company*	*Indication (Date of U.S. Approval)*
Actimmune Interferon γ-1b	Genentech† (S. San Francisco, CA)	Management of chronic granulomatous disease (December 1990)
Activase Alteplase, recombinant	Genentech† (S. San Francisco, CA)	Acute myocardial infarction (November 1987); acute massive pulmonary embolism (June 1990)
Alferon N Interferon α-n3 (injection)	Interferon Sciences (New Brunswick, NJ)	Genital warts (October 1989)
Betaseron Recombinant interferon β-1b	Berlex Laboratories† (Wayne, NJ) Chiron† (Emeryville, CA)	Relapsing, remitting multiple sclerosis (July 1993)
Cerezyme Imiglucerase for injection (recombinant glucocerebrosidase)	Genzyme (Cambridge, MA)	Treatment of Gaucher's disease (May 1994)
Engerix-B Hepatitis B vaccine (recombinant)	SmithKline Beecham† (Philadelphia, PA)	Hepatitis B (September 1989)
EPOGEN Epoetin α (rEPO)	Amgen† (Thousand Oaks, CA)	Treatment of anemia associated with chronic renal failure, including patients on dialysis and not on dialysis, and anemia in Retrovir-treated, HIV-infected patients (June 1989); treatment of anemia caused by chemotherapy in patients with non-myeloid malignancies (April 1993)
PROCRIT§ Epoetin α (rEPO)	Ortho Biotech† (Raritan, NJ)	Treatment of anemia associated with chronic renal failure, including patients on dialysis and not on dialysis, and anemia in Retrovir-treated, HIV-infected patients (December 1990); treatment of anemia caused by chemotherapy in patients with non-myeloid malignancies (April 1993)
Humatrope Somatropin (rDNA origin) for injection	Eli Lilly† (Indianapolis, IN)	Human growth hormone deficiency in children (March 1987)
Humulin Human insulin (recombinant DNA origin)	Eli Lilly† (Indianapolis, IN)	Diabetes (October 1982)
Intron A Interferon α-2b (recombinant)	Schering-Plough† (Madison, NJ)	Hairy cell leukemia (June 1986); genital warts (June 1988); AIDS-related Kaposi's sarcoma (November 1988); hepatitis C (February 1991); hepatitis B (July 1992)
KoGENate Antihemophiliac factor (recombinant)	Miles† (West Haven, CT)	Treatment of hemophilia A (February 1993)
Leukine Sargramostim (yeast-derived GM-CSF)	Immunex† (Seattle, WA)	Autologous bone marrow transplantation (March 1991)
NEUPOGEN Filgrastim (rG-CSF)	Amgen† (Thousand Oaks, CA)	Chemotherapy-induced neutropenia (February 1991); autologous or allogeneic bone marrow transplantation (June 1994); chronic severe neutropenia (December 1994)
Nutropin Somatropin for injection	Genentech† (S. San Francisco, CA)	Growth failure in children due to chronic renal insufficiency, growth hormone inadequacy in children (March 1994)
OncoScint CR/OV Staumonab pendetide	CYTOGEN‡ (Princeton, NJ)	Detection, staging, and follow-up of colorectal and ovarian cancers (December 1992)
ORTHOCLONE OKT 3 Muromonab-CD3	Ortho Biotech† (Raritan, NJ)	Reversal of acute kidney transplant rejection (June 1986); reversal of heart and liver transplant rejection (June 1993)
Proleukin Aldesleukin (interleukin-2)	Chiron† (Emeryville, CA)	Renal cell carcinoma (May 1992)
Protropin Somatrem for injection	Genentech† (S. San Francisco, CA)	Human growth hormone deficiency in children (October 1985)
Pulmozyme DNase (dornase α)	Genentech† (S. San Francisco, CA)	Cystic fibrosis (December 1993)
RECOMBINATE Antihemophilic factor recombinant (rAHF)	Baxter Healthcare/Hyland Division (Glendale, CA) Genetics Institute‡ (Cambridge, MA)	Hemophilia A (December 1992)
RECOMBIVAX HB Hepatitis B vaccine (recombinant), MSD	Merck† (Whitehouse Station, NJ)	Hepatitis B prevention (July 1986)
ReoPro Abciximab	Centocor (Malvern, PA) Eli Lilly† (Indianapolis, IN)	Antiplatelet prevention of blood clots (December 1994)
Roferon-A Interferon α-2a, recombinant	Hoffmann-La Roche† (Nutley, NJ)	Hairy cell leukemia (June 1986); AIDS-related Kaposi's sarcoma (November 1988)

* Adapted from Biotechnology Medicines in Development: Approved Biotechnology Drugs and Vaccines [survey], Pharmaceutical Research and Manufacturers of America, p. 20, 1995.

† PhRMA member company.

‡ PhRMA research affiliate.

§ PROCRIT was approved for marketing under Amgen's epoetin alfa PLA. Amgen manufactures the product for Ortho Biotech. Under an agreement between the two companies, Amgen licensed to Ortho Pharmaceutical the U.S. rights to epoetin α for indications for human use excluding dialysis and diagnostics.

stage of clinical testing. A detailed discussion of the various processes and methodologies involved in biotechnology and the wide array of biotechnology-derived pharmaceutical products is beyond the scope of this chapter. There are a number of reference sources[88–91] available.

Since the emphasis in this chapter is on proteins, peptides, and enzymes, the discussion of biotechnology processes and products will be limited to these topics. The various biotechnology-derived products include[87] enzymes, receptors, hormones and growth factors, cytokines, vaccines, monoclonal antibodies, and nucleic acids (genes and antisense RNA).

Biotechnology techniques are constantly changing and expanding; however, the two primary techniques responsible for the development of the majority of products are recombinant DNA (r-DNA) technology and monoclonal antibody technology. The emphasis in this chapter will be on r-DNA technology and products derived from this technology. The monoclonal antibody technology and resulting products will be discussed elsewhere in this book. Excellent references[92,93] are available for review. The following discussion of r-DNA technology assumes that the reader has thorough comprehension of the normal process of genetic expression in human cells, that is, replication, transcription, and translation. There are a number of biochemistry textbooks available for such a review.

RECOMBINANT DNA TECHNOLOGY

This technology frequently has been referred to as genetic engineering or gene cloning. A comprehensive discussion of the process and application of r-DNA technology is available in several good reviews.[88–91] The concept of genetic engineering is based on the fact that the genetic material (DNA) in all living organisms is made of the same four building blocks, that is, four different deoxy-mononucleotides. Therefore, genetic material from one organism or cell may be combined with the genetic material of another organism or cell. Since every single protein, regardless of its source, is produced as a result of expression of a specific gene coding for it, the application of this technology in the mass production of desired human proteins is obvious. A number of human diseases are caused by deficiencies of desired proteins or peptides. For example, insulin deficiency is a major cause of diabetes and human growth hormone deficiency causes dwarfism. If a human gene coding for a deficient protein is identified and isolated, then it may be combined with fast-replicating, nonchromosomal bacterial DNA, that is, plasmids. The recombined DNA is placed back into the bacteria, which are then allowed to grow in ideal media. The result is replication of the plasmids and expression of the genes within the plasmid, including expression of the human gene, resulting in large quantities of the desired human protein.

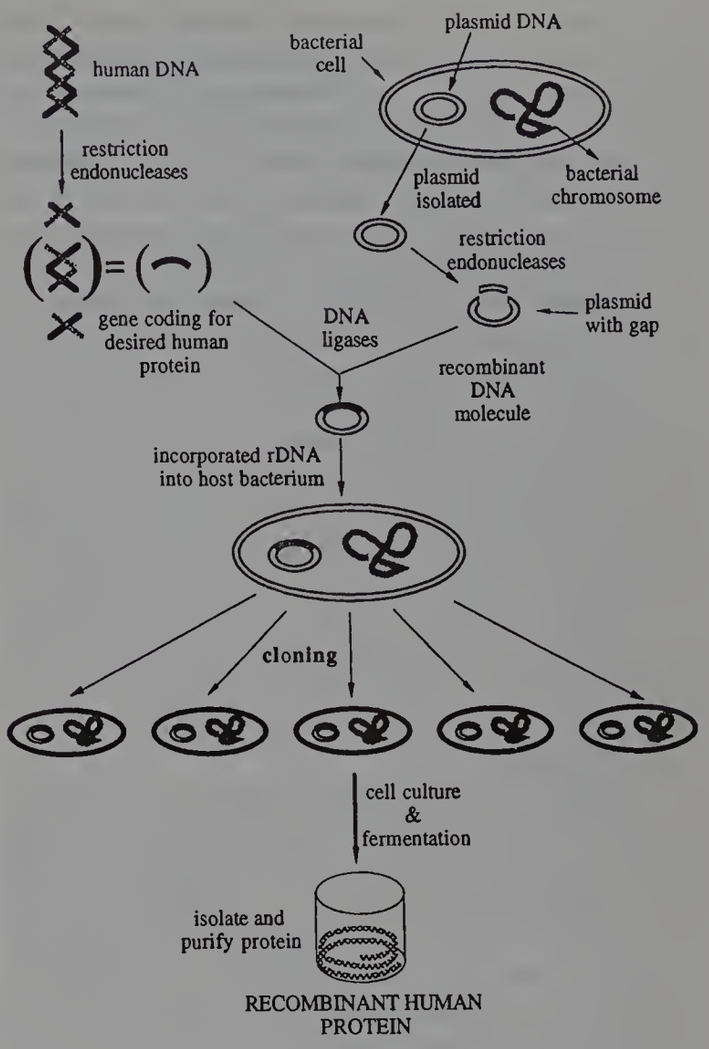


FIG. 26-7. *Summary of a typical recombinant DNA process employed in the commercial scale production of human proteins.*

The major steps in a typical r-DNA process employed in the commercial scale synthesis of human proteins are discussed below and summarized in Fig. 26-7.

1. Identification and Isolation of the Desired Gene: The possible nucleotide sequence of a desired gene can be ascertained by (1) isolating and determining the amino acid sequence of the protein expressed by the gene and then determining the possible nucleotide sequences for the corresponding mRNA and the DNA (gene), (2) isolating the mRNA and determining its nucleotide sequence, and (3) using DNA probes to "fish out" the desired gene from the genomic library (cellular DNA chopped up into 10,000 to 20,000 long nucleotide segments).

2. Constructing r-DNA: Once the desired human gene is identified and isolated, it is recombined with genes of microbial cells that are known to have rapid rates of cell division. To accomplish this task, bacterial enzymes known as restriction endonucleases are employed. Over 100 different variations of these hydrolytic enzymes, which act like scissors in hydrolyzing the phosphodiester bonds of DNA at specific sites (i.e., nucleotide sequences), are available. The use of a specific restriction endonuclease both to obtain the human

gene as well as to open a site on the microbial gene allows easy formation of the hybrid (recombined) DNA molecule because of the ''sticky'' ends on both genes. The ends of the human gene and the microbial DNA vector are ''glued'' together by enzymes known as DNA ligases. The human genes are placed in specific locations on the microbial DNA vectors to ensure expression of the human gene when the microbial cells divide. Plasmids are the most commonly used microbial DNA. These extrachromosomal circular DNA replicate independently of the chromosomes and are much smaller in size than chromosomal DNA. Plasmids are easy to manipulate and are considered to be excellent vectors to carry human genes. Other microbial cells used as hosts are yeast cells. Mammalian cells, such as Chinese hamster ovary cells, are employed when glycosylation of the r-DNA-derived protein is essential for biologic activity (e.g., erythropoietin). Nonmammalian cells cannot glycosylate proteins.

3. Cloning: The cells carrying the recombined human gene are then allowed to grow in appropriate media. As the cells divide, the r-DNA replicates and expresses its products, including the desired human protein as well as the normal bacterial proteins.

4. Isolation and Purification of r-DNA-Derived Protein: From this complex mixture containing bacterial proteins, cell components, chemicals used in preparing the media, etc., isolating and purifying the desired human protein is a daunting task indeed. This task requires sophisticated isolation techniques, such as complex filtrations, precipitations, and HPLC. The primary goal of the purification process is to ensure that the protein isolated will retain the biologic activity of the native protein in the body. The r-DNA protein is then formulated into a pharmaceutical product that is stable during transportation, storage, and administration to a patient.

BIOTECHNOLOGY-DERIVED PHARMACEUTICAL PRODUCTS

The two dozen FDA-approved biotechnology-derived pharmaceutical products are listed in Table 26-12. There are more than 200 other products in various stages of development.[87] The FDA-approved products loosely fall into five major categories: enzymes, hormones, lymphokines, hematopoietic factors, and biologicals. A detailed discussion of all of these products is beyond the scope of this chapter. Since the majority of these products are proteins or peptides, a cursory evaluation of them and their uses[94] follows.

r-DNA-Derived Enzymes

Alteplase, recombinant (Activase) was discussed earlier in this chapter.

Dornase α, rhDNase (Pulmozyme) is a mucolytic enzyme identical to the natural human DNase and is used in the treatment of cystic fibrosis. Patients with cystic fibrosis suffer from decreased pulmonary functions and infections caused by the secretion of thick mucus. Proteins contained in the mucus are bound to extracellular DNA, which are produced as a result of disintegration of bacteria in the lungs. This enzyme is involved in cleaving extracellular DNA and separates DNA from proteins, allowing proteolytic enzymes to break down proteins and consequently decrease the viscosity of mucus in the lungs.[95] Proteins bound to extracellular DNA are not susceptible to proteolytic enzymes.[96] Dornase α is a glycoprotein containing 260 amino acids that is commercially produced in genetically engineered Chinese hamster ovary cells.

Dornase α is indicated for the treatment of cystic fibrosis in conjunction with other available therapies, such as antibiotics, bronchodilators, corticosteroids, etc. Adult dosage is 2.5 mg inhaled once daily, administered via a recommended nebulizer. Dornase α should not be mixed or diluted with other agents in the nebulizer because of the possibility of adverse physicochemical changes that may affect activity. Common adverse effects include sore throat, hoarseness, and facial edema.

Imiglucerase (Cerezyme)[94] is a glycoprotein containing 497 amino acid residues and is *N*-glycosylated at four different positions. It is an analogue of the natural human enzyme β-glucocerebrosidase and contains arginine at position 495 instead of histidine in the natural enzyme. It is commercially produced in genetically engineered Chinese hamster ovary cells.

Like the natural enzyme, imiglucerase catalyzes the hydrolysis of glucocerebroside, a glycolipid, to glucose and ceramide within the lysosomes of phagocytic cells.[11] Gaucher's disease is caused by a deficiency of this enzyme, which results in the accumulation of glucocerebroside within tissue macrophages. The glycolipid-engorged macrophages are known as Gaucher cells and are responsible for the numerous clinical manifestations of Gaucher's disease. The common clinical manifestations of Gaucher's disease are severe anemia, thrombocytopenia, and skeletal complications that include osteonecrosis and osteopenia.

Imiglucerase is indicated for the long-term replacement therapy of Gaucher's disease. It is administered intravenously at the initial dosage of 2.5 to 60 U/kg, infused over 1 to 2 hours. This dosage usually is repeated every 2 weeks. However, both the dosage and the frequency of administration may be varied depending on the response.[97] Common adverse effects include dizziness, headache, abdominal discomfort, nausea, and rash.

r-DNA-Derived Hormones

Insulin Human Injection USP (Humulin R, Novolin R, Velosulin Human) and other products containing human insulin are discussed earlier in this chapter.

Growth Hormone (Somatotropin) Somatropin (Humatrope) is discussed earlier in this chapter.

Somatrem (Protropin) is discussed earlier in this chapter.

r-DNA-Derived Cytokines

Interferons.[94] Interferons are natural glycoproteins produced by virtually all eukaryotic cells; they possess immunomodulating, antiviral, and cytotoxic activities. This family of glycoproteins is produced by cells in response to a wide range of stimuli.[98] In humans, interferons bind to cellular receptors, which leads to the synthesis of over a dozen proteins that contribute to viral resistance. The antiviral effects of interferons may be caused by inhibition of the synthesis of viral mRNA or proteins or by preventing viral penetration or uncoating.[99,100] Based upon their antigenic subtypes, the interferons are classified into three major groups: α, β, and γ. Interferon-α and -β are produced by virtually all cells in response to a viral infection and various other stimuli. Interferon-γ is produced specifically by the T lymphocytes and the natural killer cells. γ Interferons have greater immunoregulatory but lower antiviral effects than α- or β-interferons.[100] More than 12 subspecies of α-interferons, one β-interferon, and two γ-interferons are known to exist. In general, the interferons are glycoproteins consisting of 165 to 166 amino acid residues. There are four r-DNA-derived α-interferons available for clinical use around the world and three available in the United States. The three available in the United States are described below. All α-interferons exhibit antiviral and antiproliferative activity, enhance phagocytic activity, and augment specific cytotoxicity of lymphocytes for certain target cells.[101] The most common adverse effects of α- and β-interferons include flu-like symptoms, bone marrow suppression, neurotoxic effects, hypocalcemia, anorexia and other gastrointestinal symptoms, and weight loss.

Interferon-α2a, recombinant (Roferon) is produced from genetically engineered *E. coli* and contains 165 amino acid residues. At position 23, interferon-α2a has a lysine residue. The pharmaceutical product contains a single α-interferon subtype. A murine monoclonal antibody is used during purification by affinity chromatography. Interferon-α2a is employed in the treatment of hairy cell leukemia and AIDS-related Kaposi's sarcoma. It is absorbed well after intramuscular or intravenous administration and has a half-life of 5 to 7 hours when administered by the intramuscular route. The solution should be stored in the refrigerator at 36° to 46°F and should not be frozen or shaken.

Interferon-α2b, recombinant (Intron A) also contains a single subtype of α-interferon. It is a glycoprotein containing 165 amino acid residues and is commercially produced from genetically engineered *E. coli*. It differs from interferon-α2a by possessing an arginine residue at position 23. It is employed in the treatment of hairy cell leukemia, condyloma acuminata (genital warts), AIDS-related Kaposi's sarcoma, hepatitis C, and hepatitis B. It is administered intramuscularly or subcutaneously with a half-life of 2 to 3 hours and via intravenous infusion with a half-life of 8 hours. The reconstituted solution is stable for 1 month when stored at a temperature of 36° to 46°F.

Interferon-αn3 (injection) (Alferon N) is a polyclonal mixture of up to 14 natural α-interferon subtypes and contains 166 amino acid residues. Its commercial production involves induction of pooled units of human leukocytes with an avian virus (Sendai virus). The purification process involves immunoaffinity and filtration chromatography. It is indicated primarily by intralesional injection for the treatment of genital warts. The solution should be stored at a temperature of 36° to 46°F and should not be shaken.

Interferon-β1b, recombinant (Betaseron) has biologic effects similar to natural interferon-β and α-interferons. The natural interferon-β is a glycoprotein containing 166 amino acid residues. The r-DNA product differs from the natural form in that it is not glycosylated, it lacks the amino terminal methionine, and it has serine in the place of methionine at position 17.[102] It is employed for a wide variety of indications via intravenous, intramuscular, subcutaneous, intrathecal, and intralesional routes. Its primary indication is in the prevention of exacerbations in patients suffering from relapsing/remitting multiple sclerosis. Recommended dosage is 8 million units, administered subcutaneously, every other day. It also is indicated in the treatment of malignant glioma and malignant melanoma. Recommended temperature for storage is 36° to 46°F, and unused reconstituted solution should be discarded.

Aldesleukin (interleukin-2) (Proleukin)[94] is an r-DNA-derived lymphokine which differs structurally from the native interleukin-2 (IL-2) but has biologic activity similar to the natural lymphokine.[103] Natural IL-2 is produced primarily by the peripheral blood lymphocytes and contains 133 amino acid residues. The immunoregulatory effects of aldesleukin include enhanced mitogenesis of lymphocytes, stimulating the growth of IL-2-dependent cell lines, enhancing cytotoxicity of lymphocytes, induction of lymphokine-activated killer (LAK) cells and natural killer (NK) cells, and induction of interferon-γ production. The exact mechanism of the antitumor activity of aldesleukin in humans is unknown.

The r-DNA process involves genetically engineered *E. coli* (pBR 322 plasmids). The gene for IL-2 was synthesized by first identifying and isolating the mRNA from the human Jurkat cell line and then preparing the complementary DNA (cDNA). The IL-2 gene was genetically engineered before it was hybridized into pBR 322 plasmid. Further manipulation of the hybridized plasmid resulted in the production of a modified IL-2, aldesleukin.[104] Aldesleukin differs structurally from the native IL-2 in that the former is not glycosylated, it lacks the N-terminal alanine residue, and it has serine in the place of cysteine at position 125. Noncovalent, molecular aggregation of aldesleukin is different from IL-2, and the former exists as a microaggregate of 27 molecules.

The primary indication of aldesleukin is for the treatment of adult metastatic renal carcinoma. It is administered via intravenous infusion in doses of 10,000 to 50,000 U/kg every

8 hours for 12 days. It is metabolized primarily by the kidneys, and no active form is found in the urine. Aldesleukin causes serious adverse effects in patients, including fever, hypotension, pulmonary congestion and dyspnea, coma, gastrointestinal bleeding, respiratory failure, renal failure, arrhythmias, seizures, and death.

r-DNA-Derived Hematopoietic Factors

Hematopoietic growth factors are glycoproteins produced by a number of peripheral and marrow cells. More than 200 billion blood cells are produced each day; and the hematopoietic factors, along with other lymphopoietic factors such as the stem cell factor and the interleukins, are involved in the proliferation, differentiation and maturation of various types of blood cell derived from the pluripotent stem cells.

Erythropoietin.[94] Erythropoietin is a heavily glycosylated protein containing 166 amino acid residues. It is produced primarily by the peritubular cells in the cortex of the kidney, and up to 15% is produced in the liver. It is the principal hormone responsible for stimulating the production of red blood cells from erythroid progenitor cells, erythrocyte burst-forming units, and erythrocyte colony-forming units.[105] Small amounts of erythropoietin are detectable in the plasma; however, the majority of the hormone is secreted by the kidneys in response to hypoxia or anemia, when levels of the hormone can rise more than 100-fold.

Decrease in erythropoietin production is one of several potential causes of anemia of chronic renal disease. Other causes of anemia of chronic renal disease include infection or inflammatory condition in the kidneys, deficiency of iron, damage to marrow, and vitamin or mineral deficiency. Regardless of the underlying disease causing renal failure, there is a decrease in erythropoietin levels in patients with renal failure. Until r-DNA technology was employed to produce commercial quantities of erythropoietin, the latter was obtained from the urine of patients suffering from severe aplastic anemia. This process of obtaining natural hormone was costly and time-consuming and produced only small quantities of the hormone.

Epoietin-α, r-EPO (EPOGEN, PROCRIT) is the recombinant human erythropoietin produced in Chinese hamster ovary cells into which the human erythropoietin gene has been inserted. These mammalian cells glycosylate the protein in a manner similar to that observed in human cells.[106]

Epoietin-α is indicated in anemic patients with chronic renal failure, including patients requiring regular dialysis and those who do not. Epoietin-α also is indicated in anemias associated with AIDS, treatment of AIDS with zidovudine, frequent blood donations, and neoplastic diseases. It is indicated to prevent anemia in patients who donate blood prior to surgery for future autologous transfusions and to reduce the need for repeated maintenance transfusions.[107] The hormone is available as an isotonic buffered solution, which is administered by the intravenous route. The solution should not be frozen or shaken and is stored at a temperature of 36° to 46°F.

Colony-Stimulating Factors.[94,108] Colony-stimulating factors are natural glycoproteins produced in lymphocytes and monocytes. These factors bind to cell-surface receptors of hematopoietic progenitor cells and stimulate proliferation, differentiation, and maturation of these cells into recognizable mature blood cells.[109] Colony-stimulating factors produced by r-DNA technology have the same biologic activity as the natural hormones. Currently, there are two colony-stimulating factors commercially produced by employing r-DNA technology. These products are discussed below.

Filgrastim, rG-CSF (NEUPOGEN) is a 175-amino-acid polypeptide produced in genetically engineered *E. coli* cells containing the human granulocyte colony-stimulating factor (G-CSF) gene. Filgrastim differs from the natural hormone in that the former is not glycosylated and contains an additional methionine group at the N-terminal, which is deemed necessary for expression of the gene in *E. coli.*

Filgrastim specifically stimulates the proliferation and maturation of neutrophil granulocytes and, hence, is considered to be lineage-specific. Accepted indications for filgrastim include the following: (1) to decrease the incidence of febrile neutropenia in patients with nonmyeloid malignancies receiving myelosuppressive chemotherapeutic agents, which lowers the incidence of infections in these patients; (2) acceleration of myeloid recovery in patients undergoing autologous bone marrow transplantation; (3) in AIDS patients, to decrease the incidence of neutropenia caused by the disease itself or by drugs used to treat the disease. The usual starting dose for filgrastim is 5 mcg/kg/day in patients with nonmyeloid cancer receiving myelosuppressive chemotherapy.

Filgrastim solution should be stored at 36° to 46°F and used within 24 hours of preparation. The solution should not be shaken or allowed to freeze. Any solution left at room temperature for more than 6 hours should be discarded. The most frequent adverse effects of filgrastim are medullary bone pain, arthralgias, and myalgias.

Sargramostim, rGM-CSF (Leukine) is a glycoprotein commercially produced in genetically engineered yeast cells. Its polypeptide chain contains 127 amino acids. It differs from the natural hormone by substitution of leucine at position 23 and variations in the glycosylation.[110] Sargramostim is a lineage-nonspecific hematopoietic factor because it promotes the proliferation and maturation of granulocytes (neutrophils and eosinophils) and monocytes (macrophages and megakaryocytes).

The primary indication for sargramostim is in myeloid engraftment following autologous bone marrow transplantation and hematopoietic stem cell transplantation. Handling, storage precautions, and adverse effects are similar to those for filgrastim.

r-DNA-Derived Miscellaneous Products

Antihemophilic Factor (factor VIII) (Humate-P, Hemophil M, Koate HP, Monoclate-P) is a glycoprotein found in

human plasma and a necessary cofactor in the blood-clotting mechanism. This high-molecular-weight glycoprotein is complex in structure and has several components (sub-cofactors).[111] The commercially available concentrates derived from blood collected from volunteer donors by the American Red Cross Blood Services are used primarily for the treatment of patients with hemophilia A. Since the commercially available products are purified concentrates derived from blood pooled from millions of donors, the major precautions in using the products relate to transmission of viruses, such as hepatitis, herpes, and human immunodeficiency virus.[112] This major problem has been alleviated mostly because of the development and marketing of r-DNA-derived antihemophilic factors.

Antihemophilic Factor (recombinant), rAHF (KoGENate, Helixate) is an r-DNA derived factor VIII expressed in genetically engineered baby hamster kidney cells.[113] KoGENate has the same biologic activity as the human plasma-derived antihemophilic factor (pdAHF). The purification process for rAHF includes monoclonal antibody immunoaffinity chromatography to remove any protein contaminants.

KoGENate is indicated for the treatment of hemophilia A and is administered by the intravenous route. In patients suffering from hemophilia A, there is a demonstrated decrease in the activity of plasma clotting factor VIII. This product temporarily prevents bleeding episodes in hemophiliacs and may be employed to prevent excessive bleeding during surgical procedures in these patients. A major advantage of the rAHF over the natural factor VIII is the lack of viral presence in the product. rAHF does not contain von Willebrand's factor; therefore, it is not indicated in the treatment of von Willebrand's disease. Patients receiving rAHF should be monitored carefully for the development of antibodies.

Bioclate is an r-DNA-derived factor VIII expressed in genetically engineered Chinese hamster ovary cells. It has the same biologic activity as pdAHF and is structurally similar. Its indications and adverse effects are similar to those for KoGENate.

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CHAPTER 27

Vitamins and Related Compounds

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Jaime N. Delgado

Vitamins traditionally have been considered to be "accessory food factors." Generally, vitamins are among those nutrients that the human organism cannot synthesize from other dietary components. Together with certain amino acids (i.e., essential amino acids), the vitamins constitute a total of 24 organic compounds that have been characterized as dietarily essential.[1] Many vitamins function biochemically as precursors in the synthesis of coenzymes necessary in human metabolism; thus, vitamins perform essential functions. When they are not available in appropriate amounts, the consequences may lead to serious disease states.

Although there are relatively few therapeutic indications for vitamin pharmaceutical preparations, diseases caused by certain vitamin deficiencies do respond favorably to vitamin therapy. Additionally, there are products indicated for prophylactic use as dietary supplements. An optimal diet provides all of the necessary nutrients; however, in some cases of increased demands, vitamin and mineral supplementation is recommended.[2]

The medicinal chemistry of vitamins is fundamental not only to the therapeutics of nutritional problems, but also to the understanding of the biochemical actions of other medicinal agents that directly or indirectly affect the metabolic functions of vitamins and coenzymes. Accordingly, this chapter includes a brief summary of the basic biochemistry of vitamins, structure–activity relationships, physicochemical properties and some stability considerations, nutritional and therapeutic applications, and brief characterizations of representative pharmaceutical products.

In 1912, Funk[2a] described a substance that was present in rice polishings and in foods that cured polyneuritis in birds and beriberi in humans. This substance was referred to as "vitamine" because it was characterized as an amine and as a vital nutritional component. After other food factors were noted to be vital nutritional components that were not amines and did not even contain nitrogen,[2b] Drummond suggested the modification that led to the term "vitamin." McCollum and Davis[3] described a lipid-soluble essential food factor in butterfat and egg yolk, and 2 years later reference was made to a water-soluble factor in wheat germ. Thus, the terms "fat-soluble A" and "water-soluble B," respectively, were applied to these food factors. Since then, many other dietary components have been discovered to be essential nutritional components (i.e., vitamins). It is traditional to classify these compounds as either lipid-soluble or water-soluble vitamins. This classification is convenient because members of each category possess important properties in common.

LIPID-SOLUBLE VITAMINS

The lipid-soluble vitamins include vitamins A, D, E, and K. These compounds possess other characteristics in common besides solubility. They usually are associated with the lipids of foods and are absorbed from the intestine with these dietary lipids. The lipid-soluble vitamins are stored in the liver and, thus, conserved by the organism, whereas storage of the water-soluble vitamins usually is not significant.

THE VITAMIN As

Vitamin A was first recognized as a vitamin by McCollum and Davis[3] in 1913 to 1915, but studies of the molecular mechanism of action of retinol in the visual process were not significantly productive until 1968 to 1972. The mechanism of action of vitamin A in physiologic processes other than vision has been very difficult to study. However, advances in molecular biology have started to identify the role of retinoids in bone growth, reproduction, embryonic development, protein synthesis, sperm production, and control and differentiation of epithelial tissues. This has led to the suggestion that vitamin A has hormone-like properties. There is convincing evidence that vitamin A performs an important function in the biosynthesis of glycoproteins and

TABLE 27-1

WEIGHT EQUAL TO 1 IU

Retinoid	*μg*
All-*trans*-retinol	0.3
All-*trans*-retinol acetate	0.334
All-*trans*-retinol propionate	0.359
All-*trans*-retinol palmitate	0.55
β-Carotene	0.6

in sugar-transfer reactions in mammalian membranes.[4] Investigations on the mechanism of this action were stymied by difficulties in elucidating definitely the nature of the biochemically active form of the vitamins, whether it is all-*trans*-retinol or retinoic acid. This question was reviewed comprehensively by Chytil and Ong.[5] Most, if not all, vitamin A actions in development, differentiation, and metabolism are mediated through nuclear receptors that bind to retinoic acid.[6]

The term "vitamin A" currently is applied to compounds possessing biologic activity similar to retinol. The term "vitamin A_1" refers to all-*trans*-retinol. The term "retinoid" is applied to retinol and its naturally occurring derivatives plus synthetic analogues, which need not have vitamin A activity.

Vitamin A activity is expressed as USP units, international units (IU), retinol equivalents (RE), and β-carotene equivalents. The USP units and IU are equivalent. Each unit expresses the activity of 0.3 μg of all-*trans*-retinol. Thus, 1 mg of all-*trans*-retinol has the activity of 3333 units. Other equivalents are listed in Table 27-1. One RE represents the biologic activity of 1 μg of all-*trans*-retinol, 6 μg of β-carotene, and 12 μg of mixed dietary carotenoids. The RE is used to convert all dietary sources of vitamin A into a single unit for easy comparison.[7]

The stereochemistry of vitamin A and related compounds is complex, and a complete stereochemical analysis is beyond the scope of this chapter. A brief summary of some stereochemical features is presented here as the basis for the characterization of the biochemical actions exerted by this vitamin. The study of the structural relationships among vitamin A and its stereoisomers has been complicated by the common use of several numbering systems, as exemplified below.

