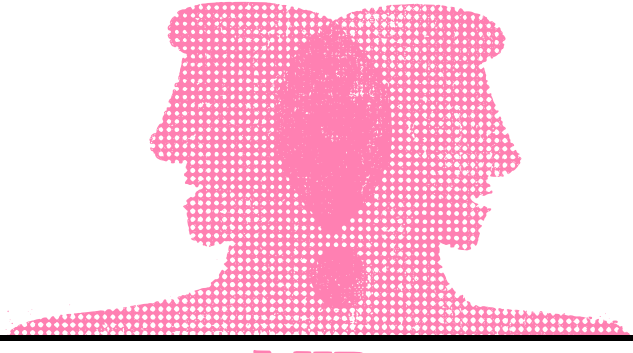
SCIENCE FOR EVERYONE

R.V. PETROV

ME OR NOT ME



MIR

Science
for Everyone

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Я ИЛИ НЕ Я

Иммунологические мобили

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Me or Not Me
Immunological Mobiles

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Two Types of Individuality

(In lieu of an introduction)

Some ten years ago Academician Vladimir Engelgardt invited Nils Jerne, a well-known Swiss scientist, to visit the Institute of Molecular Biology of the USSR Academy of Sciences. Professor Jerne delivered a remarkable lecture and talked with the professors and their young researchers. Explaining his work on the theory of network regulation Jerne pointed out that the two most exciting biological problems at present are how the human brain functions and how our defence system, immunity, works.

There are a dozen or so survival systems functioning in our bodies. The eyes provide vision, the ears hearing, the bone and muscular system motion. The digestive system supplies the blood with nutrients, the lungs saturate it with oxygen, and the heart pumps the blood along the vessels, transporting the nutrients and oxygen to every point of the body. See how every organ specializes in only one duty. Only two systems are different by not being "narrow specialists"

The first is the brain, which generates thousands upon thousands of thoughts to meet every event we encounter in life. And each time the thought, notion, or conclusion is distinct. Moreover, all of these thoughts are memorized. The memory creates intellectual experience; the brain's activity creates a person's intellectual individuality.

The immune system generates thousands upon thousands of proteins to fit all the contingencies of life, proteins that are deadly weapons, each one specific to a microorganism. There are thousands of microorganisms, hence thousands of weapons are required. And, again, a memory is needed to keep them in case of need. The immune system can remember. It remembers throughout our lives the harmful agents we've encountered, so it can deploy the weapons instantaneously. Having once suffered typhus, you will never catch it again. Immunological memory recreates an individual's immunological experience, his immunological individuality. Each of us is unique both spiritually and bodily.

Thus, there are two types of individuality—spiritual and bodily. The first is provided for by the central nervous system, the other by the immune system. One preserves the uniqueness of intellect, taste, talent, habit, and character; the other preserves the uniqueness of biological structures from which the cells of every individual are constructed. Indeed, the immune system protects each of us not only from microorganisms and viruses, but also from any alien protein, from any alien cell, even our own cancer cells!

Perhaps the reason nature gave us the immune system is to keep anything but what belongs to the organism from becoming established within it. Nothing alien is permitted to exist, only the body's own constituents are allowed. Maybe, this is why we differ from everything else. Otherwise, how would we be able to recognize a microorganism that had found its way into our bodies,

or uncover a treacherous cancer cell, if our body was not different from the enemies either invading or emerging from inside it?

Every particle of our body must bear an identification sign, or "tag", saying 'this is me'. If something does not have such a sign, or if the tag is foreign, the particle would be saying: 'this is not me' Everything 'other than me' is destroyed by the immune system.

I have compared two types of individuality—spiritual and bodily—to show the all-embracing significance of immunology, and the complexity and intricacy of the immune system. Only the future will reveal whether the mechanisms of the brain and the immune system are similar.

This book has grown out of a booklet called *Talks on New Immunology* which was published in 1976 as a part of the "Eureka" series. The talks have been expanded in volume and their form revised in an effort to convey to the reader the huge volume of scientific information that has been accumulated by a young science of immunology.

The Costs of the Apollo Programme

When people discuss works by the classics of art, literature, or the natural science, we think of Boticelli, Michelangelo, Repin, Tolstoy, Pushkin, Dostoevski, Newton, Darwin, or Lomonosov. We recall the creation of those who lived long ago and who laid the foundations of the arts, literature, and science. When "modern art", "modern literature", or "modern science" is talked about, we think of the creations and deeds of the present day. Whilst in the arts we may argue over which is better, the old or the new, there is no room for personal assessment in science, and such a discussion would make no sense. Each new formulation of a law of nature grows from an old one and prepares the grounds for those to be discovered.

Immunology is the science of immunity. It, too, is often divided into the classical and the modern, even though it is only one hundred years old. Interestingly, not all immunologists like their science to be divided in this way, feeling that such a division makes it seem that our previous understanding is obsolete, or has been cast overboard by the development of science. It would seem to me, we ought to be proud that knowledge grows and new facts and ideas are absorbed. The science is thus expanding its sphere of influence, becoming increasingly necessary.

Modern immunology has evolved from the classical science, which produced vaccines against smallpox, rabies, anthrax, and other diseases, the one which brought golden apples to mankind.

Did you know that the vaccination against smallpox, which has spared each of us from the possibility of getting this disease, resulted in the complete eradication of this terrible illness from the face of Earth? Over the last decade the UN World Health Organization has accomplished a global programme of vaccinating the entire population of the world against smallpox. And smallpox has disappeared. It no longer exists anywhere! Not even in Asia, historically a hotbed of the disease. The last registered case of smallpox anywhere was in Somali Republic in 1977.

There is even a special "rumour register" in Geneva, the location of the World Health Organization Headquarters. In the last three years it recorded 142 smallpox rumours in a number of countries. All of them were checked, but not one was confirmed. They all turned out to be chickenpox, nettle rash, etc. In May 1980 the WHO's World Assembly declared that smallpox had been eradicated all over the world! Most countries, including the Soviet Union, abolished smallpox vaccinations. Now we no longer need to disturb our babies and to put marks on their shoulders. Smallpox has simply ceased to exist in nature.

And this is not the only example.

To get more information we can turn to a book such as *Results of a Half-Century of Struggle with Infection in the USSR* by Oganes Baroyan. In 1955-1956 an efficient vaccine against whooping cough and diphtheria was introduced,

At that time at least 150 thousand children a year suffered from diphtheria. Ten years of vaccination actually put an end to this disease. From 7 to 8 hundred thousand children a year got whooping cough, while now the disease practically does not exist.

In 1959-1960 every child in the Soviet Union was immunized against poliomyelitis. By as early as 1961 the incidence of this disease had fallen from 22 thousand to 4 thousand. In 1964 only a thousand people fell ill, and around 1967 poliomyelitis was eradicated. More than 20 thousand children a year have thus been saved from death or severe paralysis. This adds up to a hundred thousand people over five years!

Dr. Cinader, a Canadian immunologist and former president of the International Immunological Society, gives even more impressive figures concerning the USA. In a rather cynical manner he converts everything into money. The total income of the state from an average American man is 226 thousand dollars and 45 thousand dollars from an average woman. If a boy or a girl dies the national budget does not get this income. In non-lethal cases paralyzed people can work, but with no more than 50 per cent efficiency. In his calculations Cinader approximated the working capacity of moderate invalids at 75 per cent, and that of slightly disabled at 90 per cent.

His calculations show that between 1955 and 1961 (vaccination in the USA started a year later than in the Soviet Union) 154 thousand people got poliomyelitis. 12 500 of them died, 36 000 were severely disabled by incurable paral-

ysis, 58 100 were moderately disabled, and 32 700 were slightly disabled. Only 14 300 recovered completely. The loss of income to the state thus came to $6.4 billion. The treatment of patients and care for invalids cost an additional $300 million, and hence the total loss was $6.7 billion.

The cost of the vaccination, including the price of the vaccine, the wages of the medical personnel and administrators, and the R&D costs amounted to 0.65 billion dollars. Even subtracting 0.65 from 6.7, more than $6 billion is added to the US national income every six years. One billion dollars a year simply due to the eradication of poliomyelitis.

The "Apollo" programme, which resulted in man's landing on the moon, cost 25 billion dollars. Immunology could well cover the expenses.

Cinader gives these figures in a textbook on immunology, so that the great contribution of the science to mankind is never forgotten. The future will bring vaccines against influenza, infectious jaundice, and many other diseases, including, I am sure, cancer. But this is the task of modern immunology.

Affiliated with Genetics

Today we can speak of "old" and "new" immunology without breaking the links between them. To understand the difference between "new" and "old" immunology, we should go back to the birth of immunological science.

The springs of knowledge originate from man's

practical activities. In the Early and Middle Ages, man was a great deal more dependent on the spontaneous forces of nature than he is today. And the main scourge of humanity even until as recently as the 19th century has been epidemics. Attacks of plague, cholera, and smallpox were raging throughout the world, carrying away more lives than the most devastating invasions of Scythians or Huns. And practice taught man how to fight against epidemics.

The results of this struggle are epitomized by man's victory over smallpox. The Chinese claim to have known a way of preventing smallpox since the early 11th century. They placed smallpox scabs from the diseased into the nostrils of healthy people. At about the same time in Persia smallpox inoculations were done in the baths, where attendants rubbed the powder of smallpox scabs into the skin cuts of the bathers. In the 18th century, Circassians and Georgians, wishing to preserve the beauty of their daughters, pricked them with needles moistened with the liquid from smallpox sores.

Long before immunology appeared as an independent scientific area, it was known that children suffer from chicken pox, measles, and mumps only once in their life. Purely practical experience indicated that, once in contact with infection, the organism acquires the ability to generate protective properties against it.

This experience paved the way for experimental immunology. Its conception is associated with the name of Edward Jenner, an English physician. He noticed that those who had been once infected with cow pox became immune to

smallpox for the rest of their lives. Being a shrewd and well-educated person, Jenner sensed that there was a rational cause for this phenomenon and started experiments to develop this method of combatting the infection.

In 1788 he published the results of his studies, showing that those infected with smallpox after inoculation with cow pox would never develop the infection. Despite the attacks of sceptics and religiously inclined laymen, Jenner's method of anti-smallpox vaccination was accepted everywhere. The essence of the method is that the cow pox virus is applied to the skin.

The prominent 19th century French scientist Louis Pasteur is the source of theoretical immunology. Pasteur's premise, which determined all his achievements, was to recognize microorganisms as the cause of infectious diseases. He boldly put forward this theory, having proved it valid for the diseases of beer, wine, silkworms, animals, and humans. He then went on to use the pathogens of the diseases in the struggle against the diseases themselves.

