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TRACE ELEMENT MEDICINE AND CHELATION THERAPY

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Preface

Health is taken for granted until one is ill and then we search out the miracles of modern science in order to gain treatment. An increasing number of people are adding many healthy years to their lives by optimizing the biochemical reactions that drive them. History has taught us that whenever nature has a difficult task to perform in biochemical evolution it has focused upon the chemistry of a metal ion or a cluster of metal ions to play a central role.

Such metal ions involve a large proportion of the Periodic Table but particular emphasis must be placed upon the trace elements associated with the first row transition metals because their low concentrations (less than 10 g for the total of several elements functioning in the biochemistry of a 70 kg person) dictates that they are pivotal and yet sensitive to environmental and pharmaceutical influences.

In medicine, chelation therapy is gaining increased acceptance in order to balance concentrations of essential metal ions in the body and to remove undesirable metal ions.

There is increasing emphasis being placed upon dietary trace elements on the understanding that 'a slow leak can sink a big ship' and having a third of our adult population now aged 60 or over it is important to have a balanced diet throughout one's life.

An ever-increasing number of healthcare agents is being offered to us by a wide range of retail outlets in addition to the pharmacist. We are under the pressure of commercial companies to buy such products and some of them may be using only partial data concerning their beneficial aspects in order to boost profits.

This book draws on our combined 60+ years of experience of researching in the area and describes important aspects of these topics listed above. It also suggests further reading where greater information can be found.

By and large the knowledge to tackle most of the modern-day problems described herein already exists. It is up to the reader to search for and to select such knowledge, to adopt that which is appropriate, and to see that it is put to good effect. Through the efforts of very many co-researchers and colleagues over the years, we hope that this small volume can facilitate that drive.

David Taylor
David Williams

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Chapter 1

Introduction

WHAT IS LIFE?

Life is a complex process that as yet defies accurate scientific definition. The eminent biochemist, and Nobel Laureate, Christian de Duve has described life as a system which is able '*to maintain itself in a state far from equilibrium, grow, and multiply, with the help of a continual flux of energy and matter supplied by the environment*'. In amplification of this description de Duve defines 'seven pillars of life' which are necessary and sufficient for all forms of life. Thus any living system must have the ability to:

1. *Manufacture* its own constituents from materials available from its surroundings;
2. *Extract energy* from its environment and convert it into the different forms of work that need to be performed to stay alive;
3. *Catalyse* the numerous chemical reactions required to support its activities;
4. *Inform* its biosynthetic and other processes about how to guarantee accurate reproduction;
5. *Insulate* itself in such a way that it keeps strict control over its exchanges with its external environment;
6. *Regulate* its activities in order to preserve its dynamic organization in the light of environmental changes;
7. *Multiply* itself.

Implicit within this statement is the fact that life is dependent on the laws of nature, which are both imperative and inescapable: these laws mean that the development of any living system, or part of any such system, is entirely dependent on the biochemical milieu in which it develops. The statement also contains a paradox in that the definition states that the living system is in a state far from equilibrium, yet 'pillars

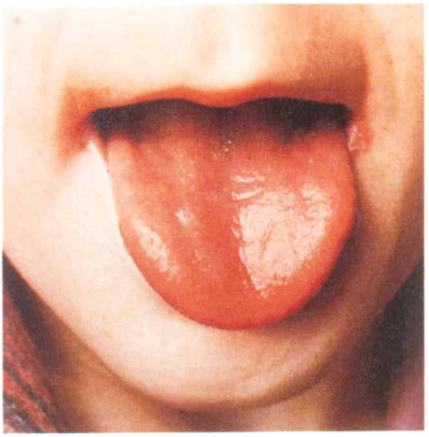

Plate 1 *Iron deficiency anaemia leads to pallor of the tongue, lips, and face.* Reproduced by permission from C.D. Forbes and W.F. Jackson, 'A Colour Atlas of Clinical Medicine', Wolfe, 1993

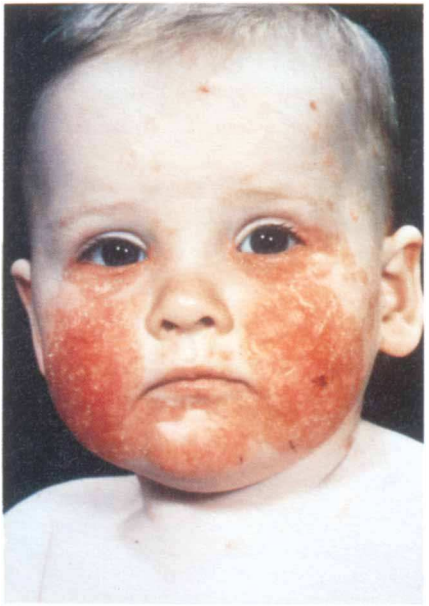

Plate 2 *An inability to acquire sufficient bioavailable zinc from the gastrointestinal tract produces acrodermatitis enteropathica which readily yields to the administration of zinc complexes.* Photograph reproduced with permission from National Medical Slide Bank

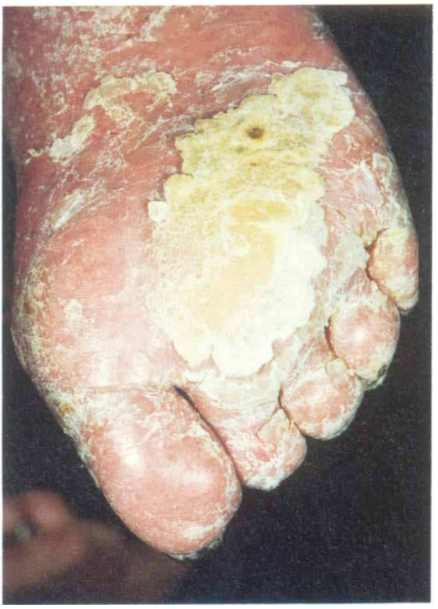

Plate 3 *Psoriasis strains the capacity of human biochemistry to supply zinc in sufficient quantities and although zinc imbalance does not necessarily cause psoriasis, symptoms such as those shown often respond to zinc administration applied topically.* James Stevenson/Science Photo Library

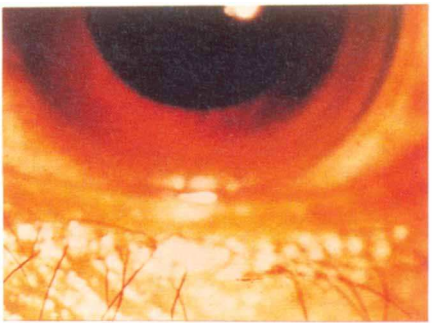

Plate 4 *Copper imbalance causing Kayser–Fleischer rings of copper deposited in the outermost region of the cornea of the eyes. A genetic fault produces an inability to turn dietary copper into ceruloplasmin which, in turn, overloads the albumin* $\rightleftharpoons$ *low molecular mass* $\rightleftharpoons$ *aquated cupric ions system and results in copper compounds becoming deposited (see Chapter 3, Table 3.3). Treatment involves chelation therapy to remove as much of this excess copper as possible.* Reproduced by permission from I.G. Barrison, M.G. Anderson, and P.B. McIntyre, 'Gastroenterology in Practice', Gower Medical, 1992

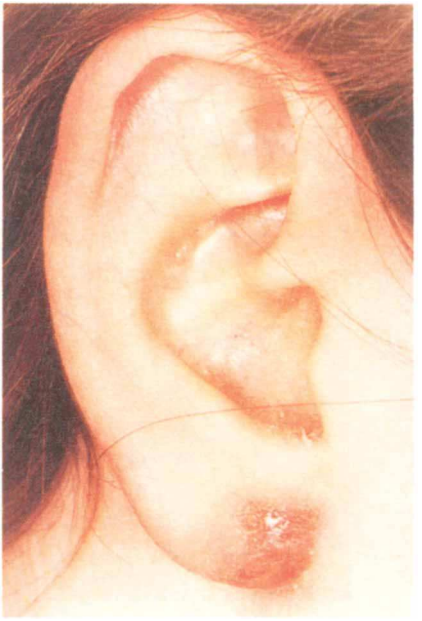

Plate 5 *Nickel allergy seen as contact dermatitis arising from the wearing of jewellery or wrist-watches,* etc. *containing nickel. Such sensitivities to nickel can affect up to 10% of the female population.* Reproduced by permission from C.D. Forbes and W.F. Jackson, 'A Colour Atlas of Clinical Medicine', Wolfe, 1993

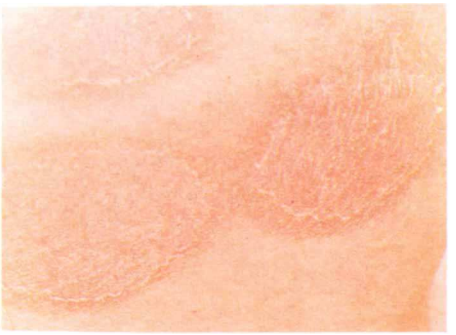

Plate 6 *Sensitivity to metallic gold or its alloys seen as a Riasiform eruption. Such gold rashes are often seen in patients suffering from rheumatoid diseases whereby levels of essential metals such as copper and zinc are also disturbed. Similarly, the use of gold compounds in the treatment of arthritis can produce such skin problems.* Reproduced by permission from C.D. Forbes and W.F. Jackson, 'A Colour Atlas of Clinical Medicine', Wolfe, 1993

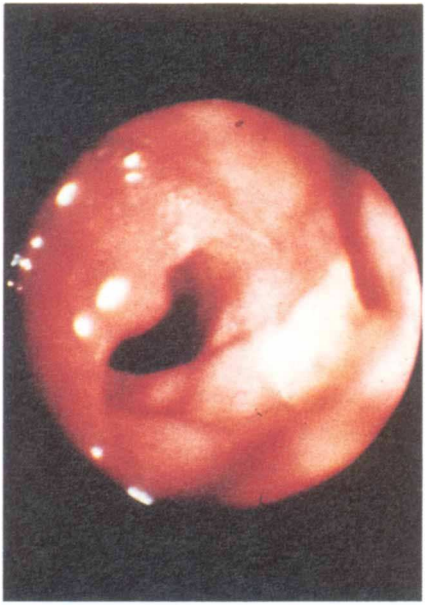

Plate 7 *Bismuth compounds administered in the treatment of stomach ulceration are seen to precipitate and form a protective patch over the ulcer.* Photograph provided by Yamanouchi Pharma Ltd.

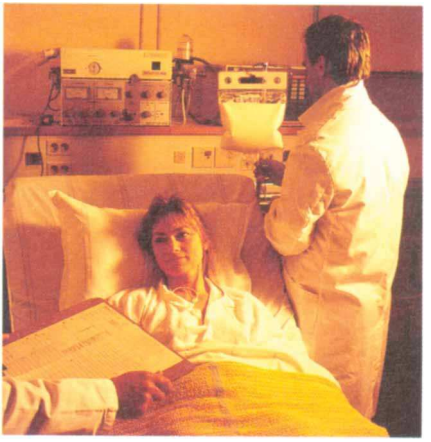

Plate 8 *Total parental nutrition wherein up to three litres of a colloidal emulsion is administered intravenously per patient per day. Trace elements which are necessary for long-term TPN include calcium, magnesium, iron, copper, zinc, cobalt, manganese, selenium, chromium, molybdenum, iodine, fluorine, phosphorus, chlorine, sodium, and potassium.* Photograph provided by Kabi Pharmacia

five and six' demand that the reactions and processes essential for life strive to maintain themselves in a *quasi-steady state*. The essential components of the biochemical milieu must be supplied from the environment in the form of foodstuffs, gases, and water.

The basic unit of life is the cell and the simplest living sytems are single cells that possess all the above capabilities, drawing their building materials from simple chemical substances in their, generally aqueous, environment. More complex life forms, for example, *Homo sapiens*, are multicellular organisms in which every cell does not possess all of the 'seven pillars of life' and life is possible only because the cells form a society whose health is dependent on the integrated activities of the different cell types within the system. In the human body there are some 10^{15} such cells.

It has been known for many years that healthy human, or animal life, requires the provision of adequate quantities of numerous organic substances (for example, proteins, sugars, fatty acids, and vitamins) and of such inorganic ingredients as calcium and iron. However, the requirement for many other inorganic elements went unrecognized for a long time because they are present in human tissues and in foodstuffs in very low concentrations. Fortunately, the natural content of these *trace* elements in food, or in soil impurities clinging to some foodstuffs, were generally sufficient to meet human needs so that health-impairing deficiencies occurred only relatively rarely. However, with people living much longer and with the often highly refined foods in our diet, long-continued, marginally sub-optimal intakes of essential or beneficial metals may over long periods lead to serious deficiency symptoms. This is not to be interpreted as a recommendation for us to become health food fanatics.

More recently, due in no small part to improvements in chemical analysis, metal deficiency diseases have become more widely recognized and this has focused the attention of laboratory researchers in biochemistry, inorganic chemistry, clinical medicine, and pharmacology upon the exact determination of elemental concentration (in order to establish the elemental status of the patient) and on the mechanisms involved in the bio-availability, assimilation, and excretion of trace elements, some of these conditions are illustrated in the colour plates (Plates 1–8, pages 2–5). Even more recently pathological conditions arising from trace element excess have come to light, perhaps because environmental pollutants have been suspected of causing symptoms in people living or working in a specific area. Trace element deficiency syndromes are not always mirrored in wild animals because, living in a natural habitat, they tend to acquire their trace elements from both

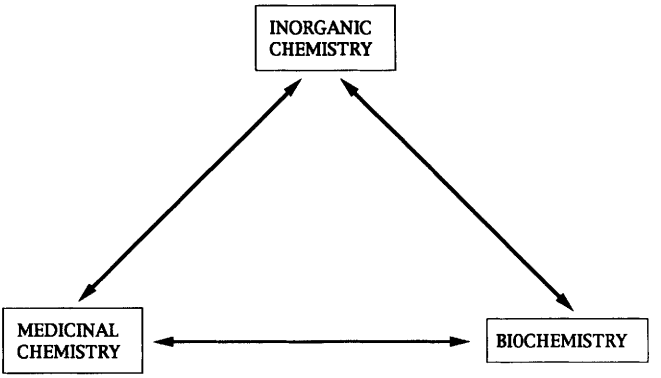

Figure 1.1 *The relationships between inorganic chemistry, medicinal chemistry, and biochemistry in human health*

their diet and from soil particles eaten with their food, thus they may avoid the deficiencies or excesses which can arise from our consumption of standardized, purified, and processed foods and can lead to the so-called 'diseases of civilization'.

The aim of this book is to consider the importance of trace elements for the maintenance of human health and to illustrate the relationships between inorganic chemistry, biochemistry, and medicine (Figure 1.1). This is achieved through discussions of how a deficiency, or an excess of metals, may cause disease; how drug-induced interference with the natural metal binding mechanisms in tissues may be used to treat illnesses; and how, in cases of metal overload, chelation therapy with powerful ligands may be used to reduce the risks of serious health effects by accelerating the natural rate of excretion of the offending metal.

EVOLUTION

The origin of life and its evolution into the life-forms we know today remains a contentious issue. This controversy arises mainly because there is a lack of hard scientific data, from fossils or other sources, on how primitive cells develop (microbial evolution being about as far back as we can go). Cosmology and geochemical data suggest that the Earth was formed as a condensation product from gas and dust particles from an immense supernova in outer space some $(4.5–4.7) \times 10^9$ years ago.

The geochemical theory of evolution suggests that the newly formed planet had a hard core and a reducing atmosphere containing H_2O, H_2S, NH_3, CH_4, and perhaps some CO_2: over the next 10^9 years these

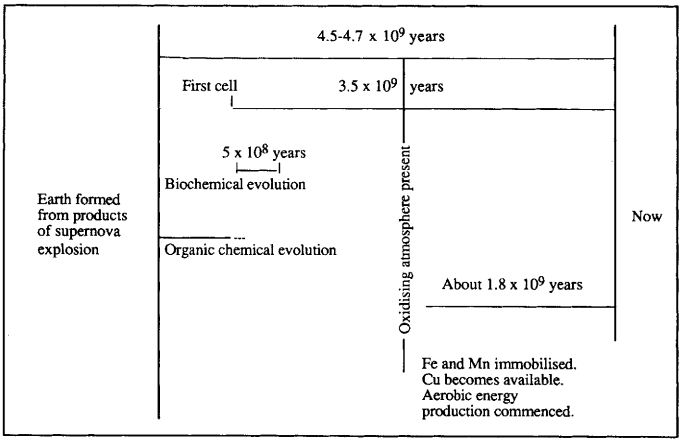

Figure 1.2 *Evolution of bio-inorganic species*

substances were bombarded with energy, for example from the sun or from nuclear changes within the Earth's crust or atmosphere, resulting in the formation of simple organic species. These then reacted with inorganic constituents to yield simple monomeric biochemicals (amino acids, sugars, nucleotides, *etc.*) and then biopolymers (proteins, starches, glycogen, and nucleic acids) and eventually to the first primitive cell which then required a further 3.5×10^9 years to evolve into life as we know it today; this evolution is illustrated diagrammatically in Figure 1.2.

Biochemical theory based on our knowledge of the rates of reaction of biological catalysts (enzymes) suggests, however, that the time from the development of protometabolism to the formation of the first oligonucleotides (nucleic acids) may in fact have required only a few thousands of years, at most a few tens of thousands of years, rather than the hundreds of millions of years implied from the geochemical data. A shorter period for the evolution of life means that there would not have been the same necessity for inordinately long periods of environmental stability and also that evolution could have been attempted in different places at different times.

Whatever the real time scale required for evolution, the process was a protracted and continuous progression from primitive, inefficient mechanisms to more complex, efficient ones. The reactions all occurred in the oceans, or along the shorelines, and metal ions must have played a critical role both in determining the compositions of the biopolymers we know today and in dictating whether the L- or D-configurations of sugars

and amino acids would be preferred. The concept that tides produced anhydrous conditions on beaches dried by the Sun and wind and that reactions occurred in the structural grooves of 'beach' crystals, such as silicates or apatites (calcium phosphates) appears logical. The condensation of monomers into polymers requires the withdrawal of water molecules and such dehydration is extremely unlikely in the ocean unless a heterogeneous catalyst is present.

The primeval cell probably contained about 100 protein molecules, in contrast to the many millions of protein molecules in modern cells, and it certainly contained a variety of metal ions some of which fulfilled structural osmotic or catalytic roles. Magnesium would have been particularly good in this latter role since it is known to catalyse condensation reactions and to have been present in high concentration in primeval oceans (it is currently present in a concentration of 50 mmol dm^{-3} in modern sea water). The close similarity of the ionic composition of many of our body fluids and those found in sea water strongly supports the view that life evolved in, or from, the oceans.

Elemental analyses of several hundred plant and some two hundred, animal species have identified the elements present in biological systems and, in Periodic Table terms, these are generally relatively light, and are those elements which are found nearest the surface of the Earth's crust (see Figure 1.3). The biologically *essential* metals are Na, K, Li, Mg, Ca, Mo, Mn, Fe, Co, Cu, and Zn and their ions all have potentially powerful complexing capabilities; some also possess important redox properties. Since the early atmosphere of the Earth was a reducing one, Mn(II) and Fe(II) would have been important in the primeval cell systems. In addition to the essential metals, some other elements are recognized as being *beneficial* to life, for example Si, V, Cr, Ni, Se, Sn, Br, and F (and possibly also As, B, Ba, Cd, and I) and these are believed to have become involved only in more recent, more highly organized life-forms.

It is signifcant that the human body has not yet evolved mechanisms to protect against overdoses of beneficial elements which are as effective as those which function to minimize or prevent the effects of overloading with many essential metals. For example, 0.1 p.p.m. of Se is beneficial while 10 p.p.m. may be carcinogenic (capable of causing cancer). Recalling that the Periodic Table is founded upon the concept of relationships between elements, we may deduce that the strongest challenges to the normal cellular processes will come from elements having Periodic Table positions adjacent to essential and beneficial elements, for example the pollutants Cd and Hg with Zn, or Pb with Sn. It appears that these dangerous metal ions interact with the *in vivo*

IA	IIA	IIIB	IVB	VB	VIB	VIIB	VIII	VIII	VIII	IB	IIB	IIIA	IVA	VA	VIA	VIIA	VIII A
H																	He
Li	Be											*B*	C	N	O	*F*	Ne
Na	**Mg**											Al	*Si*	P	S	Cl	Ar
K	**Ca**	Sc	Ti	*V*	*Cr*	**Mn**	**Fe**	**Co**	*Ni*	**Cu**	**Zn**	Ga	Ge	*As*	*Se*	*Br*	Kr
Rb	Sr	Y	Zr	Nb	**Mo**	Tc	Ru	Rh	Pd	Ag	*Cd*	In	*Sn*	Sb	Te	*I*	Xe
Cs	*Ba*	La-Lu	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac-Lr	Ku	Ns	Unh 106	Unh 107	Unh 108	Unh 109									

X Essential *Y* Beneficial

Figure 1.3 *The Periodic Table of the elements highlighting those trace elements that are* **essential** *or* beneficial *to life*

mechanisms through which essential trace elements operate and thus reduce or prevent the normal biochemical functions.

The metal ion composition of the human body has been dictated by several principles. The major role has been played by the abundance of metal ions in the hydrosphere, but, when metals possess redox properties, it is imperative to have an adequate supply of matched reducing and oxidizing ligands in order to maintain the oxidation state required for the particular biological mechanism (*e.g.* porphyrins to complex Fe(II) in the haem moiety). When oxygen entered the atmosphere, mainly from the photosynthetic activity of blue-green algae, it was very toxic to most living systems because it tended to change the oxidation states of Mn(II), Fe(II), Co(II), and Cu(I), *etc.* However, the cell soon adapted to protect itself against oxidation, principally through evolving *peroxisomes* which convert O_2 to H_2O_2 and then to water. In the early days copper was trapped mainly as insoluble Cu(I) sulfides, but once an oxidizing atmosphere developed, Cu(II) could join Mg, Fe, and Mn as one of the primitive essential trace metals. At about the same time Fe and Mn became immobilized by the reactions:

$$Fe(II) \rightarrow Fe_3O_4(s) \rightarrow FeOOH(s)$$

and

$$Mn(II) \rightarrow Mn_3O_4(s) \rightarrow MnO_2(s)$$

and the atmospheric ozone content crept up to about 1%, which is adequate to screen out the most harmful effects of the Sun's ultraviolet radiation: the latter is apparently more destructive to aerobic than anaerobic metabolism. These changes allowed aerobic metabolism to replace anaerobic metabolism, some organisms using iron and others the newly released Cu(II) for oxygen assimilation.

Certain evolutionary constraints arose because of competition for metal complexing ligands in the primitive systems. Ca^{2+} and Cu^{2+} ions provide a good example of this phenomenon. When Ca^{2+} became available to the evolving system it was rapidly assimilated into the primitive biocatalysts, the protoenzymes, through binding to carboxylate groups. However Cu^{2+} complexed far more firmly to the abundance of amino acids inside the cells (Ca^{2+}–amino acid interactions are weak) and it was only when the amino acids began to condense to form polypeptides that Cu^{2+} binding sites evolved which were powerful enough to overcome the fierce amino acid–Cu^{2+} complexing opposition that the Cu^{2+} proteins evolved. The evolution of the Cu(II)-specific binding sites was slow, requiring many generations of mutation. This principle of free amino acid 'selectivity' still operates today and forms part of the natural *detoxication mechansims* which complex polluting or contaminating metals thus rendering them less harmful to the organism than if they were bound to a biochemically critical site within an essential protein.

The main group metal ions Na^+, K^+, Ca^{2+}, and Mg^{2+} possess closed-shell electronic structures and their roles *in vivo* have evolved to exploit their electrostatic bonding properties in preference to their covalent characteristics; therefore their charges and ionic radii have been important in bio-inorganic evolution. For example, their ions are strongly aquated with the following results: (a) when they form complexes many molecules of water are liberated and the bonds to the ligand are entropy stabilized and (b) the highly ordered inner and outer solvation spheres of say Mg^{2+} and its high concentration within cells helps to produce the highly organized systems that modern cells exhibit.

It is interesting to note that Mg^{2+} ions have been present in cells from the very beginning whereas Ca^{2+} has only become essential in the later, more sophisticated species, for example those which require signal transmission along nerves and, perhaps, also a bony skeleton or a hard shell: Ca^{2+}, therefore, like the beneficial elements mentioned earlier, is one of the 'newer' elements. Despite being essential for human life, *Homo sapiens* has not yet adapted or evolved to handle completely all the chemical problems raised by the presence of Ca^{2+} in the body, and its precipitates, as phosphates, oxalates, *etc.*, all too commonly cause problems such as atherosclerosis, kidney or gall stones, and cataracts.

Some concluding comments to this brief survey of evolution are apposite:

1. The specificity of metals ions in the human body is extremely good. Why else will many of us function smoothly for 70+ years without need for 'service, lubricant change, reprogramming or spare parts'?

2. Researchers into the origins of life and chemical evolution may perhaps have become over fascinated with the organic chemical aspects, tending to lose sight of the fact that from the time when simple molecules were washed onto 'beach crystals' and condensed into stereoisomers, the organic and the inorganic aspects of chemical evolution have progressed in hand.
3. Evolution took place under relatively constant environmental conditions, but more recently, within the last 3000 years, and especially the last 200 years, human activities such as mining, large-scale utilization of metals such as lead, mercury, and cadmium and the growth of the chemical, petrochemical, and other industries, have lead to quite serious local disturbances, and to much smaller global changes of the environment that have already been, or may yet be demonstrated to be seriously detrimental to many living systems. Some of the effects of metal pollution on human health will be discussed in the next chapter.

COMPOSITION AND STRUCTURE OF LIVING SYSTEMS

This book is concerned with bio-inorganic chemistry, *i.e.* the chemistry of reactions involving metals and other trace elements with the components of living cells and tissues. In order to consider the influence of metal ions, ligands, and metal complexes on human health we should first be able to envisage the basic composition and organization of living systems and the vast differences in scale in terms of the relative sizes and masses of the biological and chemical species we are discussing. Humans are dependent on species smaller than themselves and these, in turn, are dependent on even tinier species.

As has been mentioned above the basic unit of life is the cell and *Homo sapiens* is a multicellular organisms in which many different, and often highly specialized, cell types interact with each other to make each individual human function in their own unique manner. The most abundant substance in the human body, and indeed in most living systems, is water, which accounts for about 70% of the body weight; the human body requires about 0.7 m^3 water per annum in order to stay alive. Although the tiniest cell may be loosely envisaged as a 'bag of chemicals', it is in fact a highly organized system enclosed by a highly structured, phospholipid–protein membrane. Within the cell itself there is a cytoskeleton, made up of various important proteins, in which is located the nucleus, that contains the genetic material of the cell (the DNA), and the various subcellular organelles, mitochondria, lysosomes, Golgi apparatus, *etc.* The mitochondria play a vital role in energy

metabolism, and are themselves most probably developed from a free-living bacterium; the lysosomes are concerned with the degradation of unwanted cellular materials and also play an important role in the detoxication of harmful substances. In both mitochondria and lysosomes, metals play essential roles as enzyme co-factors and in electron transport. Starting with the cell and extending to the complete organism, the human body can be envisaged as a series of compartments within which the inorganic constitutents are distributed, often in a highly specific manner. For example, about 97% of the body's K^+ is located within cells, where it acts as a co-factor for some enzymes and stabilizes internal structures, whereas about 94% of the body's Na^+, whose role is principally to maintain osmotic pressures and membrane potentials, is found in the extracellular fluids. Similarly about 98% of the body's Ca^{2+} is found in the hydroxyapatite of the mineral bone.