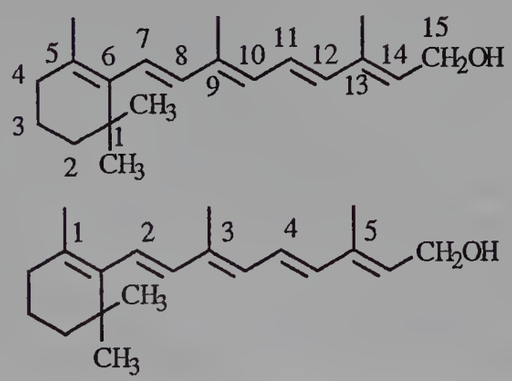
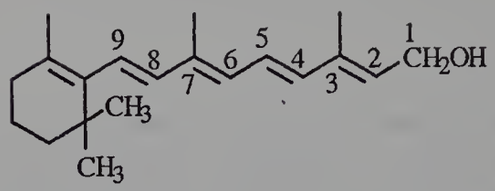


TABLE 27-2

IU PER GRAM OF SELECTED RETINOIDS

	U/g
All-*trans*-retinol	2,907,000
Neovitamin A	2,190,000
9-*cis*-retinol	634,000
9,13-Di-*cis*-retinol	688,000
9,11-Di-*cis*-retinol	679,000
All-*trans*-retinal	3,050,000
11-*cis*-retinal	3,120,000
9-*cis*-retinal	637,000
9,13-Di-*cis*-retinal	581,000
9,11-Di-*cis*-retinal	11,610,000

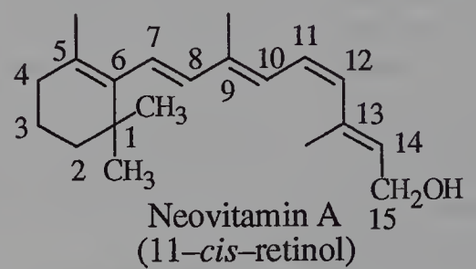


The first numbering system is the one in current use in the literature. The second system places emphasis on the conjugated π system, while the third is used by the *USP Dictionary of USAN and International Drug Names* and conforms closely to the IUPAC conventions.

For steric reasons, the number of isomers of vitamin A most likely to occur would be limited. These are all-*trans*, 9-*cis*, 13-*cis*, and the 9,13-di-*cis*. A *cis* linkage at double bond 7 or 11 encounters steric hindrance. The 11-*cis* isomer is twisted, as well as bent, at this linkage; nevertheless, this is the only isomer that is active in vision.

The biologic activity[8] of the isomers of vitamin A acetate, in terms of USP units per gram, are listed in Table 27-2.

Disregarding stereochemical variations, several compounds with structures corresponding to vitamin A, its ethers, and its esters have been prepared.[9–11] These compounds as well as synthetic vitamin A acid possess biologic activity.



Neovitamin A
(11–*cis*–retinol)

Dietary vitamin A is obtained exclusively from foods of animal origin as retinyl esters. The provitamin carotenoids are obtained from plant and dairy sources. The highest sources of natural vitamin A is fish liver oils, which vary greatly in their content of this vitamin (Table 27-3).

Most liver oils contain vitamin A and neovitamin A in the ratio of 2:1. Vitamin A occurs free and combined as the biologically active esters, chiefly of palmitic and some

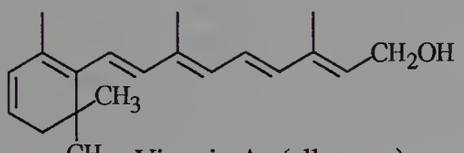
TABLE 27-3

VITAMIN A CONTENT OF SOME FISH LIVER OILS

Source	Animal Species	Potency (IU/g)
Halibut, liver	*Hippoglossus hippoglossus*	60,000
Percomorph, liver	*Percomorph* fishes (mixed oils)	60,000
Shark, liver	*Galeus zygopterus*	25,500
Shark, liver	*Hypoprion brevirostris* and other varieties	16,500
Burbot, liver	*Lota maculosa*	4,880
Cod, liver	*Gadus morrhua*	850

myristic and dodecanoic acids. It also is found in the livers of animals, especially those that are herbivorous. Milk and eggs are fair sources of this vitamin.

The livers of freshwater fish contain vitamin A_2 (3-dehydroretinol).



Vitamin A_2 (all–*trans*)
3–Dehydroretinol or Dehydroretinol

Dietary retinyl esters are hydrolyzed in the intestinal lumen by various hydrolases. The retinol is absorbed into the enterocytes by facilitated diffusion in normal concentrations. However, at pharmacologic doses, retinol can be absorbed by passive diffusion.[12] Within the enterocytes the retinol is esterified by two enzymes, acylcoenzyme A (CoA):retinol acyltransferase (ARAT) and lecithin:retinal acyltransferase (LRAT). LRAT esterifies retinol bound to an intracellular protein, cellular retinol-binding protein type II (CRBP [II]), while ARAT can esterify unbound retinol. It has been proposed that LRAT esterifies retinol at normal doses, while ARAT esterifies excess retinol.[13]

The retinyl esters are incorporated into chylomicrons, which in turn enter the lymph. Once in the general circulation, chylomicrons are converted into chylomicron remnants, which are cleared primarily by the liver. As the esters enter the hepatocytes, they are hydrolyzed. In the endoplasmic reticulum, the retinol is bound to retinol-binding protein (RBP). This complex is released into the blood or transferred to liver stellate cells for storage. Within the stellate cells, the retinol is bound to CRBP(I) and esterified for storage by ARAT and LRAT. Stellate cells contain up to 95% of the liver vitamin A stores.

Retinol is released into the general circulation from hepatocytes or stellate cells complexed to RBP. The RBP–retinol complex in turn is bound to transthyretin (TTR), which protects retinol from metabolism and renal excretion.[14]

The cellular uptake of plasma retinol remains to be fully understood. This is further complicated because chylomicron remnants also contribute retinol to the target cells. The fate of retinol after absorption is just beginning to be understood.

RBP and TTR do not enter the cell. Upon entering the cell, the retinol is bound to CRBP(I) in the cytoplasm. Most tissues contain CRBP(I) and CRBP(II). These intracellular proteins function in the transport and metabolism of retinol and retinoic acid by solubilizing them in aqueous media and presenting them to the appropriate enzymes while protecting them from catabolizing enzymes.[15] These proteins also limit the concentration of free retinoids within the cell. CRBP(I) regulates the esterification of retinol and its oxidation to retinoic acid from retinal, while CRBP(II) controls the reduction of retinal to retinol and its subsequent esterification.[16]

Retinol is susceptible to glucuronide conjugation, followed by enterohepatic recycling. It may be oxidized to retinoic acid by two enzymes, retinol dehydrogenase and retinal dehydrogenase, the former being rate-limiting.[17] Unlike retinol, there is no specific carrier for retinoic acid in the blood. Retinoic acid undergoes decarboxylation, followed by glucuronide conjugation. Normally, no unchanged retinol is excreted. However, retinal, retinoic acid, and other metabolites are found in the urine and feces.

Although fish liver oils are used for their vitamin A content, purified or concentrated forms of vitamin A are of great commercial significance. These are prepared in three ways: (1) saponification of the oil and concentration of the vitamin A in the nonsaponifiable matter by solvent extraction, the product being marketed as such; (2) molecular distillation of the nonsaponifiable matter, from which the sterols have been removed by freezing, giving a distillate of vitamin A containing 1 to 2 million IU/g; (3) subjecting the fish oil to direct molecular distillation to recover the free vitamin A, the vitamin A palmitate, and the myristate.

Pure crystalline vitamin A occurs as pale yellow plates or crystals. It melts at 63° to 64°C and is insoluble in water but soluble in alcohol, the usual organic solvents, and the fixed oils. It is unstable in the presence of light and oxygen and in oxidized or readily oxidized fats and oils. It can be protected by the exclusion of air and light and by the presence of antioxidants.

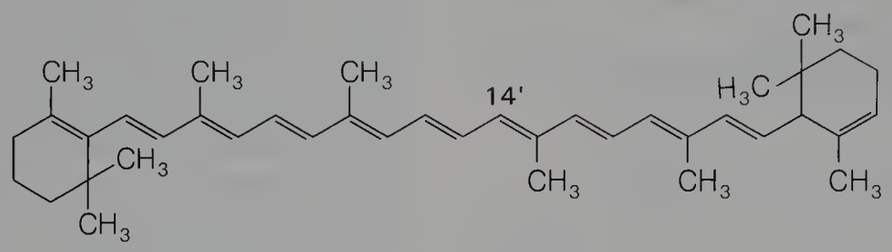
Like all substances that have a polyene structure, vitamin A gives color reactions with many reagents, most of which are either strong acids or chlorides of polyvalent metals. An intense blue (Carr-Price) is obtained with vitamin A in dry chloroform solution upon the addition of a chloroform solution of antimony trichloride. This color reaction has been studied extensively and is the basis of a colorimetric assay for vitamin A.[18]

Vitamin A (all-*trans*-retinol) is biosynthesized in animals from plant pigments called *carotenoids*, which are terpenes composed of isoprenoid units. These pigments protect plant cells for photochemical damage and transfer radiant energy to the pigments responsible for photosynthesis. Although hundreds of carotenoids have been identified, only a few function as provitamins.[19]

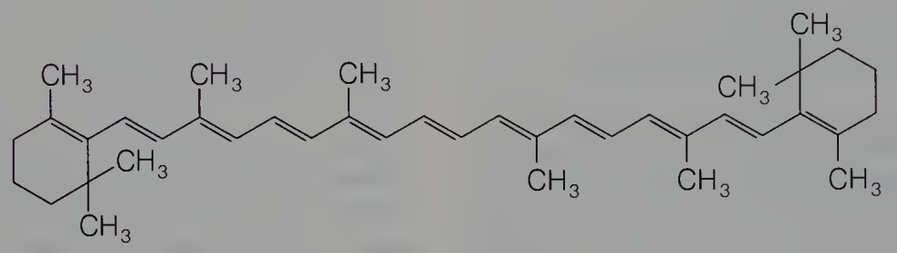
The provitamins A (e.g., β-, α-, and γ-carotenes and cryp-

toxanthin) are found in deep green, yellow, and orange fruits and vegetables, such as carrots, spinach, broccoli, kale, collard and turnip greens, mangoes, apricots, nectarines, pumpkins, and sweet potatoes. The carotenoid pigments are utilized poorly by humans, whereas animals differ in their ability to utilize these compounds. These carotenoid pigments are provitamins A because they are converted to the active vitamin A. For example, β-carotene is absorbed intact by the intestinal mucosa, then cleaved to retinal by β-carotene-15,15′-dioxygenase, which requires molecular oxygen.[20] β-Carotene can give rise to two molecules of retinal, whereas in the other three carotenoids only one molecule is possible by this transformation. These carotenoids have only one ring (see formula for β-carotene) at the end of the polyene chain, which is identical with that found in β-carotene and is necessary for vitamin A activity. This accounts for the low activity of δ-carotene.

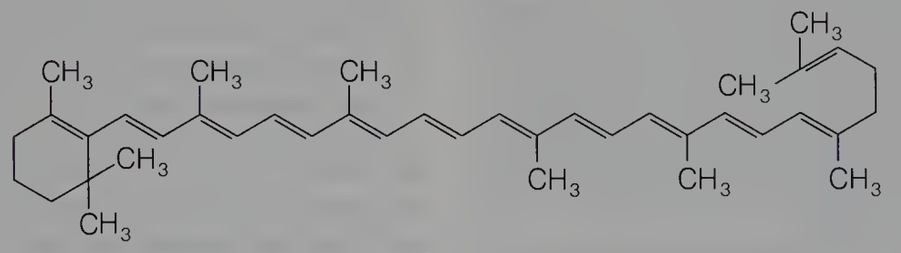
The conjugated double-bond systems found in vitamin A and β-carotene are necessary for activity, for when these compounds are partially or completely reduced, activity is lost. The β-ionone ring of retinol or the dehydro-β-ionone ring found in dehydroretinol (vitamin A_2) is essential for activity. Saturation results in loss of activity. The ester and methyl ethers of vitamin A have a biologic activity on a molar basis equal to vitamin A. Retinoic acid (vitamin A acid) is biologically active but is not stored in the liver.



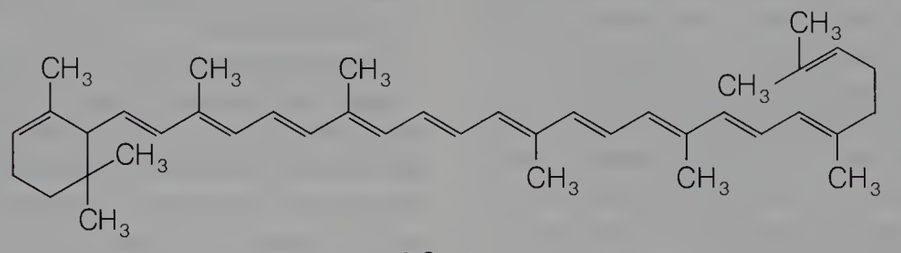
α-Carotene



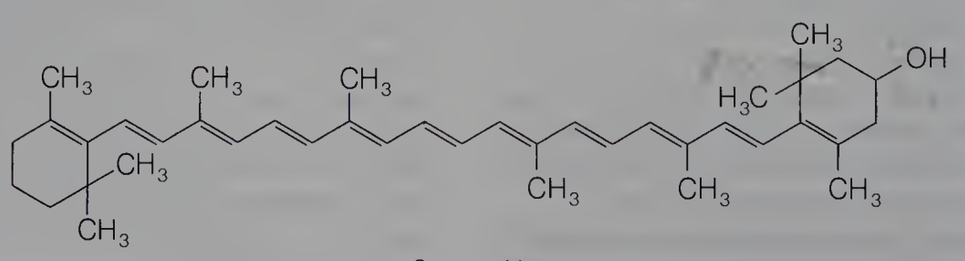
β-Carotene



γ-Carotene



δ-Carotene



Crytoxanthin

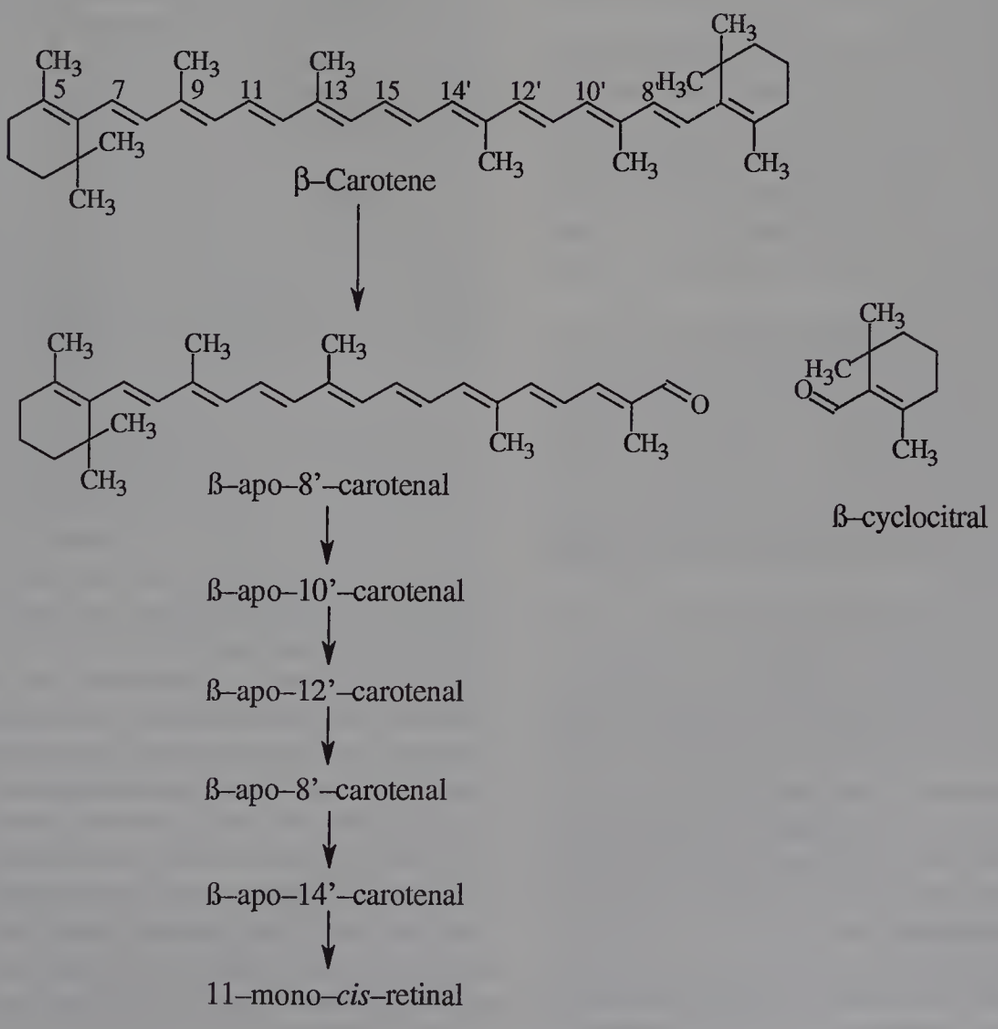
Carotenoid absorption is by passive diffusion and is dependent on absorbable fats and bile. It has been assumed that one-sixth of normal dietary β-carotene but only one-twelfth of the other provitamin A carotenoids is absorbed. The enterocytes convert the carotenes into retinol, but up to 20% to 30% are absorbed unchanged.

The intestinal mucosa is the main site of β-carotene transformation[1] to retinal, but the enzymes that catalyze the transformation also occur in hepatic tissue. Two mechanisms for the conversion to retinal have been proposed. Originally, it was proposed that cleavage occurred centrally by β-carotene-15,15′-dioxygenase.[21] However, evidence exists for the peripheral cleavage to yield apo-carotinals, which can be converted further to retinal.[22] The retinal thus formed is reduced to retinol and esterified before entering the chylomicrons for transport from the liver.

Retinoic acid, the corresponding carboxylic acid, promotes development of bone and soft tissues and sperm production, but it does not participate in the visual process. Retinoic acid is found in the bile in the glucuronide form.

Vitamin A often is called the "growth vitamin" because a deficiency of it in the diet causes a cessation of growth in young rats. A deficiency of vitamin A is manifested chiefly by a degeneration of the mucous membranes throughout the body. This degeneration is evidenced to a greater extent in the eye than in any other part of the body and gives rise to a condition known as xerophthalmia. In the earlier stages of vitamin A deficiency, there may develop a night blindness (*nyctalopia*), which can be cured by vitamin A. *Night blindness* can be defined as the inability to see in dim light.

"Dark adaptation" or "visual threshold" is a more suitable description than "night blindness" when applied to many subclinical cases of vitamin A deficiency. The *visual threshold* at any moment is just that light intensity required to elicit a visual sensation. *Dark adaptation* is the change that the visual threshold undergoes during a stay in the dark after an exposure to light. This change may be very great. After exposure of the eye to daylight, a stay of 30 minutes in the dark results in a decrease in the threshold by a factor of 1 million. This phenomenon is used as the basis to detect subclinical cases of vitamin A deficiency. These tests vary in their technique, but, essentially, they measure visual dark adaptation after exposure to bright light and compare it with the normal.[23]



Advanced deficiency of vitamin A gives rise to a dryness and scaliness of the skin, accompanied by a tendency to infection. Characteristic lesions of the human skin caused by vitamin A deficiency usually occur in sexually mature persons between the ages of 16 and 30 and not in infants. These lesions appear first on the anterolateral surface of the thigh and on the posterolateral portion of the upper forearms and later spread to adjacent areas of the skin. The lesions consist of pigmented papules, up to 5 mm in diameter, at the site of the hair follicles.

Vitamin A regulates the activities of osteoblasts and osteoclasts, influencing the shape of the bones in the growing animal. The teeth also are affected. In vitamin A-deficiency states, a long overgrowth occurs. Overdoses of vitamin A in infants for prolonged periods led to irreversible changes in the bones, including retardation of growth, premature closure of the epiphyses, and differences in the lengths of the lower extremities. Thus, a close relationship exists between the functions of vitamins A and D relative to cartilage, bones, and teeth.[24]

The tocopherols exert a sparing and what appears to be a synergistic action[25] with vitamin A.

Blood levels of vitamin A decrease very slowly, and a decrease in dark adaptation was observed in only two of 27 volunteers (maintained on a vitamin A-free diet) after 14 months, at which time blood levels had decreased from 88 to 60 IU/100 mL of blood.

Vitamin A performs numerous biochemical functions. It promotes the production of mucus by the basal cells of the epithelium, whereas in its absence keratin can be formed. Vitamin A performs a function in the biosynthesis of glycogen and some steroids, and increased quantities of coenzyme Q are found in the livers of vitamin-deficient rats. Significantly, the most well-known action of vitamin A is its function in the chemistry of vision.

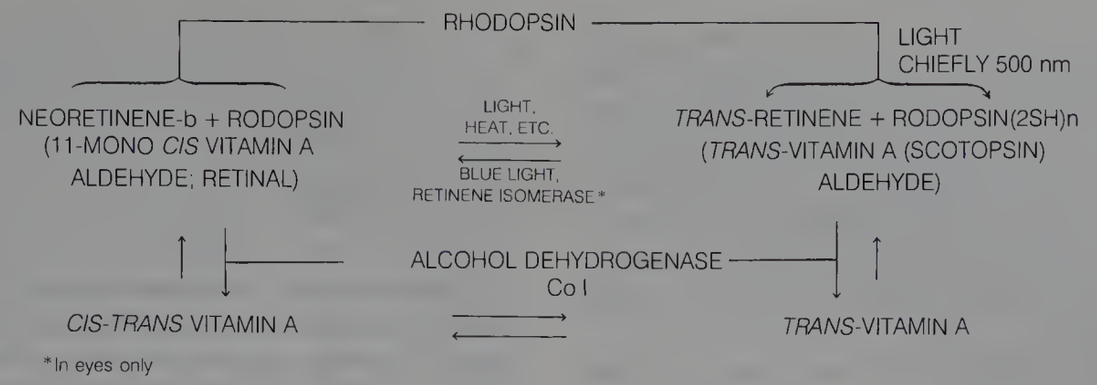
Hypervitaminosis A is associated rarely with dietary intake. Vitamin A toxicity is associated with excessive supplementation. Early signs include central nervous system symptoms (fatigue, lethargy, irritability, delirium, depression, anorexia), gastrointestinal symptoms (discomfort, nausea, vomiting), and skin disorders (dryness, scaling, pruritus, erythema). Treatment involves discontinuance of the vitamin and supportive therapy.

The molecular mechanism of action of vitamin A in the visual process has been under investigation for many years. Wald in 1968 and Morton in 1972[25a,b] characterized this mechanism of action. The chemistry of vision was reviewed comprehensively in *Accounts of Chemical Research* (1975) by numerous investigators. These reviews include theoretical studies of the visual chromophore, characterization of rhodopsin in synthetic systems, dynamic processes in vertebrate rod visual pigments and their membranes, and the dynamics of the visual protein opsin.[26–30]

Vitamin A (all-*trans*-retinol) undergoes isomerization to the 11-*cis* form in the liver. This transformation is catalyzed by a retinol isomerase. Subsequently, 11-*cis*-retinol interacts with RBP to form a complex that is transported to the retinal photoreceptor cells, which contain specific receptors for the RBP–retinol complex.

The retina has been considered[29,31] to be a double-sense organ, in which the rods are concerned with colorless vision at low light intensities and the cones with color vision at high light intensities. A dark-adapted, excised retina is rose red; when it is exposed to light, its color changes to chamois, to orange, to pale yellow; finally, upon prolonged irradiation, it becomes colorless. The rods contain photosensitive visual purple (rhodopsin), which, when acted upon by light of a definite wavelength, is converted to visual yellow and initiates a series of chemical steps necessary to vision. Visual purple is a conjugated carotenoid protein having a relative molecular mass (M_r) of about 40,000 and one prosthetic group per molecule. It contains seven hydrophobic α-helices, which are embedded in the membrane. Short hydrophilic loops interconnect the helices and are exposed to the aqueous environment on either side of the membrane. It has an absorption maximum of about 510 nm. The prosthetic group is retinene (neoretinene b or retinal), which is joined to the protein through a protonated Schiff base linkage. The function of retinene in visual purple is to provide an increased absorption coefficient in visible light and, thereby, sensitize the protein, which is denatured. This process initiates a series of physical and chemical steps necessary to vision. The protein itself differs from other proteins by having a lower energy of activation, which permits it to be denatured by a quantum of visible light. Other proteins require a quantum of ultraviolet (UV) light to be denatured. The bond between the pigment and the protein is much weaker when the protein is denatured than when it is native. The denaturation process of the protein is reversible and takes place more readily in the dark to give rise, when combined with retinene, to visual purple. The effectiveness of the spectrum in bleaching visual purple runs fairly parallel with its absorption spectrum (510 nm) and with the sensibility distribution of the eye in the spectrum at low illuminations. It has been calculated that for a human to see a barely perceptible flash of light, only one molecule of visual purple in each five to 14 rod cells needs to be photochemically transformed in a dark-adapted eye. The system possesses such sensitivity because of a biologic amplification. In vivo, visual purple is reformed constantly as it is bleached by light, and under continuous illumination, an equilibrium between visual purple, visual yellow, and visual white is maintained. If an animal is placed in the dark, the regeneration of visual purple continues until a maximum concentration is obtained. Visual purple in the eyes of an intact animal may be bleached by light and regenerated in the dark an enormous number of times.

In the resting state (dark), rod and cone membranes exhibit a steady electrical current. The membrane allows sodium ions to enter freely through specific channels. A Na^+,K^+-ATPase pump maintains the ion gradient. The closing of the pores hyperpolarizes the membrane and initiates the neuronal response.



The pores are kept open by binding to cyclic guanosine monophosphate (cGMP). The light-induced isomerization of retinal causes a conformational change in the protein part of rhodopsin, activating the molecule. One active rhodopsin activates several hundred G-protein molecules, called *transducin.* (G-proteins have the capability of binding with guanosine nucleotides.) Activation of transducin consists of an exchange of bound guanosine diphosphate (GDP) for guanosine triphosphate (GTP). Activated transducin, in turn, activates a phosphodiesterase, which hydrolyzes thousands of cGMP molecules. The decreased concentration of cGMP results in the closing of the sodium channels.

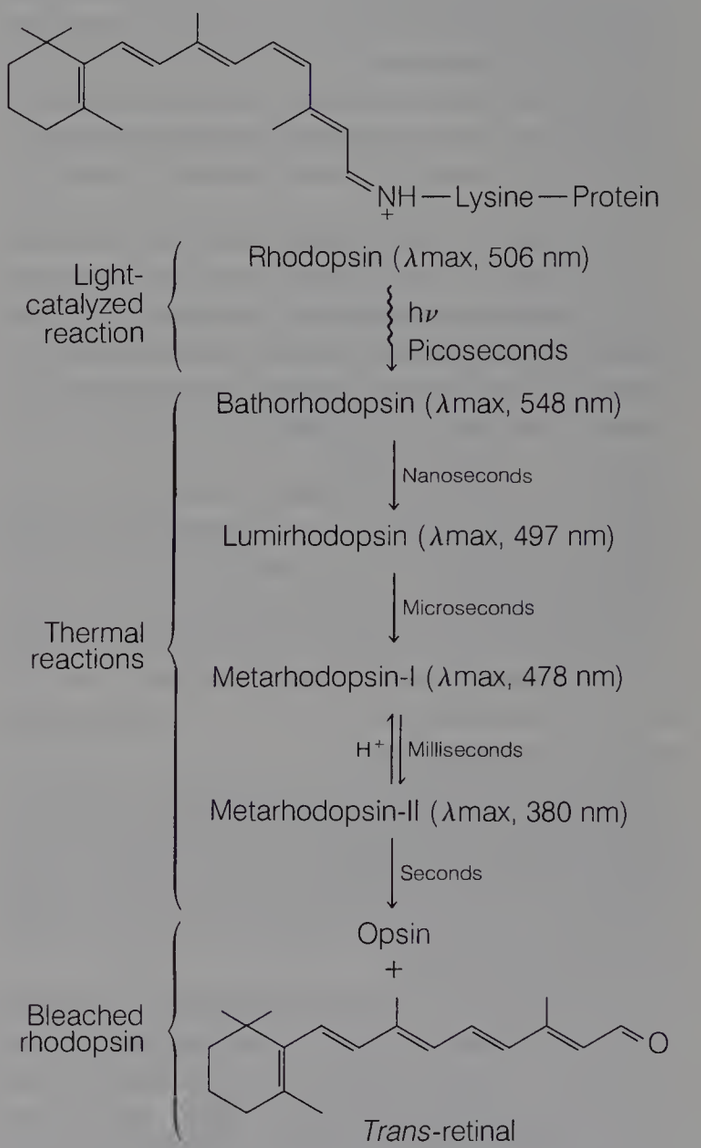
Hydrolysis of transducin-bound GTP to GDP inactivates the phosphodiesterases. However, the activated rhodopsin also must be deactivated. This is accomplished by phosphorylation of opsin by opsin kinase. Guanylate cyclase replenishes the cGMP concentration, which reopens the channels.

Visual purple occurs in all vertebrates. It is not distributed evenly over the retina. It is missing in the fovea, and in the regions outside of the fovea its concentration undoubtedly increases to a maximum in the region about 20° off center, corresponding to the high density of rods in this region. Therefore, to see an object best in the dark, one should not look directly at it.

The diagrams represent some of the changes that take place in the visual cycle involving the rhodopsin system in which the 11-mono-*cis* isomer of vitamin A is functional in the aldehyde form.[32]

Only 11-mono-*cis*-retinal can combine with opsin (scotopsin) to form rhodopsin. The isomerization of *trans*-retinal may take place in the presence of blue light. However, vision continues very well in yellow, orange, and red light, in which no isomerization takes place. The 11-mono-*cis*-retinal under these circumstances is replaced by an active form of vitamin A from the bloodstream, which obtains it from stores in the liver. The isomerization of *trans*-vitamin A in the body to *cis-trans*-vitamin A seems to keep pace with long-term processes such as growth since vitamin A, neovitamin A, and 11-mono-*cis*-retinal are equally active in growth tests in rats.

The sulfhydryl groups (two for each 11-mono-*cis*-retinal molecule isomerized) exposed on the opsin initiate the transmission of impulses in the phenomenon of vision.



Note: Bovine rhodopsin. λmax = wavelength maximum of each intermediate. (From Chem. Eng. News, Nov. 28, 1983.)

Research since the mid-1980s has taken vast strides in determining the molecular mechanism of action of vitamin A. It appears the vitamin exerts its biologic function with respect to development, differentiation, and metabolism like a steroid hormone.[33–35] The biologically active species is believed to be retinoic acid. Two intracellular retinoic acid-binding proteins have been isolated, CRABP(I) and CRABP(II). These appear to have functions similar to the

CRBPs. More importantly, several retinoic acid receptors have been identified, RARα, RARβ, and RARγ.[34] These differ in their tissue distribution and the level of expression during cell development and differentiation. After binding with retinoic acid, the complex binds to specific regulatory sequences on DNA, thus influencing the transcription of specific genes. The ultimate biologic effects of retinoic acid are mediated through various proteins which remain to be identified.

A new family of retinoic acid receptors has been identified.[36] These have been called retinoid (unknown) receptors, RXRα, RXRβ, and RXRγ. They have a different tissue distribution from the RARs. The ligand for RXR has been identified as 9-*cis*-retinoic acid.[37]

RXRs appear to be coregulators with RAR, thyroid hormone, and vitamin D receptors, increasing their affinity for DNA.[38] Several enzymes whose expression depends on RXR have been identified.[37]

The available experimental data do not provide complete evidence that these two proteins are, in fact, receptors analogous to steroid hormone receptors, but there is convincing evidence that they mediate important aspects of vitamin A function. The existence of a protein that specifically binds retinoic acid substantiates the implication of retinoic acid as a physiologic form of vitamin A.

Studies have shown a correlation between a diet high in β-carotene and a reduced risk of certain cancers. Several reviews of these studies are available.[39,40]

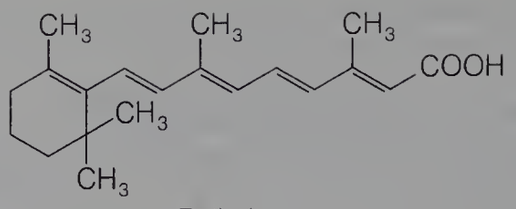
Vitamin A, USP, is a product that contains retinol (vitamin A alcohol) or its esters from edible fatty acids, chiefly acetic and palmitic acids, the activity of which is not less than 95% of the labeled amount; 0.3 μg of vitamin A alcohol (retinol) equals 1 USP unit.