Many people who visit Southern France try to call on the two small towns of Dole and Arbois. Pasteur, one of the greatest citizens of France, was born in Dole, in a small two-storied house which stands so close to the river that its back door opens directly onto the water. One can draw water or wash linen from the doorstep. The verandah extends over the river itself. A visitor feels as if he is on a ship.

Arbois was the place where Pasteur moved after his marriage, where he began his first studies. The French say that Pasteur saved

France three times. The first time was in Arbois, in 1865. Pasteur discovered the causes of the beer and wine diseases which had been ruining French vine-growers and brewers. He taught them to pasteurize wine and beer.

A vineyard of several hectares which once belonged to Pasteur, or rather to his wife, bears fruit to this day. The first wine to be pasteurized was produced here. A hundred years have passed since then. Arbois and Dole attract millions of tourists. They drink wonderful wine and feel as if they are sharing in Pasteur's great discovery. And the present owner of all the Arbois vineyards, Henry Meyer, honours the memory of Pasteur.

In 1973, when scientists from all over the world got together to celebrate Pasteur's 150th birthday, Meyer held a grand reception, more to pay homage to Pasteur than to entertain the visitors. Many scientists were awarded the title of Honorary Peer of Arbois. This is an unofficial peerage, a peerage to the glory of wine. It has its own three-coloured flag of green, yellow, and red stripes, which symbolize the vine, sun, and wine. A small fragment of this flag is on the diploma of an Honorary Peer. A similar three-coloured ribbon worn over the shoulder bears a heavy bronze medal, showing that the bearer is a Peer of Arbois. On the front of the medal there is a nymph with grape bunches for her hair; the back shows two hands with goblets and the inscription: "PAIRIE des vins d'ARBOIS".

The second time Pasteur saved France, or rather French silkworm breeders, in 1868. He found the cause for silkworm diseases which had

spread throughout the country. The third time was when he helped cattle-breeders by creating a vaccine against anthrax, which had been killing hundreds of thousands of cows, horses, sheep, and goats every year. All this happened soon after 1881, the year Pasteur founded immunology.

In 1881 Pasteur developed the general principle of preventive inoculations by introducing weakened microorganisms. He and his colleagues discovered methods of preventing vaccination not only against anthrax, but also against chicken cholera, pig German measles, and rabies. Later, vaccines against many other infections, such as plague, cholera, poliomyelitis, etc., were created.

By the end of the 19th century the main point became clear: that inoculations using cultures of infection pathogens develop immunity against these infectious diseases. However, the mechanisms of immunity and the basis for natural and acquired non-susceptibility were still not known. It was for other scientists to reveal the mechanisms of immunity.

The science of immunity emerged from the pressing need to combat infectious diseases. A tremendous army of researchers channeled their energies to study the mechanisms of resistance to infections, to learn how the organism protects itself. In the light of this, the definition of immunology went something like this: immunology is the science of the factors and mechanisms which govern humans' and animals' resistance to infectious microorganisms.

One can hardly think of a more important area of science. But still, the well-being and

longevity of any scientific trend is possible only if it does not confine itself to one specific task, but expands its sphere of influence, to penetrate into allied and even into rather remote scientific disciplines.

Figuratively speaking, the flourishing of any area of science depends on how much the "infectiousness" of one science matches the "susceptibility" of others.

Thorough studies of immunity mechanisms resulted in the union of immunology with other biological disciplines. For instance, the studies of antibody structure (antibodies are the agents used by the organism to deal with alien newcomers) allied immunology with biochemistry and molecular biology. Immunochemistry appeared as an independent area of immunology. While studying the cells which generate antibodies and take part in immune reactions, scientists entered the domains of cytology and histology, morphological subjects concerned with cell and tissue structures. This was how immunomorphology came into existence. But the most important development was that immunology became affiliated with genetics, the science of heredity.

Everything, it seemed, was against the possibility that heredity mechanisms might be involved in developing immune responses. Indeed, a person who once suffered from smallpox will never get it again. He acquires immunity for the rest of his life. But his children are as susceptible to this disease as he himself had been. We all get over the measles in our early years and become immune for the rest of our life. But our

children are not immune. They get infected and have measles. All the evidence suggests that immunity is not inherited and has nothing to do with genetics.

Still, these two sciences met. The very ability to respond to a pathogen turned out to be under rigid genetic control. By the late 1960s the genes of the immune response were discovered. They were called IR-genes, from the words Immune Response. If you have an IR-1 gene, you are able to respond to a certain alien substance should it enter your body; no gene—no ability. If you have IR-2 gene, you are able to respond to another substance, and so on. Immunogenetics came into being.

By this time fundamentally new branches in immunology began to emerge, such as graft immunology, cancer immunology, and immunopathology. These are the trends in immunology called upon to solve problems of primary importance. These trends most graphically display the principles of genetic analysis used in developing the mechanisms underlying successful organ transplants and the factors suppressing cancer growth.

The new immunology is, first and foremost, a biological discipline affiliated with genetics. Strictly speaking, the entire field of modern immunology is interlinked with immunogenetics as an integral whole.

Indeed, the reasons for rejecting grafted organs are genetic, while the mechanism is immune. The reasons for cancer cells to appear are genetic, but the mechanisms of combatting cancer growth are immune. The reasons people differ

in their susceptibility to infectious microorganisms are genetic, and the mechanisms of overcoming the infection and developing non-susceptibility are immune.

The "old", or, as it is now called, infectious immunology, has become a full and equal member in a brilliant cohort of sciences, where immunochemistry, immunopathology, graft immunology, and cancer immunology stand side by side. Note that immunogenetics is not included into this grouping. Immunogenetics is the foundation on which all these sciences are resting.

Why So Strict?

Modern immunology is referred to as "new" not only because it has new objectives, but also because it has acquired a new meaning. Today we can no longer consider protecting an organism against microorganisms and pathogens of infectious diseases as the only, or even the main, task of immunology.

A new interpretation of immunology was proposed in 1944, in the works of Sir Peter Medawar, the British researcher and Nobel Prize winner.

These were the hard war years. The Soviet people waged the sacred war against Nazi Germany, as did our allies, among them Great Britain. Londoners experienced troubled nights. At that time the allies could not intercept the fascist bombers flying above the English Channel. At night, the shells crashed into the London housing blocks. The Londoners called these weapons "flying gas mainlines". The explosions

and fires breaking out without air-raid warnings produced the impression of gas mainline accidents. Hospitals were filled with hundreds of burnt Londoners.

A young Zoology professor at London University left his chair and went to work in a hospital to treat the wounded and burnt. He tried to treat the burn victims by doing skin transplants on them using skin from donors. But the "alien" skin failed to fuse. Why?

The allies learned how to shoot down bombers above the English Channel. "Gas mainlines" stopped flying. The problem of how to treat London burns was solved by the engineers who designed radars, not by physicians. But why two skin types would not fuse together remained the basic scientific question for Medawar. In a series of experiments on rabbits the scientist proved that the rejection of transplanted skin is an immunological phenomenon.

In 1945, immunity was finally shown to use the body's resources to protect it not only from microorganisms, but also from all genetically alien cells and tissues, such as transplanted skin or organ, a kidney for instance.

According to Leslie Brent, a student of Medawar's, Medawar first plotted the rejection phenomenon on the map of immunology. Incompatibility of genetically alien tissues under transplants appeared to be an immunological problem.

During the following decade, geneticists managed to breed new species of laboratory animals, mice in particular. These species were called pure strains. All the animals within one pure

strain are identical, just like monozygotic twins —identical in everything! Tissues and organs grafted from one animal of a pure strain to another fuse because they are not genetically alien to each other.

Individuals of one pure strain are genetically alien to those of another.

Many of their genes are different. Immunity recognizes the genetically alien tissue and rejects it. The development of new pure strains led to congeneic strains that genetically differed by only one gene.

Tissue transplantations between individuals of congeneic strains resulted in rejection. It became evident that immunity responds to an alien cell or organ even when this cell or organ differs in only one gene, that is, in the minimal genetic unit.

Researchers faced the question: why so strict? Why does such a rigid censorship exist, capable of detecting even one minimal alien feature, that is one alien gene?

This question was formulated in the early 1960s and formed the basis for the new immunology. Everyone who asked himself this question inevitably came to the conclusion that this strict censorship of everything alien existed in nature for far more important purposes than putting obstacles in the way of surgeons who do transplants. These purposes were very soon understood.

The human body consists of 10^{13} cells. This is a huge community of genotypically identical cells that all originated from one fertilized cell and contain identical sets of genes. But every-

thing in nature is subject to changes, genes included.

Accidental gene changes are called mutations. The cell of a gene mutation becomes a mutant. Mutations occur seldomly, but there are always mutants among a group of cells. Their rate is about one per million, that is 1 10^6. If there are 10^{13} cells in a human body, there can be 10^7 mutants at any given moment. Ten million cells with different (and maybe dangerous) properties. Ten million traitors! And if they start to proliferate? If they start to perform the functions differing from what the organism needs? Isn't this how cancer and some other non-infectious diseases originate?

Somebody should be on guard against these traitors. We now know that this is the function of immunity. Immunity recognizes and destroys "a newcomer", even if it differs by only one gene. This is the basic aim of immunity—immunological supervision, immunological control over the inner consistency of the body.

Considering immunity on this basis, it should be ranked along with other manifestations of a not yet formulated law of protecting biological individuality: heredity preserves individuality in succeeding generations, and immunity—over the lifetime of each individual.

Distinguishing between "one's own" and "someone else's", protecting biological individuality, immunological censorship, the police function of immunity—all these are synonyms used by various scientists with one purpose: to stress the basic aim of immunity—the protection of the genetic consistency of the organism's inner me-

dia. A weakening or disturbance of this system leads to innumerable infectious diseases and autoimmune disorders, and the likeliness of cancer increases tens or even hundreds of times.

Club "Under the Integral Sign"

In April 1965 I visited the academic campus near Novosibirsk, the domain of the Siberian division of the USSR Academy of Sciences. The purpose of my visit was to deliver a course of lectures on immunology to the fourth-year students of the biological faculty of the university. Naturally enough, I started my first lecture with the question: "Who can tell me what immunology is?" "I think it's a science of preventing infectious diseases," a girl answered. "And of the processes taking place in the body when microorganisms get there," added a boy. "So," I said, "All my twelve lectures will be devoted to leading you out of this delusion and to showing that the theory of protecting against infections is but a small part of modern immunology."

The academic campus is an unusual place. This city of science is full of youth. Young scientists, post-graduates, and students are as sociable as elsewhere. But nowhere else are they so united by the spirit of learning and searching, the spirit of scientific inquisitiveness, and interest in everything. Biologists want to know mathematics, physics, and cybernetics. Physicists and mathematicians are fascinated by biology and medicine. Chemists and geneticists, economists and power engineers, all look for interesting and useful aspects in each other's fields.