We have already seen that around 70% of the human body is water and this should be no surprise since, following development of the primeval cells in the oceans, evolution has continued within an aqueous environment and exploited the unique properties of water to the best advantage to living systems. Water is the only naturally occurring inorganic liquid and is the only compound which occurs in nature in all three physical states of matter: solid, liquid, and gas. The omnipotence of the roles of water in the human body may be seen by reference to Table 1.1. Water is used to provide bulk to the body and use is also made of its unusual chemical properties.

The high specific heat of water enables it to act as a heat buffer, thus minimizing the effects on the cells of fluctuations in environmental temperature. The high latent heat of vaporization permits humans to use the evaporation of sweat as a cooling mechanism.

The hydrogen bonding between molecules of liquid water means that it has a high melting-point, boiling-point, heat of fusion, and surface tension. Hydrogen bonding is also very important in biomolecules other than water: for example, the specific configurations of some proteins which permits them to function as enzymes or metal carriers is achieved by hydrogen bonding, and the two strands of the DNA are held together as a double helix through hydrogen bonding between the complimentary base pairs.

Many important properties of macromolecules depend on their interactions with water molecules. Water, being dipolar in nature, is a very good solvent for a wide variety of solids. Most ionic compounds, of course, dissolve because the ion solvation contributes enough energy to disrupt the crystal lattice structure. Polar compounds, *e.g.* alcohols, aldehydes, and ketones, dissolve due to the ability of water to form

Table 1.1 *Some features of water in the adult human*
(Data adapted from F. Franks, *Chem. Br.*, 1976, **10**, 278 and *Reference Man*, ICRP Publication 23, Pergamon Press, Oxford, 1975)

Water content/%	
Adult human	60–70
Human embryo, 1st month	93
Liver	72
Muscle	79
Skin	62
Nervous tissue	77
Connective tissue	63
Adipose tissue	15
Plasma, saliva, gastric juice	94–99
Adult input/cm^3 day^{-1}	
Drink	1950
Food	700
Food oxidation	350
Adult output/cm^3 day^{-1}	
Respiration	850
Perspiration	650
Excretion	1500
Adult turnover/cm^3 day^{-1}	
Saliva	1000–1500
Gastric juice	1000–2000
Bile	500–1000
Pancreatic juice	600–800
Intestinal juice	200
Adult throughput/dm^3 day^{-1}	
Kidney	180
Heart	7000

hydrogen bonds to their polar groups. Molecules which contain both hydrophilic and hydrophobic groups, such as fatty acids and polar lipids, become dispersed in water as micelles.

The control of the degree of ionization of water is of crucial importance, with the following reactions being most important:

$$H_3PO_4^- \rightleftharpoons H_2PO_4^{2-} + H^+ \text{ inside cells}$$

and

$$H_2CO_3 \rightleftharpoons HCO_3^- + H^+ \text{ in the body fluids outside the cells.}$$

Biological membranes and the active sites of enzymes may be considered as special types of solvents in their own right. In the membranes, phospholipid, protein, and carbohydrate molecules make up a system through which both ionic and non-ionic species must pass, some by simple diffusion, others by some form of specific active transport. At the active sites of enzymes, exceptional conditions exist which permit substrates to be held in stereochemical forms which modify the chemical activity, but which would be extremely unstable *in aquo*.

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Chapter 2

The Elemental Composition of the Human Body

THE ELEMENTS IN THE HUMAN BODY

The important roles played by a wide variety of elements in the function of living systems has been discussed in Chapter 1 and we now turn to consider the human body and its function in more detail. *Homo sapiens* has emerged over the past three and a half million or so years as a creature that when fully grown stands 150 to 180 cm high, weighs some 45 to 90 kg and has a life-span ranging from around 60 to over 100 years. The adult human consists of some 10^{15} cells of various types, containing some 10^{27} molecules of different compounds; the vast majority of these molecules are in a continual state of turnover being broken down and resynthesized with half-times that, depending on the particular compound, may range from a fraction of a second to many years. Similarly most of the cells are in a continual state of replacement with millions of cells dying and being replaced every day for up to a century.

The average amounts of some essential and non-essential elements in the human body are shown in Table 2.1. This shows that the weights of some of the essential elements in the body vary by six or more orders of magnitude. Oxygen, at ~ 45 kg, is by far the most abundant element with the majority being present as one simple inorganic compound: water. Some of the elements listed in Table 2.1, and marked with an asterisk, have no known beneficial function and are present in the body simply because they are present in rocks and soils and find their way into water and foodstuffs and from thence into the human body; many of these elements are present in only minute quantities.

Each of the elements shown in the three groups in Table 2.1, irrespective of whether it is essential, beneficial or even potentially toxic, has its own individual pattern of intake into the body, transfer to the blood,

Table 2.1 *The elemental composition of a 70 kg 'reference' person*

Elements	*Mass*	
	g	*Moles*
	A. Main group non-metals	
Hydrogen	7000	3500
Carbon	12 600	1050
Nitrogen	2100	75
Oxygen	45 500	1425
Phosphorus	700	22.5
Sulfur	175	5.5
Fluorine	0.8	0.02
Chlorine	105	3.0
Bromine	0.2	0.025
Iodine	0.013	0.0001
	B. Main group metals	
Lithium	0.0007	0.0001
Boron	0.01	0.0009
Sodium	105	4.6
Potassium	140	3.6
Rubidium*	1.1	0.013
Caesium*	0.0015	0.00001
Aluminium	0.1	0.0037
Zinc	2.3	0.035
Silicon	1.4	0.05
Arsenic	0.014	0.0002
Antimony	0.07	0.0006
Selenium	0.02	0.003
Tin	0.03	0.0002
Lead	0.08	0.0004
Cadmium	0.03	0.0003
Magnesium	35	1.4
Calcium	1050	26
Strontium	0.14	0.0016
Barium*	0.016	0.00012
Radium*	3×10^{-11}	1.4×10^{-13}
Uranium*	9×10^{-5}	3.8×10^{-14}
Plutonium*	6×10^{-18}	$2.5.10^{-20}$
	C. Transition series metals	
Titanium	0.01	0.0002
Vanadium	0.02	0.0004
Chromium	0.005	0.0001
Manganese	0.02	0.00036
Iron	4.2	0.075
Cobalt	0.0007	0.0001
Nickel	0.01	0.0002
Copper	0.11	0.0016
Zirconium	0.3	0.003
Niobium	0.1	0.001
Molybdenum	0.005	0.00005

*Elements with no recognized physiological role.

Table 2.2 *The fractional absorption (f_1) and the notional equivalent half-time (T_{eq}) of retention in the human body of some main group and transition series metals. The f_1 is the fraction of an orally administered element which passes from the gastrointestinal tract to the blood stream; the notional T_{eq} assumes a constant rate of loss from a single compartment*

Element	f_1	T_{eq}/days
	Main group metals	
Lithium	1	1
Sodium	1	10
Potassium	1	30
Rubidium*	1	60
Caesium*	1	110
Magnesium	0.5	0.8
Calcium	0.5	10000
Strontium	0.3	10000
Barium*	0.2	10000
Radium*	0.2	10000
Uranium*	0.01	10000
Plutonium*	0.0005	10000
	Transition series metals	
Manganese	0.1	45
Iron	0.15	2000
Cobalt	0.1	40
Copper	0.5	40
Zinc	0.5	400
Molybdenum	0.8	45
Nickel	0.05	1

* Elements with no recognized physiological role.

utilization in the tissues and finally excretion from the body. For example, hydrogen taken into the body by the ingestion of water, or the inhalation of water vapour, is rapidly and completely transferred to the blood, from where it passes into the tissues to participate in many different types of reaction, before being excreted from the body with an equivalent half-time of about 10 days. The equivalent biological half-time assumes that the material is being lost at a constant rate from a single compartment, whereas in fact more that one compartment and rate of loss may be involved. In contrast iron is taken into the body in foods or drugs, but its absorption from the gastrointestinal tract is closely controlled to meet the physiological needs of the body. However, the iron which is absorbed into the blood stream is effectively all retained in the body for a very long time. Some illustrative, nominal values for the

gastrointestinal absorption and the notional equivalent half-times of excretion from the body tissues for some important elements are listed in Table 2.2.

As we saw in Chapter 1, some elements are essential for life and others, although not truly essential, contribute to our general well-being. Some elements, though apparently harmless at 'normal' concentrations, may be toxic if they are present in rather larger amounts. For example, the natural total body content of barium is about 2 mg, of which about 90% is locked up in the hydroxy apatite of the bone mineral but about 400 times this amount ($\sim$ 800 mg) could cause death. However, because it is present as a species which is not bio-available, the so-called *Barium Meal* containing up to 200 g of the highly insoluble barium sulfate (solubility product 1.07×10^{-10} mol dm^{-3}), is routinely, and safely, administered orally to humans as a contrast medium for the radiological investigation of gastrointestinal disorders.

The proper functioning of the human body requires an adequate supply of the essential and beneficial elements and this is met from the diet, and in the case of some inorganic elements to a lesser extent from drinking water. For metallic elements the adequacy of the diet with respect to the requirements for a specific metal will depend on two factors: the concentration of the element of interest in the food and water and its bio-availability. The bio-availability is the extent to which the metal concerned is transferred from the gastrointestinal tract to the blood and this depends on the chemical behaviour of the element in the gastrointestinal tract, where the pH may vary from $\sim$ 2 in the stomach to about pH 8 in the small intestine, where high concentrations of complexing ligands are also present. The bio-availability of metals is element-specific and may range from virtually 100% for Group I metals like sodium, to less than 0.1% for readily hydrolysable metals such as plutonium.

For the essential elements the amounts in the body are normally controlled by physiological mechanisms, but for the non-essential, non-beneficial elements there are no such controls and the amounts in the body generally reflect the natural occurrence of the elements in food and water. For many such elements we may consider that there is a *base load* in the human body which reflects the natural intake of the elements in the diet. For some elements, industrial, mining or other human activities, may release metals into the environment. Such activities may result in a *civilization-related load* being added to the natural base load; in some circumstances this civilization-related load may be very much greater than the base load. For example, the natural concentrations of the highly toxic metal cadmium in soils are generally quite low, yet in the

Table 2.3 *Typical recommendations for the daily intake of some inorganic nutrients by young adult males* (from Coultate, 1991). *The amounts absorbed from the gastrointestinal tract into the blood will generally be only a fraction of these quantities*

Element	*RDA*/mg	*Element*	*RDA*/mg
Calcium	500	Phosphorus	800
Magnesium	350	Zinc	15
Iron	10	Iodine	0.15
Copper	2	Selenium	0.05

vicinity of old zinc smelting works, the concentrations may be increased by factors of 100 or more. Similar observations have been made for lead and mercury. Such increased environmental concentrations will often, but not always, lead to an increase of similar magnitude in the civilization-related load of the metal, or metals, but this may well occur if a large proportion of the foods in the diet are produced locally.

SUPPLYING ELEMENTS TO THE BODY

In order to keep healthy the body has specific daily requirements for essential and beneficial elements and these may be expressed as 'recommended daily amounts' (RDAs); these are discussed in more detail in Chapter 6. Some RDAs are listed in Table 2.3.

Both essential and non-essential trace elements are taken into the body with foodstuffs, and some of the elements may be biologically incorporated into the food itself while, particularly with vegetables, another part may be taken into the body in the form of soil particles which adhere to the foodstuff; it has been estimated that an adult human may ingest as much as 100 g of topsoil per year.

It is important to recognize these two sources of trace elements; first of all because the bio-availability of a trace element incorporated into a food material may be markedly different to that from a soil particle. Therefore a diet that, on the basis of a total elemental analysis, appears to provide adequate amounts of a particular element may in fact be quite inadequate because a large fraction of the metal is present in a highly insoluble and poorly available form in soil particles. Secondly the modern practice of offering washed and packaged vegetables for sale may result in a single serving of the product providing a much smaller, and even inadequate, amount of an element than would have been provided by a serving of freshly harvested and less well washed vegetables (see Chapter 8).

METALS AND HUMAN HEALTH

It has been recognized for many years that trace concentrations of the essential and beneficial metals are necessary for good human health, while other metals, *e.g.* lead or mercury may be quite toxic at similar concentrations. Further, for some beneficial elements, *e.g.* selenium, the difference between a beneficial and a toxic concentration in the body may be quite small.

In the past quarter of a century improvements in methods of total elemental analysis and the development of methods of measuring the different chemical forms in which an element may be present in a sample of tissue or body fluid have shown that metals may play pivotal roles both in health and in disease. Changes in the metal content of the human body may contribute to ill-health in various ways. Accidental or deliberate intake of much larger amounts of metals than those present in the normal diet may cause signs, or symptoms, of acute or chronic poisoning.

The presence of complexing ligands in the diet or drinking water may dangerously affect the bio-availability of a metal in the blood or tissues: one example of this is the use of powerful chelators, such as the anions of nitrilotriacetic acid or ethylenediaminetetraacetic acid (EDTA) as substitutes for phosphate in washing powders. It has been calculated that in some parts of the world the concentration of EDTA in ground or river water may be as high as 100 μmol dm^{-3}.

Genetic or other alterations in metal absorption from the gastrointestinal tract, or in excretion may result in the accumulation of metals in the body and the development of serious disease due to metal storage in the brain, liver or other tissues; the classic examples of this are Wilson's Disease and primary haemochromatosis, which are copper and iron storage diseases, respectively. Deficiencies of essential metals may also cause disease. Some examples of metal dependent diseases are listed in Table 2.4. The observation of increased tissue concentrations of a metal or metals in a particular disease state may not always indicate that the metal plays a causative role in its onset. A good example of this has been the recent controversy about the role of aluminium in the development of the premature senile dementia which is known as Alzheimer's Disease. Observations of high concentrations of aluminium associated with so-called neurofibrillary plaques in certain parts of the brain led to suggestions that the metal caused the disease. However, after many further studies it is now considered very unlikely that aluminium does play a causative role in this disease, there being several elements present in the silting up of the neurofibrillary tangles in the brains of victims of

Table 2.4 *Some conditions in which metals have, or may have, a causative role*

Element	*Disease associated with deficiency*	*Disease associated with excess of the element*
	Essential and beneficial elements	
Calcium	Bone deformities, tetany	Cataract; gall stones; atherosclerosis
Chromium	Disturbance of glucose metabolism	Lung cancer [Cr(VI)]
Cobalt	Anaemia	Cardiac failure: ?polycythaemia
Copper	Anaemia, kinky hair syndrome	Wilson's Disease: liver cirrhosis, neuropathy
Iron	Anaemia	Primary and secondary haemochromatosis; siderosis, liver cirrhosis
Lithium	Manic depression	
Magnesium	Convulsions	Anaesthesia
Manganese	Skelectal deformities; gonadal dysfunction	Ataxia; motor neurone diseases
Potassium		Addison's Disease
Selenium	Liver necrosis; white muscle disease	Blind staggers in cattle
Sodium	Addison's Disease; stomach cramps	
	Non-essential, non-beneficial elements	
Aluminium		Encephalopathy
Cadmium		Kidney damage, hypertension, lung oedema, gastrointestinal disturbances, bone disorders
Lead		Anaemia, gastrointestinal disturbances, peripheral neuropathy, encephalopathy, decrease in learning ability in children.
Mercury		Polyneuropathy, encephalopathy, stomatitis, colitis, kidney damage, uraemia
Thallium		Gastrointestinal disturbances, polyneuropathy, paralysis, psychological disturbances

Alzheimer's Disease. It has also been observed that there is an increased incidence of the motor neurone disease, amyotrophic lateral sclerosis (ALS) in those parts of the world where there are high concentrations of manganese in the soils, *e.g.* the Northern Territories of Australia and the island of Guam, and this has led to suggestions that this metal might play a causative role in the development of ALS. However, measurements of manganese concentrations in brain and spinal cord from normal subjects and ALS victims have not shown evidence of manganese accumulation in diseased tissues. However, multi-element analysis has indicated that the elemental patterns are different between normal and ALS tissues. Whether altered elemental relationships cause the disease, or whether they are caused by some disease induced biochemical or physiological change, such as reduced blood flow, remains to be discovered. However, the ability of an excess of one metal to interfere with the normal biochemical functions of another is already well recognized, for example, the chemistry of the metabolically disruptive action of the toxic metal cadmium is that of challenging essential zinc ions.

The mechanisms by which metals induce toxic effects or diseases are not well understood. The most toxic heavy metal ions, cadmium, lead, mercury, are potent enzyme inhibitors because the ions are readily polarizable and bind to donor groups in the enzyme, the binding strengths decreasing in the electron-donor element order S > N > O. *In vivo*, phosphate and chloride ions are ubiquitous and these may lead to the formation of insoluble species such as lead hydroxophosphate or only slightly soluble mercuric chloride.

Inorganic lead is toxic only in ionic form and exerts its effects largely by disturbance of the synthesis of haem and cytochromes as a result of complex formation with the sulfhydryl groups in the enzymes. Organolead compounds, for example, the original petrol 'antiknock' compound lead tetraethyl, are more toxic than inorganic forms and, because of their greater lipid solubility, can cause more severe injury to the brain and nerves. Lead poisoning may also occur through the inhalation of lead vapour, and this was quite commonly encountered in plumbers who, in the days when lead piping was in common use, made the so-called 'wiped' or welded joints in piping. In such circumstances the lead vapour is oxidized in the lungs to yield ionic lead which is quite rapidly absorbed into the blood stream, or cleared from the lungs into the gastrointestinal tract.

Mercury vapour is also rapidly converted into ionic form in the lungs and absorbed into the bloodstream. Ionic mercury, irrespective of whether it is absorbed from the lungs or from the gastrointestinal tract,

is highly toxic and causes severe cellular damage by interaction with proteins and other cell components, especially in tissues such as the intestine, kidney, and brain (*e.g.* the phrase 'mad as a hatter' refers to the severe mental disturbances caused by the use of mercuric nitrate in the manufacture of felt hats). As with lead, organic mercury compounds, such as alkylmercury derivatives are lipid soluble and can traverse the blood–brain barrier and can be deposited in nerve cells. This leads to disastrous and permanent injuries to nerve cells caused by the formation of mercury–protein complexes in cell membranes, and results in disturbance of electric potentials and the passage of nutrients across the membranes. Alkylmercury can also cross the placental barrier and cause catastrophic damage to a developing embryo or foetus.

Acute iron poisoning may occur by the ingestion of quite large amounts of iron compounds, for example, pharmaceutical products designed to treat an iron deficiency. The ingested iron can cause severe gastrointestinal disturbances with diarrhoea and vomiting, and sufficient iron may be absorbed into the blood stream to cause severe liver and kidney damage. Chronic iron poisoning manifests itself frequently in the form of siderosis and haemochromatosis. These conditions result from saturation of the normal iron transport protein, transferrin, in the blood and the deposition of the excess iron as iron-storage compounds, such as ferritin and heamosiderin, in tissues such as liver, spleen, and bone marrow, leading to cirrhosis and other forms of liver damage, and to kidney disturbances.

Haemochromatosis is found in two forms, primary, or *idiopathic*, in which the reasons for the spontaneous iron accumulation in the tissues is not fully understood; and secondary haemochromatosis in which the excess iron arises from iron administration for medical reasons. Patients receiving frequent blood transfusions to combat severe anaemia are unable to excrete the large amounts of iron which are released from the breakdown of the transfused erythrocytes and this leads to extensive build up of iron in the tissues. A particularly tragic example of such secondary haemochromatosis is found in sufferers from sickle cell anaemia, a genetic abnormality commonly found in young people in tropical regions, especially around the rim of the Mediterranean Sea. The disease causes severe anaemia for which blood transfusions are essential to prolong life. Chelation therapy can help to remove some of the excess iron, but intravenous desferrioxamine which is the most effective chelator available, is too expensive and inconvenient for the treatment of large numbers of poor patients in developing countries; there is an urgent need for an orally active chelator of low toxicity which can be manufactured cheaply.

Wilson's Disease is another metal storage disease caused by some unknown derangement of copper transport which results in the storage of excess of the metal in liver, brain and eyes and causes progressive liver, kidney, and brain damage. This damage cannot be cured although, as we shall see later, the progression of the disease can be slowed down by chelation treatment.

Much more research is needed to understand the mechanisms which lead to the spontaneous metal deposition in the tissues of patients with Wilson's Disease and primary haemochromatosis. The development of more and more sensitive tests that permit the detection of early signs of disease, well before it has progressed to the stage of causing obvious symptoms, the so-called *occult disease*, may well help to identify more diseases in which metals, or a spectrum of metals, play an important role in their development. Already studies in persons exposed to only natural levels of cadmium have identified groups of individuals who excrete more than 2 μg of cadmium per day in their urine, as compared with generally < 0.5 μg per day, and who also show very early biochemical signs of kidney malfunction.

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Chapter 3

Metal Ions, Complexes, and Chemical Speciation

ELEMENTS AND THE PERIODIC TABLE

At their simplest level, elements are composed of neutrons, protons, and electrons, which are respectively uncharged, positively, and negatively charged particles. Traditionally, the hundred or so elements known are given a symbol which often carries as a subscript on the left-hand side its atomic number, which is equal to the number of protons in the nucleus, and a superscript on the left-hand side, the mass number, which is equal to the number of neutrons plus protons. As a superscript on the right-hand side, we have the number of charges left on the element when it becomes an ion by gaining or losing electrons. For uncharged elements, the number of electrons equals the number of protons and, therefore, these are neutral. For each electron lost, the element increases its overall charge by $+1$.

Early last century, scientists began to note a recurrence (or periodicity) of similar chemical properties amongst groups of elements and work to classify such properties by Mendeleev and Lothar Meyer (1869–70) was taken further by Thomsen and Bohr in 1885, when they compiled the Periodic Table, shown in Figure 3.1, which is familiar to most of us.

Essentially, this Table is based upon the distribution of electrons amongst four sets of orbitals labelled s, p, d, and f and is comprised of the main group elements, with the completion of s and p orbitals, the transition elements, with the completion of electron shells for the d orbitals, and then the inner-transition elements, known as the lanthanons and actinons, with the completion of f orbitals. All of these transition and inner-transition elements are metals in their native state, whereas the elements to the top right-hand side of the main group of the Periodic Table tend to be non-metals.

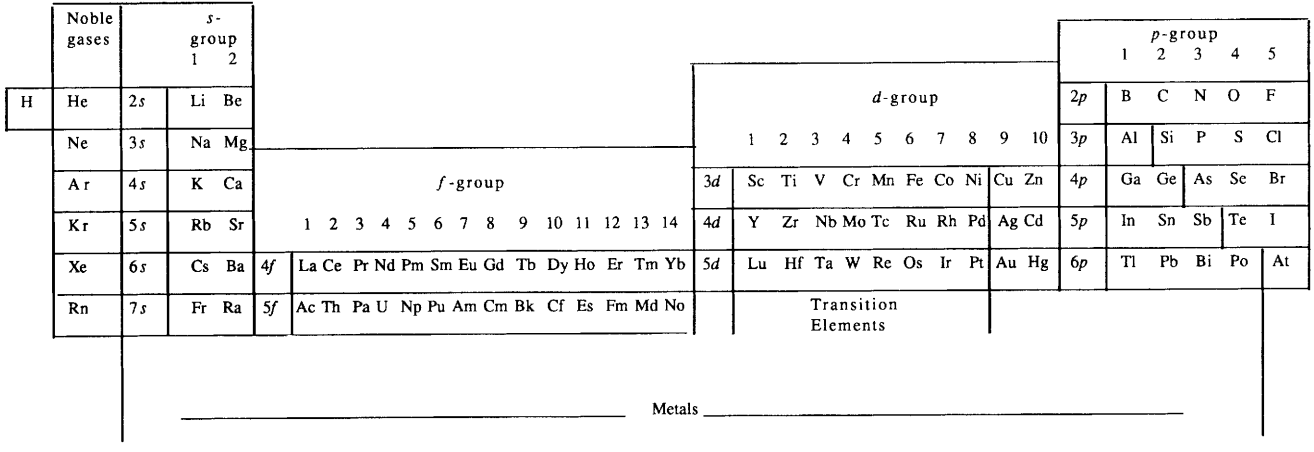


Figure 3.1 *Periodic Table showing s-, p-, d-, and f-groups of elements*

There are many intricate details of the bonds formed between combinations of elements of the Periodic Table in order to make molecules but, at its simplest level, there are three types of bonding:

1. *Ionic bonding* where an element which has lost electrons to form positively charged species is ionically bonded to another element, which has gained electrons to form a negatively charged species. This is classical electrostatic attraction.
2. *Covalent bonding* wherein two atoms of the same, or of differing elements, share one or more electron(s) from each atom in order to unite to form an electron shared bond between the two atoms.
3. *Dative covalent bonding* wherein a similar electron shared bond occurs but all of the electrons are provided by the atom at one end of the bond.

SOLVENTS *IN VIVO*

The human body consists of the order of 10^{15} cells, each having an aqueous interior trapped within a lipoprotein cell membrane. Some 70% of the human body is water and so, to a crude approximation, there are two predominating solvents to be encountered in humans. First, there is the overwhelmingly aqueous environment whereby water, being a molecule with partially negative charges on the oxygen and partially positive charges on the hydrogens, tends to prefer to dissolve metals as metal ions. These are often denoted as aquated metal ions, $M^{n+}{}_{(aq)}$. Similarly, elements in the non-metallic part of the Periodic Table tend to dissolve in water as anions, such as $Cl^{-}{}_{(aq)}$.

The second type of solvent is the lipid protein matrix of a cell membrane which approximates to that of an organic solvent, such as dioxane, chloroform, *etc.* This kind of solvent rejects charged species and prefers molecules having no residual overall charge. Thus, to negotiate metals or molecules through such cell membranes requires them to be made lipophilic, *i.e.* fat-loving, rather than hydrophilic, water-loving, materials.

Much of the main group metals and non-metals found *in vivo* are highly solvated by water and produce a pressure within capillaries, arteries, *etc.* For example, blood plasma approximates to 150 mmol dm^{-3}* sodium chloride. This accounts for a large proportion of the water in humans. Conditions which cause imbalances away from this constant pressure of aquation (called the isotonic pressure) can cause serious cramps in the patient.

* The unit mol dm^{-3} refers to the molar mass of a compound dissolved in one litre of solution.

Much of the calcium required by humans is built into solids of calcium hydroxyphosphate that forms the major matrix of bones and teeth.

COORDINATION COMPLEXES

The transition metals, essential for humans and, also, several of those found as polluting metals, are present at considerably lower concentrations than the main group elements, sodium, potassium, calcium, *etc.* mentioned above and, therefore, contribute little to the isotonic pressure via their solvation properties. They do, however, have a fascinating and essential role which involves coordination complexes. In such reactions, they pair off with a ligand and become an essential part of a biochemical process.

A ligand is loosely defined as a molecule or ion that has electrons available for donating towards a positively charged species such as a transition metal ion. It may thus be envisaged that biochemical donor groups such as RS^-, $-NH_2$, $-COO^-$, $-O^-$ *etc.*, as well as the active group of drugs (the above groups plus $-PO_4^{3-}$, $-NO_2^-$, *etc.*) can form dative (*i.e.* electron donation) covalent bonds into the metal ion's vacant orbitals.

BONDING

A classical example of how bonds are formed refers to potassium hexacyanoferrate(II) $[K_4Fe(CN)_6]$, made by mixing colourless potassium and ferrous cyanides together to form a brownish-yellow coloured product which lacks the lethal poisonous properties of potassium cyanide. A century ago, Werner explained many such phenomena by his principles that:

1. Metals have two types of valency:
   primary (which are ionizable) and
   secondary (which are non-ionizable, *i.e.* covalent);
2. Primary valencies are satisfied by negative ions such as Cl^-, NO_3^-, SO_4^{2-};
3. Every metal has a fixed number of secondary valencies, called the coordination number. For example, for Fe^{2+} it is 6 and for Cu^{2+} it is often 4 and sometimes 6;
4. Secondary valencies are directional in nature, four going to the corners of a regular tetrahedron or a square (as for Cu^{2+}) and six reaching out to the corners of a regular octahedron (as for Fe^{2+});
5. The metal ion and all its secondary valencies are written inside square brackets, e.g. $[Fe(CN)_6]^{4-}$ and this ionizes as a whole, showing none of the properties of the component ions (*i.e.* tests for Fe^{2+} and CN^- are negative).