Vitamin A is indicated only for treatment of vitamin A deficiencies. Because the vitamin is prevalent in the diet, especially with supplementation of milk, this disorder is not common. It is associated with conditions resulting in the malabsorption of fats (e.g., biliary or pancreatic diseases, sprue, hepatic cirrhosis).

Pure vitamin A has an activity of 3.5 million IU/g. Moderate to massive doses of vitamin A have been used in pregnancy, lactation, acne, abortion of colds, removal of persistent follicular hyperkeratosis of the arms, persistent and abnormal warts, corns and calluses, and similar conditions. Phosphatides or the tocopherols enhance the absorption of vitamin A. Vitamin A applied topically appears to reverse the impairment of wound healing by corticoids.

Vitamin A occurs as a yellow to red, oily liquid; it is nearly odorless or has a fishy odor and is unstable to air and light. It is insoluble in water or glycerin and is soluble in absolute alcohol, vegetable oils, ether, and chloroform.

Tretinoin, USP, retinoic acid (Retin-A). Tretinoin is a yellow to light orange, crystalline powder. It is insoluble in water and slightly soluble in alcohol.



Tretinoin

Tretinoin, indicated for topical treatment of acne vulgaris, initially was used systemically. However, therapeutic doses frequently resulted in hypervitaminosis A. It appears to exert its action by decreasing the adhesion of corneocytes and by increasing the proliferation of the follicular epithelium.[41]

Tretinoin usually is applied as a 0.05% polyethylene glycol (PEG)-400/ethanol liquid or a 0.05% hydrophilic cream. Daily application results in inflammation, erythema, and peeling of the skin. After 3 to 4 weeks, pustular eruptions may be seen, causing the expulsion of microcomedones. Treatment may then be changed to applications every 2 or 3 days.

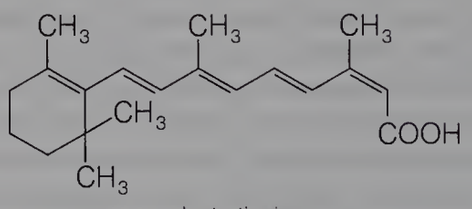
Because the horny layer is thinned, the skin is more susceptible to irritation by chemical or physical abuse. Thus, it is recommended that other kerolytic agents (salicylic, sulfur, resorcinol, benzoyl peroxide) be discontinued before beginning treatment with tretinoin. Sunscreens labeled SPF-15 or higher are recommended.

Unlabeled uses of tretinoin include the treatment of some forms of skin cancer, lamellar ichthyosis, Darier's disease, and photoaging.

Photoaging of the skin is mainly the result of excessive exposure to sunlight and is manifested by lax, yellow, mottled, wrinkled, leathery, rough skin. Once-daily application has been reported to aid in the early stages of photoaging.[42] Tretinoin is believed to exert this action by its function in regulating epithelial differentiation, cell division, and protein synthesis.[43] However, termination of treatment results in reversal within 1 year.

Tretinoin is believed to exert its antineoplastic effect by promoting cellular differentiation toward normal cells.[42]

Isotretinoin, USP, 13-*cis*-retinoic acid (Accutane). Isotretinoin is a yellow-orange to orange, crystalline powder. It is insoluble in water and sparingly soluble in alcohol.



Isotretinoin

Isotretinoin is indicated for the treatment of severe recalcitrant cystic acne. Because of the risks of adverse effects, its use should be reserved for those patients who are unresponsive to conventional acne therapies. Treatment should be individualized and modified depending on the course of the disease.

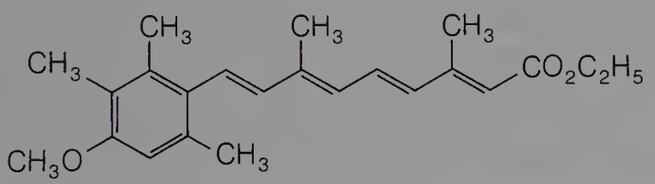
The mechanism is believed to involve inhibition of seba-

ceous gland function and follicular keratinization. Isotretinoin reduces sebum production, the size of the glands, and gland differentiation.

The initial dose is 0.5 to 1 mg/kg daily in two divided doses. Absorption is rapid, but bioavailability is low (~25%) because of degradation in the lumen, and metabolism by the gastrointestinal mucosa and the liver on the first pass. The chief metabolite is 4-oxoisotretinoin. Both isotretinoin and its metabolite are conjugated to the glucuronide and excreted in the urine and feces. The usual course of therapy is 15 to 20 weeks.

The adverse effects of isotretinoin are typical of chronic hypervitaminosis A. Because of the high potential to cause teratogenic effects, isotretinoin should be used with extreme caution in females of childbearing age. The manufacturer of the drug strongly recommends that patients should have pregnancy tests performed before the onset of therapy and utilize a form of birth control during therapy.

Etretinate (Tegison). Etretinate is indicated for the treatment of severe recalcitrant psoriasis. Because of its potential adverse effects, therapy should be limited to those diseases that do not respond to standard therapies. The exact mechanism of etretinate's action is unknown but is believed to result from some of the actions common to the retinoids.

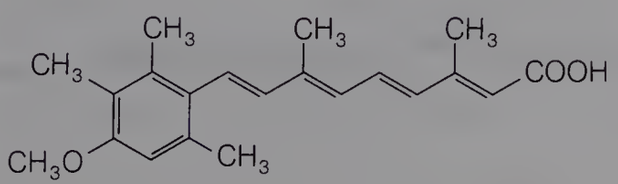


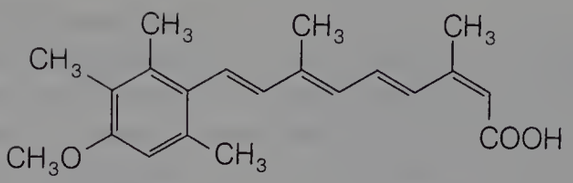
Etretinate

Oral bioavailability of etretinate is approximately 40%. Milk and lipids increase the absorption. Etretinate is converted significantly to the free acid on the first pass through the liver. The free acid (etretin) is also active. After a single dose, the half-life is 6 to 13 hours, but after long-term therapy, the half-life is 80 to 100 days. Etretinate's high lipid character results in storage in adipose tissue, from which it is released slowly. After discontinuation of therapy, etretinate can be detected for up to 1 year.

Etretin has the advantage of a shorter half-life, 2 hours after a single dose and 50 hours after multiple doses. However, it is more susceptible to conversion to 13-*cis*-etretin. Thus, it appears that the ester provides metabolic stability.

The initial dosage of etretinate is 0.75 to 1 mg/kg in divided doses. After 8 to 16 weeks, a maintenance dosage of 0.5 to 0.75 mg/kg may be started. Similar to isotretinoin, extreme caution should be exercised in the administration of etretinate.





13-*cis* Etretin

β-Carotene, USP (Soletene). β-Carotene is a red or reddish brown to violet-brown powder. It is insoluble in water and alcohol and sparingly soluble in vegetable oils. It is a naturally occurring carotenoid pigment found in green and yellow vegetables.

β-Carotene is indicated for the treatment of erythropoietic protoporphyria. It does not provide total protection against the sun, but patients who respond to its treatment can remain in the sun the same as normal individuals. Discontinuance of the drug results in a return of hypersensitivity. β-Carotene does not function as a sunscreen in normal patients and should not be used as such.

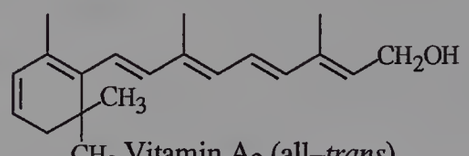
The dosage range is 30 to 300 mg/day in a single or divided dose, usually administered with food because its absorption depends on the presence of bile and absorbable fat.

Most β-carotene is converted to retinol during absorption, but the fraction that is absorbed is distributed widely and accumulates in the skin. The metabolic pathway of β-carotene is similar to that of retinol.

Several weeks of therapy are required before sufficient amounts accumulate in the skin for it to exert its protective effects. Carotenodermia, a result of accumulation in the skin, is the major side effect. However, a "tanning" capsule containing β-carotene and canthaxanthin utilizes this effect.

Vitamin A_2

Vitamin A_2 is found in vertebrates that live, or at least begin, their lives in fresh water. Vitamin A_2 exhibits chemical, physical, and biologic properties very similar to those of vitamin A. It has the structural formula shown below.



Vitamin A_2 (all-*trans*)
3–Dehydroretinol or Dehydroretinol

Vitamin A_2 has a biologic potency of 1.3 million USP U/g, which is approximately 40% of the activity of crystalline vitamin A acetate.

THE VITAMIN Ds

The term "vitamin D" originally was applied to agents with antirachitic activity. Several compounds were isolated and given the designation D_1, D_2, or D_3. D_1 was given to the

material obtained by irradiation of yeast ergosterol. This material later was found to be a 1:1 mixture of ergocalciferol and lumisterol. Upon purification and further characterization, ergocalciferol (calciferol) proved to possess the antirachitic properties and became known as vitamin D_2. Cholecalciferol was given the designation vitamin D_3.

In a classical sense, vitamin D_3, the form produced in animals, is not a true "vitamin" because it is produced in the skin from 7-dehydrocholesterol by UV radiation in the range 290 to 300 nm.[44] 7-Dehydrocholesterol is produced from cholesterol metabolism. Only when exposure to sunlight is inadequate does vitamin D_3 become a vitamin in the historical sense. Further, vitamin D_3 is now termed a provitamin because it requires hydroxylation by the liver and the kidney to be fully active.

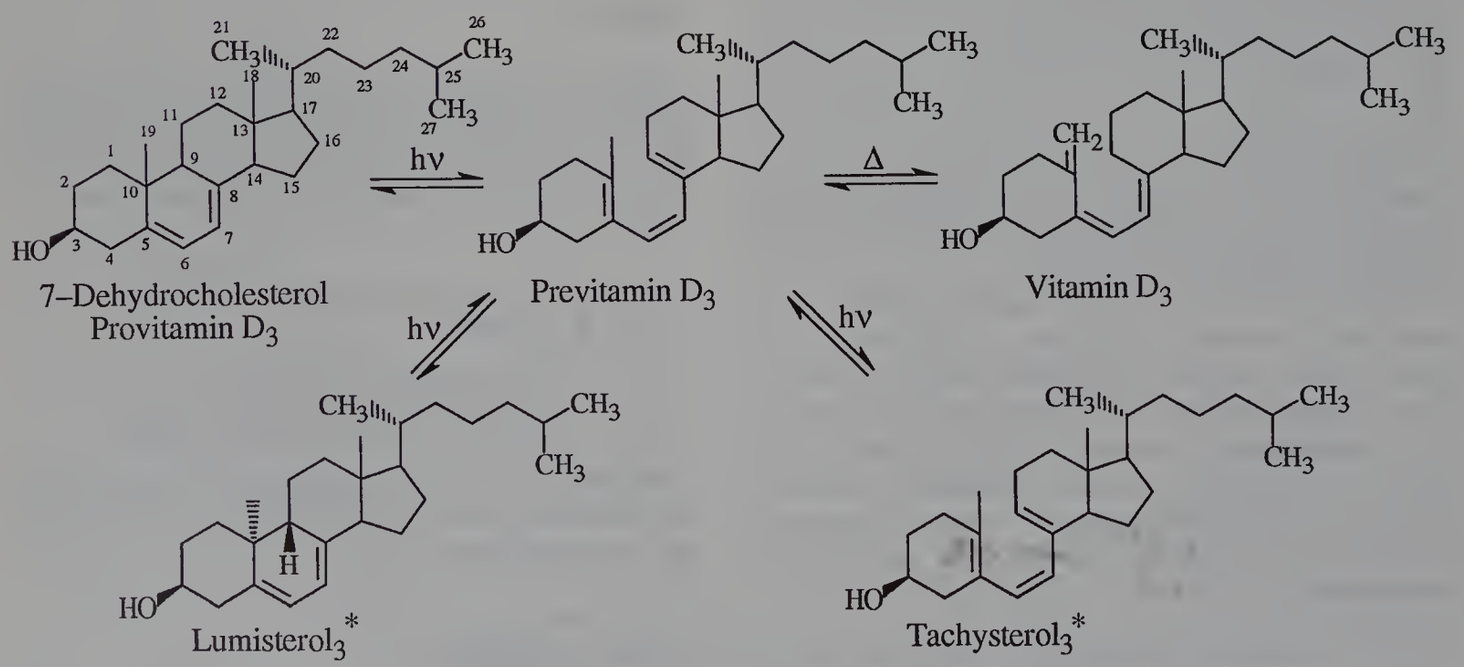
Upon UV irradiation, 7-dehydrocholesterol is converted rapidly to previtamin D_3. Previtamin D_3 undergoes a slow thermal conversion to vitamin D_3 and the biologically inactive lumisterol$_3$ and tachysterol$_3$. This provides a mechanism to prevent an overproduction of vitamin D_3 with an overexposure to sunlight, while providing a means for an adequate supply of the vitamin when exposure to sunlight is short. Excess exposure increases production of the inactive compounds. The slow conversion of previtamin D_3 to vitamin D_3 ensures adequate supplies when the exposure is brief. Further, lumisterol and tachysterol can be converted back to previtamin D_3, thus serving as a reservoir.[44] It has been estimated that a 10-minute exposure of just the uncovered hands and face suffices to produce sufficient vitamin D_3.[45]

The mechanism responsible for the movement of vitamin D_3 from the skin to the blood is not known. In the blood vitamin D_3 is bound primarily to an α-protein known as vitamin D-binding protein (VDBP). This protein selectively removes vitamin D_3 from the skin because it has low affinity for 7-dehydrocholesterol, previtamin D_3, lumisterol and tachysterol.

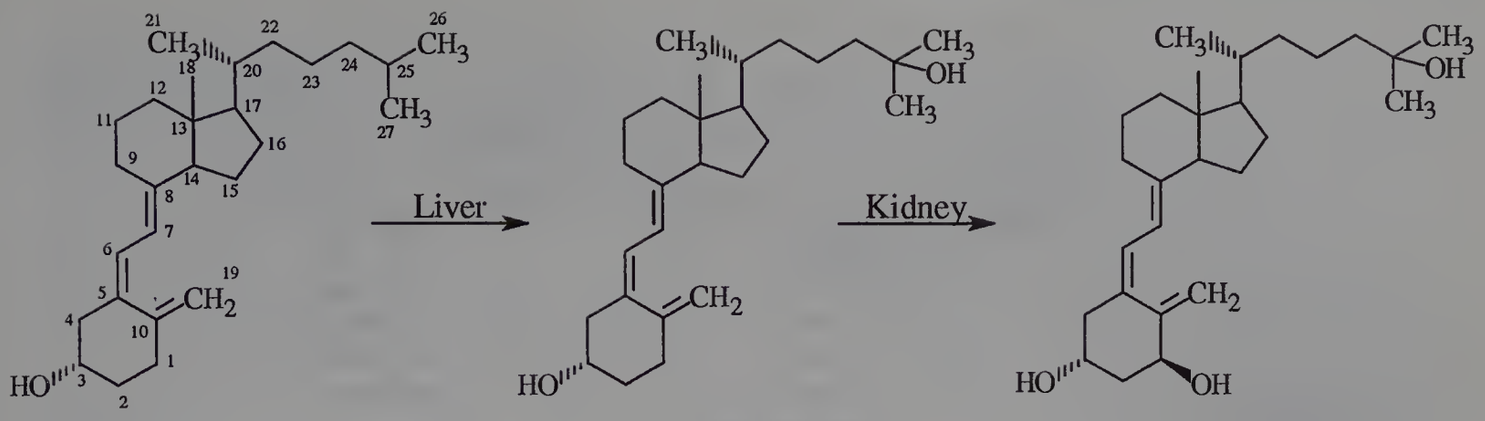
Cholecalciferol (vitamin D_3) does not perform its function directly. It must be transformed by the liver and the kidney. The first step occurs in the liver by the enzyme vitamin D_3 25-hydroxylase. This enzyme converts the provitamin to 25-hydroxyvitamin D_3 (25-OHD$_3$). This enzyme, requiring both molecular oxygen and reduced nicotinamide adenine dinucleotide phosphate (NADPH), appears to be a cytochrome P450 monooxygenase and is found in the endoplasmic reticulum and the mitochondria.[46] The rate of this hydroxylation correlates with substrate concentration.[44] The 25-OHD$_3$ thus formed is the major circulating form of the vitamin bound to VDBP.

The epithelial cells of the proximal convoluted tubules convert 25-OHD$_3$ to 1α,25-OHD$_3$ by the enzyme 25-OHD 1-hydroxylase. The activity of this mitochondrial cytochrome P450 enzyme is controlled by 1α,25-OHD$_3$ and parathyroid hormone as well as high concentrations of calcium and phosphate.[44]

Subsequently, 24-hydroxylation proceeds in the kidney, and this initiates inactivation. 1α,25-OHD$_3$ can bring about the appearance of the 24-hydroxylase system that catalyzes its metabolic inactivation. The need for calcium stimulates parathyroid hormone secretion. Parathyroid hormone in turn



The subscript 3 indicates the steroid has a C–17 side chain similar to that of 7–dehydrocholesterol and thus related to vitamin D_3.



Cholecalciferol
Vitamin D_3

Calcifediol
25–Hydroxyvitamin D_3
(25–Hydroxycholecalciferol)

Calcitriol
1,25–Dihydroxyvitamin D_3
(1,25–Dihydroxycholecalciferol)

suppresses the 24-hydroxylase and stimulates the 1-hydroxylase system. When phosphate availability is below normal, the 1-hydroxylase is stimulated and the 24-hydroxylase undergoes suppression.

As with vitamin A, most of the effects of vitamin D involve a nuclear receptor. The vitamin D receptor is a member of the steroid/thyroid hormone superfamily of receptors. When 1α,25-OHD_3 binds to its receptor, the complex forms a heterodimer with an unoccupied RXR. This heterodimer subsequently binds to the regulatory regions on specific genes in target tissue. These regions are called vitamin D response elements (VDREs). The binding to VDREs can increase or decrease expression of genes.[44] The proteins thus made carry out the functions of vitamin D.

The physiologic role of vitamin D is to maintain calcium homeostasis. Phosphate metabolism also is affected. Vitamin D accomplishes its role by enhancing the absorption of calcium and phosphate from the small intestines, promoting their mobilization from bone, and decreasing their excretion by the kidney. Also involved are parathyroid hormone and calcitonin.

1α,25-OHD_3 promotes Ca^{2+} intestinal absorption and increases Ca^{2+} renal reabsorption in the distal tubules and mobilization of Ca^{2+} from bone. The mechanism of action promoting Ca^{2+} transport in the intestine involves formation of a calcium-binding protein. 1α,25-OHD_3 promotes availability of this protein. A calcium-dependent ATPase, Na^+, and the calcium-binding protein are necessary for the intestinal Ca^{2+}-transport process. 1α,25-OHD_3 also promotes intestinal phosphate absorption, mobilization of Ca^{2+} and phosphate from bone, and renal reabsorption of Ca^{2+} and phosphate.

Vitamin D deficiency results in rickets in infants and children as a result of inadequate calcification of bones. In adults, osteomalacia most often occurs during pregnancy and lactation. Rickets is rare in the United States due to fortification of foods. However, deficiencies in the elderly are the result of underexposure to sunlight.

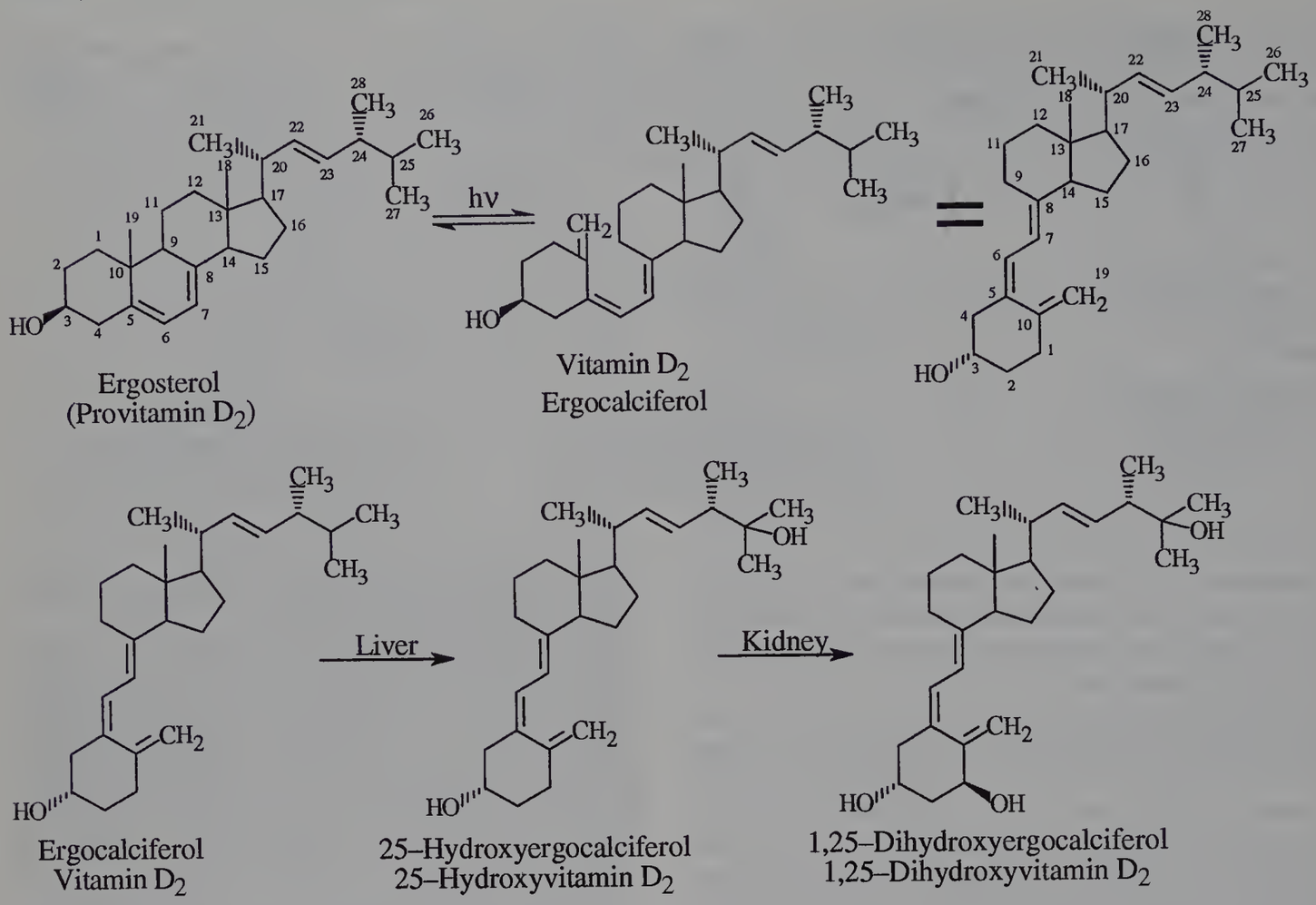
Hypervitaminosis D may result from large doses of the vitamin or from a hypersensitivity to the vitamin. Early symptoms are associated with hypercalcemia, including fatigue, weakness, nausea, vomiting, vertigo, and bone pain. Prolonged hypercalcemia may result in calcium deposits in the kidneys, vessels, heart, lungs, and skin. Treatment includes withdrawal of the vitamin and a low-calcium diet with an increase in fluids.

Ergocalciferol (vitamin D_2) is produced in plants from ergosterol upon UV irradiation. Vitamin D_2 is the form most often used in commercial products and to fortify foods. Although different in structure, its biologic activity is comparable to that of vitamin D_3 and must be bioactivated in a similar fashion.

Ergosterol (precursor of D_2) occurs naturally in fungi and yeast. Eggs and butter contain vitamin D_2 (ergocalciferol) or D_3 (cholecalciferol). Milk and bread are fortified with vitamin D_2. Cholecalciferol is found in fish liver oils.

The gastrointestinal absorption of the vitamin Ds requires bile. Vitamin D_3 may be absorbed better than vitamin D_2. The vitamin Ds enter the circulation through lymph chylomicrons. In the blood they are associated with vitamin D-binding protein (VDBP). The 25-hydroxylated compounds are the major circulating metabolites. These may be stored in fats and muscle for prolonged periods. The 24-hydroxy metabolites are excreted primarily in the bile.

The vitamin Ds are important in the therapeutics of hypoparathyroidism and of vitamin D deficiency.[2] Ergocalciferol, cholecalciferol, and dihydrotachysterol are recognized by the *USP*. Although dihydrotachysterol has relatively weak antirachitic activity, it is effective and quicker acting in increasing serum Ca^{2+} concentrations in parathyroid deficiency. Dihydrotachysterol has a shorter duration of action; hence, it has less potential for toxicity from hypercalcemia.



Vitamin D receptors have been identified in tissue not normally associated with bone mineral homeostasis. Besides the intestines, kidneys, and osteoblasts, vitamin D receptors have been located in the parathyroid gland, the pancreatic islet cells, the mammary epithelium, and the skin keratinocytes. This has resulted in many investigational uses for vitamin D, including suppression of parathyroid hormone and treatment of colon and breast cancers and psoriasis.[44]

The previously mentioned investigational treatments require high doses of vitamin D. The resultant hypercalcemia and hypercalciuria limit the use of vitamin D natural metabolites. Vitamin D analogues with a decreased tendency to cause hypercalcemia and hypercalciuria are being developed and investigated. These analogues have low affinity for VDBP but retain high affinity for the vitamin D receptors.[44] The only approved use of a vitamin D analogue is in the treatment of psoriasis with calcipotriene.

Products

Ergocalciferol, USP. 9,10-Secoergosta-5,7,10(19),22-tetraen-3-ol (3β,5Z,7E,22E); vitamin D_2; calciferol; activated ergosterol. One USP or International unit is 0.025 μg of vitamin D_3. Thus, 1 μg equals 40 USP units. Because ergocalciferol is the least expensive of the vitamin D analogues, it is the preferred drug, unless the patient is unable to activate it.

Ergocalciferol has a half-life of 24 hours (19 to 48 hours) and a duration of action of up to 6 months. After oral or intramuscular administration, the onset of action (hypercalcemia) is 10 to 24 hours, with maximal effects seen 4 weeks after daily administration.

After irradiation, the steroid undergoes fission of ring B; therefore, it is known as a secosteroid. This is indicated in the name by the "9,10-seco" portion. The "ergosta" portion indicates the presence of 28 atoms in the carbon skeleton.

The history and preparation of this vitamin have been described. Vitamin D_2 is a white, odorless, crystalline compound that is soluble in fats and in the usual organic solvents, including alcohol. It is insoluble in water. Vitamin D_2 is oxidized slowly in oils by oxygen from the air, probably through the fat peroxides that are formed. Vitamin A is much less stable under the same conditions.

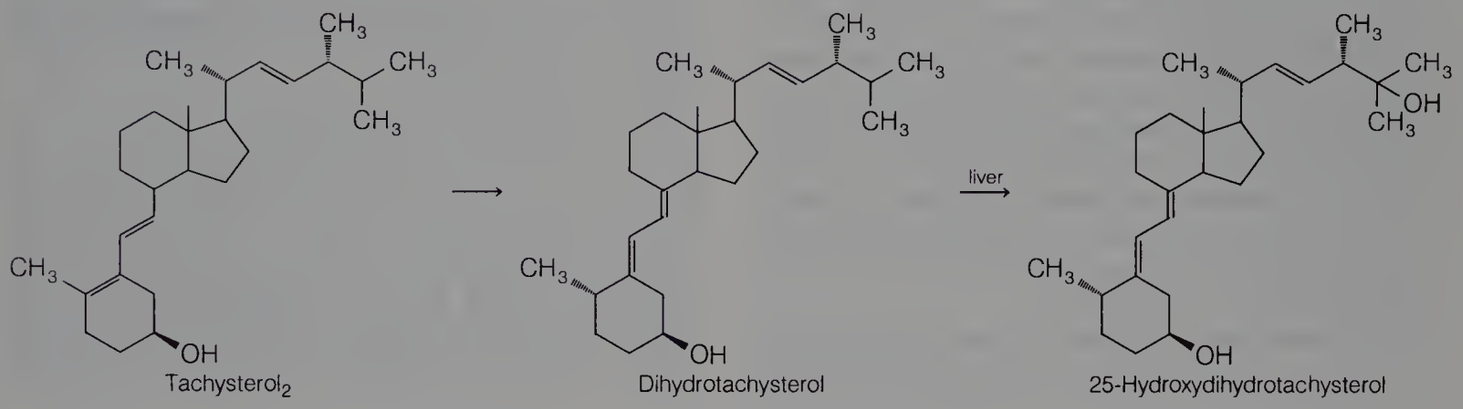
Cholecalciferol, USP. 9,10-secocholesta-5,7,10(19)-trien-3-ol (3β,5Z,7E); vitamin D_3; activated 7-dehydrocholesterol. It occurs as white, odorless crystals that are soluble in fatty oils, alcohol, and many organic solvents. It is insoluble in water.

Vitamin D_3 also occurs in tuna and halibut liver oils. It has the same activity as vitamin D_2 in rats but is more effective in the chick; however, both vitamins have equal activity in humans.

Vitamin D_3 exhibits stability comparable with that of vitamin D_2.

Epimerization of the —OH at C-3 in vitamin D_2 or D_3 or conversion of the —OH at C-3 to a ketone group greatly diminishes the activity but does not completely destroy it. Ethers and esters that cannot be cleaved in the body have no vitamin D activity. Inversion of the hydrogen at C-9 in ergosterol and other 7-dehydrosterols prevents the normal course of irradiation.

Dihydrotachysterol, USP. 9,10-Ergosta-5,7,22-trien-3-ol (3β,5E,7E,10α,22E); dihydrotachysterol$_2$; dichysterol; DHT. Tachysterol (represented below) is a by-product of ergosterol irradiation. Reduction of tachysterol led to dihydrotachysterol.



Tachysterol$_2$ Dihydrotachysterol 25-Hydroxydihydrotachysterol

Dihydrotachysterol occurs as colorless or white crystals or a white, crystalline, odorless powder. It is soluble in alcohol, freely soluble in chloroform, sparingly soluble in vegetable oils, and practically insoluble in water.

Dihydrotachysterol has slight antirachitic activity. It causes an increase of the calcium concentration in the blood, an effect for which tachysterol is only one-tenth as active.

In high doses, dihydrotachysterol is more effective than the other analogues for the mobilization of calcium. Thus, it is used in hypoparathyroidism.

After oral administration, the onset of action is seen within hours. This fast onset of action is an advantage of this drug. Maximal activity is seen in 2 weeks after daily administration. Its duration of action is 2 weeks.

Dihydrotachysterol is activated by hepatic enzymes to its 25-hydroxylated metabolite. It does not require renal activation, for the hydroxy on ring A occupies the same position as that of the 1-hydroxyl in the activated forms of the vitamin Ds.

25-Hydroxydihydrotachysterol$_3$ has weak antirachitic activity, but it is a more important bone-mobilizing agent and is more effective than dihydrotachysterol$_3$. Also, it is more effective in increasing intestinal calcium transport and bone mobilization in thyroparathyroidectomized rats. Its activity suggests that it may be the drug of choice in the treatment of hypoparathyroidism and similar bone diseases.[47a]

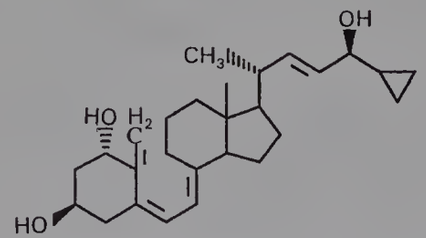
Calcifediol, USP. 9,10-Secocholesta-5,7,10(19)-trien-3,25-diol (3β,5Z,7E); 25-hydroxycholecalciferol; 25-hydroxyvitamin D_3. Calcifediol occurs as a white powder. It is practically insoluble in water and sensitive to light and heat. The half-life of calcifediol is 16 days (10 to 22 days). Its onset of action is seen within 2 to 6 hours, and its duration of action is 15 to 20 days.

Calcifediol is indicated for patients receiving long-term renal dialysis.

Calcitriol. 9,10 - Secocholesta - 5,7,10(19) - trien - 1,3,25 - triol (1α,3β,5Z,7E); 1,25-dihydroxycholecalciferol; 1,25-dihydroxyvitamin D_3. It occurs as colorless crystals that are insoluble in water. Since calcitriol does not require activation, an increase in calcium absorption is seen within 2 hours of administration. Its half-life is 3 to 8 hours, and its duration of action is 1 to 2 days.