For these reasons the club "Under the Integral Sign" was founded on the campus. Young scientists were granted a house originally meant to be a cafe from the local authorities. Here, young people can get together. Here, their interests are integrated, and everyone's striving for knowledge is satisfied. Here, the scientific issues of the day are discussed and people get acquainted with new scientific fields. Here, debates are held on the ways of scientific thinking, on the mathematization of biology, on the biologization of technology, and on many other subjects. The club is also popular with mature scientists, professors, and academicians. But not all of them are received in the same way. Only those most possessed by science manage to get along. This is a youth club.

After my lecture dealing with the immunological problems of space exploration a girl came up to me. She introduced herself just as Ira, saying that she was a board member of the club "Under the Integral Sign", and on behalf of the board she invited me to the club to tell people about immunology. "It is only just that there are almost no biologists among them," she added, "Your audience will be mainly physicists, engineers, and mathematicians. And they are very fond of arguing; and they asked to touch upon some problem of space immunology."

I could only think to speak in a special way about immunity, to speak in a scientifically reliable, comprehensible, and interesting way to be able to generate a discussion in the very specific audience of the club. Finally, and it was quite indispensable, my narration was to draw away

the inertion of thinking of immunity only as a nonsusceptibility to the pathogens of infectious diseases, to overcome the inertion of narrow understanding of immunology.

There are two halls in the club. One has a bar-stand, a stage, tables, and a space for dancing. Two bronze conventionalized masks are hanging over the stage. One is thoughtful, the other laughing. A buzz of voices. . People are drinking wine or coffee, playing chess, talking, and dancing. The organized "scientific chatter" is in the other hall. There are also tables, though much fewer of them. A lot of chairs. Some people are sitting on the window-sills. There is no stage or platform of any kind. A blackboard with chalk. The atmosphere for free talk.

"Imagine some cybernetic device," I started, walking between the chairs. "A high-level machine with a feedback. It gives accurate and expedient responses to the external conditions. The expediency is determined by self-preservation in changing outer conditions. For its internal and external connection it uses the words composed, say, of Latin letters. Our machine knows one hundred words. It was programmed with these words from the beginning. It uses these words and can even write verses. But once used, they disappear from its vocabulary forever. There is never again such a word. But without it a certain command cannot be transmitted to some part of the machine. The verses will no longer be produced."

While speaking, I was watching the audience, which never expected reasoning like this. Two young men displayed special interest. One of

them, as I found out later, was from the Computer Institute, the other was a post-graduate of the bionics laboratory.

"Can you imagine such a machine?" I asked them.

"Of course we can," the young cyberneticist answered. "Only it would not be able to sustain "an active existence" for any appreciable time. We can't feed it with an infinite number of copies for each of the hundred words. Their number should be finite. However, the machine loses every word after single use. As soon as the stock of any of these hundred words is through, a block or unit governed by this word will be switched off. The machine will stop working. It will not be able to "reasonably" respond to the outer signals, or, as you suggest, write verses."

"OK! But our machine has a special channel transporting whole phrases, or word aggregates, from the outer world. Let us call them tablets with words. In this channel the tablets are disintegrated into separate letters. Then we have a kettle filled with all the letters of Latin alphabet. These letters are used by the machine to construct its hundred words to be spent to its diverse "life" contingencies."

"But why so difficult," the same young man asked, "Isn't it more simple for the machine to borrow whole words from the outside?"

"You see," I explained, "First, it would have been an unreliable system. It could be a long time before the needed word appears. And, second, foreign words, not included in the ma-

chine's hundred, are not allowed to come inside. This is a principal rule. Redundant or wrong words that are sent as a command would at best be rejected. At worst, the response will be wrong, the verses will lose their sense, and the machine will die."

In my talk, I tried increasingly to speak of our fantastic machine as a living being. In this I was helped by the post-graduate in bionics.

"But what if foreign words and phrases, or, as you called them, the tablets, still manage to get inside, perhaps, by-passing the "natural" route, that is the channel where these tablets are broken into their constituent letter bricks? They can either get there by chance, or we can force them from the outside. In other words, we'll introduce foreign words inside the machine bypassing the processing channel."

"The machine has provisions against this possibility", I hurried to say. "There are special devices in the connection channels throughout the machine's body. They distinguish between their own and someone else's. Recognition is absolutely strict and is never off. Every tablet of inner or outer origin, passing by, goes through this "screening". The tablets are read, and, in case there is just one foreign word, or a wrong letter in the machine's own word, the command is given, and the tablet is discharged from the machine. This rule is strictly followed, since it is a vital necessity. Alien information can put a part or the entire machine out of order."

"But if we artificially introduce a tablet with any of its hundred words into the machine's connection channels, will the "screening" let it

pass?," some of the audience asked. "Yes, it will, since it does not bear any foreign information. If the tablet is blank, it will also not be discharged. It will present no danger and can be used for the machine's own notes," I finished the description of our cybernetical model. The only thing left was to kindle the active discussion of its "life"."

I started to "deploy the combat actions" "Now think, if we feed our machine, by-passing the natural route, with the tablet filled not with the Latin letters, but with Chinese hieroglyphs. Will it be passed by the "screen" or discharged from the machine?" "Passed by! ... Discharged! Passed by! ... several opinions were spoken out at the same time.

"Why do you think it will be discharged?" I asked the cyberneticist. "Just because it contains the strange signs." "But," the bionics researcher interposed, "Chinese hieroglyphs are so different from the Latin letters that the "screen" will see nothing. It will take the tablet for a blank one and pass it into the machine."

Other people joined the discussion. They started reasoning incomprehensible to me, concerning the capacities of modern machines and the ways of reading out. No common opinion was arrived at. One asserted that the tablet filled with a principally different script will be taken as an empty one and passed into the machine's connection channels. Others insisted that the tablet would be discharged.

I sat down and silently listened to the discussion. Finally someone asked me: "What are we arguing about? There are no such machines and

we are not going to build them. What is this fantasy about?"

You are wrong. There is a lot of such machines around. It is not just imagination. If you wish, we ourselves are such machines, as well as other mammals on our planet, including birds, amphibia, and fishes. Our machine is a model of a being with immunity, and the words are the main life substrate.

Proteins act as a substrate for all living matter on Earth. One hundred words are one hundred conventional proteins in a living organism. The letters composing the words are amino acids that make up all proteins. The most diverse proteins in the human body or a rabbit's body, that of a cat, a horse and a frog, an eagle or a perch are constructed of twenty basic amino acids, the alphabet of protein words. And, just as a few letters of the alphabet make up an infinite number of words and phrases varying immensely in their meaning, twenty amino acids build an infinite quantity of protein molecules which construct Earth's inhabitants, the molecules of diverse forms and properties.

Every organism reproduces its "one hundred words", the proteins typical only of this organism. The proteins are built based on gene matrices located in cell nuclei. The set of genes is unique for every individual organism, as well as the pattern for protein molecules. The organism spends them to exercise its life functions, and, having run out of them, builds them anew.

The channel that feeds the machine with the letters from the outside is a model of the animals' digestive tract. Like in our machine, the alien

proteins come from the outside with food; the words, or, as we called them, the tablets, are broken into their constituent letters, or amino acids. This is necessary just because the foreign proteins have a different "pattern" They are constructed based on alien genetic information, which is also unique, and hence different. They are built on different blueprints, different matrices. Food proteins are the first to be broken into constituent letters, amino acids, in order to make up one's own words.

If alien proteins or tablets are introduced into a human or animal's body by-passing the digestion channel, for example, directly into blood, then the guard of inner consistency, immunity, comes into force. The screening system in our machine is the body's immunological system. If instead of the proteins, their constituent parts, that is amino acids, or the tablets with letters, are introduced into the animal's blood, they will be passed over by the screening system, since the separate letters bear no signs of alien information. If a tablet with proteins is introduced, the screening system will first read this tablet and compare its protein-words with its own hundred words, to distinguish between one's own and another's. Now imagine that a protein presents a foreign word which could not have been written based on one's own genetic information. The immunological screening will instantly order the plate destroyed and discharge it from the body. It will start antibody production, phagocytosis, and rejection of the foreign invader, whether it is a microorganism, alien blood cell, alien protein, or grafted tissue or organ.

So, what is the gist of all this? It is the fact that immunity is not only the means of protection against microorganisms. Immunity is the weapon for safeguarding the inner organism's consistency against living bodies and substances bearing genetically alien information. As long as a microorganism is also a foreign biological agent, the action of immune mechanisms involves it along with other substances.

"And what about the puzzle with Chinese hieroglyphs?" Asked the bionics post-graduate.

"This is just one of the problems of space immunology. Immunity as a means of protection against everything biologically alien was a result of life's development on Earth. Life on our planet is based on proteins. Remember the tablets with the words consisting of letters—amino acids. Our "screening" knows only our planet's amino acid alphabet, and the order protection is set up in accordance with these familiar phenomena.

If life on other planets is based on different principles, if the genetic code produces some material other than amino acids and proteins (and man would face the minute, perhaps microscopic, and, surely, strange inhabitants of this planet) —would it be possible for immunological screening, century-long taught amino acid alphabet, to recognize the newcomers? This is what we are to find out. The censorship might pass them, taking the tablets with "Chinese hieroglyphs" for blank ones. And then they would multiply in the blood and tissues, becoming lethal.

Remember, in "The War of the Worlds" by H. G. Wells, the invaders from Mars die of innocent, non-pathogenic Earth microorganisms? It

is not just fiction any longer, but really existing scientific problem.

We had a long discussion on the problems of space biology. I told the audience about the facts showing that these apprehensions are well justified. Chemists joined the discussion, but they were more interested in the question whether life can be based on principles other than those on Earth. Then we spoke about the ways to study this problem. Then drank coffee and wine, and danced. Two masks were looking from the wall—one thoughtful and one laughing.

Immunology and Space

Actually, it is not quite correct to say "Immunology and Space". Immunology relates not to space *per se*, but to another scientific field. Let us not just stick to words saying that the subject of our talk will be space biology and medicine of the recent years.

In the most concise and approximate form the questions facing space medicine are: how is man affected by space flight, that is weightlessness, acceleration, and space radiation; how can the normal body activities be provided for in the hermetically closed chamber of a spaceship, and, in the future, on other planets and astral bodies.

Here we meet a host of biological problems. And immunology faces the question: how will one of most important systems of the human body, the immunological system, protect man against microorganisms in the unusual conditions of space flights? Will the body's resistance to

bacteria and viruses be as reliable as on Earth?

This might seem to be an idle question, especially since the results of space flight, known all over the world, give no grounds to be afraid of infectious complications. Astronauts have always been feeling fine in space flights, which, however, were never longer than several days, or, at most, several months.

But we should bear in mind that the time is coming when the first stage of space exploration, that is the development and research of near space, is nearing completion. The next stage is the exploration of the nearest astral bodies, specifically, the planets of Solar system. And the least possible distance between Mars and Earth is 78 million kilometres.