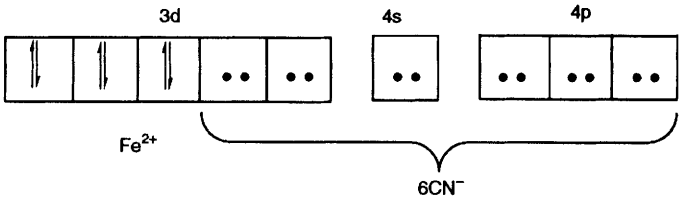


Figure 3.2 *The $[Fe(CN)_6]^{4-}$ moiety for Werner's complex, $K_4[Fe(CN)_6]$*

Thus, for Werner's complex, $K_4[Fe(CN)_6]$, the $[Fe(CN)_6]^{4-}$ moiety can be drawn as Figure 3.2.

The six lone pairs of electrons enter the unfilled $3d^2$, 4s and $4p^3$ orbitals and all the d^2sp^3 orbitals are hybridized, *i.e.* 'made equal' and spread evenly about the central metal ion as shown in Figure 3.3.

CHELATION

Large ligands can be attached to the metal ion by two or more bonds, remembering that they have to be sufficiently spaced to accommodate the spatial distribution of the metal ion's secondary valencies. The ligands are called bi-, tri-, hexa- or polydentate for 2, 3, 6 or many points of attachment and the rings formed are called chelate rings (5- and 6-membered rings are the most common for spatial reasons).

The directional nature of secondary bonds on a metal ion can act as a template to hold specific configurations (see the example from evolution in Figure 3.4) and to produce isomers which may be optically active. Such coordination compounds have given our evolution a great boost and added a whole new dimension to life's biochemistry.

Such compounds can be used to hold a metal in an unfavourable valence state, to neutralize a charge to permit the metal complex to pass through a cell membrane by becoming lipophilic, to produce exceptionally powerful bonding by multiple chelation and to design a ligand drug specific to a metal ion *in vivo*.

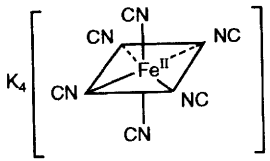


Figure 3.3 *Werner's complex, $K_4[Fe(CN)_6]$*

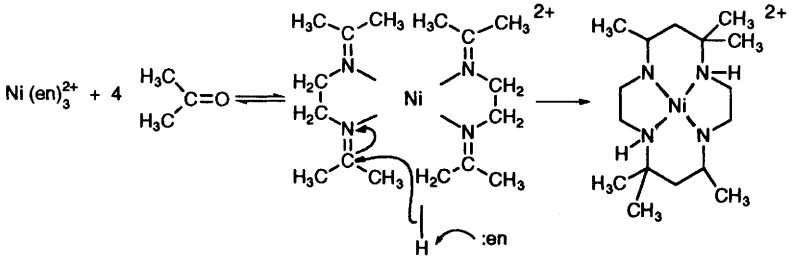


Figure 3.4 *The nickel ethylenediamine complex reacts with acetone to form a nirrin, the four planar positions of the Ni^{2+} bonds templating the reaction. In evolutionary terms, such reactions permitted great strides forward, as simple molecules such as the two above immediately became corrins surrounding the cobalt of vitamin B_{12}: chlorophyll if magnesium and haem if ferrous was at the centre* (from R.W. Hay, in 'An Introduction to Bio-inorganic Chemistry', ed. D.R. Williams, by permission of the Publishers, C.C. Thomas, Springfield, Il, 1976).

METAL–LIGAND SELECTIVITY

The extent to which metal ions and ligands react is measured in terms of a formation constant, K for stepwise, β for overall, and the rate of interaction is measured as a kinetic constant, k.

Metal ions and ligands show a definite order of affinity for one another. One method of quantifying this degree of tightness in binding is to employ formation constants (K or β) which are related to mass-action equilibria between metal ion and ligand or ligands. Thus, for the 1:1 glycinate–Cu^{2+} complex the equilibrium is

$$K_1 = \frac{[H_2N\text{–}CH_2\text{–}COOCu^+]}{[Cu^{2+}][H_2N\text{–}CH_2\text{–}COO^-]}$$

where K_1 is the formation constant. A second constant, K_2 describes the stability of the 2:1 complex. Also, cumulative formation constants, β, can be used ($\beta_2 = K_1 \times K_2$). These may be determined by potentiometric titration methods whereby the amount of $H_2N\text{–}CH_2\text{–}COO^-$ complexed is followed using a $[H^+]$ responsive glass-reference electrode pair and the known pK_a, since

$$K_a = \frac{[H_2N\text{–}CH_2\text{–}COO\text{–}H]}{[Cu^{2+}][H_2N\text{–}CH_2\text{–}COO^-]}$$

HARD AND SOFT ACIDS AND BASES

When considering the metal ion complexing of a new ligand, it would be highly desirable to be able to predict which metal ion ligand bonds will form and how strong they are, so that likely side effects and *modus operandi* can be suggested and investigated using laboratory experiments. Such predictions can be made using the hard and soft acid and base approach (HSAB). The approach assumes: (i) that if a bond exists between two atoms, one will play the role of an acid and the other a base, and (ii) that electrons hold the bonded atoms together.

The (Lewis) acid is taken as the species (atom, molecule or ion) that has vacant accommodation for electron pairs and the base has a tendency to give up electron pairs. A typical acid–base reaction is:

$$\underset{\text{Acid + Base}}{A + :B} \rightarrow \underset{\text{Complex}}{A:B}$$

This A–B bond may be any chemical bond, *e.g.* $[(H_3N)_5Co–NH_3]^{3+}$, $C_2H_5–OH$, $C_2H_5O–H$ or H–OH. Thus, we mentally dissect any species into an acid and a base fragment. We need not consider whether the fragments could exist in isolation. In this chapter, A is the metal ion and :B is the ligand.

Classification of Acids and Bases

'Softness' arises from the electron mobility or polarizability of a species. If the electrons are easily removed, the species is soft; if firmly held, the species is hard. Other descriptions of a soft base would include such terms as highly polarizable, easily oxidized or valence electrons loosely held. Clearly, this is all associated with a low density of charge on the base. A hard acid is of small size, with high positive charge density and usually does not contain unshared pairs of electrons in its valence shell. Naturally, a hard base and a soft acid are the converse of these description. These definitions have been used to produce Tables 3.1 and 3.2. The single principle behind HSAB is: *a strong bond is formed by a hard acid combining with a hard base or a soft acid with a soft base.* Hard–soft bonds are weak.

BIO-INORGANIC CHEMISTRY

1. Thousands of examples of the HSAB concept that strong bonds are only formed between hard–hard or soft–soft components have been

Table 3.1 *HSAB classification of acids*

Hard	*Soft*
H^+, Li^+, Na^+, K^+	Cu^+, Ag^+, Au^+, Tl^+, Hg^+
Be^{2+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Mn^{2+}	Pd^{2+}, Cd^{2+}, Pt^{2+}, Hg^{2+}, CH_3Hg^+, $Co(CN)_5^{2-}$, Pt^{4+}, Te^{4+}
Al^{3+}, Sc^{3+}, Ga^{3+}, In^{3+}, La^{3+}	Tl^3, $Tl(CH_3)_3$, BH_3, $Ga(CH_3)_3$
N^{3+}, Cl^{3+}, Gd^{3+}, Lu^{3+}	$GaCl_3$, GaI_3, $InCl_3$
Cr^{3+}, Co^{3+}, Fe^{3+}, As^{3+}, CH_3Sn^{3+}	RS^+, RSe^+, RTe^+
Si^{4+}, Ti^{4+}, Zr^{4+}, Th^{4+}, U^{4+}	I^+, Br^+, HO^+, RO^+
Pu^{4+}, Ce^{3+}, Hf^{4+}, WO^{4+}, Sn^{4+}	
UO_2^{2+}, $(CH_3)_2Sn^{2+}$, VO^{2+}, MoO^{3+}	I_2, Br_2, ICN, *etc.*
$BeMe_2$, BF_3, $B(OR)_3$	Trinitrobenzene, *etc.*
$Al(CH_3)_3$, $AlCl_3$, AlH_3	Chloranil, quinones, *etc.*
RPO_2^+, $ROPO_2^+$	Tetracyanoethylene, *etc.*
RSO_2^+, $ROSO_2^+$, SO_3	O, Cl, Br, I, N, RO, RO_2
I^{7+}, I^{5+}, Cl^{7+}, Cr^{6+}	M^0 (metal atoms)
RCO^+, CO_2, NC^+	Bulk metals
HX (hydrogen bonding molecules)	CH_2, carbenes
Borderline	
Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Sn^{2+}, Sb^{2+}, Bi^{3+}, Rh^{3+}, Ir^{3+}, $B(CH_3)_3$, SO_2, NO^+, Ru^{2+}, Os^{2+}, R_3C^+, $C_6H_5^+$, GaH_3.	

Table 3.2 *HSAB classification of bases*

Hard	*Soft*
H_2O, OH^-, F^-	R_2S, RSH, RS^-
CH_3CO^-, PO_4^{3-}, SO_4^{2-}	I^-, SCN^-, $S_2O_3^{2-}$
Cl^-, CO_3^{2-}, ClO_4^-, NO_3^-	R_3P, R_3As, $(RO)_3P$
ROH, RO^-, R_2O	CN^-, RNC, CO
NH_3, RNH_2, N_2H_4	C_2H_4, C_6H_6
	H^-, R^-
Borderline	
$C_6H_5NH_2$, C_5H_5N, N_3^-, Br^-, NO_3^{2-}, SO_3^{2-}, N_2	

The symbol, R, stands for an alkyl group such as CH_3 or C_2H_5.
(From R.G. Pearson, *J. Chem. Educ.*, 1963, **45**, 581, by permission)

recorded and many are listed in the Further Reading section. One of the first observations of the HSAB principle at work was made by Berzelius, who noticed that some metals occur on the Earth's surface as ores of carbonate or oxide, whereas other metals occur as sulfides. The explanation is that hard acids, *e.g.* Mg^{2+}, Al^{3+}, Ca^{2+}, form strong bonds with hard bases, *e.g.* O^{2-}, CO_3^{2-} or SO_4^{2-}. Also, the

softer acids, *e.g.* $Cu^{+/2+}$, Hg_2^{2+} or Hg^{2+} or Pb^{2+} or Ag^{+} prefer soft bases, *e.g.* S^{2-}. Hard acid–soft base or soft acid–hard base combinations, according to HSAB, will not have strong bonds and so their ores will have hydrolysed away millions of years ago.

2. *Symbiosis* is a process whereby one hard (or soft) base on a metal ion encourages other hard (or soft) bases to join it. Metal ions in biological systems are often in a state of equilibrium between two different oxidation states. The lower state can be stabilized symbiotically by adding soft ligands and the higher oxidation state by adding hard ligands. However, if we add very soft or very hard ligands the metal ion will be completely anchored in one oxidation state and so the living process (*e.g.* a redox reaction) is prevented. This, of course, is called 'poisoning' and the poisons that are most well known are usually acids or bases that are so strongly held to the active sites of an enzyme that the sites are effectively blocked off. Examples of soft acid poisons are organic mercurials and cadmium ions. As well as site blockings, poisoning by heavy metal ions usually results in precipitation of the metal–protein complex as well. These soft acids bind strongly to sulfur groups and so rob the organism of sulfur-containing proteins such as those involving cysteine residues. Very soft base poisons function by robbing our bodies of metal ions. Examples include cyanides, sulfides, trivalent arsenic compounds and carbon monoxide. In small concentrations these poisons act by blocking as they become attached to the metals in metalloporphyrins and other metallo-enzymes. At high concentrations they remove the metal ion entirely from the protein.
3. Conversely, very non-poisonous or inert metals are needed when artificial prostheses have to be introduced during surgery and so metals which, if dissolved, would give soft ions are chosen, *e.g.* gold, silver, tantalum, and platinum or their alloys. Because they give soft ions there is negligible tendency for those metals to give up electrons to form soft metal ion–hard solvent bonds (water is hard).

OXIDATION–REDUCTION REACTIONS

The number of charges on a metal ion can be altered by addition or removal of an electron. Many transition metal ions found in humans can exist in a range of oxidation states. For example, copper has + 1 and + 2 (cuprous and cupric respectively), iron has + 2 and + 3 (ferrous and ferric respectively), but some of these states are not stable in aqueous solution at neutral pH without ligands being attached to the metal ion. Thus, the oxygen-carrying protein, haemoglobin, is able to maintain the

iron in the ferrous state in spite of oxygen being present in aqueous solution. The ligand donor groups attached to the ferrous ion from the peptide chains and from the haem moiety collectively anchor the metal ion in its lower oxidation state.

Similarly, when electrical messages are passed along the nerves, it is normal for metal ions to change their oxidation states in order for the electron to pass by. A reduction in an oxidation state is coupled with an increase in oxidation state of an adjacent ion. These possibilities can only occur through metal–ligand complexing.

HYDROLYSIS OF COMPLEXES

In aqueous solution, all vacant coordination positions are fully occupied by water molecules. However, much body chemistry occurs at the pH near neutrality, *e.g.* blood plasma pH = 7.4. At such a pH, it is possible for protons to be liberated from some of these water molecules in order to produce hydroxy complexes, *e.g.*

$$[M(H_2O)_6]^{2+} \rightleftharpoons [M(H_2O)_5OH]^{+} + H^{+}$$

etc. for other complexes of five H_2O molecules. Such reactions can be encouraged by the presence of acid, which forces the metal ion back to its aquated state. This may well occur in stomach juices where the pH is considerably lower but is not possible in blood plasma. Once again, as with redox, when the coordination positions are taken over by ligands, they prevent complexing by water molecules leading to the subsequent hydrolysis products.

POLYNUCLEAR COMPLEXES

Several of these hydroxy complexes may polymerize to form a chain whereby the hydroxide ion acts in a bridging role. Such hydroxy-bridged polymers are found when heavy metals, such as plutonium, or the other actinides, enter into the lungs or blood plasma and then become deposited as insoluble metal hydroxy polymers.

UNCHARGED METAL–LIGAND COMPLEXES

Metals having a zero oxidation state can still be complexed by a similarly soft bonding ligand such as carbon monoxide. Nickel carbonyl, $Ni(CO)_4$, is a gas used in the nickel refining industry in order to volatilize and purify nickel from its ores. It is interesting to note that

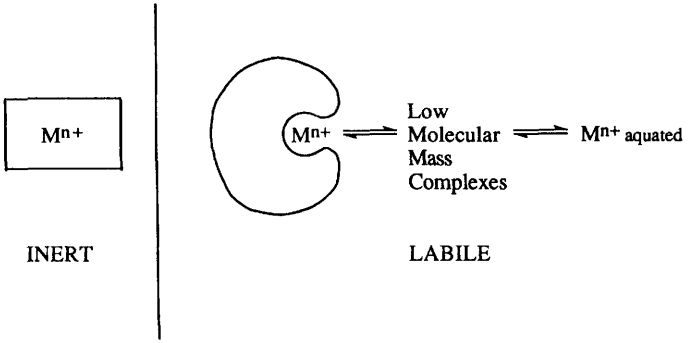
neither the metal nor the ligands are charged and that the resulting nickel carbonyl gas is extremely soluble in the lipid–protein membranes of cells and, therefore, is one of the most hazardous gases known in industry.

LABILE AND INERT COMPLEXES

A complex which is formed with thermodynamically strong bonds may, in fact, exchange its ligands quite readily when offered an identical ligand, as followed using radioactive isotopes of the same ligand. This is known as lability. The converse is the unwillingness of a thermodynamically weak metal–ligand complex to yield up one of its ligands for a preferred alternative. The whole concept depends upon the ability of the metal ion to be able to form the transition-state intermediate involving, for octahedral complexes, a seven coordinate intermediate. This involves donating lone pairs of electrons from the approaching ligand. Just as the previous six ligands were attached by dative covalent bonding if a vacant orbital is not to hand to accept this attacking ligand, then the complex will be inert. Conversely, complexes having very large formation constants can readily exchange ligands in aqueous solution because they are able to form an intermediate. There are other tricks found in nature in order to make bonds inert, such as blocking the possibility of forming a transition state intermediate species.

Metal ions in biological systems tend to be distributed between four different states *in vivo*. First, there is the inert form of the metal complex whereby it is laid down into a solid matrix, just like the calcium in the apatite of bones and teeth (Scheme 1). Secondly, there are the three states of metal–protein having reversibly bound metal ions, known as high molar mass species, that are in turn in equilibrium with low molecular mass complexes and, for purely thermodynamic reasons, there must be the occasional aquated metal ion, although this is highly unlikely with a pH of 7.4 because of the threat of hydrolysis.

Interestingly, these last three complexes have the metal ions in equilibrium such that one can deposit excess metal on circulating labile proteins or, alternatively, draw metal ions from it by ligand drugs or by depletion of lower molecular mass metal complexes. Alternatively, a rise in metal ion concentration present in the biological fluid will bring about an across-the-board increase in labile protein, low molecular mass, and aquated metal ion concentrations without necessarily increasing the amount of metal complexing to the inert protein. To build a metal into, or to extract a metal from, an inert protein usually involves a sophisticated biochemical process such as occurs in the liver or the



Scheme 1

Table 3.3 *Chemical species in humans as illustrated in Scheme 1*

Inert and/or thermodynamically non-reversible	*Labile and thermodynamically reversible*
Iron	
Haemoglobin	
Myoglobin	Transferrin $\rightleftharpoons$ Low molecular mass Fe^{3+} complexes $\rightleftharpoons$ $[Fe(H_2O)_6]^{3+}$
(Ferritin)	
Copper	
Ceruloplasmin	
(Metallothionein)	Serum albumin $\rightleftharpoons$ Low molecular mass Cu^{2+} complexes $\rightleftharpoons$ $[Cu(H_2O)_6]^{2+}$
Zinc	
α_2 Macroglobulin	
(Metallothionein)	Serum albumin $\rightleftharpoons$ Low molecular mass Zn^{2+} complexes $\rightleftharpoons$ $[Zn(H_2O)_6]^{2+}$

spleen, *etc.* Table 3.3 indicates some examples of metal ions complexed in these four different types of species *in vivo*.

CHEMICAL SPECIATION MODELLING

Speciation is the occurence of an element in separate, identifiable forms and so the term 'chemical speciation' defines the oxidation state, concentration, and composition of each of the species present in a chemical sample. Improved computing and analytical methods of late have led to an increased understanding of the exact speciation underlining many

Table 3.4 *Some examples of elements having ambivalent biological effects dependent upon their chemical speciation*

Parent element	*Predominantly beneficial species*	*Potentially toxic species*
As	As(v) compounds	As(III) compounds
Ba	Chloride	Nitrate
C	Widespread in biochemistry	Cyanide ion
Cr	Cr(III) compounds	Cr(VI) compounds
Cu	Carbonate	Chloride

chemical reactions occurring *in vivo*, in industry, in medicine, and in the environment. No organic chemist would think of expressing the analytical levels of carbon present in an ethanolic solution in water, in a calcium carbonate solution, or in a potassium cyanide solution solely in terms of the total carbon presence. Rather, the toxicity and mood-changing aspects of these three aforementioned compounds of carbon are highly dependent on the chemical speciation. Similarly, food, beverages, and contaminants are often assessed in an analogous manner in terms of their total concentrations present. Such concentrations do not reflect the chemical species and the bio-availability of the metal which, in turn, can vary markedly as concentration, pH, temperature, *etc.* change. Similarly, oxidation states can determine whether a metal is toxic or beneficial. A change in the speciation can completely modify the behaviour of the element (see Table 3.4).

The human body is a remarkably efficient organism that is able to reduce the amount of waste and ensure that energy liberated in one part of a biochemical reaction is actually used up, or stored, in another part of such a reaction. This means that we exist more or less at steady state giving out very little surplus energy. In fact, the amount of heat that we lose is equivalent to less than that of an electric light bulb. Less efficient organisms lose a great deal of heat and, hence, one sees spontaneous combustion of farmers' haystacks because of bacterial inefficiency *en masse*.

In thermodynamic terms, steady states in multi-faceted reactions occur pretty close to chemical equilibrium. Assuming that the latter is a good approximation for many biological fluids and for many medicines and administered drugs, it is possible to build chemical speciation models based upon the equilibrium formation constants described earlier.

Typically, when considering the four states in which a metal ion can

Table 3.5 *The percentage distribution of the metal ions Ca^{2+}, Cu^{2+}, Fe^{3+}, Pb^{2+}, Mg^{2+}, Mn^{2+}, and Zn^{2+} amongst low molar mass ligands in human blood plasma as found by computer simulation using the ECCLES program* (P.M. May *et al.*)

Complex	*Percentage of the total metal in the low molar mass fraction*
Ca.carbonate. H^+	9
Ca.citrate$^-$	4
Ca.lactate$^+$	3
Ca.phosphate$^-$	3
Ca.carbonate	2
Cu.histidinate.cystinate$^-$	21
Cu.histidinate.cystinate.H	17
Cu.histidinate$_2$	11
Cu.histidinate.threoninate	8
Fe.citrate.OH^-	99
Pb.cysteinate	80
Pb.cysteinate.citrate^{3-}	7
Pb.cysteinate.H^+	5
Pb.cysteinate.phosphate.H^{2-}	3
Mg.carbonate.H^+	6
Mg.citrate$^-$	5
Mg.carbonate	2
Mg.lactate$^+$	2
Mn.carbonate.H^+	24
Mn.citrate$^-$	10
Mn.carbonate	2
Mn.oxalate	2
Zn.citrate.cysteinate^{3-}	43
Zn.cysteinate$_2^{2-}$	19
Zn.cysteinate.histidinate$^-$	12
Zn.cysteinate	3
Zn.histidinate$^+$	3

exist in humans, the three labile states can be gathered together into a large chemical speciation model, which will calculate the most predominant species of complex present in the bio-fluid. As the fourth state, that of the inert protein-bound or solid matrix-bound metal ion, is not immediately available to this equilibrium model, it does not markedly affect the output data.

Typically, models of blood plasma, urine, intestinal fluid, perspiration,

saliva, and all of these fluids with added health care agents, like toothpaste, or with food and drink, have been modelled up to about 10 000 co-existing species. The technique is able to work at concentrations below those analysable by even the most sophisticated analytical techniques and to indicate the knock-on effects of administering a contaminant metal ion or of essential metal ion deficiency, or of administering a ligand drug to redistribute metal ions *in vivo*.

Figure 6.1 in Chapter 6 shows a typical example of perspiration being attacked using a blocking device based upon aluminium and a roll-on or spray-on antiperspirant. Secondly, Table 3.5 indicates the most prevalent species of a few transition metals found in blood in competition with many thousands of other species.

It is noteworthy that most of the complexes formed are actually mixed ligand complexes whereby two different ligands are associated with the same metal ion. Thermodynamically, these form the more stable complexes.

These more prevalent species are actually the building blocks used in the construction of high molar mass metal-containing species'. Very often zinc is found associated with both the anion of histidine and the anion of cysteine in hundreds of proteins that contain zinc, as this zinc ternary complex unit is far more readily available than the exceedingly low concentration of aquated metal ion at biological pH values.

The output from such computer simulation modelling of the chemical speciation can be a good indicator of whether a metal ion and its complex are bio-available (*i.e.* sufficiently lipophilic) to traverse a cell membrane and go inside cell tissue down a concentration gradient. Similarly, if charged, low molar mass species are formed in blood plasma, they will be readily excreted through the renal system and will pass out in the urine. These are means of negotiating, using metal complexing, metal ions into and out of biological tissue and, indeed, of expelling them from the body via the kidney.

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Chapter 4

Chelation, Ligands, and Drugs

BONDING TO TRANSITION METAL IONS

The bonding to transition metal ions has been described in the previous section as being predominantly that of dative covalent bonding whereby lone pairs of electrons are donated into vacant orbitals of the transition series metal ions. It was established by Werner that these bonds are directional in nature and have certain preferred angles subtended at the central metal ion.

In principle, any group that has lone pairs of electrons can participate in such bonding and this embraces most of the biochemicals present in nature, contaminating species in our environment, pharmaceuticals, and many other agents. In fact, pharmaceuticals work through certain active groups interacting with pivotal biological species such as proteins, and it is often the same pairs of electrons that are used to bring about the pharmaceutical activity which will also interact with a transition series metal ion if conveniently available.

THE CHELATE EFFECT

The number of monodentate groups such as hydroxide, halide, water molecule, *etc.*, bonded to a central metal ion, usually equates to its coordination number. However, a remarkable feature occurs if two or more of these ligand donor groups are united, possibly by a short chain of hydrocarbons: there will be a markedly enhanced preference for forming such bidentate complexes with the transition metal ion, and indeed in the ultimate, it is possible to go through tri- to tetra- to penta- to hexadentate chelate ligands such as EDTA (ethylenediaminetetra-acetic acid) which is widely described in this book.

Most chelate rings involving octahedral transition metal ions tend to be five- or six-membered since these best satisfy the 90° angle between

bonds subtended at the central metal ion. The increasing magnitude of the amount of complexes formed under such circumstances may be seen by comparing four monodentate ligands of ammonia complexing with a cupric ion, compared with two bidentate ligands of ethylenediamine. The respective log formation constants are log $\beta = 11.9$ and 20.0. This implies that if the bidentate ligand is in competition with the monodentate ligands, the bidentate ligand complexes will predominate with a concentration ratio of the order of 10^8. A hexadentate ligand such as EDTA would have an even greater advantage and be formed to the virtual exclusion of all other complexes. This explains why polydentate ligands are widely used in detoxication therapy.

Although there is a preponderance of monodentate ligands in biological systems, bearing in mind that the body is 70% water by volume and that there is much chloride present in blood plasma, it is commonly found that bidentate ligands such as the anions of amino acids or of carboxylic acids avidly complex with the essential biometals present and that monodentate ligands, in spite of their overwhelming presence, are the residual legatees in terms of being able to complex with any remaining metal ion bonds after all of the bidentate and polydentate ligands have had first choice. Later, we shall indicate that there are certain preferred bidentate ligands which are normally found associated with a given transition metal ion in humans.

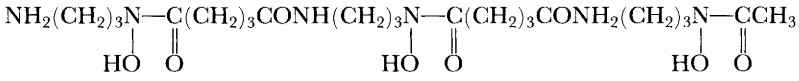
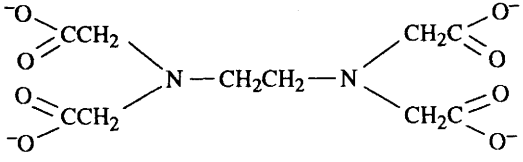
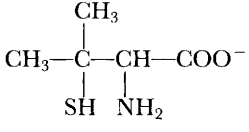
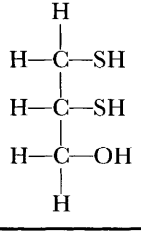
For completeness, it must be noted that the main group metal ions, because they prefer ionic bonding, are normally found in association with monodentate ligands such as the water molecule, chloride, carbonate, *etc.*, in human systems, but even they can be complexed with a well chosen polydentate ligand.

METAL–LIGAND SELECTIVITY, A COMBINATION OF THE CHELATE EFFECT AND THE HSAB APPROACH

Certain metal ions are undoubtedly preferred by specific ligands in biological systems and this occurs because of the selectivity of ligands for coordinate bond configurations and the preference of electrons overlapping from ligands into vacant metal ion orbitals, as summarized by the HSAB approach described earlier. This concept of matching donor groups to metal ions or acids to bases in the HSAB terminology, along with a knowledge of the stereochemistry of the competing metal ions involved, can lead to remarkable selectivity when one wishes to design agents for complexing undesired metal ions.