Calcitriol is the most active form of vitamin D_3. It is indicated in patients receiving long-term renal dialysis or who cannot properly metabolize ergocalciferol.

Calcipotriene. 9,10 - Secochola - 5,7,10(19),22 - tetraien - 1,3,25-trio 1,24-cyclopropyl-(1α,3β,5Z,7E,22E,24S); calcipotriol. Calcipotriene is a synthetic vitamin D_3 analogue indicated for topical application in the treatment of moderate plaque psoriasis. It has the same affinity for the vitamin D receptor as calcitriol, but its effect on calcium metabolism is 100 to 200 times less. Calcipotriene inhibits epidermal cell proliferation and enhances cell differentiation. It reduces cell numbers and total DNA content.[47] Antiproliferative effects are caused by a reduction in the mRNA levels of a cellular oncogene associated with proliferation, c-*myc*. The mechanism resulting in differentiation changes is not completely known but involves the secondary messengers inositol trisphosphate (IP_3) and diacylglycerol (DAG).[44]



Calcipotriene

VITAMIN E

Since the early 1920s it has been known that rats fed only cow's milk are not able to produce offspring. The principle from wheat germ that can rectify this deficiency in both male and female rats was named vitamin E. When the compound known as vitamin E was isolated in 1936, it was named tocopherol. Since then, several other closely related compounds have been discovered from natural sources, and this family of natural products took the generic name tocopherols.

The tocopherols are especially abundant in wheat germ, rice germ, corn germ, other seed germs, lettuce, soya, and cottonseed oil. All green plants contain some tocopherols, and there is evidence that some green leafy vegetables and rose hips contain more than wheat germ. It probably is synthesized by leaves and translocated to the seeds. All four tocopherols have been found in wheat germ oil; α-, β-, and γ-tocopherols have been found in cottonseed oil. Corn oil contains predominantly γ-tocopherol and, thus, furnishes a convenient source for the isolation of this, a difficult member of the tocopherols to prepare. δ-Tocopherol is 30% of the mixed tocopherols of soya bean oil.

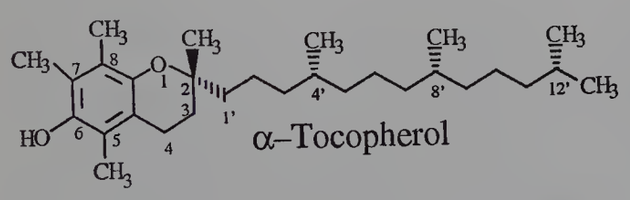
Several tocopherols have been isolated. Some have the 4′,8′,12′-trimethyltridecyl-saturated side chain, while others have unsaturation in the side chain. It has been suggested that these polyunsaturated tocols be named "tocotrienols." The most well known is α-tocopherol (vitamin E), which has the greatest biologic activity.

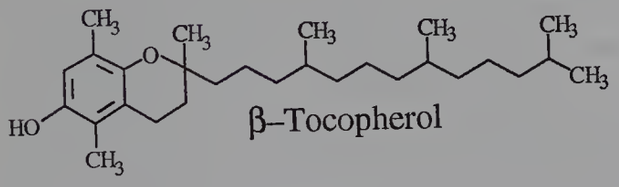
The base structure, represented below, shows that the tocopherols are methyl-substituted tocol derivatives: α-tocopherol is 5,7,8-trimethyltocol; β-tocopherol is 5,8-dimethyltocol; the γ-compound is 7,8-dimethyltocol; and δ-tocopherol is 8-methyltocol. The tocotrienols have similar substituents.

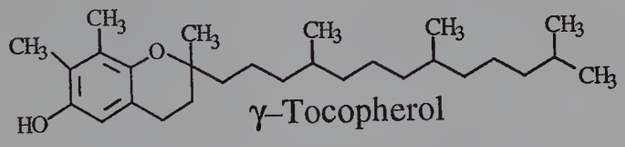
Natural α-(+)-tocopherol has the configuration 2R,4′R,8′R. The natural tocotrienols have a 2R,3′E,7′E configuration.

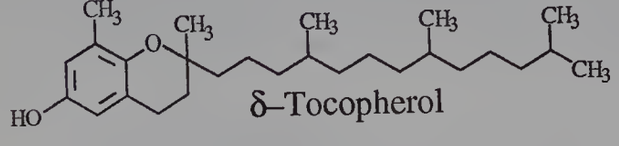
The tocopherols are diterpenoid natural products biosynthesized from a combination of four isoprenoid units; geranylgeranyl pyrophosphate is the key intermediate that leads to these compounds.[48]

The tocopherols and their acetates are light yellow, viscous, odorless oils that have an insipid taste. They are insoluble in water and soluble in alcohol, organic solvents, and fixed oils. The acid succinate esters are white powders insoluble in water and soluble in ethanol and vegetable oils. Tocopherols are stable in air for reasonable periods but are oxidized slowly by air. They are oxidized readily by ferric salts, by mild oxidizing agents, and by air in the presence


α–Tocopherol


β–Tocopherol


γ–Tocopherol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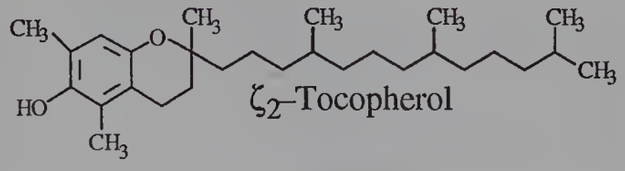
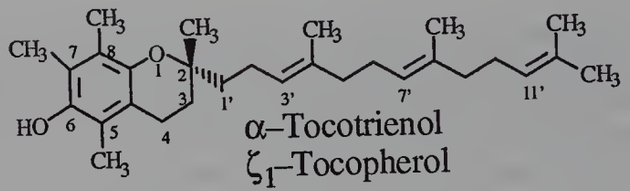
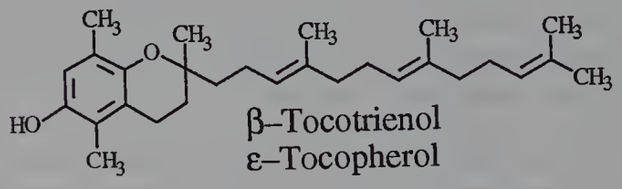

ζ2–Tocopherol


α–Tocotrienol
ζ1–Tocopherol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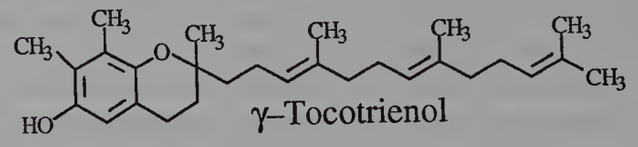
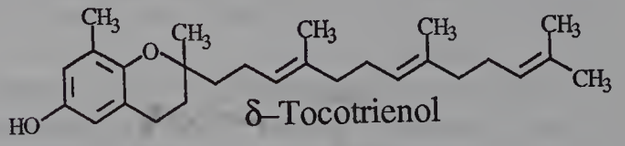
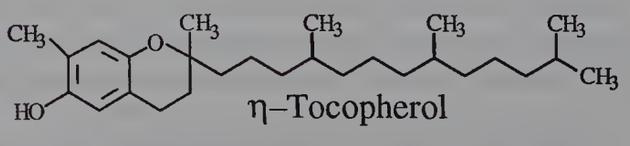
ε–Tocopherol


γ–Tocotrienol


δ–Tocotrienol


η–Tocopherol

of alkali. They are inactivated rapidly by exposure to UV light; however, not all samples behave alike in this respect because traces of impurities apparently greatly affect the rate of oxidation. Tocopherols have antioxidant properties for fixed oils in the following decreasing order of effectiveness: δ-, γ-, β-, and α-.[49] In the process of acting as antioxidants, tocopherols are destroyed by the accumulating fat peroxides that are decomposed by them. They are added to Light Mineral Oil NF and Mineral Oil USP because of their antioxidant property. Tocopherols can be converted to acetates and benzoates, which are oils as active as the parent compounds and have the advantage of being more stable toward oxidation.

(+)-α-Tocopherol is about 1.36 times as effective as (±)-α-tocopherol in rat antisterility bioassays. β-Tocopherol is about one-half as active as α-tocopherol, and the γ- and δ-tocopherols are only 1/100 as active as α-tocopherol. The esters of tocopherol, such as acetate, propionate, and butyrate, are more active than the parent compound.[50] This is also true of the phosphoric acid ester of (±)-δ-tocopherol when it is administered parenterally.[51] The ethers of the tocopherols are inactive. Oxidation of the tocopherols to their corresponding quinones also leads to inactive compounds. Replacement of the methyl groups by ethyl groups leads to decreased activity. The introduction of a double bond in the 3,4-position of α-tocopherol reduces its activity by about two-thirds. Reduction of the size of the long alkyl side chain or the introduction of double bonds in this side chain markedly reduces activity.

Vitamin E activity currently is expressed in terms of the α-(+)-tocopherol equivalents based on the former USP units and mass listed on Table 27-4. One former USP unit is equal to one former IU.

Vitamin E, USP, may consist of (+)- or (±)-α-tocopherols or their acetates or succinates, 96.0% to 102.0% pure.

(−)-α-Tocopherol is absorbed from the gut more rapidly than the (+)-form; however, absorption of the mixture of (+)- and (−)-α-tocopherol was considerably higher (about 55%) than expected from the data obtained after administration of the single compounds.

As doses increase, the fraction absorbed decreases. No marked differences were noted in the distribution in various tissues and the metabolic degradation of (+)- and (−)-α-tocopherols.[52] The liver is an important storage site.

Most of the gastrointestinal absorption of vitamin E occurs through the mucosa and the lymphatic system. Bile performs an important function in promoting tocopherol absorption. The ester derivatives are hydrolyzed by pancreatic enzymes before absorption. Although hydrolysis of the ester is not required, it does improve absorption. Vitamin E preparations are absorbed better from aqueous solutions than from oily solutions.

α-Tocopherol and γ-tocopherol are absorbed from the intestines and distributed to the liver equally well. However, γ-tocopherol is secreted primarily into the bile, while α-tocopherol enters the circulation, where it is found in much higher levels than γ-tocopherol, even though the latter predominates in the diet. This difference is attributed to a liver cytosolic binding protein which is selected for α-tocopherol.

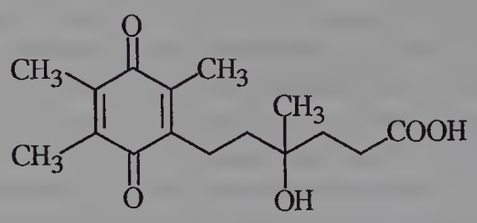
The tocopherols in lymph are associated with chylomicrons and very low-density lipoproteins (VLDLs). Circulating tocopherols also are associated mainly with the blood low-density lipoproteins (LDLs). The tocopherols are readily and reversibly bound to most tissues, including adipose tissue, and the vitamin is thus stored. The vitamin is concentrated in membrane structures, such as mitochondria, endoplasmic reticulum, and nuclear and plasma membranes.

Vitamin E is metabolized primarily to tocopheronic acid and its γ-lactone, followed by glucuronide conjugation. The terminal methyl group is oxidized to a carboxylic acid and shortened by β-oxidation to produce tocopheronic acid. The chroman ring is hydrolyzed to a quinone, which subsequently is reduced to a hydroquinone. Nucleophilic attack by a hydroxyl on the carbonyl side chain produces tocopheronolactone. These metabolites are excreted in the bile. Vitamin E may undergo some enterohepatic circulation.

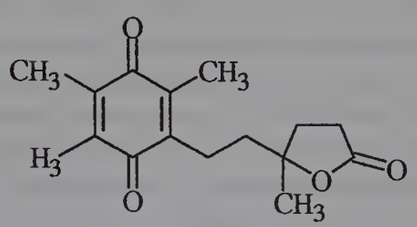
TABLE 27-4

RELATIVE POTENCIES OF VARIOUS COMMERCIAL FORMS OF VITAMIN E

Form of Vitamin E	*Potency*
Potency (in former USP units) of 1 mg	
(±)-α-Tocopherol	1.1
(±)-α-Tocopherol acetate	1.0
(±)-α-Tocopherol acid succinate	0.89
(+)-α-Tocopherol	1.49
(+)-α-Tocopherol acetate	1.36
(+)-α-Tocopherol acid succinate	1.21
Potency (in terms of (+)-α-tocopherol equivalents) of 1 mg	
(+)-α-Tocopherol acetate	0.91
(+)-α-Tocopherol acid succinate	0.81
(±)-α-Tocopherol	0.74
(±)-α-Tocopherol acetate	0.67
(±)-α-Tocopherol acid succinate	0.60



Tocopheronic acid



Tocopheronolactone

For decades, there has been significant interest in investigating the biochemical functions of vitamin E, but it is still difficult to explain many of the biochemical derangements caused by vitamin E deficiency in animals. There seems to be general agreement that one of the primary metabolic functions of the vitamin is that of an antioxidant of lipids, particularly unsaturated fatty acids. This function of preventing lipid oxidation does not, however, explain all of the biochemical abnormalities caused by vitamin E deficiency. Moreover, vitamin E is not the only in vivo antioxidant. Two enzyme systems, glutathione reductase and *o*-phenylenediamine peroxidase, also function in this capacity.[53]

It has been postulated that vitamin E has a role in the regulation of protein synthesis. Other actions of this vitamin also have been investigated, for example, effects on muscle creatine kinase and liver xanthine oxidase. Vitamin E deficiency leads to an increase in the turnover of creatine kinase. There is also an increase in liver xanthine oxidase activity in vitamin E-deficient animals, and this increase is due to an increase in de novo synthesis.[53]

Although it has been difficult to establish clinical correlates of vitamin E deficiency in humans, Bieri and Farrell[53] have summarized some useful generalizations and conclusions. These workers have noted that the infant, especially the premature infant, is susceptible to tocopherol deficiency because of ineffective transfer of the vitamin from placenta to fetus and that growth in infants requires greater availability of the vitamin. In adults, the tocopherol storage depots provide adequate availability that is not readily depleted, but intestinal malabsorption syndromes, when persistent, can lead to depletion of the storage depots. Children with cystic fibrosis suffer from severe vitamin E deficiency caused by malabsorption. Tropical sprue, celiac disease, gastrointestinal resections, hepatic cirrhosis, biliary obstruction, and excessive ingestion of mineral oil also may cause long-term malabsorption.

Vitamin E therapeutic indications include the clinical conditions characterized by low serum tocopherol levels and increased fragility of red blood cells to hydrogen peroxide or conditions that require additional amounts. The latter can be exemplified by individuals who consume excessive amounts of polyunsaturated fatty acids (more than 20 g/day over normal diet).[54]

It has been claimed that vitamin E could be of therapeutic benefit in ischemic heart disease, but evidence against this claim continues to accumulate. It also has been suggested that megadoses of tocopherol be used in the treatment of peripheral vascular disease with intermittent claudication. Although some studies support this proposal, experts in the field state that further clinical studies are necessary to make a definitive recommendation. Nevertheless, it continues to be popular and controversial to consider the beneficial effects of vitamin E and other vitamins in large (mega) dietary supplements, and investigations of megavitamin E therapy for cardiovascular disease continue to appear in the literature.[53]

The eminent vitamin biochemist R. J. Williams has emphasized that

> . . .[L]ipid peroxidation, the formation of harmful peroxides, from the interaction between oxygen and highly unsaturated fats (polyunsaturates) needs to be controlled in the body. Both oxygen and the polyunsaturated lipids are essential to our existence, but if the protection against peroxidation is inadequate, serious damage to various body proteins may result. Vitamin E is thought to be the leading agent for the prevention of peroxidation and the free radical production that is associated both with it and with radiation.[55]

Williams also notes that although exact mechanisms of action of these antioxidants are not yet known,

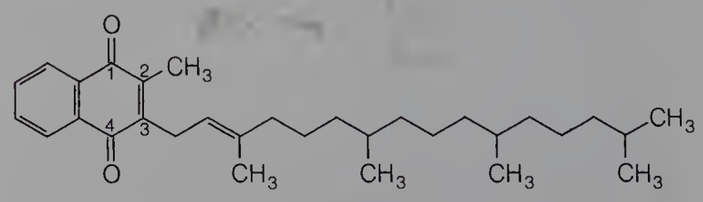
> . . .[P]roviding plenty of vitamin E and ascorbic acid—both harmless antioxidants—is indicated as a possible means of preventing premature aging, especially if one's diet is rich in polyunsaturated acids.

Considering the foregoing implication of unnecessary peroxidation of unsaturated lipids, it is interesting that atherosclerosis appears to be due to a deficiency of prostacyclin and that this deficiency is caused by inhibition of prostacyclin synthetase by lipid peroxides or by free radicals that are likely to be generated during hyperlipidemia. Although there is no direct evidence that in experimental or human atherosclerosis lipid peroxidation is the earliest sign of the disease state, lipid peroxides have been found in arteries from atherosclerotic patients and in ceroid atheromatic plaques, and, at the same time, hardly any prostacyclin is generated in human atheromatic plaques.[56]

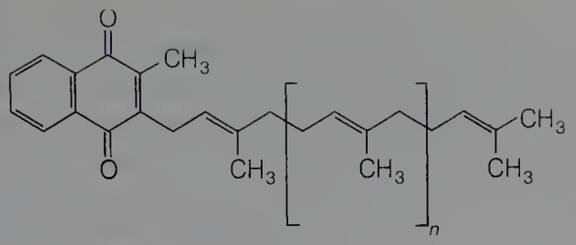
THE VITAMIN Ks

The biologic activity of vitamin K was discovered in 1929, and vitamin K was identified structurally in 1931, as represented in the following diagram. The term "vitamin K" was applied to the vitamin isolated from alfalfa, and a similar principle from fish meal was named vitamin K_2. Vitamin K_2 refers to a series of compounds called the "menaquinones." These have a longer side chain with more unsaturation. This side chain may be composed of one to 13 isoprenyl units. The most common are depicted below.

Many other closely related compounds possess vitamin K activity (e.g., menadione [2-methyl-1,4-naphthoquinone] is as active as vitamin K on a molar basis). The synthetic compounds menadione and menadiol are referred to as vitamin K_3 and K_4, respectively.



Vitamin K_1
Phytonadione
(2-Methyl-3-phytyl-1,4-naphthoquinone)



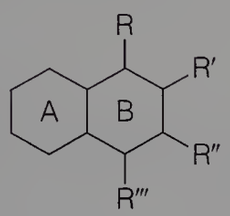
$n = 4$ = Vitamin $K_{2(30)}$ Menaquinone-6
$n = 5$ = Vitamin $K_{2(35)}$ Menaquinone-7

Vitamin K is a naphthoquinone derivative containing diterpenoid units biosynthesized by the intermediate geranylpyrophosphate.[57]

Animals depend on two sources for their intake of this vitamin, dietary and bacterial synthesis. Table 27-5 lists excellent sources of vitamin K_1.

Natural K_1 occurs as a *trans*-isomer and has an R, R, E configuration. The synthetic, commercially available form is a mixture of *cis*- and *trans*-isomers, with no more than 20% *cis*. Vitamin K_2 is synthesized by the intestinal flora, especially by gram-positive bacteria. It is not available commercially.

Numerous compounds have been tested for their antihemorrhagic activity, and significant biologic activity is manifested in compounds with the following structure when:



1. Ring A is aromatic or hydroaromatic.
2. Ring A is not substituted.
3. Ring B is aromatic or hydroaromatic.
4. R equals OH, CO, OR, OAc (the R in OR equals methyl or ethyl).
5. R′ equals methyl.
6. R″ equals H, sulfonic acid, dimethylamino, or an alkyl group containing ten or more carbon atoms. A double bond in the β, γ-position of this alkyl group enhances potency, whereas if the double bond is further removed, it exerts no effect. Isoprenoid groups are more effective than straight chains. In the vitamin $K_{2(30)}$-type compounds, the 6′,7′-mono-*cis*-isomer is significantly less active than the all-*trans*- or the 18′,19′-mono-*cis*-isomer. This also was true of the vitamin $K_{2(20)}$ isoprenolog. A vitamin $K_{2(25)}$ isoprenolog was 20% more active than vitamin $K_{1(37)}$.
7. R‴ equals H, OH, NH_2, CO, OR, Ac (the R in OR equals methyl or ethyl).

Decreased antihemorrhagic activity is obtained when

1. Ring A is substituted.
2. R′ is an alkyl group larger than a methyl group.
3. R″ is a hydroxyl group.
4. R″ contains a hydroxyl group in a side chain.

It is interesting that if ring A is benzenoid, the introduction of sulfur in place of a —CH═CH— in this ring in 2-methylnaphthoquinone permits the retention of some antihemorrhagic activity. This might indicate that in the process of exerting vitamin K activity, the benzenoid end of the molecule must fit into a pocket carefully tailored to it. That the other end is not so closely surrounded is shown by the retention of activity on changing the alkyl group in the 2-position.

Although marked antihemorrhagic activity is found in many naphthoquinone compounds, these compounds may be converted in the body to a vitamin K_1-type compound. The esters of the hydroquinones may be hydrolyzed, and the resulting hydroquinone may be oxidized to the quinone. The methyl tetralones, which are very active, could be dehydrogenated to the methylnaphthols, which are hydroxylated, and the latter product converted to the biologically equivalent quinone. Compounds with a dihydrobenzenoid ring (such as 5,8-dihydrovitamin K_1) appear to be moderately easily dehydrogenated, whereas the corresponding tetrahydrides are resistant to such a change.

Vitamins K_1 and K_2 are absorbed by an active process in the proximal small intestines. Bile of a normal composition is necessary to facilitate the absorption. The bile component principally concerned in the absorption and transport of fat-soluble vitamin K from the digestive tract is thought to be deoxycholic acid. The molecular compound of vitamin K with deoxycholic acid was effective upon oral administration to rats with biliary fistula. Vitamin K is absorbed through the lymph in chylomicrons. It is transported to the liver, where it is concentrated, but no significant storage occurs.

The entire metabolic pathway of vitamin K has not been elucidated. However, the major urinary metabolites are glucuronide conjugates of carboxylic acids derived from shortening of the side chain. High fecal concentrations are probably due to bacterial synthesis.

The only known function of vitamin K in higher animals is to maintain adequate plasma levels of the protein pro-

TABLE 27-5

VITAMIN K CONTENT OF SELECTED FOODS*

Food	*μg*
Broccoli	200
Brussels sprouts	220
Cabbage, Chinese	175
Cabbage, red	50
Cabbage, white	80
Green onions	60
Kale	750
Lettuce	120
Parsley	700
Spinach	350
Turnip greens	300
Watercress	200

* In μg/100 g.

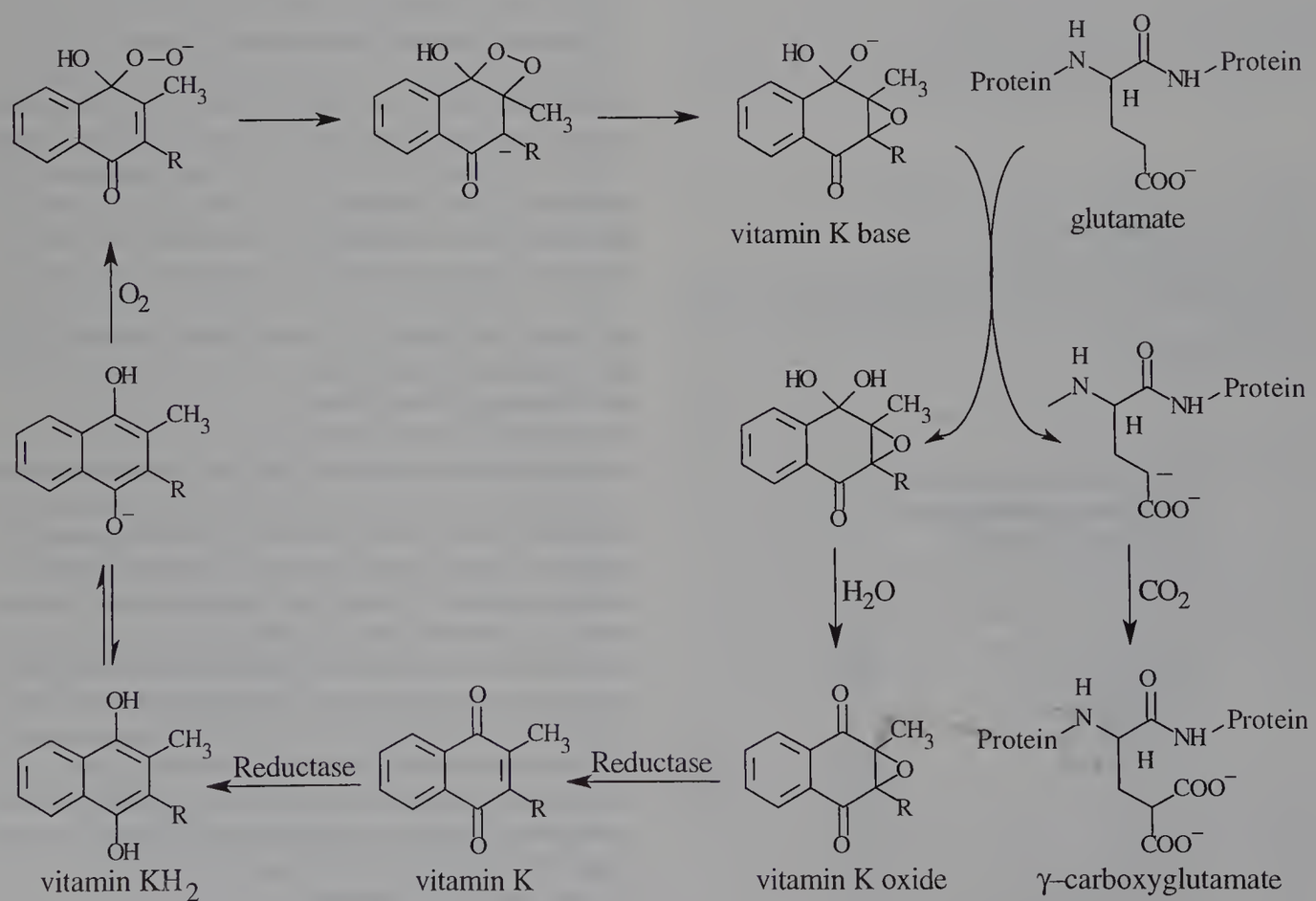
thrombin (factor II) and three other essential clotting factors: VII (proconvertin), IX (autoprothrombin II), and X (Stuart-Prower factor). It follows that any condition that does not permit the full utilization of the antihemorrhagic agents or the production of prothrombin would lead to an increase in the amount of time in which the blood will clot or to hemorrhagic conditions. Some of these conditions are (1) faulty absorption caused by several conditions (e.g., obstructive jaundice, biliary fistulas, intestinal polyposis, chronic ulcerative colitis, intestinal fistula, intestinal obstruction, and sprue); (2) damaged livers or primary hepatic diseases, such as atrophy, cirrhosis, or chronic hepatitis; (3) insufficient amounts of bile or abnormal bile in the intestinal tract; and (4) insufficient amounts of vitamin K.

Vitamin K also is involved in the production of two anticoagulant proteins, protein C and protein S. These two proteins have γ-carboxyglutamic acid residues essential to their function. Protein C is activated by thrombin bound to thrombomodulin, a cell-surface protein. Activated protein C along with protein S cleave factors V_a and $VIII_a$. Another vitamin K-dependent protein, protein Z, has been identified but its function has not.

The vitamin K-dependent carboxylase system includes a specialized microsomal electron-transport system coupled to a carbon dioxide-fixation reaction. Although the reaction does not require ATP, it uses the energy from the oxidation of reduced vitamin K to execute the carboxylation of glutamic acid.[58] The carboxylase must create a carbanion by extracting a proton from the glutamate α-carbon. This requires a base with a pK_a of 26 to 28. The anion of the hydroquinone has a pK_a of approximately 9.

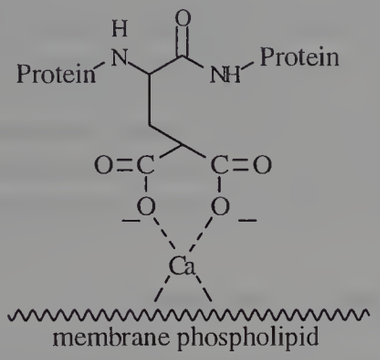
A proposed mechanism for the above carboxylation creates such a base from vitamin K.[59,60] Vitamin K is reduced to its hydroquinone form (vitamin KH_2). Molecular oxygen is incorporated into the conjugate acid form of vitamin KH_2 to form a peroxy anion, which subsequently forms a dioxetane intermediate. The peroxy bond is cleaved by the adjacent enolate anion to produce an intermediate sufficiently basic to deprotonate the α-carbon.

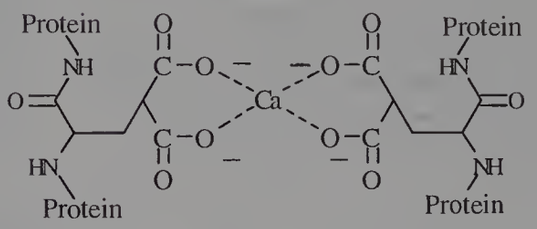
Extraction of the proton allows the carboxylase to carboxylate the glutamate residue. The vitamin K intermediate is converted to vitamin K oxide, which must be reduced back to vitamin K. It is this vitamin K oxide reductase that 4-hydroxycoumarin anticoagulants inhibit.



Vitamin K thus participates in the formation of specific γ-carboxyglutamic acid Ca^{2+}-binding sites on the above factors. It is also significant that γ-carboxyglutamic acid has been found as a residue in all vitamin K-dependent clotting factors. Moreover, the γ-carboxyglutamic acid residues in these factors are found in essentially the same region, near the amino terminus, and this region is called the "Gla domain."

These Gla residues may function as ion bridges linking the blood-clotting protein to phospholipids on endothelial cells and platelets. Binding to membrane surfaces is critical in the activation of these proteins.[61]





recover spontaneously. This transition period was and is critical because of the numerous sites of hemorrhagic manifestations, traumatic or spontaneous, that may prove serious if not fatal. This condition now is recognized as a type of alimentary vitamin K deficiency. The spontaneous recovery is perhaps due to the establishment of an intestinal flora capable of synthesizing vitamin K after ingestion of food. However, administration of vitamin K orally effects a prompt recovery.

Vitamin K_1 acts more rapidly (effect on prothrombin time) than menadione, within 2 hours after intravenous administration. However, no difference could be detected after 2 hours.[62]

Products

Phytonadione, USP. 2-Methyl-3-phytyl-1,4-naphthoquinone; vitamin K_1. Phytonadione is a clear, yellow, very viscous, odorless or nearly odorless liquid.

Pure vitamin K_1 is a yellow, crystalline solid that melts at 69°C. It is insoluble in water, slightly soluble in alcohol, and soluble in vegetable oils and in the usual fat solvents. It is unstable toward light, oxidation, strong acids, and halogens. It easily can be reduced to the corresponding hydroquinone, which in turn can be esterified.

The therapeutic use of vitamin K as a systemic hemostatic agent is based on the critical function that the vitamin performs in blood coagulation. Vitamin K_1 (phytonadione) is effective both in the treatment of hypoprothrombinemia caused by dietary deficiency of the vitamin or malabsorption and in bleeding caused by oral anticoagulants (e.g., coumadin derivatives). Phytonadione exerts prompt and prolonged action. It can be administered orally, subcutaneously, or intramuscularly; in emergencies it can be given by slow intravenous injection.

Vitamin K is administered in conjunction with bile salts or their derivatives in pre- and postoperative jaundiced patients to bring about and maintain a normal prothrombin level in the blood.

In the average infant, the birth values of prothrombin content are adequate, but during the first few days of life, they appear to fall rapidly, even dangerously low, and then slowly

The menadiones are much less active than vitamin K_1 in normalizing the prolonged blood-clotting times caused by dicumarol and related drugs.[62]

Vitamin K_1 is the drug of choice for humans because of its low toxicity. Its duration of action is longer than that of menadione and its derivatives. Vitamin K should not be administered to patients receiving warfarin or coumarin anticoagulants.

Vitamin K can be used to diagnose liver function accurately. The intramuscular injection of 2 mg of 2-methyl-1,4-naphthoquinone has led to response in prothrombin index in patients with jaundice of extrahepatic origin but not in patients with jaundice of intrahepatic origin (e.g., cirrhosis).