At the next stage of space development the flights will take much longer, and this makes all the difference from the standpoint of medics and biologists. Contemporary space medicine and biology should study and provide for long space flights that will take months or years. Until now study was mainly focused on how the organism behaved during short-term overloads and weightlessness, what were the functional possibilities and specific features of the cardio-vascular, nervous, and other systems in these conditions, on the questions of work fitness, training, and psychophysiology. The advent of long space flights brings about new, fundamental biological problems. Among others they include the immunological question of how the human body and microorganisms will interact in extra-terrestrial conditions. This gives rise to an entirely new branch of science, space immunology.

This branch is underlied by at least three factors.

First, people travel in space together with indispensable free-riding passengers, the microorganisms that inhabit our intestines, skin, mouth, etc. The ship in space is a kind of sealed vial with people inside. It is impossible for man to be sterile due to the fact that a number of microorganisms control the body's vital functions, like the enzymatic and vitamin-forming activities. To do without them would not be just hard, but next to impossible. At the same time, many representatives of our normal microflora are certainly agents of evil, either all the time or under certain conditions. These include staphylococci, streptococci, bacillus intestine, the pathogens of gas gangrene, and viruses.

The processes of circulation and elimination of microorganisms in a sealed "vial", ship capsule, will differ from those in normal Earth conditions. They will bring about changes in the microbial associations of the air, capsule surfaces, and human body. The changes in familiar microbial communities, individual for any person, can also result from the close contact between astronauts in the air-tight space. This poses a previously unknown problem on infecting one man with the microorganisms harmless for another. These can cause various pathological states in the first man.

The data of Soviet researchers on the long-term life of the people in hermetic spaces simulating flight conditions have recently been published. They showed markedly increased quantities of microorganisms, including pathogenic ones, both in the surrounding and in the human body.

Thus, the conditions of long-term space flights provide for changes in the normal microflora of the astronauts' bodies and their surroundings. The changes in common microbial associations and excessive accumulation of certain bacterial forms are expected. Mutations induced by ionizing irradiations seem to change the properties of the microorganisms as well.

Immunology is concerned with which microorganism species will dominate these new associations, and which types will be predominant within these species. Which of them might appear to be most likely and frequent pathogens? These questions are posed not only to satisfy scientific inquisitiveness. Their answers should indicate the pathogens against which the astronauts are to be vaccinated before the flight.

Another question for space immunology is how long-term space flight influences the organism's resistance to pathogens, including those belonging to usual microflora of the human body. The point is that in spaceships man will be affected by new, long-acting factors, such as weightlessness or artificial gravitation, special diet and artificial atmosphere, forced limitations of mobility, space radiation, and others. No one yet knows how these oddities will affect his immune system.

The basic approach to solving these problems is to study how these unusual conditions of space flights would affect the immunity by simulating them on Earth. The researchers try to discover how effective vaccination will be and reveal the mechanism through which these conditions will affect the basic immune processes. Space immu-

nology should not only solve these problems, but also find new ways to prevent the possible complications.

Although it is no less important than the others, the third question seems to be more pertinent to science fiction than immunology. In time it may become a principal problem of space immunology. I mean of course man's possible encounter with alternative life forms. By embarking on space flight we are setting off into an unknown world. Nobody knows what will happen in the next flight or, especially, the next visit to another planet.

Although writers are interested in contacts with reasoning creatures, immunologists are primarily concerned with encounters with microorganisms. But contact with microorganisms might be much more fantastic and unusual in their results so that the writers will regret their missed opportunities. Unknown microorganisms might help eradicate disease or even cause men to glow in the dark! This is the first which comes to mind, and using our brains we can come to some even more striking and enticing ideas.

Microorganisms will most possible be the first aborigines we'll meet. This meeting will take place sooner or later. The problems ensuing from this meeting will be most pertinent to exobiology, the science of life beyond our planet. Immunology is most interested in what happens when a man from Earth faces an entirely strange microorganism. Will the human body display the same resistance to these alien microorganisms as it does to its own Earthly ones? This is the basic question.

Immunity as a means of protecting the organism resulted from life's evolution in the specific conditions of Earth. An immune response is directed at either rejecting or neutralizing everything alien penetrating the body, such as viruses, bacteria, animal cells, tissues, and proteins. But an immune reaction is only induced if the incoming bodies (living or dead) are recognized to be alien.

The first task of our bodies' protective forces is to distinguish between "one's own" and "someone else's". Any cell or its product is considered as alien and triggers an immune response only if they bear genetically foreign information. This requires them to be built on molecules evolutionally familiar to the immune mechanisms, and their signs of alienness to be written in an Earthly "script".

The degree of immunity's universality is unknown. If extraterrestrial microorganisms and their metabolic products bear no chemical groupings identifiable as alien by our immune mechanisms, if they are not recognized and do not trigger immune responses, strange microorganisms will multiply unabated in man's blood and tissues. What happens then?

Let us once again come back to H. G. Wells's "The War of the Worlds". The invaders from Mars die from "harmless" Earthly bacteria. Today Wells's fantasy has become a real scientific problem. Even now immunology has facts that are alarming in this respect. As they say, immunology has already "got the signal"

What is quite clear now is that immunity is stimulated by alien substances, or antigens.

Scientists have synthesized very big polypeptide molecules consisting of the basic protein components, amino acids. At a certain size these artificial polypeptide molecules become antigens. The only necessary condition is that they must be constructed of amino acids optically similar to those constructing all living matter on Earth. These are amino acids that deflect a plane of polarized light to the left; they are called levorotatory isomers.

Dextrorotatory compounds are absolutely identical to levorotatory ones in their chemical structure, except that only one group is located at a different angle to the entire molecule. This is sufficient for a complex organic substance not to be taken as alien, not to stimulate immune responses. An Earthly organism based on levorotatory compounds fails to recognize (or does it very imperfectly) an alien substance composed of dextrorotatory amino acids. What, then, if the microorganisms of other worlds are based on dextrorotatory compounds? Will our immunity appear to be helpless against them?

The task of space immunology in this area is extremely complex and interesting. It includes the simulation of possible responses by mammals to various natural and artificial high polymer compounds. Irrespective of the form extraterrestrial life might take, it must always be based on high polymer compounds. The search for ways to stimulate immunity against unusual polymers, ways for transforming non-antigen compounds into antigens, and immunological studies of space objects—these are the areas for study by space immunology.

The Fathers of Immunology

Edward Jenner

A scientist usually does not know whether or not his concept is correct, whether or not his idea will be confirmed. Nevertheless he works, he believes in his concept, he believes in the idea.

Confidence generates resolution. But not only resolution to years of scientific research. Sometimes it is focused in one culmination point.

Edward Jenner was born more than two hundred years ago, in Berkley, a village of Gloucester country, in England.

When he was 21, the young doctor took seriously the popular belief that once someone has suffered from quite innocent cow pox, he would never get natural, or the so-called "black", smallpox which, in London alone, killed between one and three thousand people every year.

Jenner believed in the popular talk. This belief ripened for 26 years. For 26 years he had been observing and comparing facts. He had less and less doubts that people, most often milkmaids, who once suffered from cow pox, really never caught smallpox.

Jenner was 47.

On May 14, 1796 the doctor and scientist Edward Jenner performed an experiment which delivered mankind from smallpox, and made him the forefather of a new science—immunology. Once sure that he was right, the scientist experimented on man.

A peasant, Sarah Nelms by name, got cow pox,

and several typical bubbles appeared on her hand. The contents of one of these bubbles was to be injected by Jenner into an eight-year-old boy, James Fipps. But this was not all. Afterwards the boy was to be infected with real "black" smallpox. If the scientist was mistaken, the boy would die, and Jenner himself would not be able to live any more....

Was he sure enough? Were there enough proofs confirming the idea? What's a pity that the experiment could not be staged on the scientist himself! A person who had never before been in contact with smallpox patients was needed. Though, this was also an experiment on the scientist himself, if we recall how many opponents the anti-smallpox vaccine had in England in subsequent years.

Jenner wrote that to have more accurate observations over the course of infection, he chose a healthy boy (James Fipps), at the age of about eight years, to inoculate him with cow pox. He took the matter from the pustule on the hand of a milkmaid (Sarah Nelms) who got cow pox from her master's cows and inoculated this matter into the boy's hand on May 14, 1796 by means of two surface cuts, hardly penetrating through the skin layer, each half an inch long. On the seventh day the boy complained of pain in his armpit, and on the ninth day he got some fever, lost his appetite, and felt a slight headache. The next day he was quite well. All the signs of the disease disappeared, leaving scabs and slight scars at the point of inoculation, but otherwise not troubling Jenner or his patient. He made sure that the boy he had experimented with was

protected from being infected with real smallpox after the slight illness caused by the cow pox poison. On July 1 of the same year Jenner inoculated him with human smallpox taken directly from a smallpox pustule. Several slight pricks and cuts were made on both his hands, and the matter was thoroughly rubbed into them. No detectable illness followed.

The decisive experiment, the climax of the idea, was a success. As a result of a safe inoculation the little Fipps became non-susceptible to one of the most terrible diseases, to smallpox. This inoculation was called vaccination, from the Latin word "vacca" which means "cow" The term was accepted, and since then any preventive inoculation of a pathogenic agent is called a vaccination, even though the vaccine can be derived from the brain of an infected rabbit as for rabies, or from murine lung tissue as for exanthematic typhus.

The scientist's confidence generated resolve. The scientist's resolve led to a discovery. Shall we stress the word "scientist's"? Yes, we shall. Confidence and resolve of an ignoramus can lead to nonsense at best, and to tragedy at worst. The confidence of a scientist is a belief based on long-term observations, comparisons, and accurate knowledge. The belief of a scientist based on the strict arguments of reason is a great creative force.

Shall I explain what those days and nights spent observing the boy meant for Jenner? Shall I describe the joy which came in the end?

Edward Jenner came to love the boy as his

own son. If, in the final analysis, Jenner was an active creator of the discovery, then the boy was a co-author, though he did not even know how he helped or what he risked.

But the active creator knew and never forgot it. He loved the boy, loved the co-author. He loved his child, his embodied idea.

Louis Pasteur

Jenner's discovery, however, did not give rise to a new science. This was an observation of genius, ahead of his time by about 100 years. But it only gave mankind a means to prevent smallpox.

To be sure, that was a great gift, and humanity thanked the great Englishman even in his lifetime. His way of preventing smallpox was recognized and spread throughout many countries. The London Medical Society made a large gold medal in honour of Jenner. He was awarded £10 thousand, and then £20 thousand by British Parliament. Jenner became an honorable citizen of London. Russian Empress Elizabeth, the wife of Alexander I, endowed him with a finger-ring with a large diamond. The first vaccinated Russian child, Anton Petrov, was given the family name Vaccinov and brought up at the expense of the state. In France, Napoleon Bonaparte officially promoted vaccination against smallpox, making it obligatory in his army. They say that Napoleon was once asked to release an English captive. "Jenner asks for him," remarked Josephine. "Oh, Jenner!" Napoleon exclaimed, "To Jenner I can't deny anything."