The four, earliest, sequestering reagents used therapeutically for treating metal ion excesses have been used to illustrate the principle of

Table 4.1 *Earliest sequestering reagents for treating metal ion excesses. The chemical formulae are given below. As one descends the Table, increasing softness of donor atoms is paralleled by increasing softness of the acid removed*
(From Fiabane and Williams, 1977, with permission.)

Ligand	*Donor atoms*	*Metals removed*	*HSAB classification of acid and base*
Desferrioxamine B 	Several O	Fe(III)	Hard
EDTA 	4O,2N	Pb(II)/Co(II)	Borderline
D-Penicillamine 	S, N, O	Cu(II)/Cu(I)	Borderline/soft
British Anti-Lewisite (BAL) 	2S	Arsenicals/Au(I)/ Hg(II)/Hg(I)	Soft

HSAB: the matching of acids and bases necessary for strong bonds (Table 4.1). Clearly, there is a matching of donor group type to acceptor group type with these four agents which arose from literally decades of research. These treatments could well have been discovered using a more planned route than their accidental discoveries had the HSAB theory been available in the first half of this century. Although none of these four agents were specifically designed to remove the metal ions listed, they are now the main drugs of choice in such decorporation therapies.

METABOLIC SPECIFICITY *IN VIVO*

If one examines the speed of reaction as measured by rate constants (k) and also the strength of the bonds formed, as reflected in the formation constants (K or β), one can find many instances where, for example, the zinc-containing metalloprotein carboxypeptidase would have been kinetically and thermodynamically better placed to undertake this role *in vivo* had the zinc have been replaced with cobalt(II). Similarly, there are kinetic and thermodynamic reasons for replacing the ferrous ion in porphyrins with cobalt(II) or with copper ions.

However, there are two factors that have determined why nature has evolved to use zinc and iron rather than the apparently more advantageous alternative metal ions. First, zinc and iron are thousands of times more abundant in nature than the alternatives suggested and, therefore, clearly have an evolutionary preference. Secondly, the whole process of ingesting an element and of it being able to traverse many membranes, of it being buffered by proteins at several stages, and eventually of it being correctly inserted into a metalloenzyme in the correct oxidation state and, indeed, complexed to two or three bidentate ligands to form a building block, requires more than solely the kinetics and the thermodynamics of the final product. Rather, each of these stages has to be traversed without wastage or build-up of unused material as all metals past their biological half-lives must then commence the journey to excretion or to the liver and spleen for re-use.

Regardless of these kinetic and thermodynamic considerations, it is truly remarkable that nature has managed to design such specificity for each stage that a metal ion traverses *in vivo*, such that most of us can survive for many decades without need of therapy to assist or to remove material. Unfortunately, probably the best that we can hope for in terms of our designing ligand drugs for manipulating metal ions *in vivo* is a much lower degree of selectivity as compared with the specificity of

nature. The former word (selectivity) implies that there will probably be some slight side effects caused by the therapeutical and these could well require the administration of metal ions to top-up processes unintentionally depleted by the ligand concerned. Examples are given in a later section.

CHELATING DRUGS DESIGNED FOR MOBILIZING AND/OR EXCRETING METAL IONS FROM HUMANS

The term 'sequestration' in medical areas refers to the complete envelopment of a metal ion by a multidentate ligand drug. Such sequestering ligands can be used to remove essential metal ions which are present in excess, to move them from one part of the body to another part, and also to remove toxic metals present above their threshold levels. The field is described more fully in Chapter 7, 'Chelating Agents and Therapy'. However, in order to be quantitative about the efficacy of an agent for complexing a metal it is highly desirable that we have a quantitative scale to indicate mobilization.

Such numerical data are not available from analyses of *in vivo* solutions, since the total metal concentration may not change, but can conveniently be calculated using computer simulation of the multiple equilibria present in biological fluids. These are based upon formation constants, upon total quantities of each of the materials present, and upon large computer programmes which calculate the distribution of metal ions at concentrations below those normally available to analytical chemists.

If it is assumed that the metal ion concerned is present in all four of the states mentioned in Chapter 3, then it will be present as inert metalloprotein or a solid material, and then three equilibrium states of circulating labile protein in equilibrium with low molar mass complexes in equilibrium with exceedingly low concentrations of aquated metal ion. Such a concept can be modelled in respect of its labile equilibrium and the ability of sequestering drugs to remove the metal ion from the labile protein into low molar mass form. Thereafter its destiny is determined by whether the material has charges for its low molecular mass (l.m.m.) species or whether these are uncharged, and therefore potentially bioavailable through a cell membrane.

May and Williams have defined plasma mobilizing index (PMI) terms based upon such formation constant modelling which are able to indicate the total concentration of l.m.m. species of the metal after therapy/total concentration of l.m.m. complexes before treatment.

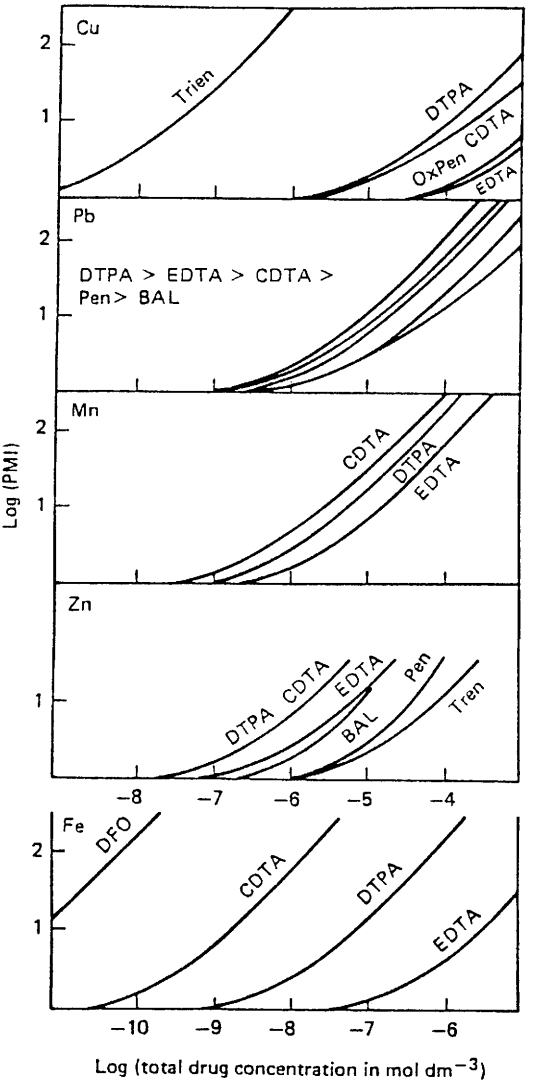


Figure 4.1 *PMI curves calculated for metal ion-chelating agents by using computer simulation. PMI is as defined in the text. Ligand symbols: Tren = triethylenetetramine; EDTA = ethylenediaminetetraacetic acid; DTPA = diethylenetriaminepentaacetic acid; CDTA = cyclohexylenedinitrotetraacetic acid; Pen = D-penicillamine; OxPen = D-penicillamine-S-S-D-penicillamine, i.e. dimer of Pen; BAL = 2,3-dimercaptopropanol; DFO = desferrioxamine*
(From P.M. May, and D.R. Williams, *FEBS Lett.*, 1977, **78(1)**, 135, by permission).

$$\text{PMI} = \frac{\text{Total low molar mass metal concentration in presence of agent}}{\text{Total low molar mass metal concentration in normal plasma}}$$

Clearly, if the l.m.m. complexes are those which are excreted or are passed into tissue and if the correct choice and administration of a ligand drug is such as to increase the PMI by a factor of 10, *i.e.* log PMI = 1, then there will be a 10-fold increase in the chances of the mobilized metal ion being excreted or passed into tissue. Some examples of log PMI curves are shown in Figure 4.1.

The usefulness of such curves can be seen if one considers a patient contaminated with lead. Clearly, at a concentration of EDTA of 10^{-8} mol dm^{-3}, there will be such a 10-fold increase, showing a log PMI of 1. Thus, at first sight, EDTA is a means of mobilizing such toxic metal ions. However, by dropping a vertical line through the manganese and zinc EDTA curves one can immediately see that co-liberalization of these two essential metals will occur with the same EDTA sequestering agent. Thus, it is necessary, on the one hand to monitor the amount of trace elements passing out in the urine and, on the other hand, to top up by placing equivalent quantities of manganese and zinc compounds into the patient via the oral route.

There is a more fundamental problem of using EDTA to remove lead ions in that the species of EDTA present in blood plasma is $EDTA^{4-}$. This means that this material is not able to pass through cell membranes and to approach the lead ions which are normally deposited or complexed in tissue.

However, a second agent shown in the PMI curves is that of D-penicillamine. Upon studying the speciation of that agent in blood plasma it is found that this is electrically net-neutral and therefore is able to penetrate into tissue. Further, the PMI curves indicate that it is able to complex with lead ions and to form a net-neutral lead-penicillaminate complex. This then passes out of the cell membrane into the blood plasma where it remains net-neutral and thus is not amenable to kidney excretion.

The next treatment stage is to administer the EDTA as previously mentioned and from the PMI curves it can be seen that it is able to win away the lead ions from the penicillamine and to form the aforementioned charged lead EDTA complex which then passes out through the kidneys.

This approach is known as synergistic chelation therapy since it involves two different drugs undertaking separate roles *in vivo* and yet together, the overall result is the desired excretion of the polluting metal ion. Applications of such synergistic chelation therapy are now being

researched, based upon speciation knowledge from computer models and from biological sampling, involving a range of essential metal overload and of polluting metal intoxication cases.

FURTHER READING

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Chapter 5

Delivery of Trace Elements to Humans

Normally, the human body receives the trace elements it needs through the diet. However, whenever, either through famine, food preferences, ageing, or disease, the quality and/or quantity of the food intake decreases to such a point that the diet can no longer meet the body's requirements, we need to consider ways of making good the trace element deficiencies. The increasing longevity of the populations in many countries, which is one of the achievements of modern chemistry and medicine, frequently leads to overt or sub-clinical deficiencies of one or more trace elements, which need to be addressed if the afflicted person is to enjoy the best possible standard of health.

The present day predilection for 'convenience' foods is making trace element deficiency a problem in industrialized societies, especially in the elderly. For example in Japan the recommended daily intake of calcium is 600 mg but surveys show that the average daily intake is only 540 mg. Most Japanese also hover on the edge of anaemia, suggesting that the daily intake of iron may also be inadequate. In addition, natural physiological events such as pregnancy pose an increased requirement for minerals that may not always be fully met from even the most sensible, normal diet; therefore, supplementation with iron and calcium may be necessary or at least desirable. The importance of a good, sensible diet which offers an adequate, but not excessive, intake of all necessary nutrients, including minerals, cannot be stressed too strongly. However, individual tastes, and financial and social pressures, often may make this ideal difficult to achieve; thus the question of the best ways in which to provide mineral supplements for the body becomes very important.

MEANS OF MINERAL SUPPLEMENTATION

There are two main ways by which substances may be administered to humans: the *enteral* and the *parenteral* routes. For enteral administration the substance is placed directly into the gastrointestinal tract by permitting a tablet to dissolve when it is placed under the tongue (sub-lingual administration), or by swallowing a tablet, capsule or a solution (oral) or by rectal administration as a suppository. In parenteral administration the substance in solution may be injected subcutaneously, intramuscularly or intravascularly, inhaled as an aerosol, applied topically to the skin as a cream or ointment, or, rarely, in the form of a pessary.

In mineral supplementation in humans the oral route is the most commonly used, the supplement being given as a tablet, capsule, solution or as a 'fortified' food. Injections are usually reserved for those cases where the patient is unable to eat due to damage to the gastrointestinal tract or other reason, *e.g.* in patients on total parenteral nutrition it may be difficult to maintain adequate levels of iron, and supplementation by intramuscular injections of iron, for example, as a sorbitol complex will often be required. In view of the importance of the oral route it is helpful to consider the more salient general aspects of gastrointestinal absorption.

ABSORPTION OF LIGANDS FROM THE GASTROINTESTINAL TRACT

In general enteral absorption may occur throughout the whole length of the gastrointestinal tract but three areas are of special importance, depending on the formation constants of the particular metal–ligand complexes. These are the mouth (pH ~ 7.4), the stomach (pH ~ 1.6) and the small intestine (pH ~ 6–6.5 in the duodenum and ~ 6.5–7 in the jejunum).

Specific carrier systems exist for the transport of some species across the intestinal wall (mucosa), *e.g.* glucose or amino acids. However, for other species the important properties for good absorption are the presence of a high proportion of a non-ionized form with a high lipid-water partition coefficient and a small atomic or molecular radius. It is generally assumed that ionized species cannot cross the mucosa whereas non-ionized forms equilibrate fairly freely, providing that they have molecular radii of less than about 3 nm, which corresponds to a molecular mass of ~ 6000 Da. For metals the most important region for absorption is the small intestine, where the pH lies between about 6 and 7.4.

Supplementing the intake of minerals into the body sounds like a deceptively simple task, but, as we shall see, it is far more complicated than it might appear at first sight. The minerals we take in from our diet are generally absorbed from the upper small intestine where the pH lies in the region of 6–7.4; therefore they must reach the site of absorption in a soluble and absorbable form. However, at this pH in the aqueous environment of the small intestine most multivalent metal ions react almost quantitatively with water to form hydrolysed insoluble, and thus non-absorbable, hydroxides and oxides. Other reactions occur with the numerous complexing ligands present in the intestinal contents to form metal complexes, the major fraction of which may be electrically charged and also be non-absorbable; the fraction of electrically net-neutral and thus absorbable species may well represent only a tiny fraction of the total metal which enters the gastrointestinal tract.

High concentrations of minerals in the diet may give foods an unpleasantly astringent taste. Thus the formulation of mineral supplements that combine good bio-availability with little, or no, undesirable side effects or spoiling of the taste of food is no easy task and calls for considerable skill and ingenuity. One possible advance may be seen in recent work from Japan that suggests that poly-γ-glutamic acid, an amino acid produced by *Bacillus natta* bacteria, appears to almost double the solubility of minerals in the small intestine and at the same time confers a 'mellow' flavour that masks the usual astringent taste of minerals.

In formulating metal supplements a suitable complexing ligand may be added with a view to enhancing the proportion of neutral metal complexes which are formed near the absorptive surfaces in the intestine. However, because such complex formation is usually very pH dependent the choice of ligand is not always easy. Figure 5.1 illustrates the variations in the total percentage of neutral complexes formed when ferrous iron reacts with ascorbic acid or with galacturonic acid in the pH range of 5–7.5, and indicates that ascorbic acid appears to be the better ligand for facilitating iron absorption. Figure 5.2 shows the proportions of neutral Fe(II) species formed at pH 6.5 when the metal reacts with five ligands sometimes used in iron supplements.

IRON, ZINC, AND COPPER SUPPLEMENTATION

These metals are the most prevalent metals *in vivo* and supplementation treatments can be traced back thousands of year; for example, in pre-Christian times, solutions of rust in acid wine were used for anaemia and zinc oxide unguents for wounds or skin conditions.

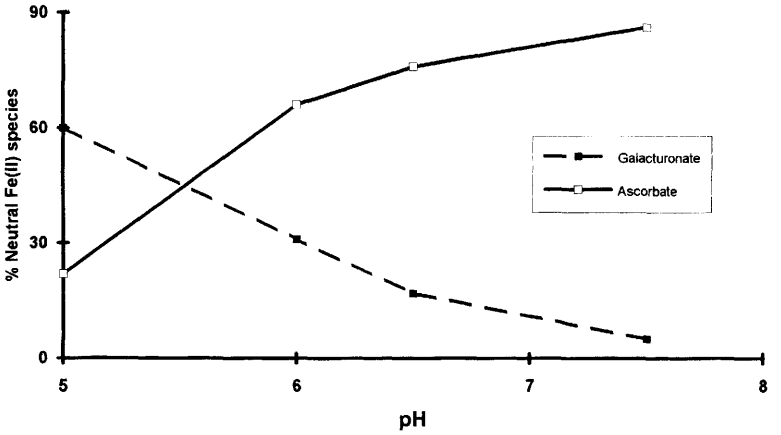

Figure 5.1 *The percentages of electrically net-neutral species formed when Fe*(II) *interacts with galacturonic acid or ascorbic acid in the pH range 5.0–7.5*

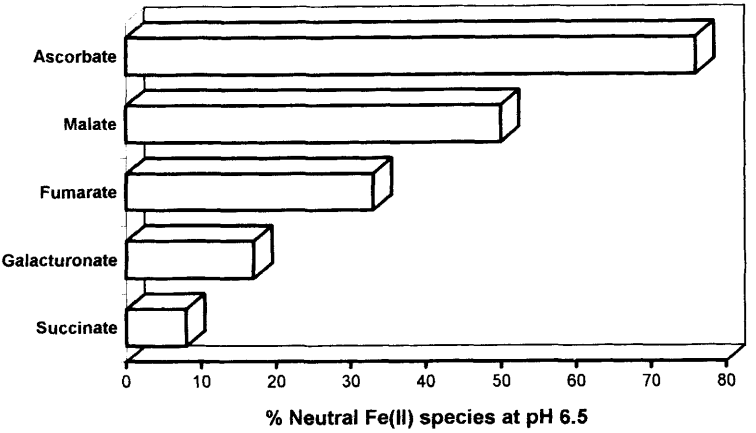

Figure 5.2 *The percentages of electrically net-neutral Fe*(II) *species formed at pH 6.5 with five physiologically important hydroxycarboxylic acids*

Modern oral supplementation therapy is a combination of scientific and commercial approaches which:

1. Aim to increase the flow of metal complexes from intestine to blood by increasing the concentration of lipid-soluble, low molecular mass complexes present in the intestinal fluids;

Table 5.1 *The types and composition of some metal supplement preparations*

Type of preparation	*Principal ingredient(s)*
Oral iron preparations	
Ferrous sulfate (tablets)	Iron(II) sulfate (40–105 mg Fe*)
Ferrous fumarate (tablets)	Iron(II) fumarate (65–10 mg Fe*)
Ferrous gluconate (tablets)	Iron(II) gluconate (35 mg Fe)
Ferrous glycine sulfate (tablets or solution)	Iron(II) glycine sulfate (25–100 mg Fe*)
Ferrous succinate (solution)	Iron(II) succinate (37 mg Fe)
Sodium ironedetate (solution)	Iron(II) chelate of ethylenediaminetetraacetic acid (EDTA) (27.5 mg Fe)
Compound oral iron preparations	
Iron and folic acid (capsules, tablets or solution): numerous proprietary preparations designed to prevent iron and folic acid deficiency during pregnancy	Iron(II) as sulfate or fumarate or glycine sulfate or iron(III)ammonium sulfate (47–110 mg Fe*) + folic acid (350–500 μg)
Injectable iron preparations	
Iron sorbitol injection	5% iron sorbitol (50 mg Fe ml^{-1})
Oral zinc preparations	
Zinc sulfate (tablets or capsules)	Zinc(II) sulfate (22.5–50 mg Zn)

*Range of iron contents found in unit doses of various preparations.

2. Aim to keep such metals in solution at intestinal pH by complexing the metal ions. For example, iron preparations may contain Fe(II) in association with complexing ligands such as ascorbate, malate, fumarate, gluconate or amino acids that promote the formation of neutral complexes at pH ~ 6–7. Gluconate may be used for the same purpose for metals such as Cu, Zn, or Co;
3. Tend to favour iron(II) rather than iron(III) compounds, since the former can be up to ~ 17 orders of magnitude more soluble (K_{sp}* $Fe(OH)_2 = 10^{-15.1}$, $Fe(OH)_3 = 10^{-38.7}$);
4. Cause least irritation to the gastrointestinal tract. For this reason ferrous sulfate is not an agent of choice;
5. Appear to avoid approaches advocated by hundreds of years of folklore medicine, so as not to undeservedly undermine confidence in either the product or its prescriber;
6. Use metal complexes that are capable of being patented.

* The solubility product K_{sp} is defined as the product of the concentrations of the ions present in a saturated solution of a substance. The units are in moles dm^{-3}.

The 'Martindale's Extra Pharmacopoeia' lists more than 40 preparations for iron, but only three for zinc; some of the most frequently used iron and zinc supplements are illustrated in Table 5.1. Irritation of the gastrointestinal tract tends to follow the Irving–Williams series of complex stability, *i.e.*

$$Mn < Fe < Co < Ni < Cu > Zn$$

for divalent ions. Thus oral supplementation with copper is exceedingly difficult and recourse is made to absorption via the skin from copper-impregnated gels used as a dressing being a modern equivalent of the copper bracelet from which the metal can be solubilized by the amino acids in sweat that form neutral lipophilic complexes that can penetrate the epidermis.

In all supplementation therapy it is prudent to assess the trace element status of the patient, by monitoring the blood plasma. For example, for iron the haematinic index can yield useful information that can be used to monitor the success of the treatment. However, simple measurement of the concentrations of the relevant metals in blood plasma is not necessarily the most useful indicator of the effectiveness of supplementation therapy, since the total concentration of metal in the plasma may not reflect the concentration of the particular metal species that is required to counteract the deficiency state.

BIOCHEMISTRY OF TRACE ELEMENT DELIVERY

There are instances in which, despite an apparently adequate intake of trace elements, either in the diet alone or from the combination of diet plus supplementation, the clinical symptoms persist. This may be due to a flaw or deficiency in the underlying, highly complicated biochemistry of trace element absorption and assimilation; such a deficiency may be because there is an inadequate presence of some essential co-factors.

Volumes have been published about the biochemistry and pharmacology of elements such as iron, cobalt, zinc, *etc.* (see, for example, the articles by P.M. May and D.R. Williams in 'Iron Metabolism' edited by A. Jacobs and M. Worwood, Academic Press, London, 1982). Indeed, life science laboratory walls often display large posters concerning the detailed biochemistry of the metal reactions occurring in a range of cells and at a variety of sites in the body.

For various reasons, for example, the hydrolytic reactions discussed above, it is sometimes necessary to 'target' the metal complex to specific organs or sites by such relatively simple arrangements as the enteric

coating of tablet so that they persist unreacted until they reach the small intestine. The fact that humans can live for almost a century on exceedingly small amounts of trace elements (see Table 2.1) underlines the highly complex biochemistry which controls, buffers and, eventually, excretes such trace element species from the different cell types in the body.

However, the weakest biochemical links in the chain may often be the non-metallic, usually, organic, molecules required as reactants. This explains why iron supplementation sometimes needs to be reinforced by concomitant administration of folic acid (see Table 5.1, Compound oral iron preparations), whereby the folate plays a vital role as a co-factor in the biosynthesis of the haem ligand to which the iron becomes attached to form haemoglobin. Similarly, cobalt is required in the form of vitamin B_{12}, cobalamin, which consists of a corrin ring surrounding a central cobalt atom. Cobalamin is unique in biochemistry since it cannot be synthesized by plants or animals, but only by micro-organisms. Thus cobalamin must be supplied by the diet, but in order to be absorbed into the circulation it must first form a complex with a glycoprotein called intrinsic factor present in the intestinal lumen. This intrinsic factor–cobalamin complex then binds to a specific receptor in the lining of the ileum from which the cobalamin is transported into the blood. Thus a deficiency of cobalamin cannot be made good by simple administration of a cobalt salt. In other situations trace element deficiency may result not so much from a shortage of the metal itself, but more from derangement of the mechanisms by which it is absorbed or transported to its sites of action.

FURTHER READING

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Chapter 6

Agents Containing Metals

RECOMMENDED DAILY AMOUNTS AND BIOLOGICAL HALF-LIVES

The 'right' amount of trace metal and even a macro-metal to be taken in one's diet varies widely and is controlled by a large number of factors. For example, the requirements of the human body range over a wide margin of concentrations depending on age, rates of tissue growth, general fitness, and the activity parameters of the individual concerned and upon other foods consumed in the diet.

To quote a few examples, the rate at which human tissue grows *in utero* or as a young baby is nothing short of spectacular in terms of the amounts of new biochemicals synthesized every day. However, our genes appear to have been programmed so that we lose half of our muscle tissue between the ages of 30 and 50 but during the same time-span we virtually double the amount of adipose tissue distributed around the body. Modern-day tendencies to fight against this pre-programmed ageing in order to acquire teenage-like figures can create serious trace element imbalances.

By and large, dietary trace elements are supplied along with much larger amounts of carbohydrates, proteins, and macro-elements by the normal diet. Physical work and physical recreation enlarges the need for such energy, whereas increasing age and lowered physical activity clearly reduces the need. This can mean that since one's appetite often also diminishes with age, there may be an inadequate supply of trace elements reaching the desired tissues. Furthermore, the ability to absorb food material through the intestinal lining seems to diminish after middle age.

Thus, it is clear that it is almost impossible to lay down a table of recommended daily dietary amounts for all the nutrients. Nevertheless, distinguished professional bodies have tabulated the average required

Table 6.1 *Recommended daily mineral intake (RDA) for a 70 kg adult*

Elements	*RDA Oral/* mmol	*% Absorbed from GI tract*	*RDA Intravenous/* mmol	*RDA Oral/*g	*RDA Intravenous/* g
Calcium	20	20–30	7–14	0.80	0.28–0.56
Phosphorus	25	–	14–70	0.77	0.43–2.17
	μmol		μmol	mg	mg
Zinc	230	~ 60	49–210	15	3.2–13.8
Manganese	45–90	~ 12	7–35	2.5–5.0	0.3–1.9
Copper	30–45	30–80	5–70	1.9–2.8	1.9–5.1
Chromium	1–4	1–25	1	0.05–0.20	0.05–0.12
Fluorine	78–208	–	49	1.5–3.9	0.93
Iron	180–324	10–20	21–70	10.0–18.1	1.2–2.3
Iodine	1.2	–	1–7	0.15	0.13–0.89
Selenium	0.6–2.6	–	1.5–5.0	0.047–0.20	0.12–0.39
Molybdenum	1.5–5.0	–	0.2	0.14–0.48	0.019

GI = gastrointestinal.

intake for many of the elements and examples are shown in Table 6.1. These data do not imply that these are the required amounts to be taken in from 'cradle' to 'grave' because of the reasons given earlier. Similarly, it ought not to be assumed that being out of balance with these inputs at a given stage of life is necessarily going to be seriously damaging overall.

In practice, clinicians and dieticians rarely aim to balance this whole spectrum of recommendations but rather they tend to relate a clinical manifestation to an imbalance of a particular metal and then, possibly to assess the intake from so-called 'food from the plate' studies. It must be stressed that the biological roles of many of these elements are interdependent and in some instances will tend to raise the apparent concentration of a sister element (this is called stimulation), and in other cases will tend to suppress the intake and bio-availability of a related element (called antagonism).

FOODS

Coultate's sister volume to this book describes our intake of food in great detail and lists the bulk minerals such as sodium, potassium, magnesium, calcium, and phosphorus. The trace minerals are listed as iron, copper, zinc, selenium and iodine. Other trace minerals described as essential by inference from their occurrence in various enzymes in human and

animal tissue are listed as boron, silicon, vanadium, chromium, manganese, cobalt, nickel, and molybdenum.

Modern-day diets are composed of foods from all five Continents and often reflect the elemental compositions of the soil used to grow those crops and to raise the animals, as mentioned in Chapter 2, it has been estimated that a human eats approximately 8 kg of soil during a lifetime. Thus, a varied and ample diet will probably protect against trace-metal deficiencies for most of a lifetime. However, the reduction in physical activity, in circulation, and in appetite in later life, may lead to less trace elements being taken in. Thus, it is often advisable to increase the concentrations of such trace metals for older persons, to counteract their lower presence in the smaller diets (see Wound Dressings, page 70).

In the early part of this century it was possible to correlate clinical conditions with imbalances of trace elements in those areas where the majority of the food intake came from the immediate surroundings inhabited. There have been many attempts to correlate the incidence of carcinoma (cancer) in the USA with the intakes of selenium which were either too low or excessive compared with the recommended selenium dietary figure.