Menadione, USP. 2-Methyl-1,4-naphthoquinone; menaphthone; vitamin K_3. Menadione can be prepared readily by the oxidation of 2-methylnaphthalene with chronic acid. It is a bright yellow, crystalline powder and is nearly odorless. It is affected by sunlight. Menadione is practically insoluble in water; it is soluble in vegetable oils, and 1 g of it is soluble in about 60 mL of alcohol. The NF has a caution that menadione powder is irritating to the respiratory tract and to the skin, and an alcoholic solution has vesicant properties.

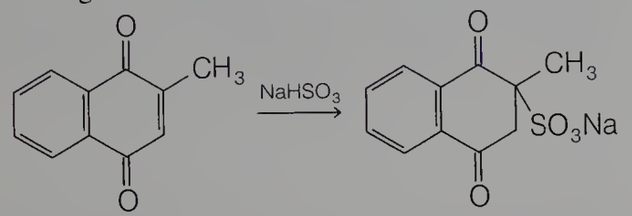
On a mole-for-mole basis, menadione is equal to vitamin K_1 in activity and can be used as a complete substitute for this vitamin. It is effective orally, intravenously, and intramuscularly. Oral absorption occurs in the distal small intestines and the colon. If given orally to patients with biliary

obstruction, bile salts or their equivalent should be administered simultaneously to facilitate absorption. It can be administered intramuscularly in oil when the patient cannot tolerate an oral product or has a biliary obstruction or when a prolonged effect is desired.

Carbon-14-labeled menadiol diacetate in small physiologic doses is converted in vivo to vitamin $K_{2(20)}$, and the origin of the side chain probably is from mevalonic acid. This suggests that menadione may be an intermediate or a provitamin K.[62]

Menadione in oil is three times more effective than a menadione suspension in water. More of menadione than of vitamin K_1 is absorbed orally, but 38% of the former is excreted by the kidney in 24 hours, whereas only very small amounts of the latter are excreted by this route in 24 hours. In rats, menadione in part is reduced to the hydroquinone and excreted as the glucuronide 19% and the sulfate 9.3%.

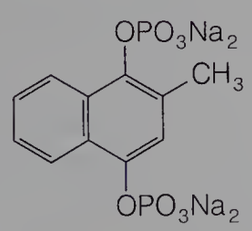
Menadione Sodium Bisulfate. 2-Methyl-1,4-naphthoquinone sodium bisulfite. Menadione bisulfite is prepared by adding a solution of sodium bisulfite to menadione.



Menadione — Menadione sodium bisulfite

Menadione sodium bisulfite occurs as a white, crystalline, odorless powder. One gram of it dissolves in about 2 mL of water, and it is slightly soluble in alcohol. It decomposes in the presence of alkali to liberate the free quinone.

Menadiol Sodium Diphosphate, USP. Tetrasodium 2-methyl-1,4-naphthalenediol bis(dihydrogen phosphate); tetrasodium 2-methylnaphthohydroquinone diphosphate. Vitamin K_4 is a white hygroscopic powder, very soluble in water, giving solutions that have a *p*H of 7 to 9. It is available in ampules for use subcutaneously, intramuscularly, or intravenously and in tablets for oral administration. Unlike the other vitamin K analogues, menadiol[42] oral absorption is not dependent on the presence of bile. Once absorbed, it is converted to menadione.



Menadiol sodium diphosphate

Menadione bisulfite and menadiol diphosphate produce hemolytic symptoms (reticulocytosis, increase in Heinz bodies) in newborn, premature infants when given in excessive doses (more than 5 to 10 mg/kg). In severe cases, overt hemolytic anemia with hemoglobinuria may occur. The increased red cell breakdown may lead to hyperbilirubinemia and kernicterus.

These compounds also may interfere with bile pigment secretion. Newborns with a congenital defect of glucose-6-phosphate dehydrogenase can react with severe hemolysis, even with small doses of menadione derivatives. However, small nonhemolyzing doses can be used in the newborn, and combination with vitamin E is not considered essential.[63]

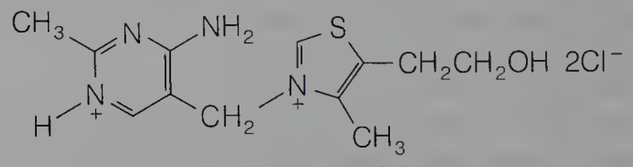
WATER-SOLUBLE VITAMINS

Although these vitamins are structurally diverse, they are characterized as a general class on the basis of water solubility to distinguish them from the lipid-soluble vitamins. This class includes the B-complex vitamins and ascorbic acid (vitamin C). The term "B-complex vitamins" usually refers to thiamine, riboflavin, pyridoxine, nicotinic acid, pantothenic acid, biotin, cyanocobalamin, and folic acid. Dietary deficiencies of any one of the B vitamins commonly are complicated by deficiencies of more than one member of the group; hence, treatment with B-complex preparations usually is indicated.

THIAMINE (VITAMIN B_1)

Thiamine was the first water-soluble vitamin to be discovered (1926), but the complete determination of its structure and synthesis were not accomplished until 1936.

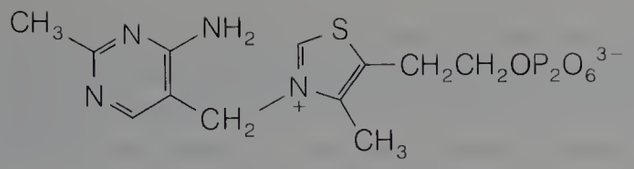
Many natural foods provide adequate amounts of this vitamin. The germ of cereals, brans, egg yolks, yeast extracts, peas, beans, and nuts usually provide enough thiamine to satisfy adult requirements. The requirement for thiamine is related directly to caloric intake, 0.2 to 0.3 mg/1000 calories. It is not economically practical to isolate the crystalline vitamin from natural sources on a commercial scale; hence, commercially available thiamine is prepared synthetically.



Thiamine hydrochloride

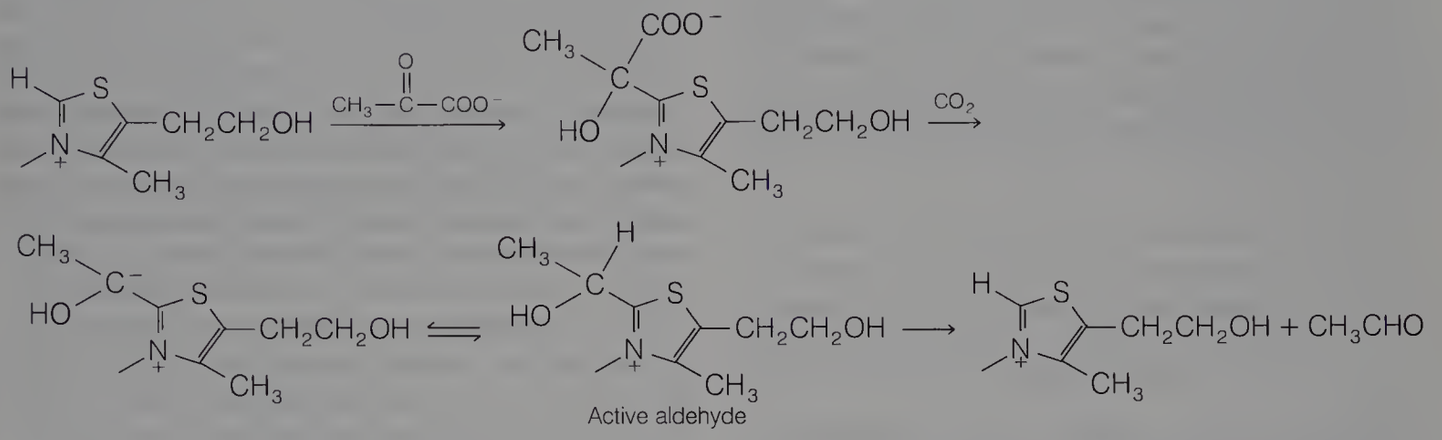
Thiamine is synthesized biologically from the pyrimidine derivative 4-amino-5-hydroxymethyl-2-methyl pyrimidine methylpyrimidine and 5-(β-hydroxyethyl)-4-methylthiazole. These two precursors are converted to phosphate derivatives under kinase catalysis, which requires ATP. The respective phosphate derivatives then interact to form thiamine phosphate. The latter reaction is catalyzed by thiamine phosphate pyrophosphorylase.

In higher mammalian organisms, thiamine is transformed to the coenzyme thiamine pyrophosphate by direct pyrophosphate transfer from ATP. This coenzyme performs important metabolic functions, for example, as cocarboxylase in the decarboxylation of α-keto acids (e.g., pyruvate to form acetyl-CoA) and in transketolases (e.g., utilization of pentoses in the hexose monophosphate shunt).



Thiamine pyrophosphate

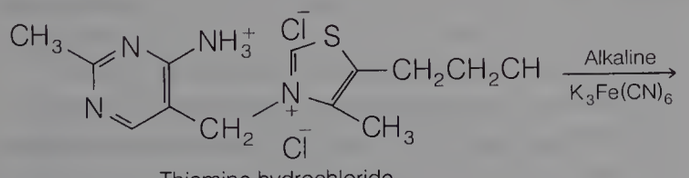
In the decarboxylation of pyruvate, the coenzyme interacts with pyruvic acid to form so-called active aldehyde, as shown below.



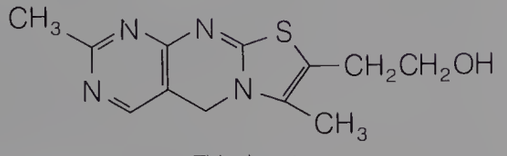
Active aldehyde

The active aldehyde intermediate then interacts with thioctic acid to form acetyl-thioctate, which is responsible for acetylating CoA-SH to form acetyl-CoA. In deficiency states, the oxidation of α-keto acids is decreased, resulting in increased pyruvate levels in the blood.

Thiamine hydrochloride is stable in acid but is unstable in aqueous solutions of *p*H greater than 5. Under these conditions, it undergoes decomposition and inactivation. Thiamine is also susceptible to oxidation. It is oxidized readily by exposure to the atmosphere or by oxidizing reagents such as hydrogen peroxide, permanganate, or alkaline potassium ferricyanide. This oxidation forms thiochrome, as represented below.



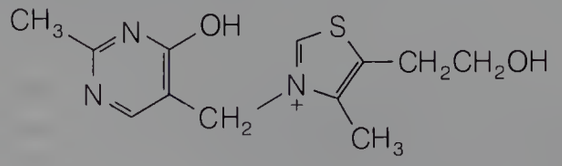
Thiamine hydrochloride



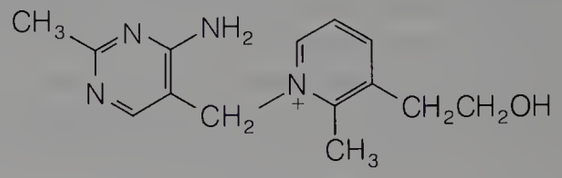
Thiochrome

Thiochrome exhibits a vivid blue fluorescence; hence, this reaction is the basis for the quantitative fluorometric assay of thiamine in the *USP*.

Oxythiamine and neopyrithiamine are antivitamins used in the study of the deficiency state. Oxythiamine is a competitive inhibitor of thiamine pyrophosphate. Neopyrithiamine inhibits the pyrophosphorylation of thiamine.



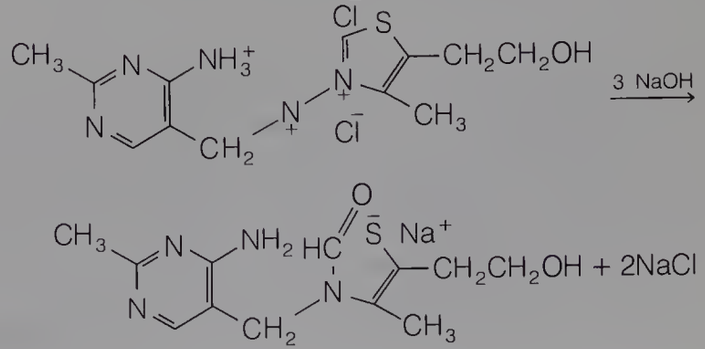
Oxythiamine



Neopyrithiamine

Thiamine Hydrochloride, USP. Thiamine monohydrochloride; thiamine chloride; vitamin B_1 hydrochloride; vitamin B_1; aneurine hydrochloride. This occurs as small, white crystals or as a crystalline powder; it has a slight, characteristic yeast-like odor. The anhydrous product, when exposed to air, will absorb rapidly about 4% of water. One gram is soluble in 1 mL of water and in about 100 mL of alcohol. It is soluble in glycerin. An aqueous solution, 1:20, has a *p*H of 3. Aqueous solutions 1:100 have a *p*H of 2.7 to 3.4. Thiamine has pK_a values of 4.8 and 9.0.

Thiamine hydrochloride is sensitive to alkali. The addition of 3 moles of sodium hydroxide per mole of thiamine hydrochloride reacts as shown below.



Thiamine hydrochloride absorption is a sodium-dependent process; thus, saturation limits absorption to 8 to 15 mg daily. Absorption is decreased in alcoholism and cirrhosis. Food affects the rate, but not the amount, absorbed. After absorption thiamine hydrochloride is distributed to all tissue. There is limited storage of the vitamin in the body (about 30 mg).

Normally, little or no thiamine is excreted in the urine. However, if doses exceed physiologic needs after body stores are saturated, thiamine can be found in the urine as pyrimidine or as the unchanged compound.

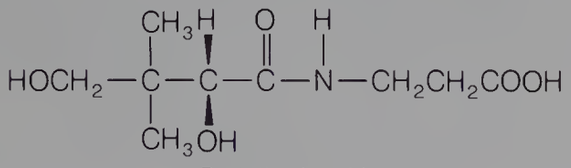
Severe thiamine deficiency is called *beriberi*. The major organs affected are the nervous system (in dry beriberi), the cardiovascular system (in wet beriberi), and the gastrointestinal tract. Thiamine administration reverses the gastrointestinal and cardiovascular symptoms. However, the neurologic damage may be permanent if the deficiency has been severe or of long duration.

Thiamine hydrochloride is indicated in the treatment or prophylaxis of thiamine deficiencies. Dietary deficiencies are rare in the United States; alcoholism is the most common cause of the disease. Alcoholics have poor dietary habits (deficient diet), and alcohol interferes with absorption of the vitamin.

Thiamine Mononitrate, USP. Thiamine nitrate; vitamin B_1 mononitrate. Thiamine mononitrate is a colorless compound that is soluble in water 1:35 and slightly soluble in alcohol. Two percent aqueous solutions have a *p*H of 6.0 to 7.1. This salt is more stable than the chloride hydrochloride in the dry state, less hygroscopic, and recommended for multivitamin preparations and the enrichment of flour mixes.

PANTOTHENIC ACID

During the 1930s, R. J. Williams and his collaborators recognized, isolated, and synthesized pantothenic acid. Because its occurrence is so widespread, it was called "pantothenic acid" from the Greek, meaning "from everywhere."



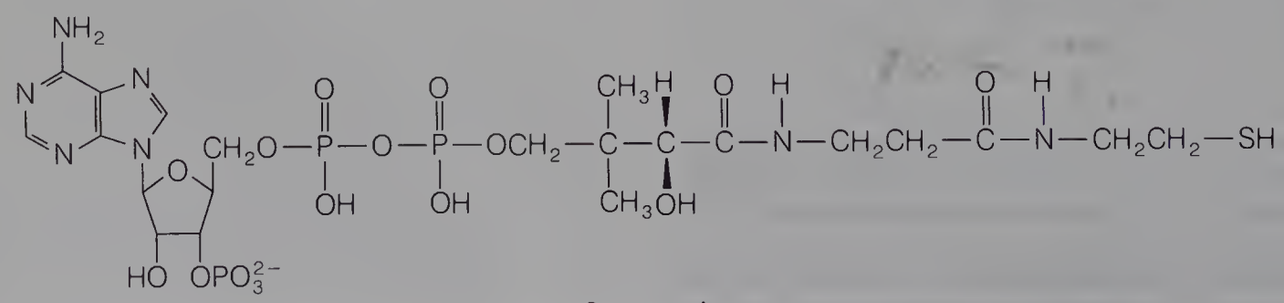
Pantothenic acid

Pantothenic acid also has been called vitamin B_5. Excellent sources of the vitamin are liver, eggs, and cereals. However, it is found in the form of CoA. This coenzyme cannot be absorbed directly from the gut. While no experiments have been conducted in humans, studies on animals indicate that the coenzyme must be hydrolyzed to panthenene and pantothenate.[28] These two are absorbed by passive diffusion. Human intestinal cells contain enzymes capable of hydrolyzing the coenzyme. Pantothenate is the major form circulating in the blood and is absorbed by individual cells. Once inside the cell, CoA is synthesized.

This vitamin is synthesized by most green plants and microorganisms. The precursors are γ-ketoisovaleric acid and β-alanine.[1] The latter originates from the decarboxylation of aspartic acid. γ-Ketoisovaleric acid is converted to ketopantoic acid by N^5, N^{10}-methylenetetrahydrofolic acid; then, upon reduction, pantoic acid is formed. Finally, pantoic acid and β-alanine react by amide formation to form pantothenic acid.

The metabolic functions of pantothenic acid in human biochemistry are mediated through the synthesis of CoA; this vitamin is a structural component of CoA, which is necessary for many important metabolic processes. Pantothenic acid is incorporated into CoA by a series of five enzyme-catalyzed reactions. CoA is involved in the activation of fatty acids before β-oxidation; this activation requires ATP to form the respective fatty acyl-CoA derivatives. It also participates in fatty acid β-oxidation in the final step, forming acetyl-CoA. The latter also is formed from pyruvate decarboxylation. In pyruvate decarboxylation, CoA participates in collaboration with thiamine pyrophosphate and lipoic acid, two other important coenzymes. Thiamine pyrophosphate is the actual decarboxylating coenzyme that functions with lipoic acid to form acetyldihydrolipoic acid from the decarboxylation of pyruvate. CoA then accepts the acetyl group from acetyldihydrolipoic acid to form acetyl-CoA. Acetyl-CoA participates as an acetyl donor in many processes and is the precursor in important biosyntheses (e.g., those of fatty acids, steroids, porphyrins, and acetylcholine).

Clinical cases of pantothenic acid deficiency do not commonly develop, unless they arise in combination with deficiencies of the other B vitamins.[2] Accordingly, pantothenic



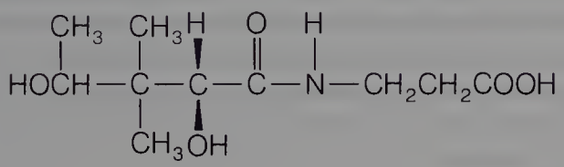
Coenzyme A

acid usually is included in multivitamin preparations. The calcium salt is commonly used in pharmaceutical preparations. Panthenol, the alcohol derivative, is another form commonly used.

Products

Pantothenic Acid. Vitamin B_5 occurs as a viscous hygroscopic oil, freely soluble in water but unstable to heat, acid, and alkali. Because of this instability, the calcium salt is used commercially.

Pantothenic acid and its derivatives have essentially no pharmacologic actions per se. Because of the ubiquitous nature of the vitamin, deficiency states usually do not develop. They have been produced by using synthetic diets devoid of the vitamin or by use of a vitamin antagonist. ω-Methylpantothenic acid has produced the following symptoms: fatigue, headache, paresthesia of the hands and feet, cardiovascular instability, and gastrointestinal problems.

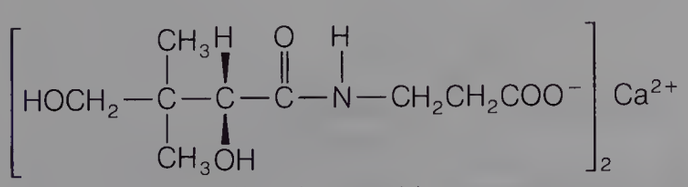


ω-Methylpantothenic acid

Pantothenic acid and its derivatives are readily absorbed and widely distributed. The highest concentrations are found in the liver, adrenal glands, heart, and kidneys. It appears that pantothenic acid undergoes little if any metabolism, for the amount eliminated approximates the amount consumed. Approximately 70% of a dose is eliminated unchanged in the urine, with the remainder found in the feces.

The only official indication of pantothenic acid is in the prevention or treatment of vitamin B deficiencies. Because a deficiency of a single B vitamin is rare, it is commonly formulated in multivitamin preparations or B-complex preparations.

Calcium Pantothenate, USP. Calcium D-pantothenate is a slightly hygroscopic, white, odorless, bitter powder that is stable in air. It is insoluble in alcohol and soluble 1:3 in water; aqueous solutions have a *p*H of about 9 and $[\alpha]_D = +25°$ to $+27.5°C$. Autoclaving calcium pantothenate at 120°C for 20 minutes may cause a 10% to 30% decomposition. Some of the phosphates of pantothenic acid that occur naturally in coenzymes are quite stable to both acid and alkali, even upon heating.[64]

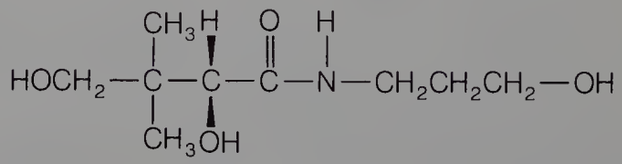


Pantothenate calcium

Racemic Calcium Pantothenate, USP, provides a more economical source of this vitamin. Other than containing not less than 45% of the dextrorotatory biologically active form, its properties are very similar to those of Calcium Pantothenate, USP.

Panthenol, USP, the racemic alcohol analogue of pantothenic acid, exhibits both qualitatively and quantitatively the vitamin activity of pantothenic acid. It is considerably more stable than pantothenic acid in solutions with *p*H values of 3 to 5 but of about equal stability at *p*H 6 to 8. It appears to be absorbed more readily from the gut, particularly in the presence of food.

Dexpanthenol, USP, occurs as a slightly hygroscopic, viscous oil freely soluble in water and alcohol. It is the dextrorotatory alcohol derivative of pantothenic acid. Dexpanthenol is converted readily in vivo to the acid form.

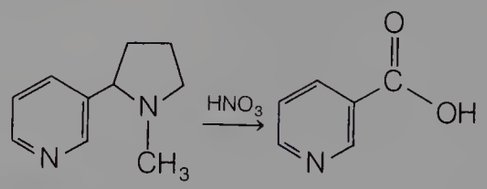


Dexpanthenol

Dexpanthenol and the racemic mixture are used in the treatment of paralytic ileus and postoperative distention. Dexpanthenol in combination with choline is used to relieve gas retention.

NICOTINIC ACID

Nicotinic acid (niacin) was prepared first by oxidation of the alkaloid nicotine, but not until 1913 was it isolated from yeast and recognized as an essential food factor (refer to the structures). In 1934–1935, nicotinamide was obtained from the hydrolysis of a coenzyme isolated from horse red blood cells. This coenzyme was later named "coenzyme II" and is now more commonly called nicotinamide adenine dinucleotide phosphate (NADP).

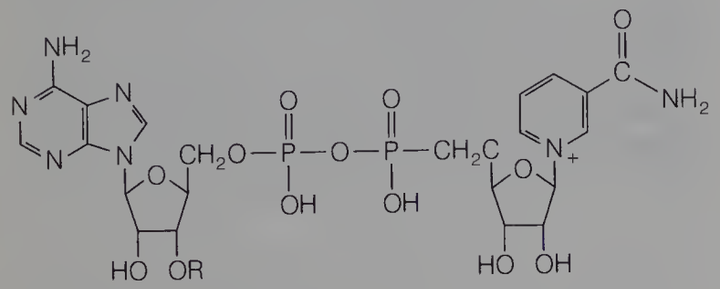


Nicotine — Nicotinic acid (Niacin)

Generous sources of this vitamin include pork, lamb, and beef livers; hog kidneys; yeasts; pork; beef tongue; hearts; lean meats; wheat germ; peanut meal; and green peas.

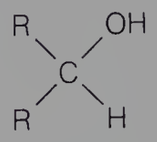
Nicotinic acid can be synthesized by almost all plants and animals. Tryptophan can be metabolized to a nicotinic acid nucleotide in animals, but the efficiency of this multistep process varies from species to species. Plants and many microorganisms synthesize this vitamin through alternative routes using aspartic acid.

In the human, nicotinic acid reacts with 5-phosphoribosyl-1-pyrophosphate to form nicotinic acid mononucleotide, which then reacts with ATP to produce desamido-NAD (the intermediate dinucleotide with the nicotinic acid moiety). Finally, the latter intermediate is converted to NAD (nicotinamide adenine dinucleotide, originally called "coenzyme I") by transformation of the carboxyl of the nicotinic acid moiety to the amide by glutamine. This final step is catalyzed by NAD synthetase; NADP is produced from NAD by ATP under kinase catalysis.[1]

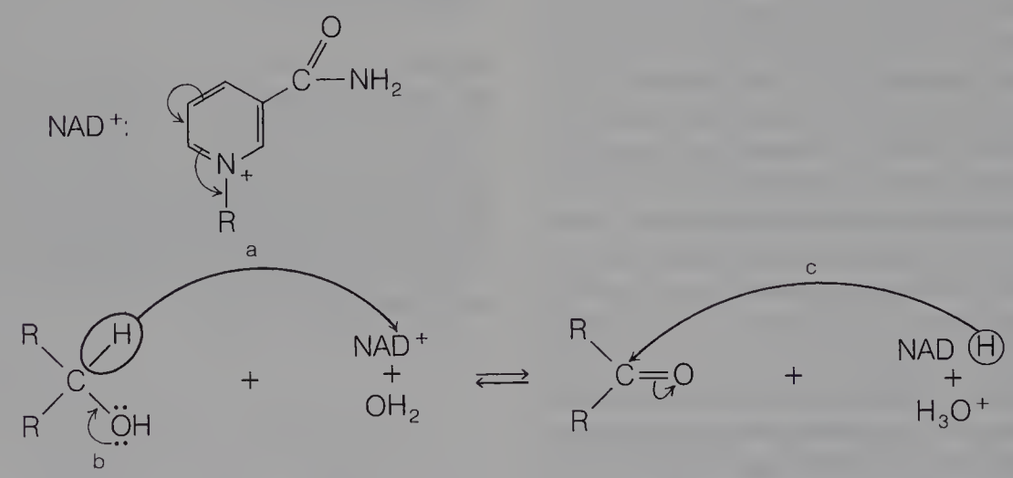


R = H = Nicotinamide adenine dinucleotide
R = PO_3^{2-} = Nicotinamide adenine dinucleotide phosphate

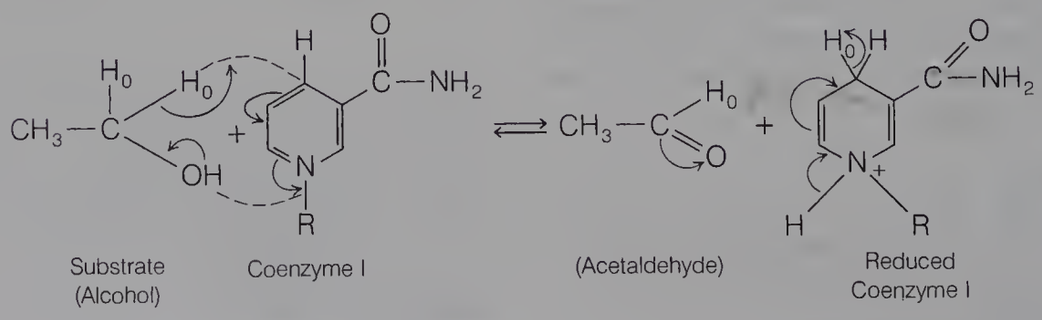
NAD and NADP participate as oxidizing coenzymes for many (more than 200) dehydrogenases. Some dehydrogenases require NAD, others require NADP, and some function with either. The following generalized representation (Fig. 27-1) illustrates the function of these coenzymes in metabolic oxidations and reductions. The abbreviation [NAD^+] emphasizes the electrophilicity of the pyridine C_4 moiety (which is the center of reactivity) and the substrate designated as



could be a primary or secondary alcohol. Arrow *a* in Fig. 27-1 symbolizes the function of NAD as oxidant in the hydride transfer from the substrate to the coenzyme forming NADH, reduced coenzyme. The hydroxyl of the substrate is visualized as undergoing deprotonation concertedly by either water or the pyridine nitrogen of NADH. Arrow *b* shows concerted formation of the carbonyl π-bond of the oxidation product. Arrow *c* symbolizes the reverse hydride transfer from reduced coenzyme, NADH, to the carbonyl carbon; and concertedly, as the carbonyl oxygen undergoes protonation, the reduction of the carbonyl group forms the corresponding alcohol. Thus, NAD and NADP function as hydride acceptors, whereas NADH and NADPH are hydride donors. Although the foregoing is a simplistic representation, it illustrates the dynamism of such oxidation-reduction reactions effected by these coenzymes under appropriate dehydrogenase catalysis. Alternatively, the reduced coenzymes may be utilized in ATP production through the electron-transport system.



Ethanol Oxidation Is Schematically Illustrated Below:



Substrate (Alcohol) | Coenzyme I | (Acetaldehyde) | Reduced Coenzyme I

FIG. 27-1. *Generalized representation of the hydride transfer reaction.*

As noted earlier, nicotinic acid (also known as niacin to avoid confusion with nicotine) can be made available in nucleotide form from the amino acid tryptophan. It has been estimated that 60 mg of tryptophan equal 1 mg of nicotinic acid. Consequently, humans and other mammals can synthesize the vitamin, provided there is appropriate dietary availability of tryptophan. (Thus, it appears that nicotinic acid is not a true vitamin according to the classic definition of the term.)

Niacin, USP. Nicotinic acid; 3-pyridine carboxylic acid; vitamin B_3. Niacin occurs as white crystals or as a crystalline powder. It is odorless or it may have a slight odor. One gram of nicotinic acid dissolves in 60 mL of water. It is freely soluble in boiling water, boiling alcohol, and solutions of alkali hydroxides and carbonates but is almost insoluble in ether. A 1% aqueous solution has a *p*H of 6. Nicotinic acid has a pK_α of 4.85.

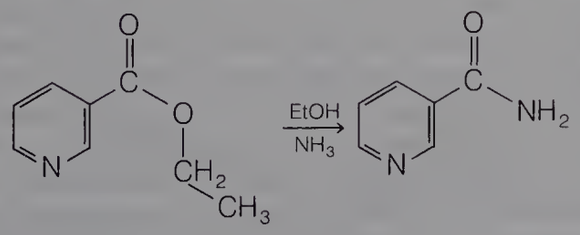
Nicotinic acid is stable under normal storage conditions. It sublimes without decomposition.

Serious deficiency of niacin or tryptophan may lead to pellagra (from the Italian, *pelle agra*, for "rough skin"). The major systems affected are the gastrointestinal tract (diarrhea, enteritis, stomatitis), the skin (dermatitis), and the central nervous system (headache, dizziness, depression). Severe cases may result in delusions, hallucinations, and dementia. In the United States, pellagra has become rare because flour is supplemented with nicotinic acid. Chronic alcoholism is the chief cause of pellagra and is associated with multiple vitamin deficiency. The symptoms of pellagra are completely reversed by niacin; therefore, it is indicated for the treatment and prevention of the deficiency.

Niacin, but not niacinamide, also is indicated in hyperlipidemia to lower triglycerides and cholesterol. Triglycerides, VLDLs, and LDLs are reduced, whereas HDLs are increased. The exact mechanism is not known. The dose required (1 to 3 g three times daily) often limits the usefulness, for niacin has a direct vasodilatory effect in high doses. This effect is believed to be mediated through the prostaglandins.

Niacin is absorbed readily from the gastrointestinal tract and distributed widely. At physiologic doses, little niacin is excreted unchanged. Most is excreted as *N*-methylniacin or as the glycine conjugate (nicotinuric acid). After administration of large doses, niacin can be found in the urine unchanged.

Niacinamide, USP. Nicotinamide; nicotinic acid amide. Nicotinamide is prepared by the amidation of esters of nicotinic acid or by passing ammonia gas into nicotinic acid at 320°C.



Ethyl Nicotinate — Nicotinamide
Niacinamide

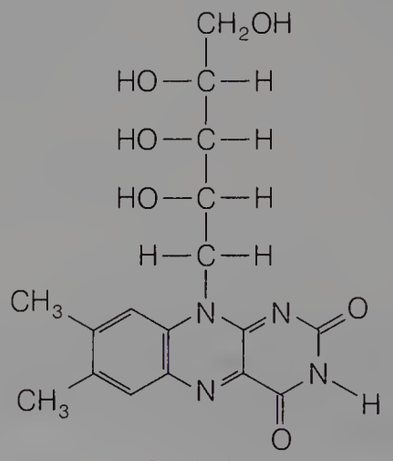
Nicotinamide is a white, crystalline powder that is odorless, or nearly so, and bitter. One gram is soluble in about 1 mL of water, 1.5 mL of alcohol, and about 10 mL of glycerin. Aqueous solutions are neutral to litmus. For occurrence, action, and uses, see nicotinic acid. Niacinamide has pK_α values of 0.5 and 3.35.