So, Jenner taught mankind not to be afraid of smallpox. But neither he nor medical science of that time developed a general method for preventing infectious diseases. There was no teaching, no theory.

The science needed to mature. Mankind needed to learn something more. Finally, Louis Pasteur needed to be born. 85 years after Jenner's discovery, he created the science of immunology and developed the principles needed to produce vaccines against any infection.

There is a memorial board on a building in Paris. It contains the landmarks of Louis Pasteur's discoveries.

"The laboratory of Louis Pasteur was here.

1857. Fermentation.
1860. Spontaneous generation.
1865. The diseases of wine and beer.
1868. The diseases of silkworms.
1881. Infection and vaccine.
1885. Rabies prevention."

1881 was the year when immunology was born. Again, everything started when a scientist believed a conjecture he glimpsed as a result of his studies, when he believed himself.

On the surface of it, the discovery came by chance. One needed the genius of Pasteur to do what seemed to be "a trifle": to notice, to check, and then to come to a deep belief in the universality of a principle.

1880. Pasteur studies chicken cholera. Hens have a special cholera, safe for humans. The microorganism living in the test tubes unfailing for

infecting the experimental birds. Death came in a day or two. Over a period of holidays the work was ceased, and the tubes were left in a thermostat with free access to air. When, three weeks later, the microorganisms from these tubes were used to infect the hens, the birds fell ill... but did not die. The failure was to be corrected; in several days the birds were infected with fresh microorganisms.

The birds did not even fall ill!

This seemingly unsuccessful experiment gave rise to a generalized idea of Pasteur. He checked what he had noticed and came to believe deeply in the universality of the principle: if the microorganisms' toxicity, or their ability to cause disease and death, is decreased, they turn into a preparation that protects against this disease. The scientist believed, though at the time he said when answering questions: "I can't say anything, I do not dare to formulate outloud all I hope will happen." As he was saying this, he was creating a new vaccine according to this new idea. This time it was not against chicken cholera, but against anthrax, which strikes both animals and humans. He prepared this vaccine by creating "terrible life conditions" for anthrax bacilli, which had been kept heated for a long time.

When the vaccine against anthrax was ready, Louis Pasteur, who was quite sure in the success, ventured a public experiment.

Louis Pasteur was great at public lectures. He could cause tears on the eyes of his listeners, he would intimidate his audience, and then show the way to salvation. He arranged scientific even-

ings, to which he invited Alexandre Dumas, George Sand, and other prominent people and high rank grandees. Pasteur would pierce the darkness of the hall with a beam of light, and, pointing at dancing specks of dust, spoke about myriads of microorganisms bearing illnesses and death. He knew the ways to stir journalists, intellectuals, snobs, bourgeois, and young people.

It is more difficult to stir scientists, especially the die-hard members of French Academy of Sciences. Not every scientist who had achieved success and took an academician's chair is inclined to accept anything new, especially something frightfully new. To add to this, it was difficult for strict, pedantic scientists to accept the ideas that were down on them by the vehement, unbelievably convinced Pasteur. But he was a genius. He was almost always right. He could have been carried away by the idea, but he never devised things from thin air.

The French Academy of Sciences was already aware of anthrax vaccine. Pasteur had reported his discovery on February 28, 1881. As usual, most of the listeners were not too enthusiastic about the new idea. But Pasteur promised a public experiment. It was decided to check his ideas, his work, and his vaccine at a cattle-breeding farm in Pouilly-le-Fort. Pasteur put his discovery to the judgement of scientists, and not only to them, but also to a crowd of dignitaries, journalists, and laymen.

This experiment, one of the most dangerous of those staged by Pasteur, was held in May 1881.

What if it would have failed? Then Pasteur's laboratory would have instantly lost its grants. It would have been difficult for him to continue his work. And this with the as yet unstarted struggle against rabies before him. He himself did not yet know what was at stake. He did not yet know what he was going to do next. But we now know what he risked. His most dramatic works were still ahead of him. But the venturesome Pasteur came to believe in his idea, tested it in the laboratory, and this gave rise to a resolve. What he was speaking about to the scientists in the Academy, plainly and without any decorations, was the principle underlying it all: the immunity.

The lecture in the Academy was not just a paper on developing vaccines against chicken cholera and anthrax. The lecture reported on the universal principle for creating artificial immunity by introducing a weakened pathogen of a disease to which non-susceptibility was to be developed. That is why the public experiment was something more than a mere testing of vaccine against anthrax. The fate of the newborn science of immunity was at stake. Many scientists in the Academy did not approve of Pasteur's decision, and reproached him of excessive self-confidence.

Still, one can imagine the burden of doubts, the strength of resoluteness, and the depth of belief experienced by Pasteur in those remarkable days.

In the beginning of May 1881, 30 sheep and 5 cows were vaccinated at the Pouilly-le-Fort

farm. The same number of animals constituted the control group. On May 31 all 70 animals were infected with anthrax. The experiment was performed in the presence of doctors, scientists, statesmen, and journalists. Two days later Pasteur and his guests were at the farm again.

All the control animals died. All the vaccinated ones survived.

Pasteur had predicted the results of the experiment beforehand. He had no doubts in the outcome of the trial.

Despite the laws of honour of that time, Pasteur would evade duels even when he insulted first. At the same time, he bravely conducted a seemingly adventurous, advertising experiment. This needs quite a bit more courage than a duel.

Pasteur discovered a general principle for stimulating immunity using vaccines. Mankind was spared a number of contagious diseases. But he did not know why the vaccination prevented a disease, what processes occurred in the body, which systems were triggered, how an organism protected itself, what were the mechanisms of immunity. He had the naive idea that when weakened microorganisms are first introduced they "eat out" nutrients needed for this very bacterial species. When the microorganisms get into a body for the second time, they have nothing to eat, so they die, and the infection does not develop. According to Pasteur, it was not the body that responded to the infection, not the immune system that worked to protect the body, but the microorganism that "ate too much" preventing the disease from being spread in the body.

Ilya Mechnikov and Paul Ehrlich

"From the most ancient to modern times it was undoubted that our body has a certain ability to react against any harmful agents entering it from the outside. This ability was given different names. Studies by Mechnikov show with certainty that this ability depends on the properties of phagocytes, mainly white blood bodies and connective-tissue cells, to eat up microscopic organisms getting into the body." This was the account of the report presented by Ilya Mechnikov in the Society of Kiev Doctors on January 21, 1884, given in the journal *Russian Medicine.*

Can we consider the day of this report the birthdate of the first scientifically founded theory explaining the mechanisms of non-susceptibility to infectious diseases?

We certainly can't. The report only formulated the thought which had appeared in the scientist's mind long before, as he worked. Certain elements of the phagocyte theory had been made public in his earlier papers and reports. What we can do is to call this the birthdate of the great discussion on theory of immunity.

The discussion lasted for 15 years. It was a severe war, in which the colours of one viewpoint were on the banner held by Mechnikov. The other banner's colours were fought for by such great knights of bacteriology as Behring, Pfeiffer, Koch, and Emmerich. This army was headed by Paul Ehrlich, the author of a fundamentally different theory of immunity.

The theories by Mechnikov and Ehrlich excluded each other. The opponents crossed their

swords at the conferences and congresses, on the pages of journals and books. Their swords were facts and only facts.

The idea appeared all of a sudden, at night Mechnikov was sitting alone over his microscope and observed the life of mobile cells in the bodies of transparent sea star larvae. He would recall later that the thought had dawned upon him that very evening, when his family went to the circus and he stayed at home alone to work. It suddenly occurred to him that these mobile cells were related to the organism's protection. (Maybe, this instant should be considered the "instant of birth".)

Dozens of experiments followed. Foreign particles, such as splinters, paint grains, and bacteria, were shown to be captured by the mobile cells. Through the microscope it could be seen how the cells were gathering round unbidden visitors. Part of a cell stretched out to form a false pedicle, a "pseudopodium" in Latin. Foreign particles were embraced by the pseudopodia, and brought into the cells, as though eaten by them. This is why Mechnikov called these cells phagocytes, or cell-devourers.

The scientist found these cells in many various animals: in sea stars and worms, in frogs and rabbits, and certainly in man. All representatives of the animal realm have specialized phagocyte cells in almost all their tissues and blood.

Bacterial phagocytosis is surely a most interesting process.

The scientist administered anthrax pathogens to the tissues of a frog. Phagocytes gathered round the point where microorganisms were ad-

ministered. Each captured one, two or even ten bacilli. The cells ate up these bacilli, and digested them.

That's it, then, the mysterious mechanism of non-susceptibility! This is how the fight proceeds with the pathogens of infectious diseases. Now it is clear why one falls ill during, say, cholera epidemics (and not only cholera), and another does not. So, the basic thing is the quantity and activity of phagocytes.

At the same time, in the beginning of the 1980s, European, especially German, scientists gave a somewhat different explanation to the immunity mechanism. They believed that the microorganisms getting into the body are destroyed not by the cells, but by special substances present in the blood and other body fluids. Their concept got the name "humoral", or liquid theory of immunity.

And the great debate started.

1887. The International Hygienic Congress in Vienna. Mechnikov's phagocytes and his theory are mentioned in passing, as something quite implausible. A Munich bacteriologist, a student of a hygienist Max Pettenkoffer, Rudolph Emmerich reports to have administered to immune, that is prevaccinated pigs, the pathogen for measles, and the bacteria died within an hour, without any interference of phagocytes which during this time did not even manage to "drift up" to the microorganisms.

What Mechnikov did was to reproduce Emmerich's experiment. The Munich colleague was shown to be mistaken. The microorganisms were alive for as long as four hours. Mechnikov in-

formed Emmerich of the results of his experiments.

Emmerich repeated his experiments and realized his mistake. Measles pathogens die in 8-10 hours, which is exactly the time needed for phagocytes to do their job. In 1891 Emmerich admitted his mistake in public.

1891. The next International Hygienic Congress, this time in London. Emil Behring, also a German bacteriologist, joins the discussion. His name will remain in our memory forever. It is connected with a discovery which saved millions of lives. Behring created anti-diphtheria serum.

A follower of the humoral immunity theory, Behring made a very logical assumption. If an animal had once suffered from some infectious disease and developed immunity, then its blood serum, the cell-free part of blood, would enhance its bactericidal properties. If this was right, people could be given weakened microorganisms or pathogens in low quantities.