Finally, we stress that it is wrong to correlate 'total amounts' data with adequacy. A classic example is that the highest concentration of zinc in most food species is to be found in the seeds of tomatoes. However, their husks are impenetrable and the seeds pass right through the eater with none of the zinc being released in the intestine. A related study has been that of trace elements such as copper and zinc being totally sufficient in terms of total amounts present in the soya protein fed to broad-breasted bronze turkey chicks and yet they exhibited severely impaired growth. It is only when the diets were spiked with complexing ligands sufficiently powerful to dissociate metal ions away from the strongly chelating phytates present in the soya protein, that the fowl were able to assimilate such complexes and to grow at a normal rate.

FOOD ADDITIVES

'Additives' are found in food for a variety of reasons. They may be agricultural additives such as fertilizers, or herbicides carried through into the food that is eaten; they may be agents specifically produced in order to give the food greater appeal, for example, by changing its colour or texture; they may be agents added in order to speed up the production of the food by, for example, reducing the mixing time required by adding flow enhancing additives; they may be agents added to speed up the cooking process or the ability to prepare a meal rapidly

(so-called 'convenience' foods). Finally, they may be added to food in order to increase the shelf, or refrigerator, lifetimes of the foods. In this last respect it must be pointed out that most micro-organisms can survive well on the diet offered to humans. The good news is that one can use micro-organisms to produce food in the form of yoghourt or beer which have compositions pretty close to those of human requirements. Several dishes involve micro-organisms as single cellular protein or as fungi. However, food manufacturers aim to control other food-borne micro-organisms so that they do not cause the food to rot too readily or to give off unpleasant smells or to take on an unpleasant appearance during storage.

The presence of food additives is carefully regulated in the European Union and many are now identifiable by their E-numbers on the packaging of foods. From a metal complexing viewpoint, some of these additives may well modify the bio-availability of trace metals present by complexing with them or even by precipitating them out of solution. Once again, the fact that most of us eat a wide range of foods tends to normalize out such features to such an extent that precious few problems arise from food additives when viewed against the background of the large amount of food eaten per year (500–600 kg per average adult).

FLAVOUR STIMULANTS

Flavour is collectively referred to as the detection of taste and aroma from the food. Taste buds in the mouth and tongue are stimulated by trace elements such as zinc and other transition series metal ions. Trace elements are eaten as micro impurities clinging to freshly prepared food from gardens and agriculture. However, with the ultra-clean processing devices which prepare fruit, vegetables, and meats, taste stimulation may often be lacking. Nature has overcome such scarcities by evolving tendencies in animals to lick metal gates and railings, cats will tend to chew grasses and bones, and, possibly, children chewing lead paint (a habit called pica) may be a response to cravings for trace element stimulation.

A crude way of satisfying such cravings has been the administration of salt to the diet. Our taste buds, however, tend to be rather more subtle and prefer a spectrum of trace element stimulations. Taste bud response can also be stimulated using sodium glutamate. Unfortunately, tasting a small amount often leads to a feeling that more is required and rather akin to salt and sugar in one's diet, this might eventually bring about excess and toxic intakes.

These overloaded and overworked taste buds ostensibly become less

sensitive to trace element stimulation. The taste buds eventually recover from overloadings and can become quite discriminating again in terms of a wide range of tastes and aromas. An interesting and encouraging trend is towards food cooked in its own juices in the absence of additives. Finally, there is a school of thought that instead of pure vacuum pan recrystallized sodium chloride one ought to use rock salt which has its origin in ancient seas from whence evolved the single cellular organisms which were the ancestral cells to those in our human bodies. Certainly, such rock salt does have an ionogram (distribution of ions) similar to that found in human blood plasma which in turn resembles the composition of ancient seas.

SUPPLEMENTS

There is a modern tendency to take trace element or vitamin supplements purchased from pharmacies or health food outlets. Furthermore, it is traditional to add elements present in low amounts to foods, for example, fortifying certain breads with iron. The important criteria which should be considered in selecting such supplementation is 'Are the metal compounds being administered in concentrations comparable to those of the recommended daily amounts given earlier? Is the speciation of the compound being used such that it is bio-available from the gastrointestinal tract into the blood stream? Is the circulation such that the metal complexes will reach their site of desired activity or if the deficiency is thought to be local, ought they to be applied topically?' and finally, but most importantly, 'How is it possible to monitor the concentration of this trace element such that overloading and related side effects do not occur?'. Often, such monitoring is in terms of the clinical response such as whether inflammation is decreased by administration of a traditional topical treatment such as wearing copper jewellery or applying a zinc and castor oil ointment for rashes.

'HEALTH FOODS'

These two words are probably tautological! Why ought we to eat anything non-healthy? In fact, considerations such as sources, processing, and appetite likes and dislikes mean that, in reality, approximately one-third of food eaten is definitely beneficial to the average person, approximately one-third has some good associated with it, and approximately one-third could be harmful above a relatively low level.

Health foods are linked in with topics such as organic farming even though it is difficult to define 'organic farming'. There is a consensus of

opinion that living in a highly Westernized society necessitates our bodies being challenged occasionally by small amounts of undesirable chemicals in order to keep our immune defence systems efficient. To be living on food which contains no traces of animal antibiotics, or man-made fertilizers, *etc.* may have theoretical advantages but there may also be a long-term weakening of our immune system. Furthermore, with the majority of the World population being defined as 'developing countries', it would not be possible to feed this increasing number of persons by an over-zealous insistence on health foods.

Quite often, an increased awareness of how exercise and regular limb movement is necessary in order to circulate the contents of our diet to the extreme parts of our body can surpass the apparent benefits from health foods consumed by an underexercised body.

FOLKLORE AND ALTERNATIVE MEDICINES

Many of our traditional Western medicines stem from centuries-old folklore remedies. For example, aspirin from witchhazel or the administration of bismuth for intestinal ulceration originating in soil deposits in central Africa.

There are now many alternative medicines which benefit the patient, sometimes from the increased encouragement and psychological uplift given by the practitioner and sometimes by the chemical contents of the material being administered. As scientists we must, as with supplements, bear in mind the total concentrations of the ingredients, the bio-availability, the efficiency of circulation, and whether overdosing is a threat. An interesting new development is the combination of therapies from both Western and traditional medicines.

HEALTHCARE AGENTS

A wide variety of metals have been used in the general area of healthcare, the field being rather loosely defined as agents purchased without prescription from a pharmacy or off the shelf of a retailer. Table 6.2 reflects some of these agents, their metal content, and their uses. Some have been handed down to us from previous generations but the vast majority have been compounded during the last half century in order to attack common but not life-threatening conditions such as dental plaque, body odour, *etc.* The trace metal may be present either because it has an integral part to play in terms of the *modus operandi* of the healthcare agent or it may be added purely as a means of counteracting negatively charged ingredients. Two examples are given to show how

Table 6.2 *Some metal-containing healthcare and therapeutic agents*

Element	*Compound*	*Prescribed as – and use*
Aluminium	Hydroxide	Aludrox – antacid
	Silicate	Kaolin – anti-diarrhoeal
Antimony	Gluconate	Pentostam – anti-leishmaniasis
Bismuth	Tripotassiumdicitrato	De-Nol – antacid and anti-ulcer
Boron	Boric acid	Monphytol – antifungal
Cobalt	Vitamin B_{12}	Ce-cobalin – pernicious anaemia
Iron	Glycine sulfate	Fe-cap – iron deficiency anaemia
Gold	Thiomalate	Myocrisin – antiarthritis
Magnesium	Sulfate, hydroxide	Epsom salts – laxative, antacid
Platinum	Dichlorodiammine	Platosin – anti-neoplastic disorders
Selenium	Sulfide	Lenium – antidandruff
Silver	Sulfadiazene	Silvadene – antibacterial
Tin	Fluoride	Toothpaste – anticaries
Zinc	Sulfate	Z-span – nutrition and would healing
	Citrate	Dental hygiene

the metal is important to the activity: the composition of a new dentifrice and the means through which perspiration is suppressed with a metal hydroxide precipitate.

The body contains a large number of pores which excrete perspiration. The intensity of these pores varies from 50 cm^{-2} in one part of the body to more than a 1000 cm^{-2} under the armpits. It is possible to block these pores with a judiciously applied aluminium compound as is found in most modern antiperspirants. Essentially, the spray-on or roll-on antiperspirant keeps the aluminium in solution as a charged complex (Figure 6.1).

When the pH of the antiperspirant is raised by the perspiration the speciation changes into an insoluble aluminium hydroxide precipitate which acts as a plug to the pore. Eventually this plug will redissolve, either by the action of soap or shower gel which changes the pH to a more alkaline value, or because the skin contains bacteria that excrete ammonia which also elevates the pH and dissolves the pore-blocking solid. Clearly, the blending of this aluminium speciation scenario with other agents such as deodorants (typically these are alcohols which cleave the long chains of foul-smelling carboxylic acids) and with other cosmetic and sweet-smelling ingredients, is a skilled art which is heavily dependent upon chemical speciation knowledge.

Toothpastes are designed with two purposes in mind. First, it must be a mild abrasive for removing calcified plaque from the surface of the

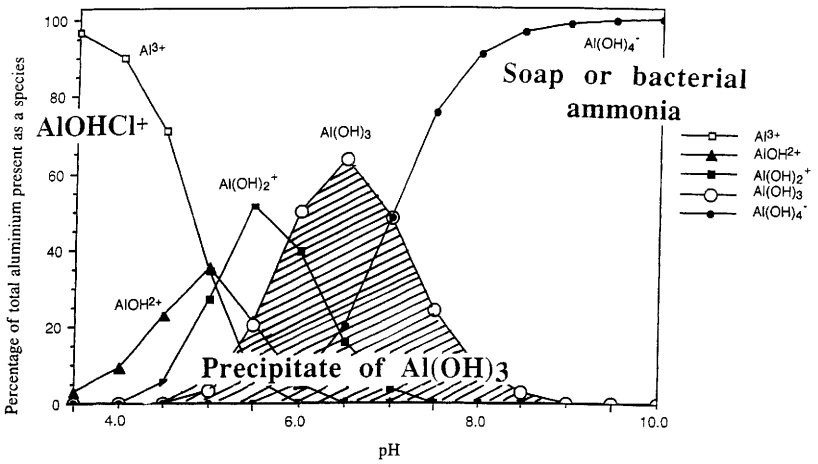


Figure 6.1 *A typical antiperspirant contains mildly acidic aluminium hydroxychloride in solution. Upon interacting with perspiration at neutral pH, aluminium hydroxide precipitate is formed which blocks the pores excreting perspiration. Eventually, washing with soap or even unwashed skin bacterial activity raises the pH value further and the aluminium in the plug becomes soluble again as a charged hydroxy complex. The number of sweat glands vary from* $50cm^{-2}$ *(buttocks) to 1000 (armpit, elbow etc.) and total 2 million per person.*

dental enamel. Secondly, it is wise to have an antibacterial agent present to discourage the growth of bacteria which eventually leads to acid liberation and dental caries. Zinc has been found to be useful in these two roles and is now fairly widely distributed in new toothpastes since a high concentration of zinc is sufficient to overload *Streptococcus mutans* microbes within saliva and to stop them from digesting sugars to liberate lactic and other acids which in turn leads to demineralization of the tooth. The selection of zinc to fulfil this role has been based upon a correlation between the positively charged species present in a mixture of toothpaste and saliva with antibacterial activity. Paradoxically, tin compounds used in dentifrices tend to exhibit activity proportional to the total amount of metal present rather than upon the positively charged species. Both these metal compounds are now widely used in toothpaste manufacturing.

COSMETICS

Cosmetics are used for more than beautifying the external body; since their inception they have been linked in with religious and cultural

habits. Metals have been used in cosmetics for many thousands of years and most of the precious metals such as gold, silver, arsenic, *etc.* are still found in cosmetic applications. More recently cosmetics have been used as a medium for applying radiation barrier creams in order to stave off the threat of UV radiation from sunlight.

THERAPEUTICALS

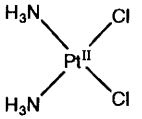
Therapeuticals which contain metals may use the metal in either a passive or an active role which corresponds to a non-specific or to a specific function, respectively (Table 6.2). Most organic therapeuticals function through electron donor groups such as lone pairs which form bonds by dative covalence to the active sites of proteins, nucleic acids, *etc.* The presence of such concentrations of electrons often means that the agent is negatively charged or negatively polarized. In order to make these agents absorbable, a counteracting positive charge is required and this is provided by the metal ion. Furthermore, the bio-availability of such organic agents often relies upon protons being available for neutralizing this negative charge and making the agent lipid-soluble, so that it passes through cell membranes *en route* to the blood plasma and tissue, *etc.*

Unfortunately, since much of human biochemistry takes place at neutral pH, a ready source of protons is not to hand and so metal ions can be used as so-called 'super acids' to provide that source of positive charge density. Related agents that are not metal ions are alkyl ammonium cations that are also used in a support role for drug manufacturing similar to protons and metal ions. Not surprisingly, one-fifth of the drugs in the pharmacopoeia in the UK contain metals in this passive, non-specific role.

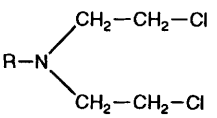
Pharmaceuticals that require metal ions for their specific activity and in which the metals have a very active role are fewer in number but hold far greater fascination. The metal ion may be present because it has the ability, coupled with its coordination chemistry properties, to direct covalent bonds or because it is a source of positive charge density or because the metal is able to form specific bonds to an active site in the biochemistry of the diseased organ. Furthermore, it is frequently necessary to raise the concentration of the total amount of the trace metal present in tissue in order to allay the symptoms of a deficiency condition. This means that the metal ion has to be made bioavailable such that it can pass from the gastrointestinal tract into the bloodstream.

CHELATION AND ANTICANCER ACTIVITY

The serendipitous discovery by Professor Barnett Rosenberg in the 1960s that the presence of a platinum wire in a culture caused severe growth disturbance of micro-organisms led to other revelations that coordination compounds of Periodic Table Group VIII in the d-block of the transition series possess anticancer (cytotoxic) activity. The difficulty encountered that such complexes tend to hydrolyse rapidly was suppressed by focussing on platinum, one of the more inert complexing metal ions. The first agent was *cis*-dichlorodiammineplatinum(II), which loses two chloride ions to form a platinum chelate with two nitrogens in the pyrimidine and purine bases of the DNA chain in the cell nucleus to form an intrastrand link that interferes with the copying of the DNA chain when the cell next attempts to divide. Other long-established cytotoxic agents, such as the nitrogen mustards, are known to form similar cross-links, but between bases on each of the two strands of the DNA molecule, *i.e.* interstrand links.



Cis-dichlorodiammine platinum(II) (Cisplatin®)

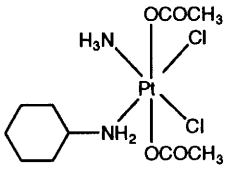


Nitrogen mustards

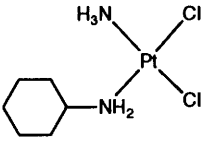
The spatial separation of the chloride ions in the *cis*-platinum complexes, 0.33 nm, and those of the chlorides at the ends of the chloroethyl arms of the nitrogen mustard, 0.80 nm, suit the formation of intra- and interstrand bridges, respectively. The cell-killing effect results from an inability, or very much decreased ability, of tumour cells to repair the intrastrand breaks.

Cis-dichlorodiammineplatinum(II) (Cisplatin®) was introduced clinically in the UK in 1979, when it was claimed to be the first heavy metal compound marketed for use in cancer treatment. Cisplatin has proved to be a very effective agent, either used alone or in combination with vinblastine and/or other cytotoxic drugs, in the treatment of ovarian and testicular cancer and also lung cancer. However, the agent suffers from serious disadvantages: first, it must be infused intravenously, and, secondly, it is extremely toxic, causing nausea and vomiting, as well as leukopaenia and renal dysfunction. Further, some tumours develop resistance to the drug. Recent research has concentrated on overcoming

these defects; attaching a 1,1–dicarboxycyclobutane molecule to the diammineplatinum(II) to produce the *cis*-1,1–dicarboxycyclobutane-diammineplatinum(II) derivative, Carboplatin®, has removed some of the disadvantages to produce a second generation agent which is now in clinical use.



JM216



JM118

More recently, an orally active platinum drug [bis-acetatoammine-dichloro(cyclohexylamine)platinum(IV)] (JM216) has been developed. This compound is completely metabolized in the body to six metabolites. The main plasma metabolite is the platinum(II) reduction product [ammine(cyclohexylamine)-dichloroplatinum(II)] (JM118) and this compound appears likely to be the active cytotoxic moiety. JM216 is now undergoing clinical trials and may well enter clinical practice by the mid-1990s. A further interesting development, that is still in the research phase, is the compound [transammine(cyclohexylamine)-dichlorodihydroxoplatinum(IV)]. This is the first *trans*-platinum compound to show any selective antitumour activity *in vivo*, and in contrast to Cisplatin it appears to form interstrand cross-links in DNA.

ULCER THERAPY USING BISMUTH COMPOUNDS

Paracelsus made the general introduction of several heavy metals into medicine in the 16th century and nowadays the particular use of bismuth compounds is focused upon the treatment of disorders of the alimentary system (14 such drugs are commonly prescribed in Britain). Several of these medicines are based upon a red viscous colloidal bismuth citrate solution containing ammonium hydroxide plus a colourant, a sweetening agent and emollients such as sorbitol or glycerine at pH = 10.

Until the 1960s, theories concerning the mechanism by which bismuth promotes ulcer healing have been largely empirical. However, the introduction of intragastric colour photography using fibre optics has made it possible to observe an ulcer before, during, and after therapy. Such observations provide support for the hypothesis that the ulcer site becomes coated with a precipitate of a bismuth compound

which isolates the underlying raw surface from the digestive action of the gastric and duodenal juices and so permits ulcer healing. Speciation research has demonstrated that the acid environment of the stomach converts the bismuth from soluble, charged bismuth(citrate)$_2^{3-}$ complexes into insoluble precipitates of bismuth oxychloride and bismuth citrate solids.

Even though most stomach ulcers are treated by gastric acid secretory inhibitors or H_2-receptor (histamine) blockers such as cimetidine, the simulation technique has led to formulations that produce bismuth patches on ulcers at other sites having other pH values. However, the optimization of conditions for ulcer healing is very difficult using clinical trials because of the vagaries of ulceration and of responses to therapy, some 80% of ulcers reoccurring within a year.

It is fascinating to note the recent re-awakening of interest in bismuth therapies since the discovery in 1982 that the intestinal bacterium *Helicobacter pylori* may well initiate ulcer formation by excreting acid. Bismuth, in common with many heavy metals, is bactericidal and so the lasting effects of bismuth citrate therapy may well be a combination of ulcer healing (from the precipitates) as well as ulceration initiator suppression (from the bacteriocidal action). *In vitro* the organism is sensitive to bismuth but results *in vivo* are feeble. However, combinations of bismuth with antibiotics such as amoxicillin or tetracycline have success rates of ~ 80%, *i.e.* four times the 20% success rate described above.

PARENTERAL NUTRITION

Hospital patients are becoming increasingly dependent upon nutrition being administered through a vein rather than through the oral route. Currently, some 5% of patients are totally dependent upon parenteral nutrition, sometimes for short-term support whilst they recover consciousness from an intestinal operation, but in the extreme, patients have been returned to a fairly active life totally supported by this means for more than ten years.

Essentially, all of the requirements for the normal diet such as water, energy sources (amino acids, glucose, protein, phospholipid, and glycerol), nitrogen source (amino acids), minerals (electrolytes, trace elements), vitamins, and even the occasional drug, such as an anticoagulant, have to be mixed into a fluid to be passed into a vein.

Often the condition leading to the requirement for parenteral nutrition has seriously depleted the trace element stores of the patient concerned and so it is vitally important that adequate amounts of trace elements are present in parenteral nutrition fluids.

Premature infants are another critical group; they often require to be kept on parenteral nutrition for up to three months until they have matured sufficiently to be able to suckle and take in more normal nutrition.

For an adult, up to three litres of fluid per day are administered into a vein. Were the patient's sole energy requirement to be given in the form of a sugar such as glucose the order of 700–800 g per day would be necessary. This would vastly exceed the sugar-handling capacity of the body insulin and so to avoid this difficulty much of the energy is administered in the form of fats and oils, typically a soya oil–protein emulsion. An emulsifying agent is required, such as egg lecithin, to hold the fluid in a fairly stable equilibrium.

Particular problems arise from the administration of the electrolytes and trace elements used to ensure that the intravenous fluid is completely isotonic and compatible with the plasma. Such charged species can cause flocculation or even coalescence of the oil–water emulsion.

Fortunately, new theories of emulsion stability have now led to such effects being predictable on a quantitative basis. Margins of safety in respect of this sort of problem and, indeed, other problems such as the precipitation of calcium phosphate during the preparation of the intravenous fluid (this stage is known as compounding) can now be avoided, the usual safety margin being a factor of at least ten. Interestingly, impurities present in materials used to make up the intravenous fluid can also lead to precipitates which block infusion lines or cause emulsions to coalesce and may thus cause thrombosis. A knowledge of the chemical speciation at each stage of preparation along with high standards of purity and sterility combines to give a safe treatment.

WOUND DRESSINGS

A wound may be defined as a defect or a break in the skin resulting from physical, mechanical or thermal damage or developing as a result of the presence of an underlying physiological disorder. The skin, which is by far the largest organ of the body, is subject to many stresses, strains, and challenges and so it is not surprising that in 1992 wound healing alone in the UK cost the Health Service in the order of £600 000 000, the 'average' leg ulcer requiring a commitment of approximately £26 000 to heal. The normal healing process goes through three general stages after blood clotting: inflammation; cell proliferation; and maturation, and each stage is trace-metal dependent.

Each cell in the human body relies upon an adequate supply of fluid, electrolytes, and nutrients from the vascular system. Often wounding

severs this supply and so cells which have been injured are starved of their normal supply of materials for self-repair. Furthermore, the three stages of wound healing just mentioned require additional biochemicals which, once again, ought to be supplied via the vascular system.

Wound management products have been used since the beginning of human life including such diverse materials as cobwebs, hair, dung, leaves, animal skin, honey, and other strange coverings. This century's materials focus more upon cotton and wool-based dressings. For example, the gauzes classically used in dressing wounds for almost a century were originally invented in order to keep mosquitoes out of soldiers' tents during World War I campaigns!

Modern wound treatment is a highly scientific and skilled speciality. In general, surgical dressings fall into three categories:

1. Passive products which protect and cover the wound, *e.g.* gauzes made of wool and cotton;
2. Interactive materials which produce a controlled microenvironment which encourages wound healing, for example, films and foams made of polymeric material;
3. Bioactive dressings which positively attempt to supply and deliver all of the normal substances required by a cell in addition to the extra biochemicals indicated under the three phases of healing above.

This last group of dressings is often based upon alginates, hydrocolloids, or hydrogels. Alginates are salts of naturally occurring polymers of alginic acid which occur in the cell walls and intracellular spaces of seaweeds such as the brown algae, *phaeophycae*. These alginates are composed of mixtures of β-D-mannuronic acid and α-L-guluronic acid. Much research has been conducted into these agents which are widespread in foods and in medicare. Essentially, they are able to form molecular cavities into which metal ions may be complexed (Figure 6.2).

Physically, when these chelating cavities have been filled by complexed calcium ions they can be spun into fibres which can then be woven into gauzes and dressings. These Ca^{2+} ions are reversibly bound and will exchange with sodium ions exuded from wound fluid, releasing calcium to the healing and clotting mechanism. When this exchange of ions occurs, the fibre-like, solid material then becomes more like a mouldable gel which is ideal for following the contours of the wound and for maintaining a moist environment inducive to the various stages of wound healing. In addition it protects against bacterial invasion without the dressing sticking to the wound or causing irritation.

An interesting extension of these studies is to consider the cells involved in the wound healing process from an intravenous nutrition

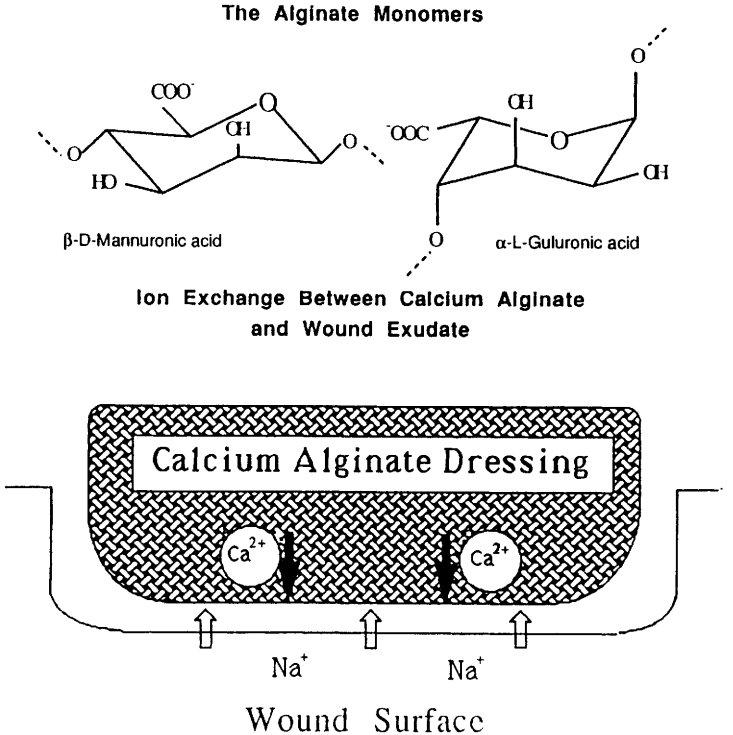


Figure 6.2 *When* β-D-*mannuronic and* α-L-*guluronic acids are polymerized to form a polymer, cavities are formed similar to an egg-tray construction. Metal ions such as* Ca^{2+}, Na^{+}, etc. *occupy these sites. The polymer is spun into a thread that is woven into a dressing and can exchange such metal ions with those present in wound fluid. Rather uniquely, when the gauze-like calcium dressing exchanges ions for sodium the alginate becomes more gel-like and closely follows the contours of the wound, thus excluding infection and contamination and also keeping the wound moist. The diagram shows the monomeric units and cation exchange.*

viewpoint. Whereas clinicians are now accustomed to supplying a large number of trace elements and other nutrients in total parenteral nutrition formulations, there are similar advantages to administering these agents through dressings such as alginates. A simple exchange of calcium for sodium could well grow to more sophisticated medications, possibly having different formulations for the different stages of wound healing. Even more sophisticated plans could involve dressings specifically designed to remove contaminating elements as well to supplement the essential ones (see later).

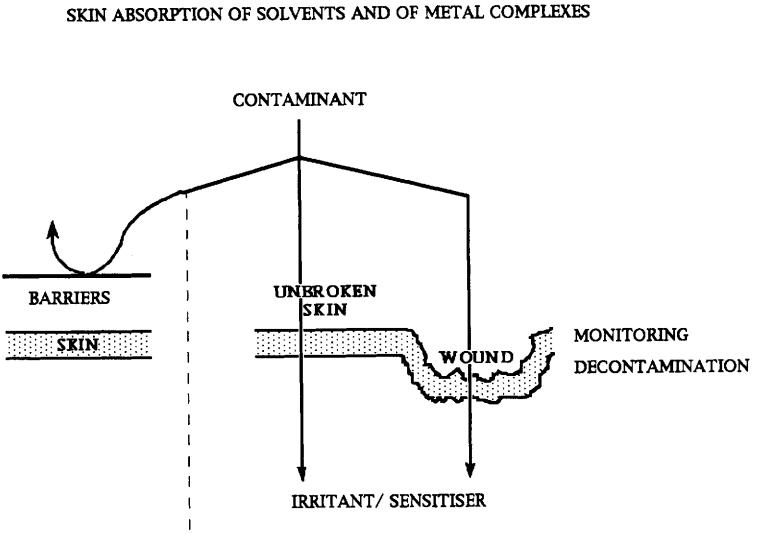


Figure 6.3 *Routes through which a contaminant chemical may irritate, sensitize, or penetrate skin.*

Wound management has come a long way since the time when it was believed that all wounds, no matter the area or depth, must be liberally overdosed with antiseptics such as iodine or cresols!