Similar to niacin, niacinamide is indicated in the treatment and prevention of deficiency states. Unlike niacin, niacinamide has no vasodilatory effect, which may be of therapeutic importance for compliance reasons. Niacinamide has no effect on triglycerides and lipoproteins. This product is formulated with potassium iodide and used as an iodine supplement.

Niacinamide hydrochloride is also available. It is more stable in solution and more compatible with thiamine chloride in solution.

RIBOFLAVIN (VITAMIN B_2)

Although the isolation of crystalline riboflavin was not accomplished until 1932, interest in this compound as a pigment dates back to 1881 in connection with the color in the whey of milk. In 1932, riboflavin was isolated as a coenzyme–enzyme complex from yeast by Warburg and Christian, and this complex was designated as *yellow oxidation ferment.*

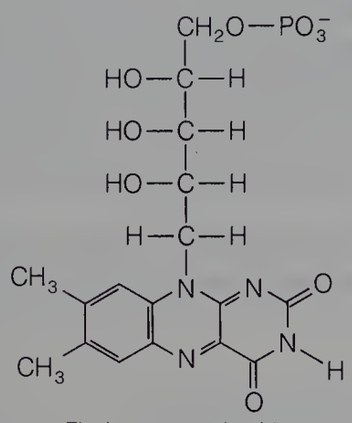


Riboflavin

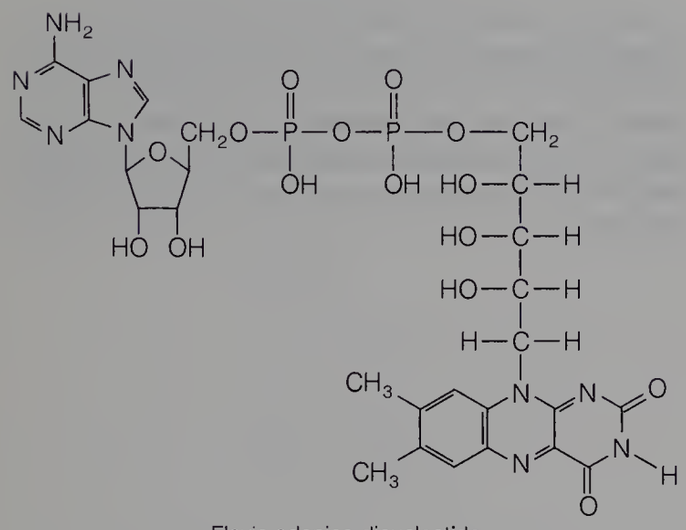
Riboflavin is synthesized by all green plants and by most bacteria and fungi. Although yeast is the richest source, eggs, dairy products, legumes, and meats are the major sources in the diet. It is known that the precursor is a guanosine phosphate derivative, but the exact synthetic steps leading to the vitamin are not understood completely.

In higher mammals, riboflavin is absorbed readily from the intestine and distributed to all tissues. It is the precursor in the biosynthesis of the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). The metabolic functions of this vitamin involve these two coenzymes, which participate in numerous vital oxidation-reduction processes. FMN, which is riboflavin-5′-phosphate, is produced from the vitamin and ATP under flavokinase catalytic action. This step can be inhibited by phenthiazines and the tricyclic

antidepressants. FAD originates from an FMN and ATP reaction that involves reversible dinucleotide formation catalyzed by flavin nucleotide pyrophosphorylase. These coenzymes function in combination with several enzymes as coenzyme–enzyme complexes, often characterized as *flavoproteins*.

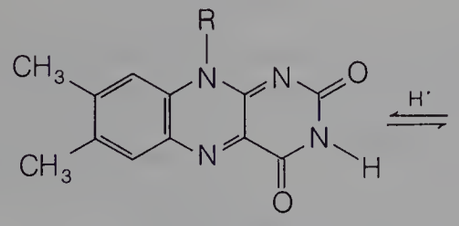


Flavin mononucleotide

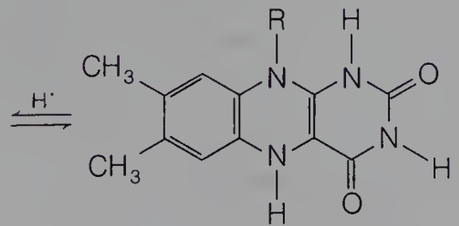


Flavin adenine dinucleotide

These flavoproteins function in aerobic or anaerobic conditions as oxidases and dehydrogenases. Examples include glucose oxidase, xanthine oxidase, cytochrome reductase, and acyl-CoA dehydrogenase.



Oxidized species

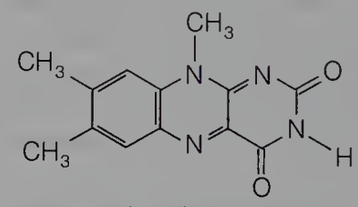


Reduced species

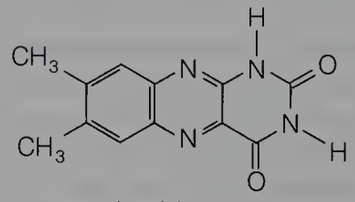
The riboflavin moiety of the complex is considered to be a hydrogen-transporting agent (carrier) functioning as hydrogen acceptors; the hydrogen donors may be NADH, NADPH, or some suitable substrate. The isoalloxazine rings accept two hydrides stepwise to form the dihydroriboflavin derivative.

Riboflavin, USP. Riboflavin; lactoflavin; vitamin B_2; vitamin G. Riboflavin is a yellow to orange-yellow, crystalline powder with a slight odor. It is soluble in water 1:3000 to 1:20,000 mL, the variation in solubility being due to differences in internal crystalline structure, but it is more soluble in an isotonic solution of sodium chloride. A saturated aqueous solution has a *p*H of 6. Riboflavin has a pK_a of 10.5. It is less soluble in alcohol and insoluble in ether or chloroform. Benzyl alcohol (3%), gentistic acid (3%), urea in varying amounts, and niacinamide are used to solubilize riboflavin when relatively high concentrations of this factor are needed for parenteral solutions. Gentistic ethanol amide and sodium 3-hydroxy-2-naphthoate are also effective solubilizing agents for riboflavin.

When dry, riboflavin is not affected appreciably by diffused light; however, it deteriorates in solution in the presence of light, and this deterioration is very rapid in the presence of alkalies, producing lumiflavin. This deterioration may be retarded by buffering on the acid side. However, under acid conditions, light can produce lumichrome. Neither of these decomposition products possesses biologic activity.



Lumiflavine



Lumichrome

The vitamin is commercially available as riboflavin, riboflavin 5-phosphate, and riboflavin 5-phosphate sodium. The phosphate esters are used commercially only in multivitamin preparations. The phosphate esters are hydrolyzed before absorption occurs. Absorption occurs through an active transport system. Riboflavin is phosphorylated by the intestinal mucosa during the absorption process. Food and bile enhance the absorption. Riboflavin is distributed widely in the body, with limited stores in the liver, spleen, heart, and kidneys. Conversion to FAD occurs primarily in the liver. FMN and FAD circulate primarily protein-bound. Only small amounts (~ 9%) are excreted in the urine unchanged. Larger amounts can be found after administration of large doses.

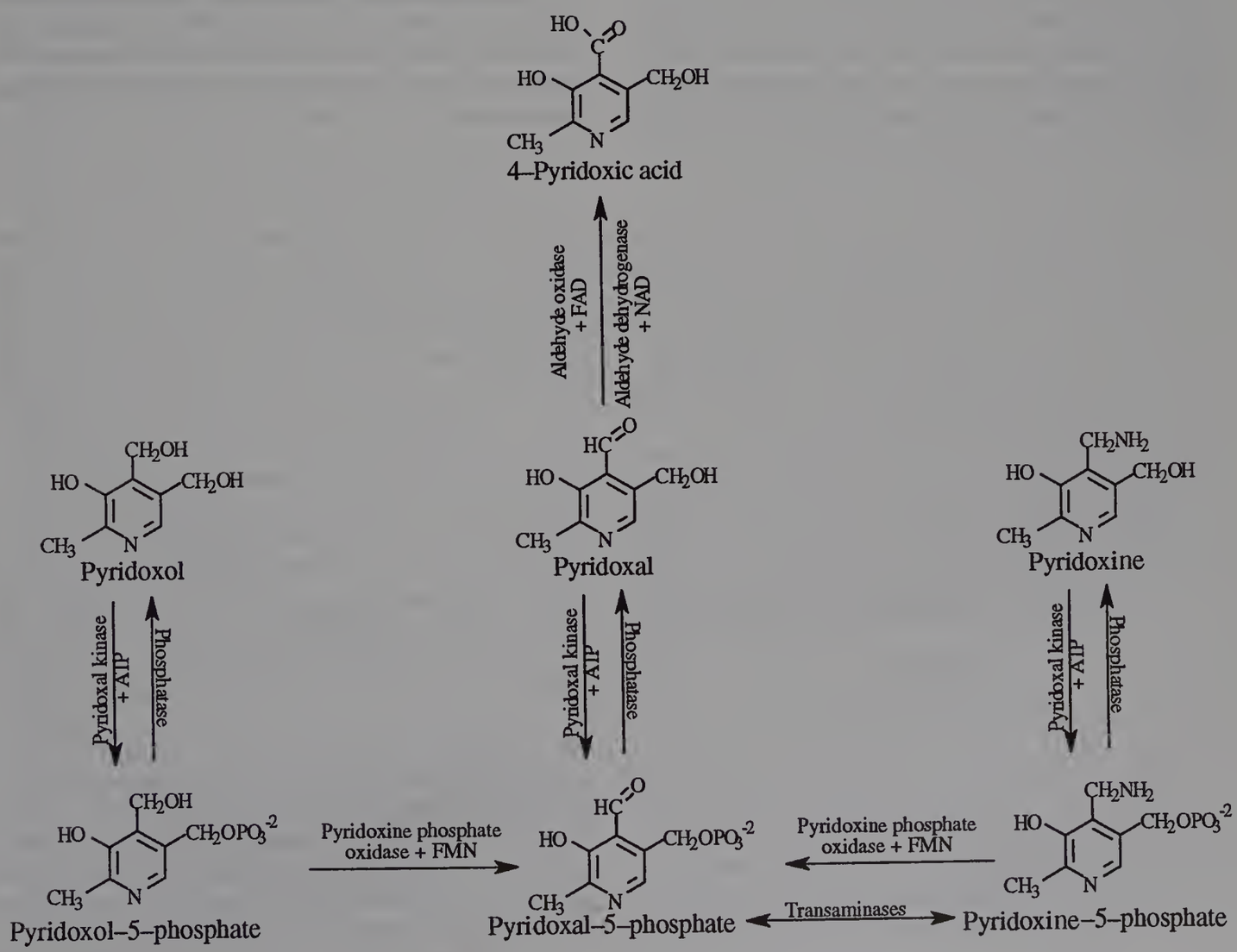
Severe riboflavin deficiency is known as ariboflavinosis. Its major symptoms include chelosis, seborrheic dermatitis, and vascularization of the cornea. Ariboflavinosis occurs in chronic alcoholism, in combination with other vitamin deficiencies. It also has resulted from phenothiazine, tricyclic antidepressant, and probenecid therapy.

Riboflavin has no pharmacologic action and is relatively nontoxic. The only approved indication is in the treatment and prevention of ariboflavinosis.

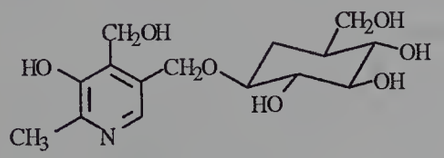
PYRIDOXINE

In 1935, the term "vitamin B_6" was applied to the principle that cured a dermatitis in rats fed a vitamin-free diet supplemented with thiamine and riboflavin. Three years later, vitamin B_6 was isolated from rice paste and yeast. In 1935, P. Gyorgy showed that rat pellagra was not the same as human pellagra but that it resembled a particular disease of infancy known as "pink disease," or acrodynia. This rat acrodynia is characterized by a symmetric dermatosis affecting first the paws and the tips of the ears and the nose. These areas become swollen, red, and edematous, with ulcers developing frequently around the snout and on the tongue. Thickening and scaling of the ears is noted, and there is a loss of weight, with fatalities occurring in from 1 to 3 weeks after the appearance of the symptoms. Gyorgy was able to cure these conditions with a supplement obtained from yeast, which he called "vitamin B_6." In 1938, this factor was isolated from rice paste and yeast in a crystalline form in a number of laboratories. A single dose of about 100 μg produced healing in 14 days in a rat having severe vitamin B_6-deficiency symptoms.

Chemical tests, electrometric titration determinations, and absorption spectrum studies gave clues to its composition. These were substantiated by the synthesis of vitamin B_6 (1938 and 1939). This vitamin also is known as pyridoxine or pyridoxol. Two additional chemical forms, pyridoxal and pyridoxamine, have been isolated from natural sources. These three compounds are interrelated metabolically and functionally. Currently, the term "vitamin B_6" is applied to all three forms of the vitamin. The interconversion between the different forms is shown in the following scheme.



Pyridoxine is available from whole-grain cereals, peanuts, corn, meat, poultry, and fish. However, up to 40% of the vitamin may be destroyed during cooking. Food sources contain all three forms, either in their free form or phosphorylated. Plants contain primarily pyridoxol and pyridoxamine, while animal sources provide chiefly pyridoxal. Many plants also contain a glycoside of pyridoxol, which is included in vitamin content determinations. While this conjugate is absorbed, it is not utilized well.[5] This may account for the lower bioavailability of plant sources when compared to animal sources.



5–O–(β–D–glycospyranosyl) pyridoxol

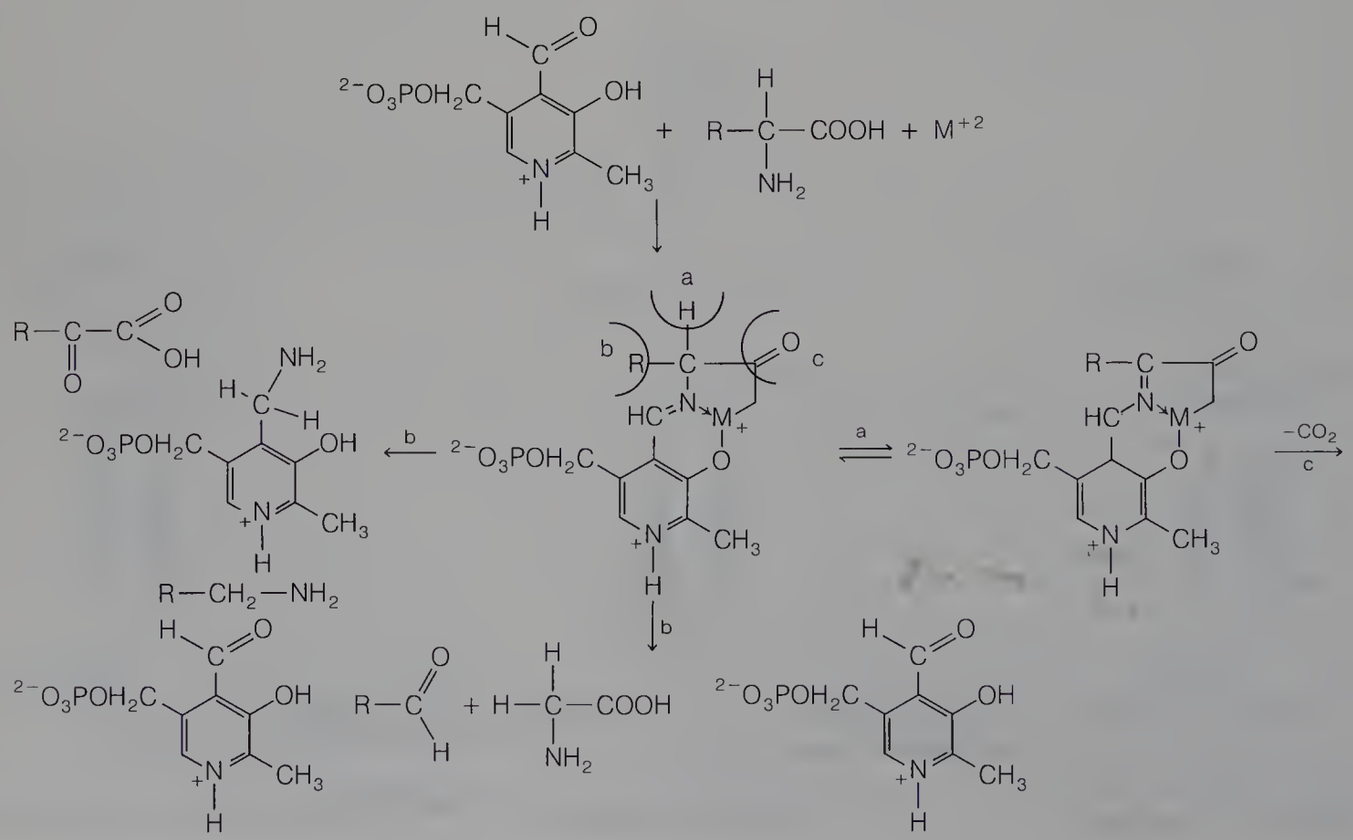
Vitamin B_6 is absorbed via passive diffusion chiefly in the jejunum and transported to the liver. The liver is a major organ of storage (16 to 27 mg), metabolism, and interconversion between the three forms. Excess B_6 is oxidized to 4-pyridoxic acid. This acid is the primary form found in the urine, accounting for up to 60% of ingested vitamin B_6.

In the liver, all three forms are converted to pyridoxal-5-phosphate. This latter form circulates in the blood bound to albumin. Before entry into cells, the phosphate must be dephosphorylated. Inside the cells, pyridoxal kinase rephosphorylates the vitamin. This kinase is found in many cell types; however, other enzymes involved in the interconversions are not expressed in all cells.[65]

Although large amounts of the vitamin are found in the liver, the muscles contain higher amounts in the form of glycogen phosphorylase.[66] However, this vitamin B_6 is not readily available during deficiency states.[67]

Pyridoxal-5-phosphate is a coenzyme[68,69] that performs many vital functions in human metabolism. This coenzyme functions in the transaminations and decarboxylations that amino acids generally undergo; for example, it functions as a cotransaminase in the transamination of alanine to form pyruvic acid and as a codecarboxylase in the decarboxylation of dopa to form dopamine. Other biologic transformations[64,68] of amino acids in which pyridoxal can function are racemization, elimination of the α-hydrogen together with a β-substituent (i.e., OH or SH) or a γ-substituent, and probably the reversible cleavage of β-hydroxyamino acids to glycine and carbonyl compounds.

An electromeric displacement of electrons from bonds a, b, or c (see diagram below) would result in the release of a cation (H, R′, or COOH) and, subsequently, lead to the variety of reactions observed with pyridoxal. The extent to which one of these displacements predominates over others depends on the structure of the amino acid and the environment (*p*H, solvent, catalysts, enzymes, and such). When this mechanism applies in vivo, the pyridoxal component is linked to the enzyme through the phosphate of the hydroxymethyl group.



Metals such as iron and aluminum, which markedly catalyze nonenzymatic transaminations in vitro, probably do so by promoting formation of the Schiff base and maintaining planarity of the conjugated system through chelate ring formation, which requires the presence of the phenolic group. This chelated metal ion also provides an additional electron-attracting group, which operates in the same direction as the heterocyclic nitrogen atom (or nitro group), thereby increasing the electron displacements from the α-carbon atom as shown above.

It also should be noted that certain hydrazine derivatives, when administered therapeutically (e.g., isoniazid), can induce a deficiency of the coenzyme (pyridoxal-5-phosphate) by inactivation through the mechanism of hydrazone formation with the aldehyde functional group.

Another hydrazine derivative, hydralazine, when administered in high doses to control hypertension, can cause similar B_6 deficiency, conceivably through a similar mechanism involving hydrazone formation.

Hypochromic anemias caused by familiar-type pyridoxine dependency respond to pyridoxine therapy. Similarly, this vitamin has been useful in the treatment of hypochromic or megaloblastic anemias that are not due to iron deficiency and do not respond to other hematopoietic agents.[2]

A review by Rose[70] summarizes studies on the effects of certain hormones on vitamin B_6 nutrition in humans, on the biochemical interrelationship between steroid hormones and pyridoxal phosphate-dependent enzymes, and on the role of vitamin B_6 in regulating hypothalamus–pituitary functions. Some of these studies have important clinical implications that are noteworthy. The use of estrogen-containing oral contraceptives has been investigated as a factor leading to an abnormality of tryptophan metabolism. This abnormality resembles a dietary vitamin B_6 deficiency and responds favorably to treatment with the vitamin. For some time, there has been clinical interest in the relationship between certain hormones and vitamin B_6 function because abnormal urinary excretions of tryptophan metabolites were observed during pregnancy and in patients with hyperthyroidism.

Estrogens and tryptophan metabolism have been studied because estrogen administration occasionally leads to the excretion of abnormally large amounts of xanthurenic acid, a metabolic product of tryptophan. This metabolic malfunction has been related to the inhibitory effect of estrogen sulfate conjugates on another pathway of tryptophan metabolism—the transamination of kynurenine from tryptophan. Consequently, xanthurenic acid formation appears to be increased abnormally owing to this estrogen effect or to B_6 deficiency.

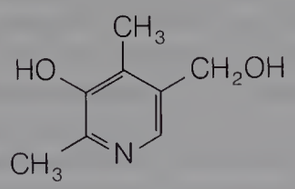
In vitro studies have been conducted to determine the effect of estrogens on kynurenine aminotransferase, which catalyzes the B_6-dependent transamination of kynurenine to kynurenic acid. Some estrogen conjugates (e.g., estradiol disulfate and diethylstilbestrol sulfate) interfere with this transamination, apparently by reversible inhibition of the aminotransferase apoenzyme. It appears that the estrogen sulfate competes with pyridoxal-5-phosphate for interaction with the apoenzyme. In contrast, free estradiol and estrone do not possess this inhibitory property.

Some women suffer from mental depression when taking estrogen-containing oral contraceptives, and this depression could be due to another malfunction in tryptophan metabolism leading to 5-hydroxytryptamine (serotonin). There is some evidence that the decarboxylation of 5-hydroxytryptophan is inhibited (in vitro) by estrogen conjugates competing with pyridoxal phosphate for the decarboxylase apoenzyme.

Other endocrine systems are interrelated. Both corticosteroids and thyroid hormones may increase the requirement for pyridoxine and affect pyridoxal-5-phosphate-dependent metabolic processes. Moreover, there appear to be associations between vitamin B_6 and anterior pituitary hormones. These associations seem to involve the hypothalamus, 5-hydroxytryptamine, and dopamine. The latter two neurotransmitters are synthesized by metabolic processes that require pyridoxal-5-phosphate.

Interestingly, studies have shown that vitamin B_6 in turn influences the function of steroid hormones.[20] Pyridoxal-5-phosphate binds to lysine residues on the steroid receptors. It has been proposed that the lysine residues are found at the site where the steroid binds to the receptor and where the receptor binds DNA.[65] Thus, pyridoxal-5-phosphate decreases the number of receptors able to bind with the steroids and the number of steroid–receptor complexes binding to DNA. The overall result is decreased expression of DNA.

Pyridoxine Hydrochloride, USP. 5-Hydroxy-6-methyl-3,4-pyridinedimethanol hydrochloride; vitamin B_6 hydrochloride; rat antidermatitis factor. Pyridoxine hydrochloride is a white, odorless, crystalline substance that is soluble 1:5 in water and 1:100 in alcohol and insoluble in ether. It is relatively stable to light and air in the solid form and in acid solutions at a *p*H of not greater than 5, at which *p*H it can be autoclaved at 15 lb at 120°C for 20 to 30 minutes. Pyridoxine is unstable when irradiated in aqueous solutions at *p*H 6.8 or above. It is oxidized readily by hydrogen peroxide and other oxidizing agents. Pyridoxine is stable in mixed vitamin preparations to the same degree as riboflavin and nicotinic acid. A 1% aqueous solution has a *p*H of 3. The pK_{a1} values for pyridoxine, pyridoxal, and pyridoxamine are 5.00, 4.22, and 3.40, respectively, and their pK_{a2} values are 8.96, 8.68, and 8.05, respectively.

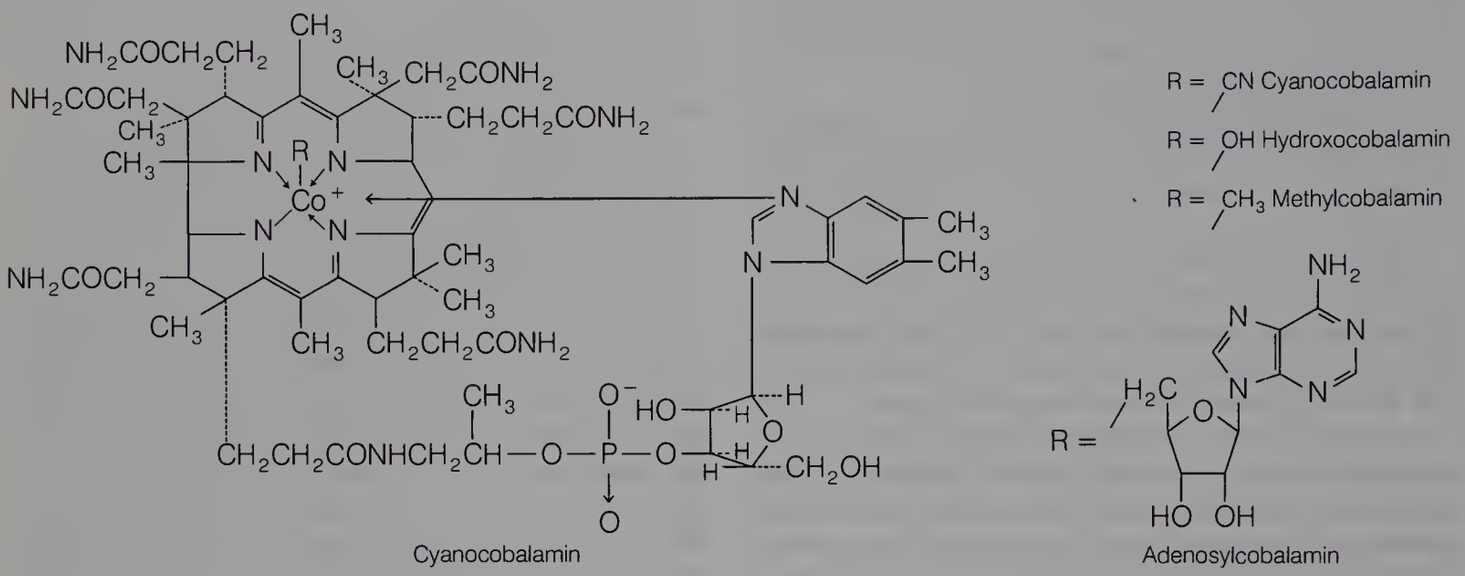


4-Desoxypyridoxal

Pyridoxine deficiencies have been studied by the use of antivitamins such as 4-desoxypyridoxal. As with the other B vitamins, dietary deficiencies are rare and are associated

mainly with alcoholism. The symptoms involve the skin, nervous system, and erythropoiesis.

Because of inadequate diets, some infants suffer from severe vitamin B_6 deficiencies that can lead to epilepsy-like convulsive seizures, and the convulsions can be controlled by treatment with pyridoxine.[1] It is believed that the convulsions are due to a below normal availability of the central nervous system neurohormone γ-aminobutyric acid (GABA), from glutamic acid decarboxylation, which is effected by the coenzyme pyridoxal-5-phosphate.



Pyridoxine hydrochloride is indicated in the treatment and prevention of vitamin deficiency. It also is approved for concurrent administration with isoniazid and cycloserine to decrease their toxicity. Concurrent administration of pyridoxine hydrochloride with levodopa is not recommended. The decarboxylation of levodopa to dopamine in the periphery is increased by pyridoxine. This results in decreased amounts reaching the central nervous system.

THE COBALAMINS

Vitamin B_{12}, cyanocobalamin, occurs in nature as a cofactor that originally was isolated as cyanocobalamin and vitamin B_{12b} (hydroxocobalamin). In April 1948, Rickes et al.[71] isolated from clinically active liver fractions minute amounts of a red, crystalline compound that was also highly effective in promoting the growth of *Lactobacillus lactis.* This compound was called vitamin B_{12}, and in single doses, as small as 3 to 6 μg, it produced positive hematologic activity in patients having Addisonian pernicious anemia. Evidence indicates that its activity is comparable with that of Castle's extrinsic factor and that it can be stored in the liver.

Vitamin B_{12} is found in commercial fermentation processes of antibiotics, such as those of *Streptomyces griseus, S. olivaceus, S. aureofaciens*, sewage, milorganite, and others. Some of these fermentations furnish a commercial source of vitamin B_{12}. Excellent dietary sources are meats, eggs, seafood, dairy, and fermented products. However, de novo synthesis of vitamin B_{12} is restricted to microorganisms. Animals depend on intestinal flora synthesis or, as in humans, consumption of animal products that have already obtained vitamin B_{12}. The only dietary plant products containing the vitamin are legumes, owing to their symbiosis with microorganisms.

Since dietary vitamin B_{12} is protein-bound, the first step in absorption is its release in the stomach. The release is enhanced by gastric *p*H and pancreatic proteases. The freed vitamin is bound immediately to a glycoprotein, the *intrinsic factor*, secreted by parietal cells of the gastric mucosa. The vitamin B_{12}–intrinsic factor complex is carried to the intestines, where it binds with receptors in the ileum. Absorption is mainly by an active process, which can be saturated by 1.5 to 3 μg of vitamin B_{12}. Excess amounts may be absorbed passively.

In the intestinal cells, the complex is broken and vitamin B_{12} is absorbed into the blood, where it binds to transcobalamin II, a β-globulin, for distribution. In the liver, the vitamin is converted to the active form and stored as such. Up to 90% of the vitamin (5 to 11 mg) is stored in the liver. Excretion of vitamin B_{12} is through the bile. Vitamin B_{12} undergoes extensive reabsorption.

In the biosynthesis of the coenzymes[72] derived from vitamin B_{12}, cobalt is reduced from a trivalent to a monovalent state before the organic anionic ligands are attached to the structure. The two types of cobamide that participate as coenzymes in human metabolism are the adenosylcobamides and the methylcobamides. These coenzymes perform vital functions in methylmalonate–succinate isomerization and in methylation of homocysteine to methionine. Methylcobalamin is the major form of the coenzyme in the plasma,

whereas 5-deoxyadenosylcobalamine is the major form in the liver and other tissues. The enzyme system methylmalonyl-CoA mutase requires 5′-deoxyadenosylcobamide, and this enzyme system catalyzes the methylmalonyl-CoA transformation to succinyl-CoA, which is the major pathway of propionyl-CoA metabolism. Propionyl-CoA from lipid metabolism has to be processed through this pathway by succinyl-CoA to enter the Krebs citric acid cycle to be either converted to γ-oxaloacetate, leading to gluconeogenesis, or oxidized aerobically to CO_2, with production of ATP. The methylation of homocysteine to form methionine requires methylcobalamin, and it is catalyzed by a transmethylase that is also dependent on 5-methyltetrahydrofolic acid and reduced FAD.[72]

A deficiency of vitamin B_{12} leads to anemia. Pernicious anemia is due to a lack of the intrinsic factor. The symptoms involve systems that have rapidly dividing cells and the nervous system.

Herbert and Das[72] have reviewed the biochemical roles of vitamin B_{12} and folic acid in hemato- and other cell poiesis; they emphasize that vitamin B_{12} and folic acid are essential for normal growth and proliferation of all human cells. It is further noted that vitamin B_{12} has a function in the maintenance of myelin throughout the nervous system. Deficiency of either vitamin leads to megaloblastic anemia involving below-normal DNA synthesis. Interference with DNA synthesis results in the inability of cells to mature properly. These symptoms are readily reversed by vitamin B_{12} supplementation; however, the nerve damage is irreversible. It has been postulated that the myelin damage is a result of low levels of *S*-adenosylmethionine caused by a methionine synthetase deficiency.[73] Most vitamin B_{12} deficiencies are due to malabsorption (lack of intrinsic factor, alcoholism) or increased need (pregnancy). It has been postulated that vitamin B_{12} deficiency is largely a conditioned folic acid deficiency caused by below-normal transformation of 5-methyltetrahydrofolic acid to tetrahydrofolic acid (THFA) by the B_{12}-dependent homocysteine methyl transferase reaction and defective cellular uptake of 5-methyl-THFA in vitamin B_{12} deficiency. This so-called methyl-THFA trap hypothesis seems to rationalize a mechanism for the pathogenesis of megaloblastic anemia in vitamin B_{12} deficiency, and investigations continue to provide evidence that supports this rationalization.