If this type of immunity could be obtained artificially, then the serum derived from such an animal should kill the corresponding microorganisms. Behring made anti-tetanus serum. To produce this serum, he administered the poison of tetanus bacilli to rabbits in progressively increasing doses. Then the strength of this serum was tested. A rat, rabbit, or mouse was infected with tetanus and then treated with anti-tetanus serum, that is blood serum of an immunized rabbit.

No disease developed when this was done. The animals survived. Behring did the same thing with diphtheria bacilli. This is the way to treat diphtheria in children, used even now. This was

what Behring was awarded a Nobel prize for in 1901.

But what does it all have to do with the "devourer" cells? The substance administered was serum, the cell-free part of blood, and this serum helped to fight the microorganisms. No cells, no phagocytes were put into the body yet it still got some "weapons against microorganisms" Consequently, it has nothing to do with cells. There is something in the cell-free part of blood. If so, the humoral theory was true, and phagocytal untrue.

This induced Mechnikov to new work. Again, he answered with experiments. His results indicated that it was not serum that kills diphtheria and tetanus pathogens. The serum detoxicates toxins, the poisons secreted by these bacilli, and stimulates phagocytes. Activated with the serum, phagocytes easily do away with the disarmed bacteria, whose poisonous secretions are neutralized with antitoxins present in the same serum.

The two theories started to draw together. Mechnikov was presenting new convincing proofs that the basic role in the fight with microorganisms is played by phagocytes, since, in the final analysis, it is the phagocyte which makes the decisive step and devours microorganisms. However, Mechnikov had to finally accept certain elements of humoral theory.

Humoral mechanisms do take part in the fight with microorganisms. After Behring's studies everyone had to admit that the body's contact with microorganisms results in accumulating antibodies that circulate in the blood. (A new concept of the antibody appeared, which will be dis-

cussed in detail later.) Affected by antibodies, certain microorganisms, such as cholera vibrions, are killed and dissolved.

Does this cancel the cell theory? By no means. The point is that, just as every agent produced by the organism, antibodies must be generated by cells. And, certainly, phagocytes do the basic job of capturing and destroying the bacteria.

1894, Budapest, the new International congress, and, again, vehement polemics of Mechnikov, this time with Paul Pfeiffer. Different cities, different subjects. The discussion led further into the deep, complex interrelations between animals and microorganisms.

The intensity of the debate, the passion, and the heat of the polemics remained the same. Ten years later, celebrating Mechnikov's anniversary, Emil Roux recalled these days:

"Even now I clearly see you as you were at the Budapest congress, objecting to your opponents. Your face was burning, your eyes gleaming, and your hair all tangled. You looked like a demon of science, but your words, your irrefutable proofs caused the applause of the audience. New facts, which first seemed to contradict phagocytal theory, soon appeared to harmoniously match it."

This was the debate. Who won it? Everybody. The Mechnikov theory became orderly and all-embracing. Humoral theory found its basic active factors, the antibodies. In 1901, having unified and analysed the data of humoral theory, Paul Ehrlich developed the theory of generating antibodies.

15 years of debate. 15 years of mutual denials

and refinements. 15 years of argument and mutual assistance.

1908. The highest recognition of a scientist, the Nobel Prize, is given to Ilya Mechnikov, the creator of phagocytal theory, and Paul Ehrlich, the creator of antibody formation theory, that is the humoral part of the general immunity theory.

Mechnikov and Ehrlich developed the theory of immunity. They argued and won. Everyone appeared to be right, even those who had seemed to be wrong. After all, who won this argument? Science did. Mankind did. In a scientific argument everyone is a winner.

The Army of Immunity

The Soldiers and the Weapons

What are the weapons and who are the soldiers in the invincible army of immunity? No denying it, invincible is the word. Don't give as examples the terrifying and devastating epidemics of "black death" (plague) in Western Europe in the 14th century, or cholera, which left India in 1823 to march through all Europe and America, or influenza, which killed about 20 million people in 1918-1919 and is not tamed even now. They all happened. And still, the army of immunity is basically invincible.

Each death resulting from infection is a victory for the disease pathogens (those of plague, smallpox or influenza) over the immunity system of a person. Each healing is a victory for immunity. The history of life on Earth is a chronicle of the struggle between living organisms and

disease pathogens. Species whose army of immunity was not reliable enough have died away. It is this invincible army that protected the survivors. If it were not for it, there would have been no animals, nor people on Earth, only microorganisms.

In every epidemic there were some people who did survive. The pathogens surrendered, and the immunity army left each battle armed with new weapons against a specific microorganism, against a very specific disease.

Certain individuals can be defeated, but the immunity army as a whole is invincible. And what about these individuals? Well, there's nothing to be done: "A la guerre comme a la guerre", war is war. But let us get back to science. Every manifestation of life is somehow linked with its basis, that is with the cell. There are a lot of cells in an organism. Man consists of about 10 000 000 000 000 different cells (or, as it is commonly put in more accurate sciences, 10^{13}). Each of these cells has its own duties. Like in our own life, one grows corn, others extract coal, or make clothes, some cells digest food, others transport oxygen or build skin integuments. Their duties are strictly divided.

Special cells are gathered in small glands to produce saliva; still smaller ones generate tears. Special organs make sexual cells unique in their properties, which contain all the information coded in a special way. It controls the development of the would-be organism by repeating all the basic features of the parents.

All cells can resist microorganisms, but to varying extents. Like in a state, all its population

is in this or that way capable of resisting enemies. But we all know that this is not enough. The state keeps special troops. Something similar holds true for the body.

All the body cells have substances capable of killing or inhibiting the multiplication of microorganisms. For instance, the cells secrete, say, saliva and tears, and at the same time produce a substance capable of dissolving microorganisms. This substance is called lysozyme. Blood also contains antimicrobial substances. One of them is called complement. Skin secretions can also kill bacteria. To demonstrate the bactericidal properties of skin, a suspension of some microorganism culture is applied to clean skin and in 10-15 minutes the number of microorganisms becomes dozens of times lower as compared to the initial level. These antimicrobial properties are related to the natural content of specific substances in the body fluids.

Unfortunately the humoral (or fluid) factor of natural immunity is not so strong as the weapons. Many microorganisms are not affected by either lysozyme or complement, feeding perfectly on the skin and multiplying in the blood.

Special "troops" are needed to combat these microorganisms.

The soldiers of immunity who protect our organism from microorganisms are the ubiquitous cells already known to us by the common name of phagocytes. "Phagos" is Greek for "devouring". Phagocytes can be found everywhere: in circulation, in blood vessel walls, in the lungs and liver, in subcutaneous connective tissue. The troops protecting us, phagocytes, are always in

the number one combat readiness state in every corner of our body. They are different in their form and size: some are mobile and can move in liquids and tissues, penetrate through vascular walls as some spectres from fairy tales; others are attached to a certain spot and fight to the last man. Some are 5 to 8, others 15 to 20 microns. They have one common property, phagocyte activity: they devour, by capturing and digesting foreign particles, including, most importantly, bacteria.

So, phagocytes are divided into two big groups, free and fixed ones. In other words, those that wander and those that stand at the same place. The free ones include white blood cells, leucocytes, and certain connective tissue cells which in an alert immediately head for the foreign stimulus. These connective tissue cells got the name "macrophages" meaning "big phagocytes".

Not all macrophages, however, are capable of wandering. All organs contain immobile, fixed phagocytes. There are many of them especially in the spleen, liver, lympth nodes, bone marrow, and vascular cells. The cells of the first group attack the enemy that has penetrated inside. The second wait till the enemy floats by in the blood or lymph flow. They lurk in ambush as knights who bar the way of the enemy hordes.

They stand on the routes which cannot be bypassed by anything getting into blood. If you administer several dozens or hundred million microorganisms into an animal's circulation, you'll find none of them after several hours. They will all be captured by the phagocytes of the liver, spleen, and other organs. If the bacteria are ad-

ministered subcutaneously, a host of blood leucocytes and mobile macrophages from the adjacent tissues can be observed to start for the hotbed of infection, surround it, and start the battle. The analogy with troops is quite complete. But the important thing is that the immune troops wage only a defensive war, only on their own territory.

There are special plasmatic cells in the immunological troops. They are the basic arms factory —the antibody factory. There are few of them. But when microorganisms get into the blood and the organism's tissues, these cells grow rapidly in number. Plasmatic cells originate from their precursors, lymphocytes, to which dozens and dozens of pages of this book will be devoted.

Antibodies display an amazing ability to bind to the very microorganism in response to which they were generated, and not to anything else. A rabbit infected with a pathogen of human cholera will not die, since this microorganism is not lethal for him. In a few days molecules of serum protein capable of binding with cholera vibrion will appear in the rabbit's blood. These are antibodies.

An antibody binding with a microorganism can be seen in the following way. Blood is taken from rabbits, and, when it is coagulated, the serum is removed with a pipet. Cholera pathogens are then added to it. Antibodies bind the vibrions and glue them together. The flakes of glued microorganisms settle down to the bottom of the tube, and are then dissolved by the antibodies attached to them. This can also be seen without any device, when a previously turbid, microbial suspension becomes transparent. No

matter what other microorganisms we choose, they will not be affected by these antibodies, they will not be glued together and dissolved.

If diphtheria bacillus toxin is administered to a rabbit, into the blood, subcutaneously or intramuscularly, diphtheria antitoxins appear in the serum. Added to the toxin of diphtheria pathogen, this serum will neutralize its poisonous properties. This is done by the antibodies against diphtheria toxin that appeared in the rabbit's blood, but only against diphtheria toxin, and nothing else. This is the specificity of immunity. Special weapons are produced against each aggressor.

There are thousands upon thousands of antibodies that fight against the most diverse alien agents which have penetrated or which are going to penetrate an organism. They are "floating" in the blood of every animal and human. Antibodies account for about one percent of a body's weight. This means 10^{20} protein molecules! 10^{20} units of weapons. This is an astronomical figure.

The Construction of the Basic Weapons

The structure of an antibody molecule was decoded by two researchers, Rodney Porter at Oxford and Gerald Edelman in New York. The first results were published in 1959. By 1965 the molecular structure was in general decoded. By 1970 immunologists knew not only the general design, but also the sequence of "bricks" (amino acids that constitute every protein molecule). In 1972 Porter and Edelman were awarded the Nobel Prize.

The course of events flowed like this. In 1958

Porter isolated the protein of antibodies from blood. This protein is called immunoglobulin. The researcher treated pure immunoglobulin with papain, a plant enzyme that breaks up proteins. It is capable of cross-cutting protein molecules.

At the same time, across the Atlantic, "simultaneously and independently", as they say in science, Edelman treated the immunoglobulin molecules isolated from blood with 6-mercaptoethanol, which could longitudinally cut the protein molecules. (Papain saws protein trunks into firewood, and 6-mercaptoethanol into planks.)

Let us digress here from antibodies to remember how proteins are constructed, what they are based on.