SKIN PROTECTION AND DECONTAMINATION

Metal ions are usually unable to penetrate the skin and to gain access to the circulation. However, when the skin has been broken by a wound, and when the metal ions are made net-neutral in the form of a metal complex, access from the exterior of the body to the internal circulation is a distinct possibility (Figure 6.3). Similarly, when such metal complexes are present on unbroken skin in the presence of fluids such as perspiration or organic solvents, their ability to penetrate the epidermis and reach the circulation may be greatly increased. Thus, a consideration of skin protection, of cleansing, and of decontamination is important.

Protection

The scheme indicates absorption through the skin and through a wound under various protective circumstances. Protection is widely used in

industry since skin diseases account for up to a quarter of reported occupational illnesses and usually involves masks to cover the mouth and nose, eye protection to cover the upper part of the face, gloves to cover the hands, overalls covering the majority of the body, and the use of skin barrier creams. Such barrier creams often need to be used when the worker is exposed to chemicals which irritate or sensitize the skin. Although the creams may be barriers to some irritant chemicals, they may actually contain a solvent which permits faster uptake of net-neutral metal complexes.

In considering the best methods for protection and occlusion, timing is important. For example, a rubber glove placed on an already contaminated hand or finger will accelerate uptake. Similarly, excessive perspiration within the glove produces a fluid that can accelerate uptake. Before using protective clothing it must always be considered whether the use, or possible misuse, can actually promote uptake rather than protect against it. This is especially so when neutral organic solvents are being used on the outside of the gloves. Each laboratory threat ought to be considered on its merits and strict safety rules implemented in conjunction with case conferences.

Cleansing

Similarly, skin cleansing by frequent washing is clearly desirable but it should be noted that many lanolin-based soaps can increase skin absorption if not completely purged from the skin after washing.

Decontamination

The amount of decontamination necessary depends on how much metal, be it heavy metal or metallic radionuclide, is present on the skin or in the wound. Clearly, any monitoring data that can be acquired in this respect is pivotal to the decisions being considered.

In an ideal situation, and assuming a wound has freshly occurred, which is possibly contaminated with a radionuclide, and whose presence can be verified and monitored by judicious monitoring, it is now feasible to apply blocking ligands in the form of sequestering agents bound to a polymer as part of the first stage of dressing. This rapid treatment may arrest the uptake from the wound into the circulating blood; it is wise that such treatment is instigated within 30 minutes of wounding and contamination.

Thus, a promising new area for future research is that of resin-bound ligands complexed to essential trace metals but in such a form that

contaminating metal ions can selectively displace the essential metals and become bound to the immobilizing resin built into the dressing.

METALS AS RADIOPROTECTIVE AGENTS

Our bodies are subject to continuous attack by ionizing radiation from cosmic rays from outer space, from γ-rays from the natural radionuclides in the upper layers of the Earth's crust, and α, β, and γ-rays from the radionuclides, ^{40}K, ^{14}C, ^{222}Rn, *etc.*, that are naturally present in our tissues. People may well receive additional radiation from *X*-rays or other types of radiation source used in medicine or industry and, rarely, from the release of large amounts of radioactivity due to accidents in nuclear facilities.

When ionizing radiation passes through an aqueous system, such as the human body, the radiation energy causes excitation and ionization that results in the production of free radicals, including $H^{\bullet}$, $O^{\bullet}$, $HO^{\bullet}$ and the superoxide radical $^{\bullet}O–O^{\bullet}$. These free radicals are highly reactive, very short-lived species that can interact with proteins, DNA, and lipids in cell tissues causing molecular changes which may kill or seriously damage the cells and lead to serious long-term injury such as cancer. In addition, some free radicals can interact with each other to form oxidizing species such as H_2O_2 or other peroxides that may also cause tissue damage.

Natural radiation levels are generally very low. In the UK, for example, the average annual radiation dose to the human body is about 2.2 millisieverts (mSv), corresponding to the absorption of about 150 mJ of radiation energy by a 70 kg person. However, occasionally, a person may be exposed to very much larger doses of radiation, and therefore it has been felt necessary to look for agents that could prevent, or reduce the severity of damage following accidental exposure to large amounts of radiation.

Over the years, many substances tested as radioprotective agents have been free-radical scavengers, including dithiols and dithiocarbamates. Some years ago, Schubert pointed out an interesting and curious fact, namely, that many of these agents under physiological conditions can react only with copper, and only copper in its distinctive oxidation states. Thus, interest was aroused in copper compounds as radioprotective agents, and a number of copper complexes were shown to be good scavenging agents for the superoxide radical, which is believed to play an important role in the induction of radiation damage. Superoxide probably exerts its toxicity through its ability to reduce metals ions *in*

vivo, for example Cu(II) → Cu(I) and to form $OH^{\bullet}$ radicals which combine to form toxic H_2O_2.

One compound, copper(II)di-isopropylsalicylate (Cu-DIPS), has been shown in mice to be very effective in reducing the severity of radiation damage when it is injected up to a few hours after irradiation, *i.e.* long after the radiation-induced free radicals would have disappeared. The mechanism by which Cu-DIPS exerts its protective effect is not known with certainty, but it is thought likely that it facilitates the *de novo* synthesis of superoxide dismutase, a copper and zinc-dependent enzyme that catalyses the dismutation of superoxide, and possibly other copper-dependent enzymes that may play important roles in tissue repair.

MEANINGFUL THERAPIES

There is always the possibility that extensive supplementation of the human body with the material that we normally acquire from our diet and manage by a system of cellular buffering could well damage the cells by overload and render the natural system inefficient or even ineffective. Responsible advice from a trained clinician is always advisable.

CONCLUDING REMARKS

It ought to be clear from this and previous chapters that virtually all therapeutic and healthcare agents will have a trace metal dependence, either as part of their *modus operandi*, or as side effects, or indeed as counterions. However, the subject has been late in developing, partly because of the scarcity of analytical techniques extendable down to the exceedingly low concentrations through which certain chemical species act *in vivo* and also partly because of the preponderance of organic chemists among pharmaceutical researchers. It has been pointed out many times that life is as much inorganic as organic.

Finally, nature does not differentiate between metals and non-metals and inorganic or organic, but rather the whole of nature's biochemistry is one of electron energy manipulation. The human body does not recognize the elements of the Periodic Table as letters of the alphabet, but rather these elements are present as active groups having specific electron distributions that give rise to bonds. These electron, energy-rich groups in biochemicals, in contaminants, and in pollutants are equally capable of forming bonds with electron acceptor species, such as the active sites of enzymes, as they are of forming bonds with metal ions *in*

vivo. It is wise to keep an open mind when considering human biochemistry and to remember that the majority of the elements of the Periodic Table are present in humans.

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Chapter 7

Chelating Agents and Therapy

INTRODUCTION

Chelation therapy has a unique place in medical science. Although the clinical need to treat the undesirable effects of excess metal ions in the human body is relatively rare, a partial knowledge of the role of metal chelation is widely held. The ubiquitous involvement of metals ions in biological systems and their pivotal roles in biochemistry illustrate this. The biological consequences of administering agents which alter metal ion distribution *in vivo* impinge on an extraordinarily wide variety of scientific disciplines. Nutritionists may use chelating agents to try to ensure an adequate dietary intake of essential elements; toxicologists may use them to minimize toxicity or to treat metal poisoning, and physicians may use metal complexes with either radioactive or stable metals as diagnostic aids in nuclear medicine or magnetic resonance imaging.

Most medical specialities have an interest in trace elements and this reflects their integrated and intrinsic participation in almost all aspects of biochemistry. Promising medical advances may well derive from the development of agents or techniques that manipulate the concentrations of metal ions *in vivo*.

Such research into inorganic medicinal chemistry will attract increasing attention, but research into trace element removal using chelation therapy will not decrease. Indeed, reliable experimental data and a sound theoretical understanding of how administered agents modify metal ion distributions *in vivo* will become more important than ever.

The conceptual criteria for synthesizing or selecting a chelating agent aimed specifically at removing toxic metals from the human body is an area of growing importance for many reasons. First, it is widely acknowledged that with the increasing population of the world, which is more than paralleled by an increasing deterioration in the quality of the

environment, there is an enhanced possibility of undesirable metals being present in foods. Thus it may be necessary to develop methods for limiting the uptake of such metals into foods, or of otherwise limiting their absorption from the gastrointestinal tract into the blood. This raises an important perceptual question akin to the long running debate concerning mass 'chemicalization' of our water supplies (chlorine, fluoride, iron, aluminium, *etc.*) or of fruit (herbicides, insecticides, sulfur dioxide, *etc.*).

Secondly, rare metals have been mined, concentrated, and used in a variety of applications that find them interfacing with the human body in greater amounts. This has become increasingly so as the industrial era has changed into the communications era, with more and more of the rarer metals finding widespread use in semiconductors and other devices that drive the computers and microprocessors that we find increasingly in the equipment in our homes, offices, and motor cars.

Thirdly, the expected average life-span of a baby at birth has almost doubled over the last couple of centuries. This means that the cumulative effects of such non-essential metals become an increasing challenge to an elderly person. Furthermore, such elderly patients may well be deficient in essential or beneficial trace elements, thus magnifying the threat from extraneous metals present as contaminants.

Fourthly, an increasing number of hereditary, and other, disorders have been traced to metal imbalance scenarios, for example the copper imbalance in Wilson's disease.

Finally, today we have a broad armamentarium of clinical and biochemical procedures for diagnosis and monitoring the progress of disease. This means that previously unattempted therapies can now be launched into with optimism and, mercifully, many diseases can now be treated successfully given the right knowledge and guidance in terms of optimizing trace element concentrations in various parts of the body; or of the controlled harnessing of the toxic properties of metals. For example, iron administration in some types of anaemia, or the use of arsenicals for the treatment of syphyllis or of platinum complexes for cancer.

It is scientifically easier to get to grips with the thermodynamics of the metal ion complexing that actually occurs *in vivo* rather than with the kinetics. This arises because what may well be thermodynamically feasible may not in reality be achieved because, due to slow kinetics, the time required for complex formation is longer than the effective residence time of the complexing molecule in the region of the metal. Conversely, the manifold presence of enzymes *in vivo* can well speed up a reaction involving a trace element by many orders of magnitude. Thus,

Table 7.1 *Important criteria to be considered on selecting a therapeutic chelating agent*

The formation constants for the complexes with the metals to be removed in comparison with those for H^+, calcium, and other essential metals.
The rate of reaction with *in situ* toxic metal deposits.
Net charge on the chelating agent and its metal complex at physiological pH.
Partition coefficient of the ligand, and its metal complexes, between water and lipid protein cell membranes.
Total concentration of the ligand achievable at the desired site of action.
Chemical stability of the ligand and its metal complexes.
Biochemical stability of the ligand and its metal complexes.
Toxicity of the ligand and its metal complexes.
Route of excretion of the metal–ligand complex.
In vivo distribution of the unexcreted metal complex.
Chirality of the ligand.
Molar mass of the ligand and its metal complexes.
Water solubility of the ligand.

many of the conclusions about cell membrane solubility and ion selectivity based on fundamental inorganic biochemical considerations involving equilibria and steady states are best used to indicate feasibility and possible side effects. However, they may also be useful in selecting the most appropriate and cost-effective method for initial screening for biological activity, *e.g.* the decision of whether an *in vitro* technique such as tissue culture will yield useful information or whether only animal experiments can yield the required data. An important contribution to such research plans is that many suggestions for a metal–ligand complexing scenario can easily be eliminated from further consideration through it not being thermodynamically feasible upon the basis of the multiple, competitive equilibrium involving the presence of other metal ions and ligands.

Several research workers, including M.M. Jones, A.E. Martell, and

D.R. Williams have discussed in great detail a selection of ligands specifically targeted at metal ions in biological fluids and tissues. An overview of the considerations taken on board by such deliberations is given in Table 7.1. The further reading references would permit one to delve more deeply into this fascinating field. There are now some eight registered or potentially clinically useful chelating agents in the field of medicine and healthcare, and only some of these can be discussed in depth in this book. However, as many pivotal considerations in the field have been based upon EDTA a fairly full discussion of this fascinating ligand, progressing from laboratory to industry to medicine more than three decades ago, is given later in this chapter. It must be stressed that the principles listed therein apply equally well to the development of other agents. However, first let us look at some more general considerations.

SELECTIVITY OF LIGAND DRUGS FOR METAL IONS USING FORMATION CONSTANT DATA

Naturally, all metal ions and all ligands are in fierce competition *in vivo* but the exact matching of the strongest bond formation can be based upon the hard and soft acids and bases considerations mentioned in Chapter 3. If, having established that the right type of electron donor groups have indeed been chosen for the metal ion and its environment under consideration, it must be established whether the ligand has the ability to select that metal ion and to pull it away from its natural biochemical ligands (often amino acids, carboxylates, or sulfydryl groups) and from labile protein sites.

This is achieved using large computer models that permit many thousands of competitive complexing equilibria to be considered and rationalized. Part of this consideration involves the protonation constant for the ligand donor groups concerned. Protons that are firmly attached to a ligand will need to be ejected before the metal ion complexing can occur. A corollary to this consideration is the fact that a fully protonated ligand may well be a neutral ligand and able to penetrate lipoprotein cell membranes to reach the offending metal ion localized within tissue. This is why, for example, aspirin is able to pass through the stomach lining because it is fully protonated under the neutral and acid conditions present. Most other agents (including aspirin to a lesser extent) reach the bloodstream from the small intestine.

A related feature that is rolled up in the overall formation constant is that of the ability of the chosen ligand to chelate the metal ion under consideration. Once again, formation constants can be used to predict this likelihood.

Upon perusing the large number of features desirable in any therapeutic chelating agent that are listed in Table 7.1, one may well find an agent that fits many of the criteria but is lacking in a few respects. Such favourable ligands can be further matched to the metal in a particular location or bio-fluid by research based upon a direct structure–efficacy correlation (DSEC).

DSEC has many attractive features that permit the optimization of therapy; for example, agents may be modified such that they can be administered orally instead of intravenously. There are now exciting possibilities, in the case of severe metal poisoning, where the offending metal could be cleared from the circulation by passing the blood over a matrix of ligands trapped on a column of resin; or, as mentioned previously under 'Wound Dressings', agents immobilized on resins could be used to remove toxic metals ions from a contaminated wound.

These considerations are in addition to the usual point of focus of such deliberations that bears upon the lipid solubility of the different forms of an agent. There are many examples in the literature of agents being made more lipid soluble, or indeed more water soluble, by the judicious choice of groups to be appended to the molecule.

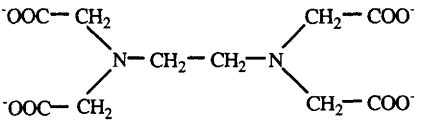
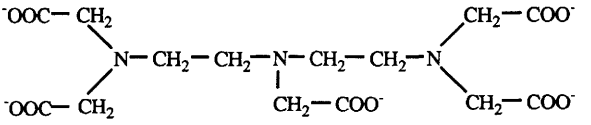
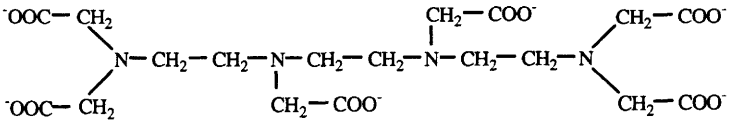
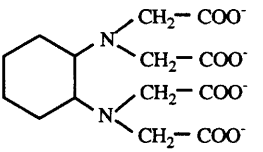
Enhancing the lipid solubility of a ligand or of a metal complex to penetrate cell membranes which may appear desirable from the point of view of enhancing the rate of removal of the metal from the body may, vicariously, increase the toxicity of that ligand or its metal complex. For example, lipid soluble derivatives of EDTA are usually more toxic than the parent acid.

Other side-effect issues centre around the words 'specificity' and 'selectivity'. Being realistic, probably the best one can achieve with a laboratory-made reagent is that of selectivity which infers that, in addition to the effect on the offending metal, certain biologically essential elements, such as zinc or copper, may well also be mobilized from their natural sites of deposition and excreted in greater amounts following administration of the agent. This problem can be tackled either by administering the agent as an appropriate essential metal salt, *e.g.* the zinc salt, or, alternatively, by using topping-up therapy in which essential metal supplements are administered coupled with careful monitoring of the levels of these critical metals in the blood and urine.

EDTA, ITS EVOLUTION, AND USE

EDTA may be regarded as the first member of a group of synthetic polyaminopolycarboxylic acids that have proved to be one of the successes of the initial basic chemical complexation research. In this section,

Table 7.2 *Some of the more common polyaminopolycarboxylic acids used in chelation therapy. The active form is the negatively charged anion, but the agent is administered in the acid or salt form having protons and sodium, zinc,* etc. *cations added to neutralize the anion*

Compound	*Structure*
EDTA	 Ethylenediaminetetraacetate^{4-}
DTPA	 Diethylenetriaminepentaacetate^{5-}
TTHA	 Triethylenetetraaminehexaacetate^{6-}
CDTA	 Cyclohexane - 1,2 - diaminetetraacetate^{4-}

we trace the introduction of EDTA and the evolution of derivatives and homologues that show greater chelation efficacy. Those polyaminopolycarboxylic acids most frequently encountered in chelation therapy are listed in Table 7.2, together with their formulae; however, by far the most widely used compounds are the calcium and sodium salts of EDTA.

The first introduction of the term chelation into the scientific literature was in 1920 when Morgan and Drewe used it to describe the

incorporation of a metal ion into a heterocyclic ring structure. EDTA was synthesized in Germany in the late 1930s by Munz, originally as a substitute for the expensive imported chemical citric acid, used in the printing industry to prevent calcium stains from hard water reacting with certain mordant dyes. EDTA was patented for this use in 1935.

The importance of EDTA in chelation therapy, its specificity and selectivity, together with some aspects of its toxicity will be discussed here. However, clinical chelation therapy began with another agent when in 1945 British Anti-Lewisite (BAL), see later, was introduced as an effective antidote for certain types of heavy metal ion toxicity. The increasing risk of nuclear fission products entering the human body led to a greater demand for chelating agents and Pfeiffer, Schwarzenbach, and others introduced EDTA to medical researchers, also in 1945.

The first recorded administration to human beings was by Geschickter who administered nickel EDTA for the treatment of breast cancer, but found it to be excreted unchanged in the urine. The ligand was then used in attempts to remove renal and bladder calculi; and in 1952 it was used against lead poisoning. Many compendia on the use of EDTA have since been published and these provide reviews of the physiological chemistry of the agent. Almost immediately after the initial research on the chemistry of EDTA, the impact which it, and its associated complexes, could have upon metal intoxication, atherosclerosis, and related conditions became obvious.

In the USA EDTA has been widely used for more than 30 years and some 100 000 patients have been treated with over 2 million injections, often to remove the calcium-containing plaques which clog up the arteries in the prevalent disease atherosclerosis. More than 5000 references to EDTA therapy are to be found in the literature.

Other than for chelation therapy, in which the ligand is generally administered by slow, intravenous infusion, EDTA has been used in creams and ointments, pharmaceuticals, oils, soaps, bath preparations, cosmetics, and in hair dyes and permanent waving solutions. A novel application of an EDTA chelate is the use of the dicobalt chelate (dicobalt edetate) as an antidote in cyanide poisoning; the CN^- ion forms a strong ionic complex with the Co^{2+} ions in the chelate to form a relatively non-toxic and readily excretable species.

Outside the fields of health and beauty care, EDTA finds many applications, for example, in foods, soft drinks, flavourings, fungicides, germicides, animal feeds, plant nutrients, herbicides, metal cleaning solutions, scale removers, emulsion stabilizers, photographic agents, organic systems, pulp and paper processing, leather processing, textile production, rubber polymerization, and water softening. In addition

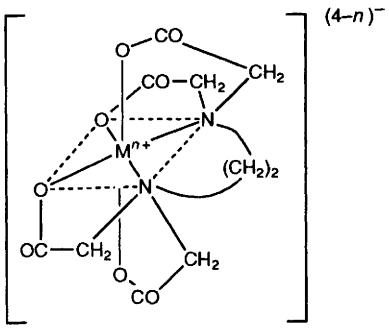


Figure 7.1 *Complex formed between hexadentate EDTA^{4-} anion and an octahedral six-coordinate metal ion^{n+}. The six dative covalent bonds point towards the corners of a regular octahedron. The complex ion formed will, in all probability, be water soluble because of the residual negative charge left on the complex*, i.e. $(4\text{-n})^-$, *becoming aquated*

EDTA is invaluable in the nuclear industry where it finds applications as a decontamination agent.

CHEMICAL PROPERTIES OF EDTA

Stable complexes with EDTA and its related compounds are formed with most polyvalent metal ions, hence their wide use in chelation therapy. Pure EDTA is a white crystalline solid with a relative molar mass of 292.1. It is a weak, tetrabasic acid which is sparingly soluble in water. EDTA is not metabolized in the human body and is effectively non-biodegradable in the environment.

Each of the nitrogen atoms (Table 7.2) has an unshared pair of electrons and the four acidic hydrogens (the pK values for the ionization equilibria being $pK_1 = 2.0$; $pK_2 = 2.67$; $pK_3 = 6.16$; $pK_4 = 10.26$) means that one EDTA molecule has six potential sites for metal ion bonding and hence may be described as a hexadentate ligand.

The $(EDTA)^{4-}$ ion is capable of complexing almost every metal cation in the Periodic Table. The number of bonds formed by the central atom or metal ion is called its coordination number. The $(EDTA)^{4-}$ ion, as described above, has six electron donating groups, *i.e.* four carboxylates and two amine groups. These are able to occupy four, five or six coordination sites around a central metal ion. Thus the $(EDTA)^{4-}$ ion is able to form one-to-one stoichiometry with the majority of metal cations. These complexing groups effectively enclose the cation, isolating it from the outside environment, hence forming very

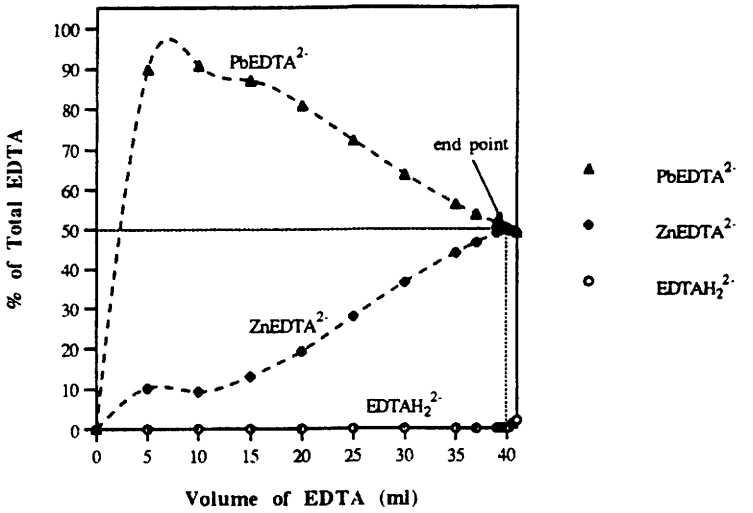


Figure 7.2 *Chemical speciation for a titration of a solution containing lead and zinc ions with EDTA. Speciation of EDTA in the first stage of the titration of 10.0 ml of 50.0 mmol* dm^{-3} *lead + 10.0 ml of 50.0 mmol* dm^{-3} *zinc with EDTA (25.0 mmol* dm^{-3}*) at pH = 5.25*

stable metal–ligand complexes. Figure 7.1 shows a typical metal–EDTA complex and also demonstrates six-coordination, as the central metal ion has six bonds.

The speed and precision with which EDTA is able to donate its spare electron pair to the central metal cation has meant that it has uses not only in chelation therapy and in the commercial areas listed previously, but also in complexation titrations in analytical chemistry.

EDTA–Metal Complexation

The degree of EDTA–metal complex formation normally depends upon the hydrogen ion concentration, *i.e.* the pH of the surrounding environment, and the stability of the particular metal–ligand complex. Effectively, there is competition between the hydrogen ions and the metal ions in the system. The pH and the amount of ligand in the system will effectively dictate which of the complexes are most likely to be formed. Figure 7.2 shows the order of complexation of EDTA with lead and zinc ions as the ligand concentration is increased. As the volume of EDTA and the pH changes the disfavoured zinc complex gains parity with that of the preferred lead complex.

Formation Constants

As we learned in Chapter 3, formation constants are mathematical expressions that relate the concentration of products and reactants in any equilibrium reaction. Thus the reaction between a metal ion and EDTA can be illustrated as:

$$M^{n+} + (EDTA)^{4-} \rightleftharpoons M(EDTA)_x^{n-4x)+}$$

and the stability constant (β) as:

$$\beta = \frac{[M(EDTA)_x^{(n-4x)+}]}{[M^{n+}]\,[(EDTA)^{4-}]}$$

The concentration of $(EDTA)^{4-}$, and thus the ability to complex metal ions, will depend upon the pH. A decrease in pH results in an increase in the deprotonation of EDTA and hence an increase in the concentration of the $(EDTA)^{4-}$ ion. The effect of this is that only metal ions with a very high affinity for EDTA will be able to form stable complexes. The stability constants for the $EDTA^{4-}$ and [diethylenetriaminepentaacetic acid]$^{5-}$ ($DTPA^{5-}$) complexes with some important metal ions that are of particular interest for chelation therapy are listed in Table 7.3. It is important to note that the stability of the EDTA and DTPA complexes with toxic metals, such as lead, mercury, cadmium, or plutonium are quite similar to those with essential metals such as zinc, cobalt or copper; however, the Ca complex is many orders of magnitude lower. This has important implications for chelation therapy. First, the mobilization and excretion of zinc and other essential metals are likely to be increased, along with that of the toxic metal during EDTA treatment; and secondly, the chelation of the ionic calcium in the blood, that can cause tetany and even death, can be avoided by administering the chelator as the calcium salt.

IN VIVO CHELATION OF RADIONUCLIDES

The widespread use of radionuclides in the nuclear industry and in other areas of work, means that there is a risk that accidents involving the intake of quite large amounts of one or more radionuclides may occur. Some radionuclides, notably those of lead, strontium, the lanthanides, uranium, thorium, and plutonium are removed quite rapidly from the blood and deposited in bone, where they may be retained for many years. This deposition of non-physiological and potentially toxic

Table 7.3 *Formation (=Stability) constants for EDTA and DTPA complexes with some essential and toxic metals* (from Smith and Martell, 1974)

Metal	*Formation constant (log β)*	
	EDTA	*DTPA*
Essential		
Calcium	10.69	10.75
Cobalt(II)	16.26	19.15
Manganese	13.81	15.51
Zinc	16.44	18.29
Copper(II)	18.70	21.4
Iron(III)	25.0	28.0
Toxic		
Mercury	21.5	26.4
Cadmium	16.36	19.0
Lead	17.88	18.66
Yttrium	18.08	22.05
Gadolinium	17.35	22.46
Thorium	23.22	28.78
Plutonium	24.6	29.4
Americium	23.2	28.78

metals in bone may be seen as a form of detoxication; however, even if they are locked up chemically in a non-toxic form, the metals continue to emit radiation which may cause serious damage to the cells and tissues of the contaminated person. In such circumstances it is considered desirable to remove the radioactive metal from the body as rapidly as possible and chelation therapy offers this possibility. Some of problems encountered in such chelation therapy can be illustrated by reference to plutonium, which deposits extensively and tenaciously in bone, emitting α-particles that can induce bone cancers.