PRODUCTS

Cyanocobalamin, USP. Vitamin B_{12} is a cobalt-containing substance usually produced by the growth of suitable organisms or obtained from liver. It occurs as dark red crystals or an amorphous or crystalline powder. The anhydrous form is very hygroscopic and may absorb about 12% water. One gram is soluble in about 80 mL of water. It is soluble in alcohol but insoluble in chloroform and in ether.

Vitamin B_{12} loses about 1.5% of its activity per day when stored at room temperature in the presence of ascorbic acid, whereas vitamin B_{12b} (hydroxocobalamin) is very unstable (completely inactivated in 1 day). This loss in activity is accompanied by a release of cobalt and a disappearance of color. The greater stability of vitamin B_{12} is attributed to the increased strength of the bond between cobalt and the benzimidazole nitrogen by cyanide. Unusual resonance energy is imputed to the cobalt–cyanide complex, giving a positive charge to the cobalt atom and, thereby, strengthening the Co—N bond. The protective action of certain liver extracts of vitamin B_{12b} toward ascorbic acid and its sodium salt is, no doubt, due to the presence of copper and iron. Iron salts will protect vitamin B_{12b} in 0.001% concentration. Catalysis of the oxidative destruction of ascorbate by iron is well known. On exposure to air, liver extracts containing B_{12} lose most of the B_{12} activity in 3 months. The most favorable *p*H for a mixture of cyanocobalamin and ascorbic acid appears to be 6 to 7. Niacinamide can stabilize aqueous parenteral solutions of cyanocobalamin and folic acid at a *p*H of 6 to 6.5. However, it is unstable in B-complex solution. Cyanocobalamin is stable in solutions of sorbitol and glycerin but not in dextrose or sucrose.

Aqueous solutions of vitamin B_{12} are stable to autoclaving for 15 minutes at 121°C. It is almost completely inactivated in 95 hours by 0.015 *N* sodium hydroxide or 0.01 *N* hydrochloric acid. The optimum *p*H for the stability of cyanocobalamin is 4.5 to 5.0. Cyanocobalamin is stable in a wide variety of solvents.

Hydroxocobalamin, USP. Cobinamide dihydroxide; dihydrogen phosphate (ester) mono(inner salt); 3′-ester with 5,6-dimethyl-1-α-D-ribofuranosyl-benzimidazole; vitamin B_{12b} is cyanocobalamin in which the CN group is replaced by an OH group. It occurs as dark red crystals or as a red, crystalline powder that is sparingly soluble in water or alcohol and practically insoluble in the usual organic solvents.

Under the usual conditions, in the absence of cyanide ions, only the hydroxo form of cobalamin is isolated from natural sources. It has good depot properties but is less stable than cyanocobalamin.

Cyanocobalamin Co 57 Capsules, USP, contain cyanocobalamin in which some of the molecules contain radioactive cobalt (^{57}Co). Each microgram of this cyanocobalamin preparation has a specific activity of not less than 0.02 MBq (0.5 μCi).

The *USP* cautions that in making dosage calculations one should correct for radioactive decay. The radioactive half-life of ^{57}Co is 270 days.

Cyanocobalamin Co 57 Solution, USP, has the same potency, dosage, and use as described under Cyanocobalamin Co 57 Capsules, USP. It is a clear, colorless to pink solution that has a *p*H range of 4.0 to 5.5.

Cyanocobalamin Co 60 Capsules, USP, is the counterpart of Cyanocobalamin Co 57 Capsules in potency, dosage, and use. It differs only in its radioactive half-life, which is 5.27 years.

Cyanocobalamin Co 60 Solution, USP, has the same potency, dosage, and use as Cyanocobalamin Co 60 Cap-

sules. It is a clear, colorless to pink solution that has a *p*H range of 4.0 to 5.5.

These four preparations must be labeled "Caution—Radioactive Material" and "Do not use after 6 months from date of standardization."

Cobalamin Concentrate, USP, derived from *Streptomyces* cultures or other cobalamin-producing microorganisms, contains 500 μg of cobalamin per gram of concentrate.

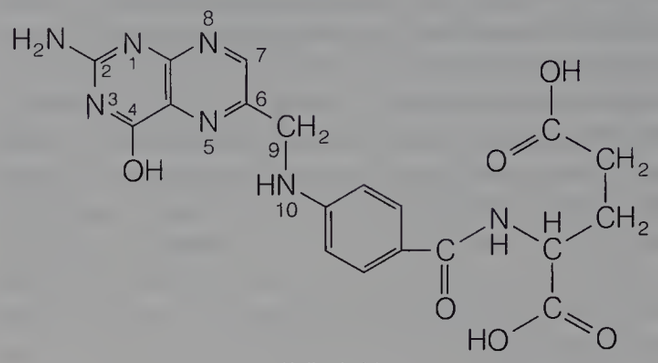
A cyanocobalamin zinc tannate complex can be used as a repository form for the slow release of cyanocobalamin when it is administered by injection.

Commercial products containing liver extract for oral and parenteral use are available. Liver extract is assayed to contain 10 to 20 μg of cyanocobalamin activity per milliliter. Crude liver extract contains 2 μg of activity per milliliter.

Vitamin B_{12} with intrinsic factor is a mixture the potency of which is expressed in terms of oral units of hematopoietic activity. One unit has no more than 15 μg of cyanocobalamin activity. The intrinsic factor is obtained from dried hog stomach, pylorus, or duodenum. One unit contains not more than 300 mg of dried product.

FOLIC ACID

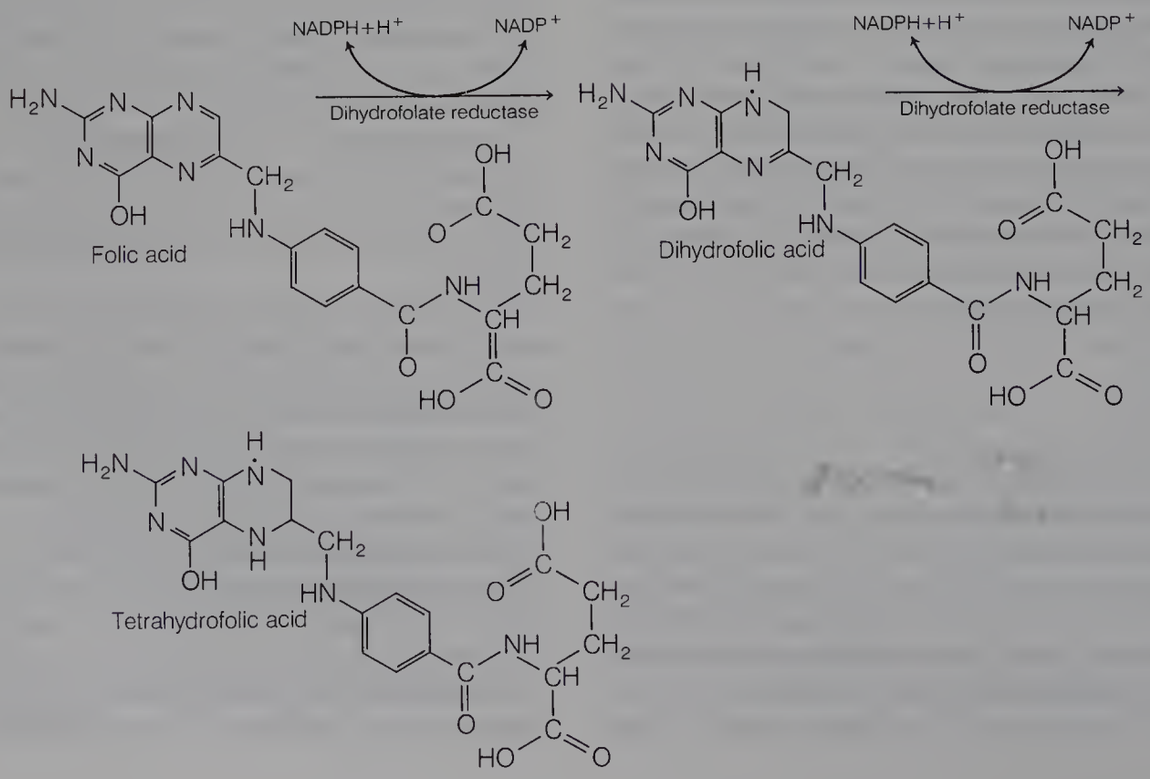
In the early 1940s, R. J. Williams et al.[74] reported the term "folic acid," in referring to a vitamin occurring in leaves and foliage of spinach, from the Latin for leaf (*folium*). Previously, it was called vitamin M and vitamin B_9. Since then, folic acid has been found in whey, mushrooms, liver, yeast, bone marrow, soybeans, and fish meal, all of which serve as excellent dietary sources. The structure (see diagram below) has been proved by synthesis in many laboratories (e.g., see Waller et al.[75]).



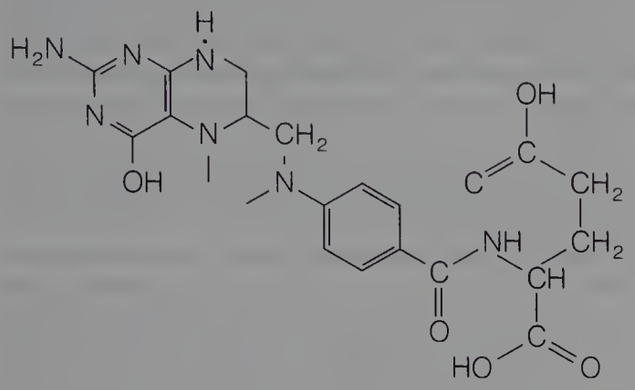
Folic Acid

Folic acid is a pteridine derivative (rings A and B constitute the pteridine heterocyclic system) synthesized by bacteria from GTP, *p*-aminobenzoic acid, and glutamic acid. Accordingly, the structure of folic acid is composed of three moieties: the pteridine moiety, derived from GTP; the *p*-aminobenzoic acid moiety; and the glutamic acid moiety. (It is interesting to relate that antibacterial sulfonamides [see Chap. 8] compete with *p*-aminobenzoic acid and thereby interfere with bacterial folic acid synthesis.) Of course, humans are not able to synthesize folic acid.

In the human, dietary folic acid must be reduced metabolically to THFA to exert its vital biochemical actions. This reduction, which proceeds through the intermediate dihydrofolic acid, is catalyzed by a reductase. This reductase enzyme system has been implicated as the catalyst in both reaction steps—folic acid reduction and dihydrofolic acid reduction. The coenzyme THFA is converted to other cofactors by formulation of the N-10 and/or N-5 nitrogen.



These coenzymes, derived from folic acid, participate in many important reactions, including conversion of homocysteine to methionine, synthesis of glycine from serine, purine synthesis (C-2 and C-8), and histidine metabolism.



N^5	$-CH_3$	N^5-Methyltetrahydrofolic acid
N^5	$-CHO$	N^5-Formyltetrahydrofolic acid (folinic acid)
N^{10}	$-CHO$	N^{10}-Formyltetrahydrofolic acid
$N^{5\ 10}$	$=CH-$	$N^{5,10}$-Methenyltetrahydrofolic acid
N^{5-10}	$-CH_2-$	$N^{5,10}$-Methylenetetrahydrofolic acid
N^5	$-CH=NH$	N^5-Formiminotetrahydrofolic acid
N^1	$-CH_2OH$	N^5-Hydroxytetrahydrofolic acid

The most critical "one-carbon" transfer that is involved in DNA synthesis requires N^5, N^{10}-methylene-THFA as the methylating coenzyme responsible for converting uridylic acid to thimydilic acid. Interestingly, some folic acid antagonists useful in cancer chemotherapy (e.g., methotrexate; see Chap. 12) interfere with DNA synthesis by inhibiting this methylation step.[76]

There is a fundamental relationship between folic acid metabolism and vitamin B_{12}. The reduction of methylene-THFA to 5-methyl-THFA is essentially irreversible; hence, there is only one pathway for the regeneration of THFA from 5-methyl-THFA. The THFA is regenerated by the B_{12}-dependent methyl group transfer from 5-methyl-THFA to homocysteine. This biochemical interrelationship has been implicated in the etiology of megaloblastic anemia (see corresponding discussion under vitamin B_{12}[72]).

Folic Acid, USP. *N*-[[(2-Amino-4-hydroxy-6-pteridinyl) methyl]amino]benzoylglutamic acid; pteroylglutamic acid (Folacine, Folvite). Folic acid occurs as a yellow or yellowish orange powder that is only slightly soluble in water (1 mg/100 mL). It is insoluble in the common organic solvents. The sodium salt is soluble (1:66) in water.

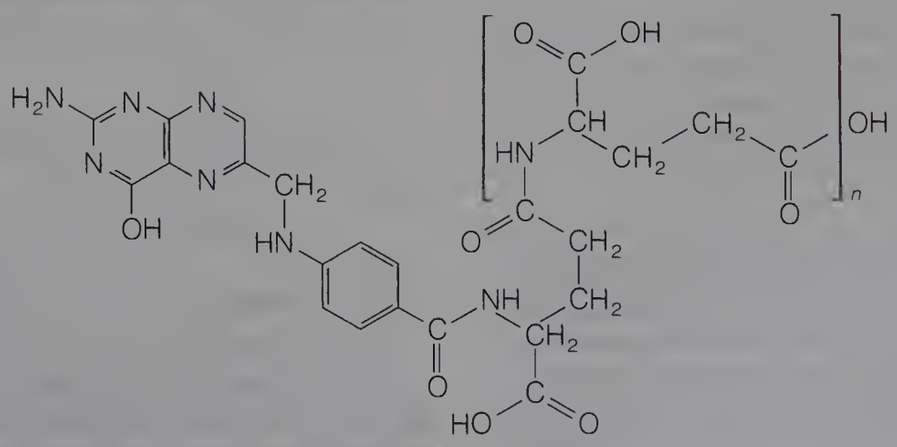
Aqueous solutions of folic acid or its sodium salt are stable to oxygen of the air, even upon prolonged standing. These solutions can be sterilized by autoclaving at a pressure of 15 lb in the usual manner. Folic acid in the dry state and in very dilute solutions is decomposed readily by sunlight or UV light. Although folic acid is unstable in acid solutions, particularly below a *p*H of 6, the presence of liver extracts has a stabilizing effect at lower *p*H levels than is otherwise possible. Iron salts do not materially affect the stability of folic acid solutions. The water-soluble vitamins that have a deleterious effect on folic acid are listed in their descending order of effectiveness as follows: riboflavin, thiamine hydrochloride, ascorbic acid, niacinamide, pantothenic acid, and pyridoxine. This deleterious effect may be overcome, to a considerable degree, by the inclusion of approximately 70% of sugars in the mixture.

Folic acid in foods is destroyed more readily by cooking than are the other water-soluble vitamins. These losses range from 46% in halibut to 95% in pork chops and from 69% in cauliflower to 97% in carrots.

Folic acid occurs in the diet as pteroylpolyglutamates that must be hydrolyzed to the monoglutamates before absorption. The hydrolysis is catalyzed by pteroyl-γ-glutamyl carboxy-peptidase, found in the membrane of the intestinal mucosa. The monoglutamates are absorbed actively in the jejunum and upper duodenum. This absorption is *p*H-sensitive and is facilitated by the slightly acidic conditions found in these regions. The vitamin is transported as monoglutamates bound to albumin.

Although the mucosa in these regions possess dihydrofolate reductase, most reduction and methylation occur in the liver. THFA is distributed to all tissues, where it is stored as polyglutamates. The N^5-methyl derivative is the main transport and storage form in the body. The body stores 5 to 10 mg, approximately 50% in the liver.

The major elimination pathway for the vitamin is biliary excretion as the N^5-methyl derivative. Extensive reabsorption occurs. Only trace amounts are found in the urine. How-



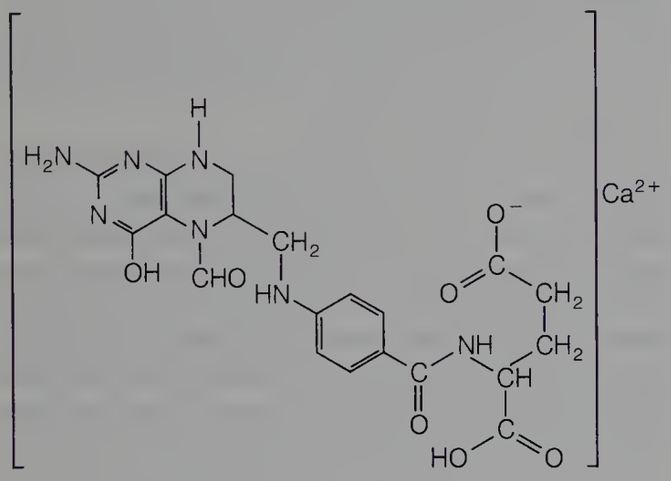
n = 1 = Pteroylglutamic acid
n = 6 = Pteroylpolyglutamic acid

ever, large doses that exceed the tubular reabsorption limit result in substantial amounts in the urine.

As with vitamin B_{12}, folic acid deficiencies are mainly the result of malabsorption or alcoholism. No neurologic abnormalities are associated with folic acid deficiency. The resulting megaloblastic anemia is indistinguishable from that caused by vitamin B_{12} because both vitamins are involved in the critical biochemical step.

Folic acid has the ability to correct the anemia caused by a vitamin B_{12} deficiency, but it has no effect on the neurologic damage. Thus, only small amounts are found in over-the-counter preparations.

Leucovorin Calcium, USP. N[4-[[(2-Amino-5-formyl-1,4,5,6,7,8-hexahydro-4-oxo-6-pteridinyl)-methyl]amino] benzoyl]-L-glutamic acid, calcium salt, calcium 5-formyl-5,6,7,8-tetrahydrofolate; calcium folinate occurs as a yellowish white or yellow, odorless, microcrystalline powder that is insoluble in alcohol and very soluble in water.



Leucovorin calcium
Calcium folinate

This product is used in chemotherapy concurrently with dihydrofolate reductase inhibitors to prevent damage to normal cells. It is not indicated for use in folic acid deficiencies.

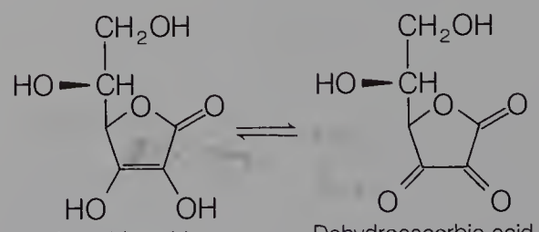
ASCORBIC ACID

The historical significance of vitamin C was summarized eloquently by the eminent medicinal chemist and pharmacist Professor Ole Gisvold, and the following direct quotation from the seventh edition of this textbook is an appropriate introduction to the significance of ascorbic acid in medicinal chemistry and basic biochemistry:

> The disease scurvy, which now is known as a condition due to a deficiency of ascorbic acid in the diet, has considerable historical significance.[77] For example, in the war between Sweden and Russia (most likely the march of Charles XII into the Ukraine in the winter of 1708–1709) almost all of the soldiers of the Swedish army became incapacitated by scurvy. But further progress of the disease was stopped by a tea prepared from pine needles. The Iroquois Indians cured Jacques Cartier's men in the winter of 1535–1536 in Quebec by giving them a tea brewed from an evergreen tree. Many of Champlain's men died of scurvy when they wintered near the same place in 1608–1609. During the long siege of Leningrad, lack of vitamin C made itself particularly felt, and a decoction made from pine needles played an important role in the prevention of scurvy. It is somewhat common knowledge that sailors on long voyages at sea were subject to the ravages of scurvy.The British used supplies of limes to prevent this, and the sailors often were referred to as "limeys."
>
> Holst and Frolich,[78] in 1907, first demonstrated that scurvy could be produced in guinea pigs. A comparable condition cannot be produced in rats.
>
> Although Waugh and King[79] (1932) isolated crystalline vitamin C from lemon juice and showed it to be the antiscorbutic factor of lemon juice, Szent-Gyorgyi[80] had isolated the same substance from peppers in 1928, in connection with his biological oxidation–reduction studies. At the time, he failed to recognize its vitamin properties and reported it as a hexuronic acid because some of its properties resembled those of sugar acids. Hirst et al.[81] suggested that the correct formula should be one of a series of possible tautomeric isomers and offered basic proof that the formula now generally accepted is correct. The first synthesis of L-ascorbic acid (vitamin C) was announced almost simultaneously by Haworth and Reichstein,[82] in 1933. Since that time, ascorbic acid has been synthesized in a number of different ways.

This vitamin is now better known as ascorbic acid because of its acidic character and its effectiveness in the treatment and prevention of scurvy. The acidic character is due to the two enolic hydroxyls; the C-3 hydroxyl has a pK_a value of 4.1, and the C-2 hydroxyl has a pK_a of 11.6. The monobasic sodium salt is the usual salt form (e.g., Sodium Ascorbate, USP).

Ascorbic acid can be synthesized by nearly all living organisms, plants, and animals; but primates, guinea pigs, bats, and some other species are not capable of producing this vitamin. The consensus is that organisms that cannot synthesize ascorbic acid lack the liver microsomal enzyme L-gulonolactone oxidase, which catalyzes the terminal step of the biosynthetic process. Sato and Udenfriend[83] summarized studies of the biosynthesis of ascorbic acid in mammals and the biochemical and genetic basis for the incapability of some species to synthesize the vitamin. Because humans are one of the few animal species that cannot synthesize ascorbic acid, the vitamin has to be available as a dietary component.



Ascorbic acid Dehydroascorbic acid

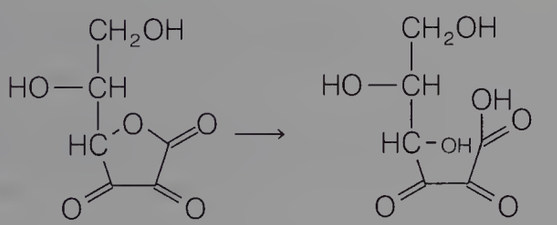
Ascorbic acid performs important metabolic functions, as evidenced by the severe manifestations of its deficiency in humans. It has been demonstrated that this vitamin is involved in metabolic hydroxylations in numerous important

metabolic processes (e.g., the synthesis of steroids and of neurotransmitters and in collagen and drug metabolism). Ascorbic acid also has been implicated as an important factor in other critical oxidation–reduction processes in human metabolism.[1]

Although it is well known that ascorbic acid is an effective reducing agent and antioxidant, the biochemical functions of this vitamin are not well understood. It is controversial to consider ascorbic acid to be an antiviral agent, but some scientists will argue that ascorbic acid is an effective cure or preventative of "common colds."[84] One study provides some evidence that ascorbic acid appears to help the organism recover from viral infections through an indirect mechanism on the body's immune system.[85] Ascorbic acid also has received attention as a possible anticancer agent. It has been demonstrated in cell culture studies that ascorbic acid, both alone and in combination with copper ions, is selectively toxic to melanoma cancer cells.[86]

Ascorbic Acid, USP. Vitamin C, L-ascorbic acid (Cevitamic Acid, Cebione). Ascorbic acid occurs as white or slightly yellow crystals or powder. It is odorless, and on exposure to light it gradually darkens. One gram dissolves in about 3 mL of water and in about 30 mL of alcohol. A 1% aqueous solution has a *p*H of 2.7.

Aqueous solutions are not very stable. The ascorbic acid in such preparations undergoes oxidation, particularly under aerobic conditions. Oxidation to dehydroascorbic acid is followed by hydrolytic cleavage of the lactone. The effect of *p*H on the aerobic degradation of ascorbic acid aqueous solutions has been studied by various investigators. Rogers and Yacomeni[87] concluded that the degradation rate shows a maximum near *p*H 4 and a minimum near *p*H 5.6. It also was noted that if a preparation of ascorbic acid develops acidity on storage and if its initial *p*H is between 5 and 5.6, the rate of degradation will increase as the *p*H decreases; hence, an initial *p*H in the range of 5.6 to 6 is recommended.



L-Ascorbic acid 2,3-Diketo-L-gulonic acid

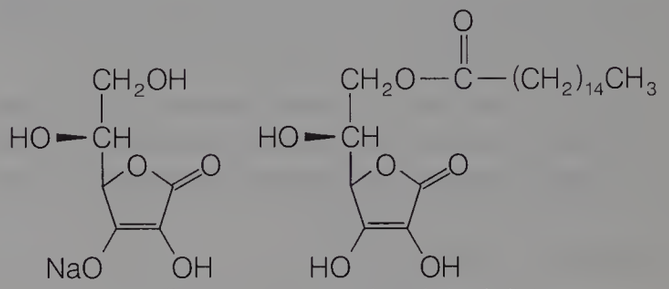
Dietary sources of ascorbic acid include citrus fruits, tomatoes, and potatoes. Although the sources of some commercial products are rose hips and citrus fruits, the largest amount of ascorbic acid is prepared synthetically.

Ascorbic acid is readily absorbed by an active process. Large doses can saturate this system, limiting the amounts absorbed. Once absorbed, it is distributed to all tissue. The vitamin is metabolized to oxalic acid before excretion. Ascorbic acid-2-sulfate is also a metabolite found in the urine. Large doses result in the excretion of substantial amounts of unchanged ascorbic acid. The resultant acidification of the urine is the basis for most of the vitamin's undesirable side effects.

The vitamin is indicated for the treatment and prevention of ascorbic acid deficiency. Although scurvy occurs infrequently, it is seen in the elderly, infants, alcoholics, and drug users. Ascorbic acid (but not the sodium salt) frequently is administered with methenamine to improve the effectiveness of this antibacterial agent. Because ascorbic acid increases the chelation of iron by deferoxamine, it is used in the treatment of chronic iron toxicity. It also finds usefulness as an adjunct in the treatment of methemoglobinemia.

Ascorbic Acid Injection, USP, is a sterile solution of sodium ascorbate that has a *p*H of 5.5 to 7.0. It is prepared from ascorbic acid with the aid of sodium hydroxide, sodium carbonate, or sodium bicarbonate. It may be used for intravenous injection, whereas ascorbic acid is too acidic for this purpose.

Sodium Ascorbate, USP, is a white, crystalline powder that is soluble 1 : 1.3 in water and insoluble in alcohol.

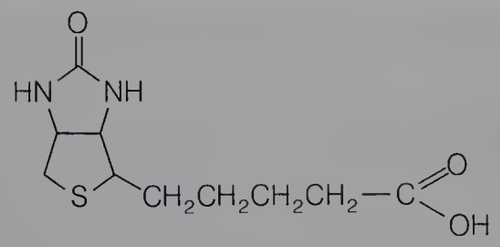


Ascorbate sodium Ascorbyl palmitate

Ascorbyl Palmitate, NF. Ascorbic acid palmitate (ester) is the C-6 palmitic acid ester of ascorbic acid. It occurs as a white to yellowish white powder that is very slightly soluble in water and in vegetable oils. It is freely soluble in alcohol. Ascorbic acid has antioxidant properties and is a very effective synergist for the phenolic antioxidants, such as propylgallate, hydroquinone, catechol, and nordihydroguaiaretic acid, when they are used to inhibit oxidative rancidity in fats, oils, and other lipids. Long-chain, fatty acid esters of ascorbic acid are more soluble and suitable for use with lipids than is ascorbic acid.

BIOTIN

Biotin was discovered, isolated, and identified structurally in the 1930s. Previously, it was known also as vitamin H. Since then, it has been noted that small amounts of biotin can be detected in almost all higher animals. The D-isomer possesses all of the activity. The highest concentrations have been discovered in liver, kidney, eggs, and yeast, as a water-insoluble complex. Considerable quantities are found both free and in the complex form in vegetables, grains, and nuts. Alfalfa, string beans, spinach, and grass are fair sources of this vitamin.



Biotin

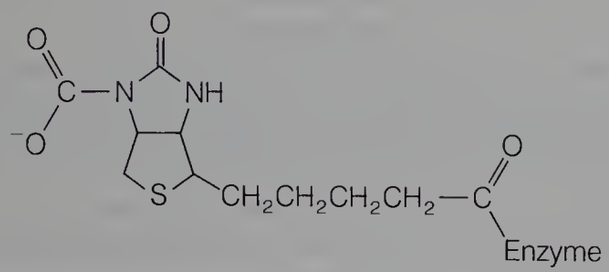
Microorganisms synthesize biotin from the fatty acid oleic acid. The biosynthetic process involves numerous complex reactions that remain to be better understood. The final reaction step requires formation of the sulfur heterocycle, but the source of the sulfur is not yet known.

Although this vitamin is known to perform essential metabolic functions in the human, the minimal nutritional requirement has not been established because it has been difficult to quantify the amounts of the vitamin made available by intestinal microorganisms. Nevertheless, deficiency states may develop owing to prolonged feeding of large quantities of raw egg white. Raw egg white contains avidin, a protein that complexes biotin and minimizes its absorption from the gastrointestinal tract. The symptoms of biotin deficiency include dermatitis, hyperesthesia, and glossitis.

Biotin performs vital metabolic functions in important carboxylation processes in the form of carboxybiotin, which is in combination with a carboxylase, as represented below.

$$\text{Biotin-enzyme} + HCO_3^- \xrightleftharpoons{Mg^{2+}} CO_2 - \text{biotin enzyme} + ADP + Pi$$

The oxygen for ATP cleavage is derived from bicarbonate and appears in the Pi.



CO_2–Biotin enzyme

Purified preparations of acetyl-CoA carboxylase contained biotin (1 mole of biotin per 350,000 g of protein [enzyme]). It catalyzed the first step in palmitate synthesis as follows:

$$CH_3COSCoA + HCO_3^- + ATP \xrightleftharpoons{Mg^{2+}} {}^-OOCCH_2COSCoA + ADP + Pi$$

Other enzymes with which biotin appears to be associated intimately in carboxylation are β-methylerotonyl-CoA carboxylase, propionyl-CoA carboxylase, pyruvate carboxylase, and methylmalonyloxalacetic transcarboxylase.

Biotin also is joined in an amide linkage to the ϵ-amino group of a lysine residue of carbamyl phosphate synthetase (CPS) to form biotin–CPS, which participates with two ATPs, HCO_3^-, and glutamine in the synthesis of carbamyl phosphate. This takes place stepwise as follows:

1. Biotin CPS + ATP + HCO_3^- $\leftrightarrows$ carbonic phosphoric anhydride biotin CPS (CPA biotin CPS) + ADP
2. CPA biotin CPS $\leftrightarrows$ ${}^-OOC$ biotin CPS + Pi
3. ${}^-OOC$ biotin CPS + glutamine $\leftrightarrows$ H_2NOC biotin CPS + ATP $\leftrightarrows$ biotin CPS + carbamylphosphate + ADP

Carbamyl phosphate can participate in amino acid metabolism and some nucleic acid syntheses.

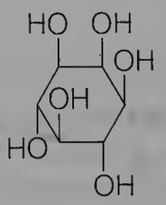
Biotin is absorbed readily from the gastrointestinal tract. The body appears unable to break the fused imidazolidine and tetrahydrothiophene ring system. Biotin appears in the urine, predominately as the unchanged molecule. Only small amounts of the metabolites biotin sulfoxide and bisnorbiotin appear in the urine.

MISCELLANEOUS CONSIDERATIONS

Some dietary components are difficult to characterize as essential nutritional factors in human metabolism because the organism has the necessary chemistry to produce these compounds from other dietary components. (Consider vitamin D and nicotinic acid, which have been discussed earlier.) Vitamin D and nicotinic acid, however, generally are considered among the classic vitamins. Moreover, there is no clear consensus on the necessity for inositol, choline, and *p*-aminobenzoic acid. Nevertheless, such dietary components do perform important metabolic functions; hence, a brief characterization of these should be noted.

Inositol. 1,2,3,5-*trans*-4,6-Cyclohexanehexol; *i*-inositol; *meso*-inositol (*myo*-inositol, mouse anti-alopecia factor) is prepared from natural sources, such as corn steep liquors, and is available in limited commercial quantities. It is a white, crystalline powder that is soluble in water 1:6 and in dilute alcohol. It is slightly soluble in alcohol, the usual organic solvents, and fixed oils. It is stable under normal storage conditions.