The structure of all proteins is based on peptide chains. A protein can be composed of several of these chains found in sequence or parallel to each other. The links of each chain are amino acids. Take, for example, a piece of the peptide chain of insulin, a well-studied protein, whose lack causes the severe disease, diabetes. It has the sequence cysteine-alanine-serine-valine-cysteine. Polypeptide chains are composed of various combinations of 20 amino acids, and they form the entire multitude of proteins on our planet.

Amino acids are bound into peptide chains by carbon and nitrogen atoms. These are called peptide bonds, and it is they that are broken by papain. Not all of them, of course, are broken at once; the first to be affected lie in most accessible sites of the molecule.

If the peptide chains composing a protein molecule are located as two strands parallel to each

other, they are connected by two sulphur atoms. These are called disulphide bonds. They are broken by 6-mercaptoethanol. This results in longitudinal cutting of molecules comprised of parallel peptide chains.

So, Porter split the antibody molecule crosswise and Edelman lengthwise.

The molecular weight of the whole molecule was somewhat above 150 thousand. Longitudinal splitting yielded three fragments each with a molecular weight of about 50 thousand. Porter obtained three fragments of about equal size. He designated them as I, II, and III. Their size was about equal, but their properties....

Fragments I and II appeared to be identical to each other. Each of them displayed the main property of antibodies, the ability to bind an antigen, the alien substance against which this antibody is directed. Fragment III lacked this quality.

Edelman got four fragments, or, rather, four chains, since he divided the protein molecule into peptide chains. Two chains were identical to each other and had a molecular weight of about 25 thousand. He called them L-chains (from the word "light"). The two others were also identical and had the weight of 50 thousand. He called them H-chains (from the word "heavy"). Neither of these chains had the basic quality of antibodies to bind antigens. However, an H-chain rebound with an L-chain made a structure, representing half a molecule, and restored the binding ability.

So the researchers faced a problem requiring quick wits.

Given: a crosswise cutting of the molecule results in 3 fragments. Denoting molecular weight in thousands as a subscript, and antibody activity with an asterisk as a superscript, we come to the equation of antibody structure:

$$AB^{*}_{150} = I^{*}_{50} + II^{*}_{50} + III_{50}$$

Lengthwise cutting results in 4 fragments with the equation:

$$AB^{*}_{150} = 2L_{25} + 2H_{50} = (L_{25} + H_{50})^{*} + (L_{25} + H_{50})^{*}$$

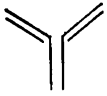
Required: determine the spatial structure of the peptide chains in the molecule, and localize the active centres: the sites where antigens are bound. Let's simplify the problem still further. From two short and two long chains compose a figure which, after crosscutting, would give three equal fragments, two of them bearing specific antigen-binding sites composed of a short and a long chain.

The resulting construction will look like a capital Y, something like a boys' slingshot. The places where a piece of elastic is attached are the active centres. The two sides are Porter fragments I and II, and the handle, fragment III. Papain cuts the construction straight at the fork point.

Two long chains located next to each other form the handle, and in the fork point they make up the inner sides of the slingshot. Short chains adjoin the long ones after the fork, forming the outer sides of the slingshot. Its end points, composed of the terminals of both type chains, determine the molecule's specificity. So, each antibody has two active centres. Like with two hands, it binds alien antigen particles, making

them inactive, insoluble, incapable of exerting adverse effects on the organism.

This construction is based not only on logical speculations. It is confirmed by special physico-chemical methods. Finally, it was seen under electron microscope. An antibody molecule really looks like this:



Sometimes two antibody molecules are bound together at their "handles" and then they are called dimers. Thus, they have four active centres to bind antigens. This is how class A immunoglobulins behave. Other molecules are united by five (pentamers), forming a star with ten active centres looking outside. These are class M immunoglobulins. Most antibodies, though, are the usual monomer type and are called class G immunoglobulins.

By 1970 the antibody structure was understood not only in general but the actual number of amino acids in each of the four chains was determined.

The light chains of human immunoglobulins turned out to be composed of 214 amino acids each, while the heavy ones contained 428 amino acids. A molecule of the most widespread class G is composed of 1284 amino acids. Not all of them form each of the two active centres of the molecule. Specific sites, by which the molecule recognizes alien antigen and binds it, are formed by no more than a dozen amino acids. However, to construct them in a spatially correct way, first

107 amino acids from each of the four chains are used. They are located on the ends of these chains. These chain sites are called variables, since their amino acid sequence varies for different molecules. Every antigen is characterized by its sequence. These are the sites used for recognizing alien subtances, firmly binding them, and thereby preventing them from harming the organism.

Specialized Troops—Lymphocytes

In 1948, the Swedish researcher Astrid Fagräus suggested that antibodies are produced by plasmatic cells, which are so called due to their high protoplasma content. In 1956 an American immunologist Albert Coons proved that the suggestion by Fagräus was true. The origin of plasmatic cells was still not known.

The opinions in this point varied greatly. It was assumed that, once having swallowed up a microorganism or an alien substance, a macrophage turns into an antibody producer, or plasmatic cell. Had this been confirmed, macrophages would have been titled the principal cell of the immune system: both a devourer of enemies and a forger of weapons. But this was not confirmed, and soon the basic specialized arms of the cell service, the lymphocytes, were discovered. They are the ones that can decode the details of alien substances, and, having transformed into plasmatic cells, start intensive antibody synthesis. They can do many other things as well.

All blood cells, except for red blood cells that transport oxygen, are white in color. They are

called leucocytes, that is white blood cells. 30 per cent of them are accounted for lymphocytes, which means "lymph cells"

Along with blood, lymph circulates in all the tissues of our body. From lymph vessels, it goes to lymph nodes, and from there to one large vessel, the thoracic duct, which falls into the blood alveus just near the heart. There are no red blood cells in the lymph system, only white blood cells, or lymphocytes.

Three hundred years ago a famous Dutchman, Anton Leuwenhoek, designed the first microscope. The first objects he observed were a drop of rain water and a drop of blood. He discovered red blood balls, or erythrocytes, which comprise the main bulk of blood cells. Less than a hundred years passed, and white blood cells (leucocytes) were discovered. Their content is one thousandth that of red blood cells, but they are also very numerous. One gram of blood contains 4-5 billion red blood cells and 6-8 million white blood cells.

Leucocytes are divided into two basic groups. The first group of cells, leucocytes proper, account for 2/3 of the whole population; they are characterized by segmented (in contrast to round) nuclei. The cells of the second group, which were called lymphocytes, have perfectly round nuclei, occupying most of the cell space.

At the end of the last century, Mechnikov found out that leucocytes protect the organism by devouring alien particles. In contrast to big tissue phagocytes, macrophages, he called them small phagocytes, microphages. However, lymphocytes'

duties in the organism became known only about 15 years ago.

How easily we look through the book of history! Three hundred years ago the first blood cells, that is red blood cells, were discovered, two hundred years ago, leucocytes, one hundred years ago, lymphocytes. Strenuous work, search, inventiveness, debates, ten generations of researchers! And it all takes only half a page of printed text.

Every gram of blood contains two million lymphocytes. What is their job? This was the question hundreds of researchers asked themselves. Professor James Gowans at Oxford, who has done more than others to find out these cells' functions, quotes the words of a well-known pathologist, Arnold Ritch: "Lymphocytes are phlegmatic observers of the vigorous activity of phagocytes." This was one of the popular opinions. Indeed, they are very small cells, 6-8 microns in diameter, a bit bigger than their own nucleus (almost completely nucleus), with no active mobility of their own, which almost always gather round the inflammation locus, where phagocytes are working, devouring everything alien or dying off.

There was yet another opinion. Lymphocytes were thought to be a source of food for other cells. They were even called trophocytes, feeding cells.

Many scientists considered that lymphocytes acted as an origin for other cells, such as connective-tissue, liver, lung cells, and others. According to Gowans, "Old literature is full of conflicting proofs to the effect that small lymphocytes

can turn into erythrocytes, granulocytes, monocytes, fibroblasts, and plasmatic cells. As it was once cynically remarked, all the cells, but for the nervous system cells, were once considered as lymphocyte derivatives!"

Lymphocyte is indeed a mysterious cell that preserved its secret until the 1960s. The early 60s brought ample evidence that all specific immune reactions, that is antibody production, rejection of grafted tissues and organs, antiviral protection, and other functions, are performed by lymphocytes.

Let us try and look into this question using for an example the research by James Gowans. At that time he had a tiny laboratory at Oxford University. There is a small room with old dim windows, and a machine designed by Gowans himself stands at the table in the centre of the room. The main part of the machine is a plastic cylinder where a rat is ingenuously fixed. The rat has a cut on its throat, through which a thin transparent tube is going inside the body. Small white drops are continuously falling from the tube.

Dr. Gowans had introduced the tube into the main lymph vessel, the thoracic duct, and "pumps out" the lymph. He leaves the rat without any lymphocytes. After that he immunized it with alien cells, sheep red blood cells. The antibodies against sheep red blood cells should be produced. He examines the rat's blood, once, twice, three times.... No antibodies! Then he takes another "lymphocyte-free" rat and gives its lymphocytes back to it, immunizes it, and detects a normal level of antibodies.

This means that antibodies cannot be produced without lymphocytes.

The second trial. Gowans irradiates a rat with X-rays, which affect many systems, including the immune system. The animal fails to produce antibodies. The irradiated rat is given sheep red blood cells, and no antibodies are generated. Another irradiated rat is given sheep red blood cells together with lymphocytes from a healthy rat and produces antibodies.

So, lymphocytes give another body the ability to produce antibodies. They also transfer the memory of the antigen. If these cells are taken from an animal preimmunized with sheep red blood cells, they provide intensive antibody production in the irradiated animal, as though it were repeatedly immunized.

The third study concerned the mechanism of rejecting grafted, alien tissues. By the early 1960s it was well known that the first skin graft immunizes the organism, and the next scrap is rejected twice as soon as the first one. But why? It was thought to be the work of antibodies. But the blood serum from such an animal which contained antibodies, when introduced into another animal, does not accelerate the rejection of transplanted skin, while the lymphocytes accelerate it exactly two-fold. That means that it is lymphocytes that deal with the rejecting of grafted alien tissues, without any help of antibodies.

Lymphocytes that after their first contact with a foreign antigen are specially directed against this antigen got the name of sensitized lymphocytes. These cells and antibodies are the two basic types of specialized immunity weapons.

Killer Lymphocytes

In 1882 Robert Koch, one of the successful "microorganism hunters", discovered the pathogen for tuberculosis. Even now, this microorganism is called Koch bacilli.

To be sure, he tried to find a way to treat tuberculosis. Following the path paved by the great Pasteur, Koch cultured the bacillus he has discovered in a nutrient broth for a long time. In 6-8 weeks, when the culture grew old, he passed it through a filter that microorganisms could not penetrate to obtain a transparent liquid which he called tuberculin.