In the body plutonium exists predominantly in the 4+ oxidation state, and can be envisaged as being distributed between four general fractions.

Insoluble deposits such as oxides, hydroxides, and polymeric aggregates, often in bone, plus non-labile protein complexes, *e.g.* ferritin and haemosiderin	Transferrin ⇌ low molar mass ⇌ Pu(IV) (α, β-globulin) as citrate or carbonate aqueous
INERT	**LABILE**

The most widely used polyaminecarboxylic acid used for chelation therapy of plutonium and americium poisoning is the higher homologue

of EDTA, DTPA, although EDTA has found some application. If administered to experimental animals, within a few minutes after an intravenous injection of plutonium, or americium, DTPA can promote the excretion from the body of more than 80% of the injected radionuclide, but the efficacy drops off with increasing time interval between contamination and the start of chelation therapy.

Some computer modelling research has suggested that CDTA, that has larger stability constants than EDTA for many of the metals of interest, would have greater complexing power than EDTA. However, the few animal experiments that have been carried out with various radionuclides indicate that CDTA is no more, and may be less, effective than EDTA and is considerably less effective than DTPA.

Both the Pu–EDTA and Pu–DTPA complexes are charged and hence the radionuclide that is chelated in the extracellular space will either remain there or be excreted via the kidneys; being charged it cannot pass through cell membranes to deposit in cells. In contrast the Pu–CDTA complex is electrically neutral and could pass through the cell membrane by passive diffusion; this is advantageous in the sense that plutonium chelated inside cells could be transported through the cell membrane back into the blood. However, it could also have the disadvantage that, if present in the blood, the complex could perhaps re-deposit in sensitive cells, thus increasing the risk of radiation damage or cancer induction.

TOXICITY OF EDTA

Despite its beneficial effects as a metal chelator and its use in the treatment of heavy metal intoxication, EDTA and its related compounds are still chemical agents and, as was pointed out by Paracelsus almost 500 years ago, all chemicals are toxic if given in sufficient quantities. As with any medical procedure, the risk of the treatment has to be balanced against its beneficial effects. For realistic risk–benefit analyses it is necessary to understand all of the risks involved.

It is axiomatic that the toxicity of the ligand selected for the treatment and any side effects, such as co-liberating essential metals during therapy, ought, collectively, to add up to less than the residual toxicity of the element that has been deposited in the body. However, factors other than simple toxicity or unpleasant side effects need to be considered. For example, about 90% of the lead accumulated in the human body is sequestered in non-toxic form in bone. Incautious chelation therapy with an agent like EDTA, or administration of large amounts of

calcium ions, can mobilize this detoxified lead and release it into the bloodstream as ionic lead, thereby producing symptoms of acute lead poisoning. Similar problems have been reported with the chelation therapy of arsenic.

Side Effects

The major side effect of chelation therapy, particularly with EDTA, is hypocalcaemia, a condition caused by too rapid administration of the chelator. The result is a rapid drop in the ionized calcium in the blood plasma that causes muscle and abdominal cramps, convulsions, and even death. The condition is usually controlled by infusion of calcium gluconate, or prevented when the metal to be removed complexes with a much higher stability constant with EDTA than that of the Ca–EDTA, by administration as the Ca–EDTA complex.

Insulin shock may also occur due to the EDTA treatment lowering the serum glucose. Regular food intake during the chelation treatment will help to prevent this.

It may also be necessary during treatment to supplement the diet with those trace metals which are also chelated by EDTA. Computer simulation models of the selectivity for lead(II) ions revealed, later to be verified by urine monitoring, that some manganese and zinc ions are co-excreted. Once having been established it is easy to rectify such phenomena by orally topping up with the appropriate salts.

Nephrotoxicity and EDTA

Many workers studying the effects of EDTA in chelation therapy have found that following treatment, with quite large doses of the agents, the patient developed symptoms resembling those of damage to the kidney, for example, sucrose nephrosis. Histological studies in animals have shown clear evidence of damage to the renal tubules. It has been established that EDTA is not metabolized in the body and that some 95% of the agent is excreted unchanged through the kidneys, the remainder being lost via the intestines.

It has been shown that Ca–EDTA fed in graded doses to weanling rats as part of a nutritionally adequate diet, produces no adverse side effects, thus suggesting that there is no nephrotoxicity after long-term intake at these levels. In other studies rats that were given a 1 millimolar solution of Zn–DTPA in place of drinking water from about six weeks of age showed no evidence of toxicity, as measured by decreased life-span or body weight, as compared with control animals.

NEPHROTOXICITY AND HEAVY METAL CHELATES

Patients treated unsuccessfully with large doses of EDTA for severe lead poisoning have, prior to death developed anuria and uraemia and autopsies have revealed that the patients had suffered kidney damage. This nephrotoxicity may have been due to *in situ* mobilization of specific metals that exchange for calcium, thus impairing the function of the metal-controlled or metal activated enzyme systems in the kidney.

Thus EDTA, and also its homologue DTPA, is potentially nephrotoxic; however, this can be controlled and minimized by careful attention to the dose administered and the treatment regime.

RATE OF ADMINISTRATION AND DOSAGE OF EDTA

If the maximum benefit is to be derived from chelation therapy, it is necessary to select a treatment regime that combines effective mobilization of the toxic metal with minimal nephrotoxicity.

When EDTA is administered orally, less than about 5% of the agent is absorbed into the blood stream, therefore intravenous administration has been employed as the most efficacious route of administration. However, nephrotoxicity is more likely to be encountered following intravenous administration.

The majority of early treatment regimes used excessively high doses of EDTA, administered by rapid intravenous infusion, and this resulted in a number of cases of serious nephrotoxicity. The general rule today is that a patient should not receive more than 3 g of EDTA or DTPA within a 24 hour time period; this corresponds to ~40 mg (~150 μmol) of EDTA kg^{-1} of body weight for a 70 kg subject. In practice a dose of 1 g day^{-1} of either EDTA or DTPA would be more usual; this corresponds to ~50 and ~30 μmol kg^{-1}, respectively. The agent is administered by intravenous infusion, in a vehicle such as 5% dextrose-saline, over a period of several hours.

It is also generally recommended that chelation treatment should not be continuous; thus for serious cases of metal intoxication the patient may be 'chelated' three to five times a week, while for less serious cases the agent may be administered once a week or even less frequently.

SULFUR-CONTAINING AGENTS

During World War II, it was feared that an organo-arsenic chemical warfare agent, Lewisite, might be used. The biochemist Sir Rudolph Peters and his co-workers developed an antidote, 2,3,-dimercapto-

propan-1-ol, which became known as British Anti-Lewisite or BAL, and, historically this was the first chelating agent to be demonstrably effective and used in humans. In addition to arsenic decontamination, BAL has now been used for removing lead, mercury, gold, and even copper. It has the distinct advantage of being lipid-soluble and thus, can mobilize heavy metals even after they have been deposited within cells. However, the pungent, garlic-like odour permeating all the tissues of the patient, and the unpleasant and painful side effects, including hypertension, nausea, vomiting, headaches, burning sensations, pains in the chest, hands, and throat, conjunctivitis, excessive perspiration, abscesses at the site of injection made BAL an unpopular prescription.

Less toxic and more water-soluble derivatives of BAL have been sought, these include glycoside derivatives, the sulfonate derivative dimercaptopropanesulfonate (DMPS or Unithiol®) and dimercaptosuccinic acid (DMSA), which have been used clinically.

REMOVAL OF AN EXCESS OF AN ESSENTIAL METAL, *e.g.* IRON

As has already been mentioned, therapies designed to remove a contaminating metal often have a side effect of essential ion removal. However, this generally unwanted feature can be turned to good advantage in cases of severe overload of an essential metal, for example, in the iron overload of haemochromatosis.

Chelation of iron is a necessary treatment in cases of thalassaemia. This is a genetic disorder affecting more than 100 000 babies per annum, mainly in developing, sub-tropical areas of the world. Essentially, the unfortunate sufferer is unable to synthesize haemoglobin correctly and, in order to alleviate the ensuing anaemia, repeated blood transfusions are given; this then leads to the build up of toxic amounts of iron in the body. Since, under normal circumstances, virtually no iron can be excreted from the human body the most logical treatment for iron overload is to administer a chelating agent to sequester the iron in an excretable form.

For the last quarter of a century the only selective iron chelator available has been desferrioxamine, Desferal®. This material is a siderophore secreted by the fungus *Streptomyces pilosus* in order to scavenge iron from the environment to provide for its nutritional and reproductive needs. The ligand is extremely powerful in respect of picking up ferric iron. Rather fortunately, it has little affinity for other essential biometals such as copper, zinc, calcium or magnesium.

Regretfully, Desferal® is very expensive and cannot be administered

orally, but must be administered either subcutaneously or by slow intravenous infusion over periods of up to 12 hours per day. This requires sophisticated pumps and a mobile energy source to drive them, together with good clinical care, all of which are also expensive. These are very serious disadvantages when the pressing clinical need is to treat large numbers of young people in poor countries; the urgent requirement is for an inexpensive, orally active iron chelator. Numerous talented scientists are tackling the problem of a replacement drug and, currently, the most promising agent, code-named L_1 (Deferiprone), is based on 3-hydroxypyridin-4-one. Apparently, this is sufficiently powerful to mobilize ferric iron from the intracellular sites of deposition, predominantly the iron-storage protein, ferritin (relative molar mass ~ 2 million). Hider has suggested that whereas Desferal® is unable to enter into the so-called tunnels in the ferritin molecule to remove ferric iron, 3-hydroxypyridin-4-ones are sufficiently small to penetrate such channels to gain access to the iron and sequester it. Furthermore, these compounds have been shown to be able to mobilize iron from a degradation product of ferritin, haemosiderin that accumulates in the tissues of thalassemia patients.

Currently, derivatives of hydroxypyridones are being synthesized that have lipophilic characteristics that permit them to dissolve in cell membranes and thus penetrate the cells overloaded with iron.

EXCRETION AND 'TOPPING-UP' THERAPY

The main routes through which metals are excreted as a result of drug administration are the urine and faeces. Perspiration and exhalation do not figure as significant routes for excreting inorganic materials, other than sodium, from blood, even though they are important for some organics (*e.g.* the use of breath alcohol and the breathalyser traffic laws).

The kidneys use a dialysis process to remove low molar mass, charged species (either positive or negative) from the blood. In contrast, biliary excretion captures uncharged, low molar mass complexes and secretes them through the bile duct into the small intestine. If they are not reabsorbed these metal complexes eventually appear in the faeces.

Excesses of iron in the diet may cause the epithelial cells lining the small intestine to slough off, thus preventing absorption and increasing faecal iron.

CRITERIA OF SUCCESS

To achieve a body that is analytically perfect is often neither desirable nor achievable, rather the quality of life is more important. Similarly,

with chelation therapy, in most cases it cannot be expected to remove all contaminating metal from the body or to repair irreversible damage that may have already begun. However, it can improve the quality of life by alleviating unpleasant symptoms and by slowing, or even preventing, the progress of the damage. Thus in Wilson's disease chelation therapy with Penicillamine, or a more modern agent such as triethylenetetramine (TREN), cannot reverse the existing damage caused by copper deposition, but it can slow its progress and improve the quality of the patient's life. The occasions when chelation therapy is truly life-saving are, sadly, probably rare. In acute heavy metal poisoning the quantity of the metal in the body may be so large that the amount of chelator required to remove it far exceeds the toxicity of the agent. One classic case of life-saving chelation treatment is to be found in the case of Harold McCluskey, a worker in a nuclear facility who, as the result of an explosion, became very heavily contaminated both externally and internally with americium. Chelation treatment, started within two hours of the accident using first Ca–DTPA and then Zn–DTPA, was successful in reducing the amount of americium in his body, especially from the liver, about a 1000-fold, thereby preventing very early death from radiation-induced liver damage. The chelation therapy in this case, which involved administration of almost 500 g of DTPA over a period of years, restored Mr. McCluskey's quality of life and some ten years later he died in his seventies from complications of the heart disease that was already present at the time of the accident.

METAL COMPLEXES USED IN DIAGNOSIS AND CHEMOTHERAPY

Modern science is aware of several hundred isotopes of which many are radioactive. The evolutionary process has usually used non-radioactive isotopes to define the composition of the human body. However, there are sources of natural radiation within our bodies, for example ^{14}C, ^{40}K, and ^{222}Rn.

Since the 1940s a wide range of radioactive isotopes have been made available to medical research scientists and a number of metal complexes have been developed for use in the diagnosis of disease, and for monitoring the progress of treatments. Particular interest lies in the use of γ-ray emitting radionuclide complexes that can be used to obtain static or dynamic images of organs, tissues, and tumours by means of external detectors, such as the gamma camera. As for chelation therapy, the development of metal containing radiopharmaceuticals demands careful matching of metal ion and ligand to try to ensure that the agent

Table 7.4 *Some radionuclides used diagnostically in nuclear medicine*

Radionuclide	*Energy(ies)*/keV	*Half-time*
Gallium-67	1001	78 h
Technetium-99m	140	6 h
Indium-111	172 and 247	67 h
Indium-113m	392	104 min
Iodine-123	1320	13 h
Ytterbium-169	207	32 days
Thallium-201	482	3 days

localizes in the desired target and provides some indication of the functional state of that organ or tissue. Radionuclides for use in scintigraphic imaging of organs need to have relatively short half-times and emit monoenergetic γ-rays, ideally with energies in the region of 100–300 keV, but not α- or β-particles since these emissions serve only to increase the total radiation dose received by the patient. A list of some of the radionuclides used in nuclear medicine is given in Table 7.4, but because of its near ideal nuclear properties ^{99m}Tc finds remarkably widespread use.

A complete understanding of why specific metal complexes target certain tissues has yet to be achieved, but would be extremely valuable for the rational design of new radiopharmaceuticals. Complexes of ^{99m}Tc with diphosphonates, *e.g.* methylenediphosphonate, localize in bone and especially in areas of disturbance of bone metabolism, such as tumours; thus they may be used to map the presence and location of bony metastases from various types of cancer. Chelates such as ^{99m}Tc–DTPA may be used to study differential renal function in the kidneys or to monitor the function of a transplanted kidney. Attachment of chelating groups, often based on DTPA, to antibody proteins permits the formation of ^{99m}Tc- or ^{111}In-labelled antibodies for tumour localization.

A recent development is the use of radioactive metal–ethylenediaminetetramethylenephosphonate (EDTMP) complexes (Figure 7.3) for the relief of pain from tumour deposits in bone. These complexes localize in bone, especially in areas infiltrated with tumour, and the pain relief results from the radiation dose delivered by the radioactive metal which has co-located at the sites with the EDTMP. Both ^{153}Sm and ^{166}Ho seem appropriate ($t_{1/2}$ = 46 and 27 h, and β-particle emission 0.7 and 1.9 MeV, respectively). However, the ^{166}Ho–EDTMP complex has a much poorer bone uptake than the corresponding ^{153}Sm complex (18% *versus* 79%, respectively) and this is reflected in the pain alleviation

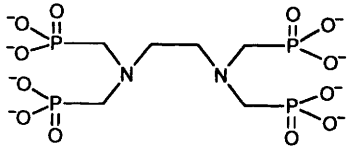


Figure 7.3 *Ethylenediaminetetramethylenephosphonate (EDTMP)*

results. Interestingly the extensive urinary excretion of zinc ions forming charged Zn–EDTMP complexes as predicted by speciation studies in blood plasma is also seen in reality.

Magnetic resonance imaging of the human body also utilizes metal chelates, albeit with non-radioactive isotopes. The presence of a paramagnetic metal ion can markedly modify the relaxation times of water proton resonances within a tissue, and thereby markedly improved the contrast of the images of adjacent organs. A classic example is that of manganous salts becoming localized in normal heart tissue, but avoiding damaged areas around blocked coronary arteries. More recently, iron(III), manganese(II) and, especially, gadolinium(III) as the $[Gd(DTPA)H_2O]^{2-}$ complex, are being used clinically for the localization of brain tumours. Many new stable isotope chelates are being screened with a view to their use in tissue imaging.

CONCLUDING REMARKS

Chelation therapy has found increased application during the last half of this century and so it is important to continue researching in order to extend its applicability, but especially to gain more knowledge of both the adverse as well as the beneficial effects of such therapy. Essentially the objective must be to optimize the treatment regime so that the best possible therapeutic effect is achieved with minimal adverse side effects.

Current successful chelators such as D-penicillamine, EDTA and Desferal®; must be used in even more effective therapeutic regimes. For example, better selectivity might be achieved by esterifying polar functional groups on ligands, or delivery might be targeted at specific organs by using liposome-encapsulated, or even antibody-coupled ligands. Nevertheless, it seems that the field has not truly fulfilled its potential so far. Future research will require increasingly detailed chemical speciation knowledge, especially of specific metals within the tissues in order to proceed towards successful new regimens or new agents.

Whereas many talented scientists have researched promising new chelators over the last three or four decades, rarely have they been able

to take their drugs as far as the clinic. The difficulties that they have encountered arise from two fundamental reasons.

1. The incidence of severe metal overload, with the exception of thalassaemia, or even of cases of severe contamination with radionuclides is relatively rare, and so pharmaceutical companies are not encouraged to put up venture capital in order to take promising chelators from the laboratory to the clinic as the sales revenue envisaged is rather thin. This is perhaps not very surprising when one realizes that the costs of the necessary toxicity testing, scaling-up of synthetic processes and applications for product registration with the relevant national or international drug authorities may cost millions of pounds.
2. To date new chelating drugs have been identified by seeking a ligand having a large formation constant for the metal ion that needs to be removed. For example, Desferal® has a formation constant of 10^{30} with ferric iron, whereas its affinity for calcium is a mere 10^2. However, there are many stages involved in getting an agent into the patient and achieving the excretion of the ligand–metal complex. The agent has to survive different pH values between saliva, stomach fluid, intestinal juices, and blood. Further, there is wide-ranging competition for other metals by the chelator, as well as by other ligands for the metal ion that is targeted for removal. Chemical speciation knowledge, often based on computer models, is the only reliable means of estimating pH, ionic strength, competition, and redox influences.

Over and above chelation therapy there are many areas of human biochemistry and medicine in which the principle of metal complexing and chelation can be applied. As Mahler observed more than 30 years ago, all enzyme-catalysed reactions are subject to the influence of trace metal ions. The therapeutic potential of chelating agents as a class of compounds is unparalleled. Not only are newer agents being sought but also new formulations involving combinations for use in synergistic chelation therapy. The future design of therapeuticals that are selective for the active sites of enzymes and for other critical metal complexing ligands *in vivo* is an exciting prospect.

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Chapter 8

Dietary and Environmental Aspects

DIET

As long ago as 1936, Sir Robert McCarrison, in his Cantor Lecture to the Royal Society of Arts, Manufactures and Commerce, said:

> *'If I have convinced you of the fundamental importance of food in relation to public health, it will have become obvious that one of the most urgent problems of our time is how to ensure that each member of the community shall receive a diet that will satisfy his or her physiological needs. It is clear that to achieve this "much to be desired end", many barriers — poverty, unemployment, apathy, ignorance, prejudice, habit — must be surmounted and many interests — agricultural, industrial, and economic — readjusted. To do so is, in the main, a primary function of government'.*

The situation has not changed markedly since Sir Robert's most revealing statement quoted above except that the pressures for such decisions have increased by many orders of magnitude, not the least of which arises because of our increased life expectancy at birth, our increasingly stressful lifestyle, desires to prepare food rapidly, and by the fact that many life-threatening conditions from the early part of this century are treatable, thanks to modern therapy.

Last century, chemists involved in the food industry were primarily interested in compiling analyses of major components of foods such as carbohydrates, fats, and proteins. Improved analytical techniques paralleled by a greater awareness from physiologists and physicians, led to breakthroughs in terms of diet and cancer, obesity and heart disease, *etc.*

Other food components present at far lower concentrations such as trace elements, flavour stimulants, colouring agents, vitamins, required

far more sophisticated laboratory techniques which became readily available only in the latter half of this century and it is now possible to draw up total analyses of 'food from the plate' samples contributed by volunteers. Furthermore, careful analyses of the waste products passed out from the body in the form of perspiration, urine, faeces, *etc.* permits whole-body metabolic studies to be achieved, although it is extremely difficult to analyse and quantify such products.

However, there are two important points to be registered here. First, it is not possible to design a perfect diet for the average human. What is a good diet for today's sport-playing, car-repairing, gardening at the weekend person is probably not appropriate for his or her role tomorrow when they revert to their normal sedentary lifestyle in an office or a vehicle. When one considers that vastly differing amounts of major components such as carbohydrates, proteins, fats, are required for babies, adolescents, middle-aged, and senior citizens and that each of these groups must be linked in to the active lifestyle point made earlier, it is understandable why different organizations have come up with different recommended daily amounts for the 'average' diet.

Secondly, the quality of life is very much linked to the 'quality' of food which commences with the attractiveness of its presentation and packaging through to the aromas produced during cooking, to the taste stimulation upon ingestion and through to the nutritional benefits. Much of this behavioural aspect of food is linked in with the exact chemical speciation of the material being imbibed.

As Coultate, in a sister volume, has pointed out, the ability of the human race to adapt to extremes of climate, poverty, and over-supply of food, although permitting us to survive throughout the surface of the earth, has a disadvantage in so far as the normal cravings for food, for trace-element stimulation of taste buds, *etc.* can soon be dampened down by such adaptability. Thus, the personal decision to 'eat what I fancy' is insufficient for persons who are going to survive in a healthy lifestyle for the best part of a century.

The reduced craving or desire for trace elements to stimulate the diet, somewhat suppressed by our adaptability, is also challenged by persuasive advertizing sometimes based upon less than complete science in order to further sales figures. The extent of this challenge is reflected in the fact that approximately 10% of UK national expenditure on 'food' goes to the confectionery industry. Regrettably, trace elements essential for a healthy lifestyle may not be efficiently taken in by such foods. Rather, trace elements tend to be eaten accompanying far larger volumes of carbohydrates, fats, proteins, *etc.* responding to hunger pangs. However, confectionery is particularly low in terms of trace

elements, except perhaps those undesirable industrial metals arising from the manufacturing process and carried through as impurities.

'Food from the plate' studies reported by Cannon *et al.* indicate that modern foods are particularly low in potassium, which protects against high blood pressure and stroke, zinc, which prevents distortion of appetite, selenium, which has a protective effect against cancer, and chromium, which protects against adult-onset diabetes.

A good indicator is the adage that 'good food goes bad'. In other words, good food is likely to rot, spoil, and decay unless eaten before it does so. Invasive organisms such as lice, moles, microbes, rats, do not survive well on many processed foods possibly because the foodstuff has been drained of an essential nutrient to extend its shelf-life. However, many traditional foods such as beers and cheeses have stood the test of time because living organisms are able to thrive therein. 'Yeast is to the health of beer what canaries were to the safety and health of coalminers!' Unless the trace elements and vitamins and other micro-nutrients are more or less correct in a batch of beer, the yeast will not ferment and produce the alcohol desired by the customers. This indicates that there is at least a rudimentary spectrum of all the necessary nutrients in the fluid and that these could well be of benefit to the drinker.

By and large, there is approximately one-third of our diet which is good for health. A second third has something good about it, and then the final third of our diet may not be of benefit to us but may well contain undesirable materials leading to the conditions mentioned in Table 2.4. For example, there may well be additives introduced during the processing of the food, agricultural materials carried through during harvesting, excessive flavours, and colouring agents.

NATIONAL FOOD SURVEYS

Purified diets analysed over many years have indicated that the following elements are found in our diet: oxygen, carbon, nitrogen, calcium, magnesium, phosphorus, iron, sodium, potassium, chlorine, sulfur, iodine, zinc, copper, selenium, manganese, molybdenum, vanadium, fluorine, cobalt, chromium, nickel, silica, aluminium, tin, and possibly traces of arsenic, are essential or beneficial to human health.

The bio-availabilities of these elements through the gastrointestinal tract vary markedly. Molybdenum, iodine, fluorine, and arsenic are apparently highly bio-available, whereas medium uptake occurs with haem iron, cobalt, zinc, chromium in the presence of a glucose tolerance factor, selenium, either as selenate or as organo selenium compounds, whereas only low bio-availability is experienced with non-haem iron,

copper, nickel, manganese, inorganic forms of chromium, selenium other than those forms above, vanadium, silicon, lead, aluminium, and tin.

The whole balance of intake *versus* uptake of a trace element is decided by the speciation prevailing in the stomach, and then in the small intestine. Computer programs have been developed in order to assess the benefits from a range of foods as they pass through the gastrointestinal tract. These function not only in terms of total amounts to ensure adequacy but also in terms of speciation that determines the bio-availability. Unfortunately, many of the validation experiments required in order to support such work have tended to be done on small animals rather than by doing metabolic studies on humans. There are, however, some keymark papers in the literature referring to complete food balances for humans.

Two points should be made concerning the link between wealth, health, and 'perfect' nutrition. First, some 85% of the world's population by the end of this century will be currently defined as developing peoples and so devices for producing far larger amounts of food than would occur naturally are highly desirable since there is insufficient food left over from the wealthy 15% to feed the desires of the vast majority. Secondly, poverty is not directly linked with malnutrition and wealth need not be linked with a perfect diet. On the one hand, the relatively cheap food such as pizza has been rated by Saltman to be close to nutritionally perfect, whereas on the other hand, it is claimed that the greatest incidence of trace element malnutrition in the western world is amongst post-menopausal women in the State of California, it being believed that their drive for healthfood supplements and for maintaining a figure related to that of their teens leads to chronic trace element malnutrition. As noted in Chapter 6, the human body is designed to double the amount of adipose tissue between the ages of 30 and 50 years and during the same interval to halve the amount of muscle present. Fighting against this with somewhat extremist diets can often do more harm than good, due to the poor bio-availability of essential nutrients. Saltman is of the opinion that red meat is a near perfect source of trace elements, such as iron and vitamins. However, he opines that to satisfy one's RDA for iron totally from greens would require a daily intake of 4 kg of broccoli per person! Indeed, less than 1% of iron from rice is absorbable, 3% from beans, 8% from soya, but 20% from red meat such as veal.

In terms of advocating the total amounts and balance of nutrients required for the standard diet, the National Advisory Committee on Nutrition Education was set up in the UK in 1979 and the fourth draft

Table 8.1 *NACNE recommended dietary intakes compared with 1983*

Dietary constituent	*1983 intake*	*Risk factors*	*Recommended intake (NACNE)*
Energy		High intake→obesity	Sufficient to maintain optimum body weight
Fat	40% of total energy	High intake (especially saturated fat) → coronary heart disease	Reduce to 30% of total energy (with saturated fat 10% of total energy)
Sucrose (sugar)	38 kg per person per year	Frequent and/or high intake → tooth decay, obesity	Reduce to 20 kg per person per year
Fibre	20 g per person per day	Low intake → constipation diverticular disease, cancer of the colon	Increase to 30 g per person per day
Salt	12 g per person per day	High intake → high blood pressure?	Reduce to 9 g per person per day
Alcohol	6% of total energy	High intake → liver damage, obesity, alcoholism, malnutrition	Reduce to 4% of total energy
Protein	12% of total energy		Unchanged but greater proportion of vegetable protein

of the NACNE report was delivered in 1983. The Report was designed to encourage a more nutritious national diet and makes the point that food ought to be enjoyable, and that eating and drinking can and ought to be socially valuable exercises. Recommendations from the NACNE Report are reflected in Table 8.1.

The UK Committee On Medical Aspects (COMA) of food policy reports of 1984 and 1994, in pointing out that coronary heart disease and cerebral vascular disease (stroke) account for more than one-third of the deaths in the UK and that one in two adults (*i.e.* 20 million) in the UK are clinically overweight, suggest dietary changes. The main recommendations are that blood cholesterol levels are kept below 5.2 units (a figure currently exceeded by 70% of the UK adult population) and that more starchy foods, vegetables, fruit, and oily fish are eaten to replace reduced intakes of salt and of fatty and sugary foods.