Inositol is one of nine different *cis–trans* isomers of hexahydroxycyclohexane and usually is assigned the following configuration:



Inositol

Inositol has been found in most plant and animal tissues. It has been isolated from cereal grains, other plant parts, eggs, blood, milk, liver, brain, kidney, heart muscle, and other sources. The concentration of inositol in leaves reaches

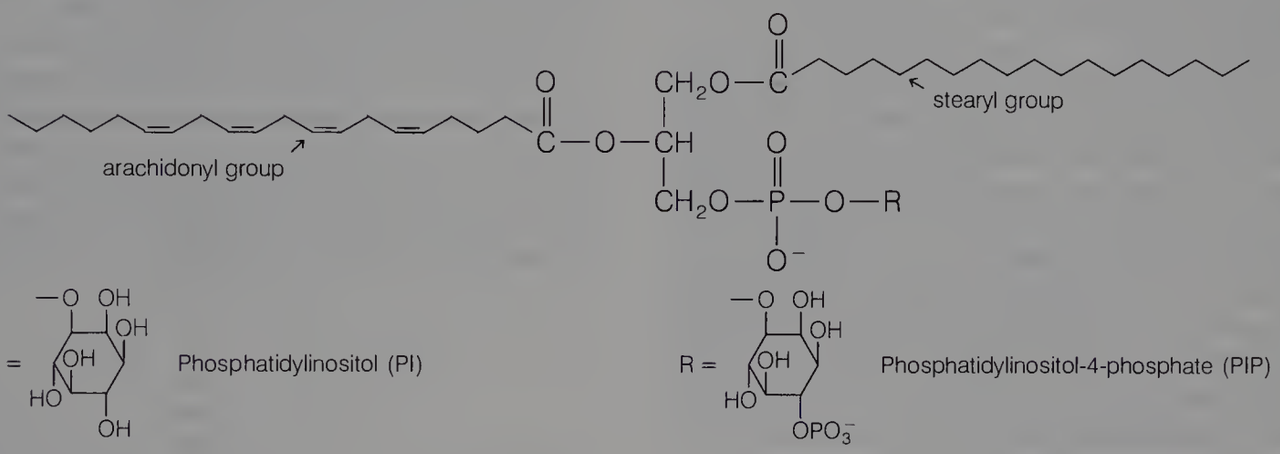
a maximum shortly before the time that the fruit ripens. Good sources of this factor are fruits, especially citrus fruits,[88] and cereal grains.

Inositol occurs free and combined in nature. In plants, it is present chiefly as the well-known phytic acid, which is inositol hexaphosphate. It is also present in the phosphatide fraction of soybean as a glycoside. In animals, much of it occurs free.[89]

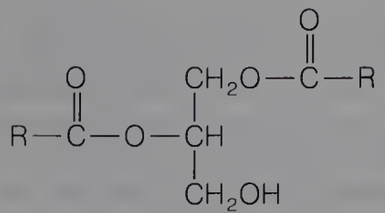
Inositol in the form of phosphoinositides is almost as widely distributed as inositol, and some of these forms are more active metabolically. Phosphatidylinositol (monophosphoinositide) is the most widely distributed of the inositides, and the chief fatty acid residue is stearic acid.

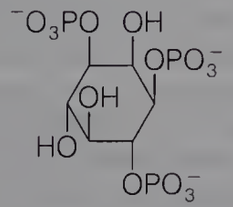
The binding of many different hormones, neurotransmitters, and growth factors to the cell surface results in activation of the polyphosphoinositide receptor system.[91] Binding to the specific receptor activates the enzyme phospholipase C through the intermediacy of a G-protein. Phospholipase C converts phosphatidylinositol-4,5-bisphosphate (PIP_2) into IP_3 and DAG. IP_3 releases calcium ion, which in turn affects many cellular responses in the target cells.

IP_3 also is converted to inositol-1,3,4,5-tetrakisphosphate (IP_4). IP_4 also acts as an intracellular messenger, resulting in an influx of extracellular calcium. IP_4 is converted to IP_3(1,3,4), which is dephosphorylated stepwise to inositol-1,4-diphosphate, inositol-1-phosphate, then inositol. The inositol is then incorporated into DAG to form phosphatidylinositol (PI). PI is sequentially phosphorylated to form phosphatidylinositol-4-phosphate (PIP) and PIP_2.



Phosphoinositides serve as storage forms for secondary messengers. Phosphoinositides compose only a minor fraction (2% to 8%) of the lipids in cell membranes, yet they can be converted to at least three intracellular messenger molecules: arachidonic acid, inositol-1,4,5-trisphosphate (IP_3), and 1,2-DAG. The functions of arachidonic acid derivatives are discussed in Chap. 24. IP_3 releases intracellular calcium. DAG acts as an essential cofactor in the activation of protein kinase C.[90]


Diacylglycerol
(DG or DAG)


Inositol-1,4,5-trisphosphate
IP_3(1,4,5)

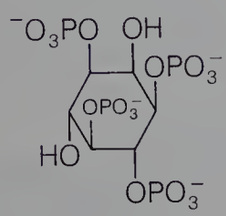
The DAG released has several fates. It can be converted to PI, as mentioned, or it can be hydrolyzed further to release the arachidonic acid component. DAG in conjunction with calcium ion stimulates protein kinase C. By phosphorylating proteins, kinases regulate many cellular activities.

The cycle is completed by the phosphorylation of DAG to phosphatidic acid, which in turn is converted to PI. The complete system remains to be fully understood. Cyclic phosphoinositol derivatives appear to function also as secondary messengers.

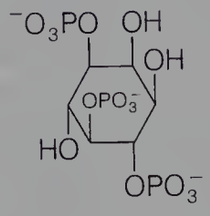
Inositol is a growth factor for a wide variety of human cell lines in tissue culture. It is considered a characteristic component of seminal fluid, and the content is an index of the secretory activity of the seminal vesicles.

Evidence is accumulating to indicate that inositol will reduce elevated blood cholesterol levels. This in turn may

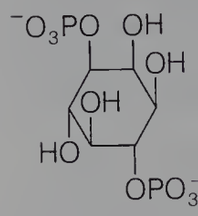
prevent or mitigate cholesterol depositions in the intima of blood vessels in humans and animals and, therefore, be of value in atherosclerosis.



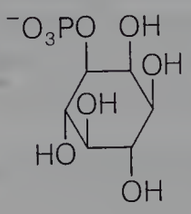
Inositol-1,3,4,5-tetrakisphosphate
IP_4



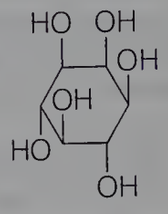
Inositol-1,3,4-trisphosphate
$IP_3(1,3,4)$



Inositol-1,4-bisphosphate
IP_2



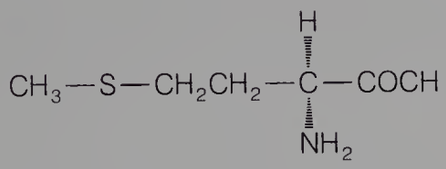
Inositol-1-phosphate
IP



Inositol

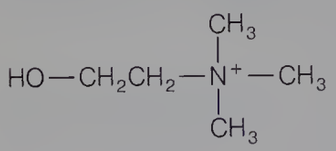
Inositol also has been considered as a lipotropic agent. Because humans can synthesize inositol, the need for it as a nutritional requirement has not been proved.[2]

Methionine, USP. An adequate diet should provide the methionine necessary for normal metabolism in the human. Methionine is considered to be an essential amino acid in humans. It is the precursor in the biosynthesis of *S*-adenosylmethionine, which is an important methylating coenzyme involved in a variety of methylations, for example, in *N*-methylation of norepinephrine to form epinephrine and *O*-methylation of catecholamines catalyzed by catechol-*O*-methyltransferases. Adenosylmethionine also participates in the methylation of phosphatidylethanolamine to form phosphatidylcholine, but this pathway is not efficient enough to provide all of the choline required by higher animals; hence, adequate dietary availability of choline is necessary.[2]

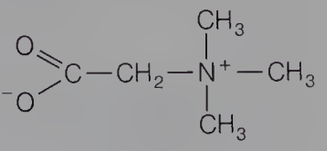


Methionine

Choline is a component of many biomembranes and plasma phospholipids. Dietary sources include eggs, fish, liver, milk, and vegetables. These sources provide choline primarily as the phospholipid lecithin. Lecithin is hydrolyzed to glycerophosphorylcholine by the intestinal mucosa before absorption. The liver liberates choline. Choline can be biosynthesized by humans; consequently, it cannot be considered a true vitamin. Biosynthesis involves methylation of ethanolamine. The methyl groups are provided by methionine or by a reaction involving vitamin B_{12} and folic acid. Therefore, deficiencies can occur only if all methyl donors are excluded from the diet.



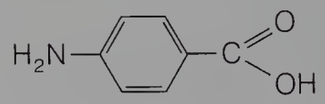
Choline



Betaine

The therapeutic uses of choline depend on its physiologic functions. Because it is involved in the formation of plasma phospholipids, it is used as a lipotropic agent to alleviate fatty infiltration of the liver, cirrhosis. It has been used, in large doses, in certain central nervous system disorders (e.g., tardive dyskinesia, presenile dementia) because it is a precursor of acetylcholine. Choline also serves as a methyl donor in some reactions after it is converted to betaine.

***p*-Aminobenzoic Acid, USP** (PABA) has been mentioned as a biosynthetic component of folic acid in bacteria, but it is well known that higher mammalian organisms cannot synthesize folic acid from its precursors. Nevertheless, it seems that PABA performs certain metabolic functions in some animals. In the early 1950s, PABA was reported to be an essential factor in the normal growth and life of the chick.



p-Aminobenzoic acid

Since these original developments in this field, various claims[92] have been made for the chromotrichial value of PABA in rats, mice, chicks, minks, and humans. The problem of nutritional achromotrichia is a complex one that may involve several vitamin or vitamin-like factors and is complicated by the synthesis and absorption from the intestinal tract of several factors produced by bacteria.

PABA is a white, crystalline substance that occurs widely over the plant and animal kingdoms. It occurs both free and combined[93] and has been isolated[94] from yeast, of which it is a natural constituent. It is soluble 1 : 170 in water and 1 : 8 in alcohol and freely soluble in alkali.

PABA is thought to play a role in melanin formation and to influence or catalyze tyrosine activity.[95] It inhibits oxidative destruction of epinephrine and stilbestrol, counteracts the graying of fur attributable to hydroquinone in cats and mice, exhibits antisulfanilamide activity, and counteracts the toxic effects of carbarsone and other pentavalent phenylarsonates.[96]

When given either parenterally or in the diet to experimental animals, PABA will protect them against otherwise fatal infections of epidemic or murine typhus, Rocky Mountain spotted fever, and tsutsugamushi disease.[97] These diseases have been treated clinically with most encouraging results by maintaining blood levels of 10 to 20 mg/100 mL for Rocky Mountain spotted fever and tsutsugamushi disease. The mode of action of PABA in the treatment of these diseases appears to be rickettsiostatic rather than rickettsicidal,

and the immunity mechanisms of the host finally overcome the infection.

PABA appears to function as a coenzyme in the conversion of certain precursors to purines.[98] It has been suggested as an effective sunscreen as a 5% solution in 55% to 75% ethyl alcohol on excessive sunlight-exposed areas of the skin.[99]

The historical significance of the effect of PABA on the antimicrobial action of sulfonamides and sulfones has been reviewed by Anand.[100]

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APPENDIX

pK_as of Drugs and Reference Compounds

Name	pK_a*	Reference
Acenocoumarol	4.7	1
Acetaminophen	9.9 (phenol)	2
Acetanilid	0.5	3
Acetarsone	3.7 (acid)	4
	7.9 (phenol)	
	9.3 (acid)	
Acetazolamide	8.8 (acetamido)	5
Acetic Acid	4.8	6
α-Acetylmethodol	8.3	7
Acetylpromazine	9.3	8
N^4-Acetylsulfadiazine	6.1	9
N^4-Acetylsulfametiazole	5.2	9
N^4-Acetylsulfamethoxypyridazine	6.9	9
N^4-Acetylsulfapyridine	8.2	9
N^4-Acetylsulfisoxazole	4.4	9
Allobarbital	7.5	10
Allopurinol	9.4	11
Allylamine	10.7	12
Allylbarbituric Acid	7.6	13
Alphaprodine	8.7	14
Alprenolol	9.6	15
Amantadine	10.8	16
Amiloride	8.7	15
p-Aminobenzoic Acid	2.4 (amine)	17
	4.9	
Aminocaproic Acid	4.4	18
	10.8	
6-Aminopenicillanic Acid	2.3 (carboxyl)	19
	4.9 (amine)	
Aminopterin	5.5 (heterocyclic ring)	20
Aminopyrine	5.0	6
Aminosalicylic Acid	1.7 (amine)	17
	3.9	
Amitriptyline	9.4	21
Ammonia	9.3	12
Amobarbital	8.0	22
Amoxicillin	2.4 (carboxyl)	23
	7.4 (amine)	
	9.6 (phenol)	
Amphetamine	9.8	24
Amphotericin B	5.7 (carboxyl)	25
	10.0 (amine)	
Ampicillin	2.7	26
	7.3 (amine)	
Aniline	4.6	6
Anisindione	4.1	27
Antazoline	10.0	28
Antifebrin	1.4	29
Antipyrine	2.2	29
Apomorphine	7.0	30
Aprobarbital	7.8	10
Arecoline	7.6	31
Arsthinol	9.5 (phenol)	4
Ascorbic Acid	4.2	18
	11.6	
Aspirin	3.5	7
Atropine	9.7	30
Barbital	7.8	32
Barbituric Acid	4.0	32
Bemegride	11.2	33
Bendroflumethiazide	8.5	34
Benzilic Acid	3.0	12
Benzocaine	2.8	30
Benzoic Acid	4.2	7
Benzphetamine	6.6	35
Benzquinamide	5.9	36
Benzylamine	9.3	12
Biscoumacetic Acid	3.1	1
	7.8 (enol)	174
Bromodiphenhydramine	8.6	37
p-Bromophenol	9.2	38
Bromothen	8.6	28
8-Bromotheophylline	5.5	13
Brucine	8.0	6
Bupivacaine	8.1	39
Butabarbital	7.9	15
Butethal	8.1	40
Butylparaben	8.4	41
Butyric Acid	4.8	12
Caffeine	14.0	42
	0.6 (amine)	
Camphoric Acid	4.7	12
Carbinoxamine	8.1	43
Carbonic Acid	6.4 (1st)	6
	10.4 (2nd)	
Cefazoline	2.3	44
Cephalexin	3.6	45
Cephaloglycin	2.5	45

*pK_a given for protonated amine.

Continued

Name	pK_a*	Reference
Cephaloridine	3.4	46
Cephalothin	2.4	45
Cephradine	2.6 (carboxyl)	47
	7.3 (amine)	
Chlorambucil	5.8	48
Chlorcyclizine	7.8	37
Chlordiazepoxide	4.8	49
Chlorindione	3.6	27
Chloroquine	8.1	50
	9.9	
8-Chlorotheophylline	5.3	51
Chlorothiazide	6.7	52
	9.5	
Chlorpheniramine	9.2	37
Chlorphentermine	9.6	35
Chlorpromazine	9.2	53
Chlorpromazine Sulfoxide	9.0	44
Chlorpropamide	5.0	54
Chlorprothixene	8.4	55
Chlortetracycline	3.3	56
	7.4	
	9.3	
Cinchonidine	4.2 (1st)	3
	8.4 (2nd)	
Cinchonine	4.0 (1st)	30
	8.2 (2nd)	
Cinnamic Acid	4.5	12
Citric Acid	3.1 (1st)	57
	4.8 (2nd)	
	6.4 (3rd)	
Clindamycin	7.5	58
Clofibrate	3.0 (acid)	15
Clonazepam	10.5 (1-position)	59
	1.5 (4-position)	
Clonidine	8.0	60
Cloxacillin	2.7	19
Cobefrin	8.5	24
Cocaine	8.4	3
Codeine	7.9	42
Colchicine	1.7	18
o-Cresol	10.3	17
m-Cresol	10.1	17
p-Cresol	10.3	61
Cyanic Acid	3.8	12
Cyanopromazine	9.3	62
Cyclizine	8.2	63
Cyclopentamine	3.5	64
Cyclopentolate	7.9	65
Dantrolene	7.5	66
Debrisoquin	11.9	67
Dehydrocholic Acid	5.0	68
Demeclocycline	3.3	56
	7.2	
	9.3	
Desipramine	10.2	21
Dextromethorphan	8.3	43
Diatrizoic Acid	3.4	69
Diazepam	3.3	49
Dibucaine	8.5	70
Dichloroacetic Acid	1.3	42
Dicloxacillin	2.8	26
Dicumarol	4.4 (1st)	71
	8.0 (2nd)	
Diethanolamine	8.9	72
Diethylamine	11.0	12
p-Diethylaminobenzoic Acid	6.2	73
p-Diethylaminosalicylic Acid	3.8	73
Dihydrocodeine	8.8	3
3,5-Diiodo-L-tyrosine	2.5 (amine)	32
	6.5	
	7.5 (phenol)	
Dimethylamine	10.7	12
p-Dimethylaminobenzoic Acid	5.1	73
p-Dimethylaminosalicylic Acid	3.8	73
Dimethylbarbituric Acid	7.1	12
Dimethylhydantoin	8.1	3
2,4-Dinitrophenol	4.1	74
Diperodon	8.4	75
Diphenhydramine	9.0	37
Diphenoxylate	7.1	76
Doxorubicin	8.2	77
	10.2	
Doxycycline	3.4	78
	7.7	
	9.3	
Doxylamine	9.2	37
Droperidol	7.6	79
Ephedrine	9.6	7
Epinephrine	8.7 (phenol)	80
	9.9 (amine)	
Equilenin	9.8	61
Ergotamine	6.3	81
Erythromycin	8.8	82
Erythromycin Estolate	6.9	83
17 α-Estradiol	10.7	61
Estriol	10.4	61
Ethacrynic Acid	3.5	18
Ethambutol	6.6	84
	9.5	
Ethanolamine	9.5	72
Ethopropazine	9.6	28
Ethosuximide	9.3	15
p-Ethoxybenzoic Acid	4.5	73
p-Ethoxysalicylic Acid	3.2	73
Ethylamine	10.7	12
Ethylbarbituric Acid	4.4	12
Ethyl Biscoumacetate	3.1	1
Ethylenediamine	6.8 (1st)	72
	9.9 (2nd)	
Ethylparaben	8.4	41
Ethylphenylhydantoin	8.5	85
Etidocaine	7.7	39
β-Eucaine	9.4	3
Fenfluramine	9.1	35
Fenoprofen	4.5	86
Flucytosine	10.7 (amide)	87
	2.9 (amine)	
Flufenamic Acid	3.9	74
Flunitrazepam	1.8	59
p-Fluorobenzoic Acid	4.2	38
Fluorouracil	8.0	88
	13.0	
Fluphenazine	8.1 (1st)	53
	9.9 (2nd)	
Fluphenazine Enanthate	3.5	89
	8.2	
Flurazepam	8.2	90
	1.9	
Formic Acid	3.7	12
Fumaric Acid	3.0 (1st)	42
	4.4 (2nd)	
Furaltadone	5.0	91
Furosemide	4.7	92
Gallic Acid	4.2	38
Glibenclamide	6.5	54
Gluconic Acid	3.6	18

*pK_a given for protonated amine.

Name	pK_a*	Reference
Glucuronic Acid	3.2	93
Glutamic Acid	4.3	12
Glutarimide	11.4	16
Glutethimide	11.8	94
Glycerophosphoric Acid	1.5 (1st)	42
	6.2 (2nd)	
Glycine	2.4	42
	9.8 (amine)	
Glycollic Acid	3.8	12
Guanethidine	11.9	67
Guanidine	13.6	3
Haloperidol	8.3	95
Heroin	7.8	14
Hexachlorophene	5.7	74
Hexetidine	8.3	96
Hexobarbital	8.3	85
Hexylcaine	9.1	70
Hippuric Acid	3.6	97
Histamine	9.9 (side chain)	98
	6.0 (imidazole)	
Homatropine	9.7	3
Hydantoin	9.1	17
Hydralazine	0.5 (ring N)	99
	6.9 (hydrazine)	
Hydrochlorothiazide	7.0	18
	9.2	
Hydrocortisone Hemisuccinate Acid	5.1	100
Hydroflumethiazide	8.9	101
	10.5	
Hydrogen Peroxide	11.3	12
Hydromorphine	7.8	3
Hydroxyamphetamine	9.6	24
p-Hydroxybenzoic Acid	4.1	38
o-Hydroxycinnamic Acid	4.7	38
m-Hydroxycinnamic Acid	4.5	38
p-Hydroxycinnamic Acid	4.4	38
Hydroxylamine	6.0	6
p-Hydroxysalicylic Acid	3.2	73
Hydroxyzine	1.8	102
Ibuprofen	5.2	103
Idoxuridine	8.3	104
Imidazole	7.0	72
Imipramine	9.5	21
Indomethacin	4.5	18
Indoprofen	5.8	105
Iodipamide	3.5	106
Iophenoxic Acid	7.5	1
Isocarboxazid	10.4	107
Isomethadone	8.1	14
Isoniazid	10.8 (pyridine)	108
	11.2 (hydazide)	
Isophthalic Acid	3.6	12
Isoproterenol	8.7 (amine)	109
	9.9 (phenol)	
Kanamycin	7.2	15
Ketamine	7.5	110
Lactic Acid	3.9	18
Leucovorin	3.1	18
	4.8	
	10.4 (phenol)	
Levallorphan Tartrate	6.9	111
Levarterenol	8.7 (phenol)	112
	9.7 (amine)	
Levodopa	2.3 (carboxyl)	113
	8.7 (amine)	
	9.7 (1st phenol)	113
	13.4 (2nd phenol)	
Levomepromazine	9.2	64
Levorphanol	8.9	14
Levulinic Acid	4.6	12
Lidocaine	7.9	114
Lincomycin	7.5	115
Liothyronine	8.4 (phenol)	116
Lorazepam	11.5	117
	1.3	
Malamic Acid	3.6	12
Maleic Acid	1.9	12
Malic Acid	3.5 (1st)	57
	5.1 (2nd)	
Malonic Acid	2.8	12
Mandelic Acid	3.8	97
Mecamylamine	11.2	7
Meclizine	3.1	118
	6.2	
Medazepam	6.2	119
Mefenamic Acid	4.3	74
Mepazine	9.3	53
Meperidine	8.7	14
Mephentermine	10.3	35
Mephenytoin	8.1	120
Mephobarbital	7.7	85
Mepivacaine	7.6	39
Mercaptopurine	7.8	121
Metaproterenol	8.8	15
Methacycline	3.5	15
	7.6	
	9.2	
Methadone	8.3	14
Methamphetamine	9.5	43
Methapyrilene	3.7	3
	8.9 (side chain)	
Methaqualone	2.5	122
Metharbital	8.2	85
Methazolamide	7.3	18
Methenamine	4.9	29
Methicillin	2.8	19
Methopromazine	9.4	62
Methotrexate	4.8	124
	5.5	
Methohexital	8.3	123
Methoxamine	9.2	64
Methoxyacetic Acid	3.5	12
o-Methoxybenzoic Acid	4.2	38
m-Methoxybenzoic Acid	4.2	38
o-Methoxycinnamic Acid	4.7	38
m-Methoxycinnamic Acid	4.5	38
p-Methoxycinnamic Acid	4.9	38
Methyclothiazide	9.4	125
Methylamine	10.6	12
1-Methylbarbituric Acid	4.4	17
Methyldopa	2.2	126
	10.6 (amine)	
	9.2 (1st phenol)	
	12.0 (2nd phenol)	
N-Methylephedrine	9.3	127
Methylergonovine	6.7	81
N-Methylglucamine	9.2	126
Methylhexylamine	10.5	64
Methylparaben	8.4	41
Methylphenidate	8.8	128
Methylprednisolone-21-phosphate	2.6	129
	6.0	
Methylpromazine	9.4	62
Methyprylon	12.0	130

*pK_a given for protonated amine.

Continued

Name	pK_a*	Reference
Methysergide	6.6	81
Metopon	8.1	14
Metoprolol	9.7	15
Metronidazole	2.6	131
Miconazole	6.9	132
Minocycline	2.8	15
	5.0	
	7.8	
	9.5	
Molindone	6.9	133
Monochloroacetic Acid	2.9	42
Morphine	8.0	32
	9.6 (phenol)	
Nafcillin	2.7	26
Nalidixic Acid	6.0 (amine)	134
	1.0	
Nalorphine	7.8	14
Naphazoline	3.9	64
1-Naphthol	9.2	38
2-Naphthol	9.4	38
Naproxen	4.2	135
Narcotine	5.9	3
Nicotine	3.1	136
	8.0	
Nicotine Methiodide	3.2	136
Nicotinic Acid	4.8	18
Nitrazepam	10.8	117
	3.2	
o-Nitrobenzoic Acid	3.2	38
m-Nitrobenzoic Acid	3.6	38
p-Nitrobenzoic Acid	3.7	38
Nitrofurantoin	7.2	91
Nitrofurazone	10.0	137
Nitromethane	11.0	12
o-Nitrophenol	7.2	38
m-Nitrophenol	8.3	38
p-Nitrophenol	7.1	38
8-Nitrotheophylline	2.1	51
Norhexobarbital	7.9	123
Norketamine	6.7	110
Norparamethadione	6.1	85
Nortrimethadione	6.2	85
Noscapine	6.2	18
Novobiocin	4.3	18
	9.1	
Ornidazole	2.6	131
Orphenadrine	8.4	15
Oxamic Acid	2.1	12
Oxazepam	1.8	138
	11.1	
Oxyphenbutazone	4.5	93
	10.0 (phenol)	
Oxytetracycline	3.3	56
	7.3	
	9.1	
Pamaquine	8.7	3
Papaverine	5.9	42
Penicillamine	1.8 (carboxyl)	18
	7.9 (amino)	
	10.5 (thiol)	
Penicillin G	2.8	42
Penicillin V	2.7	19
Penicilloic Acid	5.2	139
Pentachlorophenol	4.8	74
Pentobarbital	8.0	32
Perphenazine	7.8	21
Phenacetin	2.2	29
Phenadoxane	6.9	14
Phendimetrazine	7.6	35
Phenethicillin	2.7	19
Phenformin	11.8	140
Phenindamine	8.3	37
Phenindione	4.1	27
Pheniramine	9.3	37
Phenmetrazine	8.5	35
Phenobarbital	7.5	13
Phenol	9.9	7
Phenolsulfonphthalein	7.9	18
Phenoxyacetic Acid	3.1	12
Phentermine	10.1	35
Phenylbutazone	4.4	141
Phenylbutazone (isopropyl analog)	5.5	142
Phenylephrine	9.8 (amine)	143
	8.8 (phenol)	
Phenylethylamine	9.8	24
Phenylpropanolamine	9.4	109
Phenylpropylmethylamine	9.9	24
Phenyramidol	5.9	18
Phenytoin	8.3	144
o-Phthalamic Acid	3.8	12
Phthalic Acid	2.9	6
Phthalimide	7.4	12
Physostigmine	2.0	3
	8.1	
Picolinic Acid	5.3	38
Picric Acid	0.4	42
Pilocarpine	1.6	3
	7.1	
Piperazine	5.7	30
	10.0	
Piperidine	11.2	6
Pirbuterol	3.0 (pyridine)	145
	7.0 (pyridol)	
	10.3 (amine)	
Plasmoquin	3.5	146
	10.1	
Polymyxin B	8.9	15
Prazepam	3.0	147
Prilocaine	7.9	39
Probarbital	8.0	22
Probenecid	3.4	1
Procainamide	9.2	148
Procaine	9.0	149
Procarbazine	6.8	150
Prochlorperazine	3.6	64
	7.5	
Promazine	9.4	53
Promethazine	9.1	64
Propicillin	2.7	19
Propiomazine	6.6	151
Propionic Acid	4.9	42
Propranolol	9.5	15
i-Propylamine	10.6	12
n-Propylamine	10.6	12
Propylhexedrine	10.5	64
Propylparaben	8.4	41
Propylthiouracil	7.8	152
Pseudoephedrine	9.9	35
Pyrathiazine	8.9	64
Pyrazinamide	0.5	18
Pyridine	5.2	6
Pyridoxine	2.7	153
	5.0 (amine)	153
	9.0 (phenol)	154
Pyrilamine	4.0	3
	8.9	
Pyrimethamine	7.2	1

*pK_a given for protonated amine.

Name	pK_a*	Reference
Pyrimethazine	9.4	53
Pyrrobutamine	8.8	37
Pyruvic Acid	2.5	18
Quinacrine	8.0	3
	10.2	
Quinidine	4.2	3
	8.3	
Quinine	4.2	3
	8.8	
Reserpine	6.6	42
Resorcinol	6.2	12
Riboflavin	1.7	3
	10.2	
Rifampin	1.7 (C-8 phenol)	155
	7.9 (piperazine N)	
Saccharic Acid	3.0	12
Saccharin	1.6	42
Salicylamide	8.1	32
Salicylic Acid	3.0	6
	13.4 (phenol)	
Scopolamine	7.6	3
Secobarbital	8.0	156
Serotonin	4.9	18
	9.8	
Sorbic Acid	4.8	12
Sotalol	9.8 (amine)	157
	8.3 (sulfonamide)	
Spectinomycin	7.0	18
	8.7	
Strychnine	2.5	3
	8.2	
Succinic Acid	4.2 (1st)	57
	5.6 (2nd)	
Succinimide	9.6	158
Succinuric Acid	4.5	12
Sulfacetamide	5.4	159
	1.8	
Sulfadiazine	6.5	32
Sulfadimethoxine	6.7	159
	2.0 (amine)	
Sulfadimethoxytriazine	5.0	9
Sulfaethidole	5.4	32
Sulfaguanidine	2.8	32
Sulfamerazine	7.1	42
Sulfameter	6.8	18
Sulfamethazine	7.4	32
Sulfamethizole	5.4	32
Sulfamethoxypyridazine	7.2	9
Sulfanilamide	10.4	160
Sulfanilic Acid	3.2	6
Sulfaphenazole	6.5	159
	1.9 (amine)	
Sulfapyridine	8.4	42
Sulfasalazine	0.6 (amine)	161
	2.4 (carboxyl)	
	9.7 (sulfonamide)	
	11.8 (phenol)	
Sulfathiazole	7.1	42
Sulfinpyrazone	2.8	93
Sulfisomidine	7.5	159
	2.4 (amine)	
Sulfisoxazole	5.0	32
Talbutal	7.8	10
Tartaric Acid	3.0 (1st)	57
	4.3 (2nd)	
Tetracaine	8.5	70
Tetracycline	3.3	56
	7.7	
	9.5	
Thenyldiamine	3.9	3
	8.9	
Theobromine	8.8	32
	0.7 (amine)	
Theophylline	8.8	32
	0.7(amine)	
Thiamine	4.8	153
	9.0	
Thiamylal	7.3	123
Thioacetic Acid	3.3	12
Thioglycolic Acid	3.6	12
Thiopental	7.5	123
Thiopropazate	3.2	64
	7.2	
Thioridazine	9.5	53
Thiouracil	7.5	152
Thonzylamine	8.8	37
L-Thyronine	9.6 (phenol)	116
L-Thyroxine	2.2 (carboxyl)	162
	6.7 (phenol)	
	10.1 (amine)	
Tolazamide	5.7	15
Tolazoline	10.3	163
Tolbutamide	5.3	32
p-Toluidine	5.3	7
Trichloroacetic Acid	0.9	42
Triethanolamine	7.8	72
Triethylamine	10.7	29
Trifluoperazine	4.1	53
	8.4	
Triflupromazine	9.4	64
Trimethobenzamide	8.3	164
Trimethoprim	7.2	165
Trimethylamine	9.8	12
Tripelennamine	9.0	37
Triprolidine	6.5	166
Troleandomycin	6.6	18
Tromethamine	8.1	72
Tropacocaine	9.7	30
Tropic Acid	4.1	12
Tropicamide	5.2	167
Tropine	10.4	12
Tubocurarine Chloride	7.4	168
Tyramine	9.5 (phenol)	109
	10.8 (amine)	
Urea	0.2	169
Uric Acid	5.4	170
	10.3	
Valeric Acid	4.8	42
Vanillic Acid	4.5	12
Vanillin	7.4	17
Vinbarbital	8.0	22
Vinblastine	5.4	171
	7.4	
Viomycin	8.2	18
	10.3	
	12.0	
Warfarin	5.1	172
Xipamide	4.8 (phenol)	173
	10.0 (sulfonamide)	

*pK_a given for protonated amine.

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