CROP PROTECTION

The world demand for food is increasing and is currently inadequately supplied. Crop yields in the UK in the 19th century were less than 50% of those achievable today because of the limitation of soil nutrients and because of the overriding competition from weeds, birds and rodents, and insect attack, not only in the fields but also in the storage barns.

Nitrogen, phosphorus, potassium, and magnesium fertilizers have been used as supplements since the 1850s and have increased in use as synthetic sources of these agents have been produced by the chemical industry.

However, the major improvement in crop yields has arisen as a result of pesticides, particularly during the last half-century. They alone account for a 40% increased yield. Many of these contain inorganic compounds of metals or phosphorus or of sulfur. Table 8.2 indicates the approximate dates of introduction of insecticides and fungicides involving inorganic elements into the crop protection scenario. It is noteworthy that many of them have been with us for over 100 years and therefore, on the one hand, cannot be dubbed as 'modern' approaches to factory farming and, on the other hand, may well have been introduced and escaped the tougher legislation now prevailing in terms of permitted contaminants on food, *etc.*

The work of Fairweather-Tait and co-workers into the bio-avail-

Table 8.2 *Approximate dates of introduction of crop-protecting agents involving metals*

Agent	*Decade*
Insecticides	
Copper arsenate	1870s
Lead arsenate	1900s
Organophosphorus compounds	1940s
Fungicides	
Bordeaux mixture (copper)	1880s
Aryl mercuric salts	1930s
Organophosphorus compounds	1980s
Herbicides	
Bipyridyls (iron chelators)	1960s
Sulfonyl ureas	1980s
Organophosphorus compounds	1980s

Table 8.3 *Bio-availabilities of a range of trace elements from the gastro-intestinal tract*

High	*Medium*	*Low*
Arsenic	Cobalt	Copper
Fluorine	Chromium: glucose-tolerant factor	Inorganic chromium
		Inorganic iron
Iodine	Haem iron	Manganese
Molybdenum	Organic selenium	Lead
	Selenites/selenates	Nickel
	Zinc	Selenium/ides
		Silicon
		Tin
		Vanadium

Table 8.4 *Agents that influence iron absorption*

Enhancers	*Inhibitors*
Amino acids	Bran
Alcohol	Clay
Ascorbic acid	Phosphates
Citric acid	Tannates
Sugars	Other transition elements

ability of trace elements is of explicit interest to the adequacy of our diets. The point is made that where an element is widely distributed in foods and where there is no obvious sign of nutritional deficiency, even to certain vulnerable groups of the population, to establish the bio-availability of such elements is of mere academic interest. However, when explicit signs of trace element deficiencies exist which suggest a nutritional deficiency *per se*, it is most important to have a thorough knowledge of all of the factors such as intake, bio-availability, uptake, excretion, and biological half-lives of different chemical species of the metal concerned.

Table 8.3 shows the bio-availability of certain trace elements and Table 8.4 indicates agents that enhance (or inhibit) the uptake of iron by producing species that are lipid-soluble through their overall charge being neutral and also lists agents that are inhibitors by either precipitating or absorbing the metal concerned, or indeed by converting it into species that are charged and therefore repelled by the membranes of the cells lining the small intestine. For example, bran suppresses iron

Table 8.5 *Sherlock's data for total dietary intake of lead in 1981*

Food group	*Consumption* (kg day^{-1})	*Mean lead concentration* (mg kg^{-1})*	*Mean lead concentration* (μg)
Bread and cereals	0.240	< 0.05	12
Meat and poultry	0.058	< 0.05	3
Offal	0.003	< 0.13	0
Meat products	0.077	< 0.05	4
Fish	0.015	0.08	1
Oils	0.090	< 0.05	5
Sugars and preserves	0.095	< 0.05(5)	5
Green vegetables	0.046	< 0.06(5)	3
Potatoes	0.159	< 0.05	3
Other vegetables	0.068	< 0.05	3
Canned vegetables	0.043	0.13(5)	6
Fruit	0.060	< 0.05	3
Fruit products	0.024	0.15(5)	4
Beverages	0.118	< 0.01(5)	11
Milk	0.360	< 0.02	7
Totals	1.456		75

* Means calculated from the results of the analyses of 16 total diets.

absorption both from the high fibre content (absorption of the metal onto the polymers that make up the fibres) and from its phytate content (phytates complex and precipitate the metal ions). Radioisotopes are often used in such work since food labelled with ^{59}Fe or ^{65}Zn emit gamma radiation which can be used to trace them throughout the body.

Similar considerations concerning a wide range of bio-availabilities applies to the dietary intake of heavy metals. Table 8.5 indicates the consumption of lead as reported by Sherlock and co-workers from a

Table 8.6 *Sherlock's data for dietary intake of metals at Shipham* [mg week^{-1} person^{-1}, average (mean) value and range]*

	Lead	*Cadmium*	*Copper*	*Zinc*
Diary estimate† (based on records kept by 74 families during periods of 4 weeks in May and September 1979)	0.77 (0.66–1.15)	0.25 (0.14–0.52)	10.3 (9.1–13.4)	72 (64–110)
Duplicate diet estimate (based on the analysis of duplicates of a single week's diet, provided by 65 individuals during September 1979)	0.48 (0.28–1.20)	0.20 (0.04–1.08)	7.7 (3.8–33.0)	62 (38–140)
National average estimate (based on 1978 data reflecting average food consumption in the UK)	0.73 (0.42–1.33)	0.14 (0.09–0.18)	11.3 (7.8–14.6)	70 (56–86)

* These estimates include the contribution from all dietary components, including milk and beverages.

† These results are overestimates because of assumptions made in the calculations.

wide range of foodstuffs. Table 8.6 shows data from Shipham where the soil was found to contain rather high concentrations of cadmium. Villagers in the area were advised to reduce consumption of home-grown vegetables so that their cadmium intake was lowered to that of the average of the UK. This leads on to the so-called pockets of disease such as leukaemias which could be correlated with trace element imbalances in areas where the population was very dependent upon 10 to 20 acres of farming land that provided the vast majority of food for their families. Poland had several such areas in the 1960s and the situation was exacerbated by contemporaneous forced farming with potassium, nitrogen, phosphorus, and magnesium fertilizers.

Nowadays, food producers trade worldwide and there is a massive normalization process occurring on our supermarket shelves such that, by and large, we can expect that food high in heavy metals will be eaten only rarely and that other foods from other sources will be used in between these doses. Estimation of extreme intakes of such heavy metals can lead to consideration of the worst possible cases or, if radionuclides are concerned, of critical groups. Not unsurprisingly, the press occasion-

Table 8.7 *Some sources of iron in use for food 'fortification'*

Compound	*Iron content/%*	*Relative value*
Ferric phosphate	28	3–46
Ferric pyrophosphate	25	45
Ferric sodium pyrophosphate	15	14
Ferric ammonium citrate	17	100
Ferrous fumarate	33	95
Ferrous gluconate	12	97
Ferrous sulfate	32	100

ally has alarmist stories concerning the high levels of aluminium or of lead in our drinking supply. In the former instance it must be realized that aluminium is the second most abundant metal on the surface of the earth and is ubiquitous and that no causal link has been established between aluminium and senile dementia.

Lead, although used in some water supplies, by and large, is coated with calcium deposits which means that the element does not flow with the water supply. Only in instances of new plumbing modifications to the lead pipes do lead levels in drinking water rise. There have been many instances where dormant lead piping has been ripped out and replaced with fresh copper piping throughout a large institution and the soldering used to join the copper pipes has been based upon heavy metals such as lead and cadmium. Thus, the analysed heavy metal content of the water after the refurbishing of the building was found to be considerably higher from the older lead pipework previously used.

FOOD FORTIFICATION

Occasionally, food is fortified because of a lack of a trace element or the fact that it is being unintentionally removed during processing and a variety of food fortifying agents based upon iron in its various oxidation states is shown in Table 8.7. Pickford has listed the metallic constituents found as contaminants in processed foods as studied in Luxembourg (Table 8.8). This does not necessarily indicate that the presence of such metals is a threat. From Figure 8.1 it can be seen that as long as the metal species is below that of threshold concentrations (T) there will be little risk involved. However, the multiple presence of a range of metal complexes can be more than additive and so it is important to study the whole spectrum of elements present in modern diets. Furthermore, animal studies over three decades have shown an interrelationship

Table 8.8 *Pickford's selected data for typical levels* (mg kg^{-1}) *of trace inorganic constituents in food*

As	0.001	→	0.1
Cd	0.003	→	0.3
Cr	0.01	→	3.0
Cu	0.1	→	30
Hg	0.003	→	0.1
Pb	0.03	→	1.0
Sn	0.03	→	10 (up to > 300)
Zn	1.0	→	100

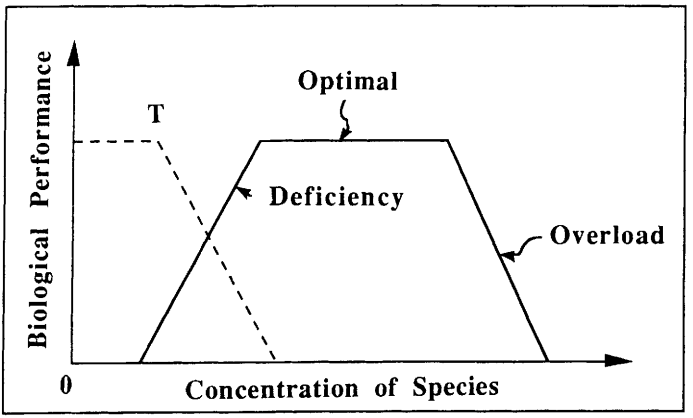

Figure 8.1 *Biological performance* versus *concentration relationships for a toxic/contaminating metal* (----) *and for an essential/beneficial metal* (———). T *is the threshold concentration below which there is usually no adverse effect. In certain allergic or sensitive individuals this may be lower. Concentrations approaching zero are probably* (a) *unmeasurable and* (b) *unachievable. The shapes of these curves vary from metal ion to metal ion and are chemical speciation dependent. The optimal plateau is sometimes described as the healthy concentration window. It commences at a concentration just sufficient to avoid nutritional deficiency*

between chemically similar elements in terms of their uptake from diet. An increasing concentration of zinc given orally can reduce the absorption of copper to less than 50%. In addition to such antagonism between metal ions, there are also stimulation effects that are dependent upon the chemical speciation prevailing. Similarly, feeding calcium to rats having a high lead content in their diets markedly decreases the amount of lead absorbed from the diet.

FOOD INTOLERANCE

Intolerance to certain foods to a greater or lesser degree is actually quite widespread and occurs in about 2% of the population at large. The figures are higher for children under the age of five but many of them tend to grow out of this condition. The presence of a low tolerance threshold in susceptible children has sometimes been linked with trace elements such as lead or chromium. Nowadays a food intolerance databank has been established in the UK and considerable information is available to enable parents to avoid trace element-containing foods.

The demand for 'natural foods' in the western world is linked in with the concept of 'unprocessed' and 'free from any artificial ingredients'. In actual fact, virtually all foods are processed nowadays, not the least of which is to maintain the freshness demanded by the customer and to provide sufficient vitamins from chemically manufactured sources. For example, it has been calculated that to provide sufficient vitamin C from a natural source such as a lemon for the population of the UK would require lemon groves covering a third of the area of the UK; and a more conducive climate! Paradoxically, the concept that all natural substances are harmless whereas all artificial substances are harmful is often misguided since laboratory-made substances used as additives are often chemically identical to the natural substance and have invariably been tested against a wide battery of screens before being introduced up to carefully controlled levels into food.

The first food legislation in the world was introduced in the UK in the 1860s to prevent trace elements such as alum being introduced into flour and lead acetate (so-called sugar of lead) being introduced into wine in order to sweeten it. Nowadays, food regulations control the use of additives, processing aids, and materials used to fortify foods. The laws are as effective as their means of enforcement, local authorities being the front-line troops in that they regularly sample food from the shelves in retail outlets.

FOODS LINKED WITH HEALTH AND A LONGER LIFE

Whereas children used to be indoctrinated that 'greens are good for you', 'spinach makes you superhuman', and 'carrots help you see in the dark', adults are now being persuaded that some foods have biological roles beyond their nutritive values; the so-called 'nutraceuticals'.

In the USA, the Food and Drug Administration and the Nutrition Labelling and Education Act permit foods to carry a health claim if a clear relationship exists between any nutrient or other substance in the

Table 8.9 *Food–disease links accepted in the USA**

Food content	*Disease influenced*
High calcium intake in teenage girls	Reduced risk of osteoporosis
High sodium levels	Causes hypertension
Dietary fats	Increased cancer risk
Dietary saturated fats and cholesterol	Increased coronary heart disease
Fibre-containing grains, fruit, and vegetables	Decreased risk of cancer
Soluble fibre	Increases risk of coronary heart disease
Antioxidant-containing fruits and vegetables	Decreased risk of cancer

* Foods containing up to one-tenth of the RDA of these agents may carry health claims such as 'Low-fat diets rich in fibre-containing grain products, fruits, and vegetables may reduce the risks of some types of cancer, a disease associated with many factors'.

food and a 'disease or health-related condition for which most people or a specific group of people are at risk'. The FDA stipulates that there must be 'significant agreement among qualified experts supported by the totality of publicly available evidence'. To date, some seven food–disease links have been accepted (Table 8.9). There are many disqualifying aspects to be resolved. For example, whole milk is prevented from being labelled with the beneficial aspects of calcium because it contains more than one fifth of the daily intake of fat. However, skimmed milk can be labelled as beneficial.

ENVIRONMENTAL ASPECTS

Humans are an essential feature of the environment and it is false logic to view them as opposing or draining benefits from the environment. Of the total volume of the Earth and its atmosphere, the space available for animal and plant life is an exceedingly small portion. This is essentially the aqueous top layer of the Earth's surface, although there is, of course, an interaction in the form of gases with the atmosphere above the surface.

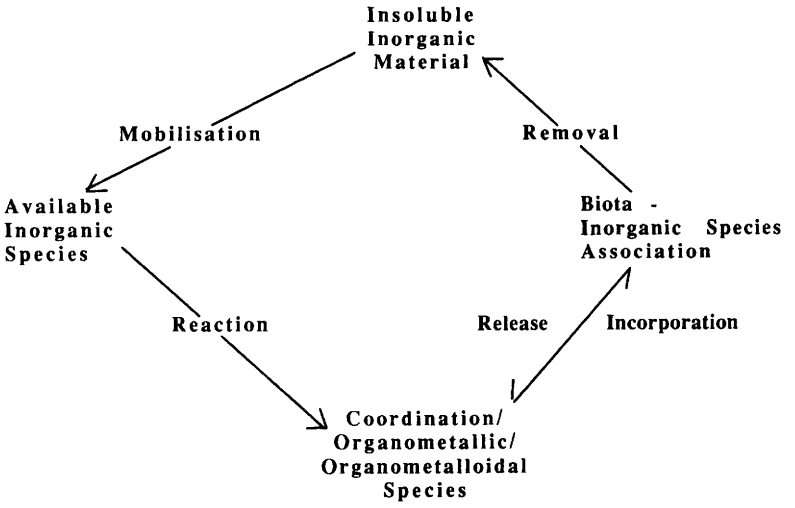

Figure 8.2 *Schematic representation of the biological cycle for a metal in nature. All metals essential, and contaminating, have such cycles that are very chemical speciation-dependent* (after K.J. Irgolic).

To date, some half a million plant species and 1.1 million animal species have been identified and some of these have been analysed in terms of their elemental content (see Chapter 7). From the broad occurrence of many of the lighter elements of the Periodic Table across many of these species, and from experiments on element-sterile diets, some elements have been designated as essential. Functions such as reproduction, growth, and healthy survival are dependent upon their presence in the correct chemical species. However, there are some elements for which species would probably function better in their absence. These are the contaminating or the polluting elements and are often from the heavier end of the Periodic Table.

All elements have cycles in nature, as per Figure 8.2. Sometimes elements are locked in as insoluble organic material in rocks or as inorganic precipitates and it may well be that mobilization can be achieved by the presence of other materials such as an organic ligand.

By and large, of the 86 naturally occurring elements in nature, some 20 or so are deemed to be essential as trace elements and approximately 10 are envisaged as toxic. Most of these essential trace elements are metals and are able to form simple ionic compounds or complex coordination compounds. However, some of the metallic elements such as cobalt, palladium, platinum, gold, mercury, germanium, tin, lead,

antimony, bismuth, and polonium are able to form metalloids in which they have a bond between a carbon atom and the element. Such metalloids do not fall into the classical considerations of coordination compounds and labile equilibria but do have the important property of being cell membrane soluble. This means that they can be used as therapeuticals because of their bio-availability or, alternatively, that they can be found as environmental contaminants that challenge the healthy living system.

The more conventional metal complexes found *in vivo* prefer the aqueous solutions in our environment. These become bio-available by the classical concepts of net-neutral low molar mass species discussed earlier.

In addition, a considerable amount of solid matter is present in the environment which, on the one hand, can provide catalytic surfaces and, on the other hand, may extract metal complexes from the natural cycle by absorption. Indeed, the micas on the beds of the oceans are regarded as a large buffer system for metal ion compounds present in sea water.

The influence of inorganic chemicals upon the metal complexes occurring in the environment is profound. It is estimated that approximately 1000 organic chemicals are being produced in the world, predominantly by so-called industrialized countries, and, in one way or another, a fraction of these eventually appear in the environment. Given that they may well react with the 30 essential and toxic elements present in living systems, it can be envisaged that several million coordination compounds could well be formed involving essential biometals and industrial organic chemicals.

Health and safety watchdogs have been well aware of this possibility and so most organic chemicals are now well tested in terms of toxicity. The approximate cost of determining reliable toxicity data for each new organic compound is of the order of £75 000. However, the speciation of a metal–ligand compound once it gets into the natural system can markedly affect the threat and introduce risk factors of the order of a thousand or even a million.

Many of the environmental properties of metal compounds occur when they are present at nanomolar or even picomolar concentrations. Clearly, these are usually well below those determinable by laboratory analysis and so one reverts to the species distributions as determined using formation constant and equilibrium considerations mentioned earlier.

Large, multinational projects have been established in the European Union in order to agree databases and reliable software for modelling

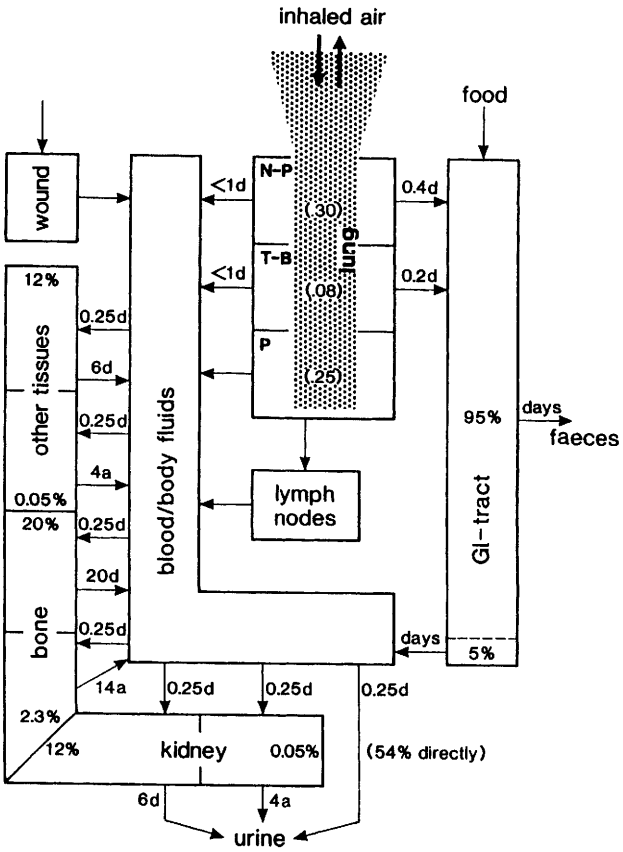

Figure 8.3 *Detailed scheme of how uranium* (IV) *is distributed amongst body compartments dependent upon transfer half-lives. Numbers in lung air space denote fractions of inhaled activity retained in the different areas for* 1 μm*-diameter particles. N–P (naseopharyngeal), T–B (tracheobronchial), P (pulmonary), and lymph nodes are compartments of the respiratory tract. For the per cent values in tissues, the fraction reaching the blood is set at* 100%. Reproduced by permission from E. Merian, (ed.), 'Metals and Their Compounds in the Environment', VCH, Weinheim, 1991.

the disposal of materials such as radioactive wastes or industrial pollutants into the geosphere. Naturally, such computer modelling has to be checked out with validation and verification exercises.

Organometallic and organometalloidal compounds can lead to unexpected species that are a serious challenge to cellular systems. For example, Figures 8.2 and 8.3 show the different forms in which uranium can appear in the environment and pathways into humans.

Table 8.10 *Sillén's estimates of the increased turnover of metals involved in the global titration since humans started to mine for minerals* (kg annum^{-1})

	Geological dissolution by rivers	*Additions from industrial mining*
Essential metals		
Copper	3.9×10^8	4.6×10^9
Iron	2.5×10^{10}	3.3×10^{11}
Manganese	4.5×10^8	1.6×10^9
Molybdenum	1.3×10^7	5.8×10^7
Zinc	3.8×10^8	4.0×10^9
Beneficial metal		
Tin	1.5×10^6	1.7×10^8
Contaminant metal		
Silver	5.0×10^6	7.0×10^6
Polluting metals		
Lead	1.8×10^8	2.3×10^9
Mercury	3.0×10^6	7.0×10^6

The resistance of different organisms to challenges from these various poisonous species can change markedly. For example, the rat responds to arsenic overdoses by binding the element selectively to vicinal thiol groups on serum proteins. The rabbit, however, responds by reducing the arsenate to arsenite and then methylating the arsenite to dimethylarsenic acid which is rapidly excreted through the kidneys. Clearly, generalizations cannot be made across species which all adds to the difficulties of designing reliable antidotes to environmental challenges.

Sillén (1960s) has likened our environment to a large titration which is cyclical in nature. However, during the last 200 years or so there have been additional inputs into this titration through the large amount of minerals that have been mined and used to make commodities that are eventually recycled back into the environment through waste disposal. The amounts that have been estimated as being involved in this large global titration, and the additional amounts introduced by industrialization are shown in Table 8.10. Interestingly, we are increasing the load on the environment by a factor of 10 in some extreme instances by our high-tech practices. This means that the buffering capacity of the environment is probably exceeded and we must give thought to this

before automatically disposing of metal-containing waste into the environment.

Waste disposal is an immense problem. The largest man-made objects in the world, far larger than the mega pyramids called the Sun and Moon, in Mexico, are domestic refuse tips in our westernized countries. Special research units concerning garbage archaeology have been set up in some USA universities and one of the leading authorities, Professor Rathje, has researched the degradation of ostensibly biodegradable materials such as vegetables and newspapers in such tips by core sampling. Rather startlingly, the cored materials revealed non-degraded vegetables such as carrots, and even newspapers disposed of some 30 years ago but which were still easily readable. Thus, the introduction of bacterial degradation of waste needs to be encouraged throughout the heterogeneous mass of a waste disposal tip rather than leaving it entirely to nature to spread in all directions.

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Chapter 9

The Future

The previous chapters have, hopefully, convinced the reader that all chemical components of the environment, all the compounds involved in human, animal, and plant biochemistry, as well as very many pharmaceuticals, to a greater or lesser extent, have relationships with trace metal ions. These metal–ligand relationships may provide the *modus operandi* for the substance, for example as counterions (such as cations), or as the electron donors necessary for drug action; or their presence in the system may lead to unwanted side effects.

The mechanisms by which metal ions complex with physiological, pharmacological or environmental ligands in order to influence the onset, progression or treatment of disease is still far from understood. However, it seems most likely that the nub of all such mechanisms will be based upon a knowledge of the pattern of chemical speciation rather than upon the total concentration of the metal present. Even now our knowledge is sufficient to permit the administration of chelating agents in order to adjust metal concentrations, or to mobilize metals from some depots and to facilitate and accelerate their transfer from one bio-fluid through to others and out into the excreta.

Knowledge of chemical speciation in biological systems is accruing rapidly. Through this increasing body of data we are beginning to be able to tackle successfully such problems as, *e.g.* the differentiation between threat and safety, between malnutrition and the wise fulfilment of appetite, or the balance between the efficacy and the side effects of pharmaceuticals.

Many future advances in healthcare may be expected to arise from an increased understanding of the correlations between chemical speciation and clinical effects. An essential pre-requisite for such advances is that researchers are able to deliver high quality data that are well validated by experimental and other means. With sound, well validated

information, causal links, for example, between an environmental contaminant, a food component or a drug, and specific conditions, can be identified and some future ill-health can be avoided. This will require a much more detailed understanding of the relationships between exposure, dose, and risk and the delivery of reliable information that will permit governments and enforcement agencies to make cost–benefit analyses and to put timely checks on such exposures when they appear to be approaching unacceptable levels; it is pleasing to note that legislation currently being developed is increasingly involving speciation criteria as well as total metal content criteria. The concept of intake compared with the actual uptake of metal complexes into the systemic circulation is now more widely understood, as, indeed, is bio-availability *versus* total elemental presence.

Society is slowly moving towards maximizing the beneficial aspects of human activities, *e.g.* in industrial development, and in the delivery of healthcare and especially in the areas of clinical medicine and the increasingly large armamentarium of pharmaceuticals now in use, whilst minimizing their disturbing side effects. Ideally, such risk–benefit considerations should be taken on board at the planning stages of introducing new processes, new materials, new techniques or new drugs, *etc.* as optimization can be achieved more cheaply *ab initio*, rather than by a retrospective campaign to tackle a problem that ought to have been designed out at an earlier stage.

Humans are increasingly being called upon to play a role in plotting their own destinies and we hope that this book has increased the reader's awareness of the influence of trace metals in a wide variety of health-related matters. Clearly there are other aspects to consider such as energy input, dietary habits, and vitamin sufficiency. However, increasing the awareness of society about the factors that predispose individuals to acute or chronic disease also places a heavy duty on scientists to present their information in a sufficiently complete and understandable form so that individual members of society may make informed valuable judgements concerning risks and benefits. Awareness of the factors that cause or influence disease is only one part of the self-protection of our health. Another essential part is the setting aside of sufficient resources to afford a healthy and long life-span, to ensure that the Cinderella area of good data acquisition is not neglected, and to guarantee that sufficient experts are trained in these manifest fields such that they do not suffer from a skills famine. In this time of rapid economic and technological changes, it is indeed true to state that, while knowledge and resources do not necessarily have the absolute power to cure, they certainly help.

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This book discusses, in relatively simple language, the importance of even minute amounts of certain trace elements for the protection of human health and how insufficiency or excess may produce serious diseases. It also examines the use of metal chelators in the treatment of such diseases.

Trace Element Medicine and Chelation Therapy looks at the role of metal ions in the evolution and development of living systems and reviews the elemental composition of the human body, the essential biochemistry of metal ions, metal complexes and the concepts of chemical speciation, as well as the interactions of metals with chelating agents, ligands and drugs. The problems of delivering adequate amounts of trace elements to the human body and the roles of metal containing drugs are also discussed, while the role of chelating agents in the treatment of both chronic and accidental overload and the dietary and environmental aspects of such treatment are both comprehensively reviewed.

Trace Element Medicine and Chelation Therapy will assist chemists in understanding more about these metal ions and the influences of industrialization and pollution, and will assist physicians involved in administering chelation therapy. It illustrates the interdisciplinary nature of the subject and will be of immense interest to students and researchers in chemistry, biochemistry, nutrition and food science, environmental sciences, pharmacology and medicine, as well as to school science teachers and scientifically oriented members of the public.

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