Administered subcutaneously to a healthy man, tuberculin causes no harm, except for slight temporary reddening. But in someone suffering from tuberculosis, this reddening will start to grow in 6 hours, after a day it will grow thicker, in two days it will become bigger and might ultimately result in pitting. Tuberculin is the only agent to cause such a response in a tuberculosis patient.

As a child, each of us was subjected to the Pirquet reaction to be tested for tuberculosis infection. This is a skin test with tuberculin. In 1907, a Vienne professor Clements Pirquet noticed that it is unnecessary to introduce tuberculin subcutaneously, it can be just rubbed into a small scratch, which is not at all painful.

The reaction is strictly specific, as all reactions in immunology. It was called the delayed type hypersensitivity reaction. It is not related to antibodies. Antibodies against tuberculin are not formed at all.

The scientists failed to explain the nature of tuberculin reaction for about 70 years. They only knew that lymphocytes head for the point where the tuberculosis bacilli filtrate is introduced, and form the inflammation causing thickening. In a way they prevent tuberculin from spreading all over the body. But this is true only for lymphocytes from a human or animal infected with tuberculosis; one who has already contacted with alien antigens of this microorganism, and triggered their immune system for specific resistance.

In the 1960s immunologists understood the gist of tuberculin and other like reactions. Similar tests are staged at brucellosis (the reaction to brucellin). The same tests appeared to be positive at grafting other organs and tissues. For instance, if a subject A has rejected the first graft, say, a piece of skin taken from a subject B, he will display a positive delayed hypersensitivity (tuberculin) type reaction, only not to tuberculin or brucellin, but to the filtrate from the skin of the subject B, and only B, no one else.

This demonstrates the specificity of the immune response.

Now remember one of the experiments by Dr. Gowans at Oxford. Delayed type hypersensitivity reactions can be transferred into a different organism if this organism is given lymphocytes from the first one. Lymphocyte not only triggers, but also effects this type of response.

There are specific receptors revealed on the surface of effector lymphocytes, which, like antibodies, detect alien antigen and bind it. Thus, lymphocyte acts as though it sticks onto an alien

object, be it a microorganism, grafted cell, or cancer cell. In contrast to antibodies, it not only keeps and binds these cells, but also secretes an enzyme that dissolves them. If need be, several lymphocytes, sometimes fifty or hundred of them, go for an alien cell. Sometimes they die to secrete as much lethal enzymes as possible, but kill the enemy.

Lymphocyte receptors demonstrate the great wisdom of Nature to use a universal structural design for different objects. These receptors are like immunoglobulins, kind of special immunoglobulins. They are often called T-immunoglobulins. They are closely linked with other structures on the cell surface, but, at the same time, they represent familiar boys' slingshots driven with their handles into the surface of lymphocyte. But the point is that they have not been drawn by blood; they have nothing to do with humoral immune response. The receptors are produced by lymphocytes themselves and constitute a part of their body. Part of the body of specialized cells which act as effectors performing the immune response of the second, cell type.

Since lymphocytes are armed with receptors against various alien cells and are capable of killing these cells, they were called killer lymphocytes. This is how they are termed in scientific papers: killers. So, it goes like this: "Cell-mediated immune response was shown to be characterized by the accumulation of killer lymphocytes", or: "The origin of killer lymphocytes was studied".

Just Discovered and Not Yet Discovered Islands

The Organ in the Organ

If you ask a geographer a naive question: "Are there any undiscovered islands?", you can easily imagine his negative answer. The last romantic hopes to discover an island unknown to mankind disappeared after sputniks took pictures of every secluded corner of Earth and applied a system of coordinates to them all. Anatomy is like geography, except that instead of Earth it describes the human body, its organs and tissues. Like geography, everything seems to have been discovered long ago in anatomy. The first works in anatomy with descriptions of the outer and inner organs of the human body are dated in the 5th century B.C. Anatomists have been studying the human body for thousands of years. All these organs are long known. Yet, nevertheless, the discovery of islands in the human body is still under way.

There was an interesting method of diagnosing, or identifying a disease, used by medicine of the last century. It was called *diagnosis ex juvantibus,* diagnosis by healing. It is used even now, when all the other methods fail. If a patient is ill, but his illness is unknown. If there are hundred analyses, but no diagnosis. What is to be done? A doctor suggests, say, that the patient is lacking vitamin A, and prescribes this vitamin. The illness is not cured; the diagnosis is wrong. A second suggestion: tuberculosis. Anti-

tuberculosis treatment is prescribed, and it helps! This means that the diagnosis was right.

This was also the principle for determining the organs' functions, which is used even now. Surely, the experiments are staged on animals. For instance, take away the thyroid gland and see what happens. The metabolism decreases, edemas develop. This means that the thyroid gland controls the metabolism and water balance. Remove the parathyroid glands, small "peas" near the thyroid gland, and the calcium level in blood falls, convulsions appear. Hence, calcium exchange is governed by these "peas".

One, however, should be careful with these conclusions, not to take them as in the joke:

"Where are cockroach's ears?" "In his legs."

"How did you prove it?" "He ran away from a shout, and when the legs were torn off, he stopped to run away, no matter how loudly we shouted."

Each organ has its own function, its own business. The heart pumps blood. The lungs process oxygen. The eyes see. The ears hear.

But sometimes it is different.

Let us remember. From the stomach food goes to the duodenum, which is small, only a dozen fingers long, but very important. The ducts from two organs flow into it like streams. On the right, there is the bile tract from the liver. On the left, the duct from the pancreas, bearing digestive juice that contains mainly the enzymes for protein digestion. Bile enzymes digest fats.

When a man eats a piece of meat, the ducts open, the liver enzymes get to the fats, and the pancreatic ones go to the proteins. It was thought

for many years that the pancreas has only one function, that is to produce digestive juice and to send it along the duct to the duodenum. This was thought up to 1889.

In 1889 German researchers Oscar Minkovski and Johann Mehring put a dog on an operation table and ectomized its pancreas. A day later the animal showed increased sugar level in blood, which kept growing. The dog developed a state similar to a human disease called diabetes, and died in two weeks.

Certainly, the conclusion that the pancreas contains a special device controlling blood sugar level was not made immediately after these experiments. Would it have been made directly, the researchers would have acted like the discoverers of the cockroach's ears. There could have been many reasons why the blood sugar level jumped higher and death came. It could have been because the digestive juice stopped flowing into the duodenum, or due to severe surgical trauma caused by the pancreas ectomy.

To prove the supposition, the pancreas was removed almost completely, together with a duct. Just a small slice of the organ, one eighth of it, was left in the dog's body. No diabetes developed. But as soon as this slice was taken away, even without a hard operation, disease and death resulted. Hence, digestive juice gets into the duct leading to the duodenum, and some other, more important substance, gets into blood, bypassing the duct.

In 1900 a Russian researcher Leonid Sobolev designed an elegant operation. He ligated the duct; the pancreas swelled and died away. It

stopped producing the digestive juice. The cells disintegrated and dissolved, but not all of them. Islands of special cells located between the thin passages which make up the duct, did not die. On the contrary, having gained "vital space", they grew more abundant.

These cell accumulations were described in 1869 by Langerhans and since then are called Islets of Langerhans. Sobolev suggested that these islets do not produce the digestive juice, but the hormone that controls the blood sugar level. This proved to be correct. Several years later these very islets, which grew bigger after ligating the duct, were used as a source of insulin.

It is now written in anatomy textbooks that the pancreas is an anatomic unit, though in reality it contains two basically different organs, a digestive gland with a very active external secretion and a gland of internal secretion.

This is how a new organ was discovered inside an old one. A new apparatus was discovered which produced insulin, a most important hormone for life. Disturbances in the function of this organ located in the pancreas do not affect digestion; they cause a disease quite widespread nowadays, that is diabetes.

How the Thymus Was Discovered Anew

Everybody knows what appendicitis is. It is a disease when a worm-like sprig of the large intestine, or appendix, gets inflamed. Everybody knows that a surgical operation is needed to remove this appendix, and this operation does no harm. One can live very well without the appen-

dix. This means that it is not needed by the organism and was created by nature only so that a man should suffer appendicitis. Is this really so? No one knows. We know seemingly everything about this worm-like sprig, but do not know what it is for. No one knows. Another organ, the thymus, kept its secret until 1961.

The story of the thymus must start in Melburn, Australia, 1960, at the Walter and Eliza Hall Institute for Medical Research. An institute which had been world-famous for several years, and in the following years its fame grew. The discoveries in immunology made at the Hall Institute surprised the world. Even then, in 1960, the Institute was the focus of attention for the scientific public. Not only its director, a Nobel Prize winner in immunology Frank Burnet, but also his students, beginners in science, Gustav Nossal and Jack Miller, had already established themselves as known scientists. They will become world-known in a few years. Nossal will take a post as the Institute's Director. He will be dealt with on many pages of this book. Miller will later become the head of the Institute's largest department, and now it's time to speak of his works.

In 1960 Jack Miller was sent to the London National Institute of Medical Research to study the role of the thymus in immunity. Why the thymus, not another organ? Were there any reasons to pose such a task?

Now this question sounds naive. It is now common knowledge that the thymus is the central organ of the immune system. But in 1960 the information on the thymus was rather scarce. This small organ was known to be located in the low-

est part of the neck, directly after sternum; it was known to have a shape of a two-pointed fork. This fork is almost completely atrophied in grown-ups, while in newborns it is quite big.

The weight of the thymus in a newborn child is 15 grams. If a baby weighs 3 kilos the thymus accounts for 0.5 per cent of its total weight. In a 40-year-old man the weight of thymic tissue does not exceed 3 grams, that is 0.005 per cent of total body weight; this is 100 times lower. It is practically absent, while the immunity does exist, and forty-year-olds are probably the most resistant to any microbial vermins.

So the preliminary reasoning was against the thymus playing a role in the activities of the immune system. But there were also some "pros" True, with the benefit of hindsight, they are clearly seen, but then they were described only by Burnet.

In early 1960 he looked in at the laboratory of Jack Miller.

"I'd like to ask you one thing. Look through the literature and pick out arguments for and against the thymus playing a role in immunity. If these arguments seem convincing to you, think about the most effective experimental approach to confirm or disprove the idea."

"How soon should I do it? The problem is that I'm leaving for London on a study program and there is a lot of urgent things to do."

"Put them all aside. I want the subject of your study program to be the evaluation of the "thymic version".

In a week Miller presented the main arguments.

Pros: 1. Most thymocytes, cells of the thymus, are outwardly identical to lymphocytes, which are cells of lymph nodes that play the central part in immunity.

2. In the course of an organism's development lymphocyte-like cells appear first, even before birth, in the thymus, and then in the lymph nodes, spleen, and blood.

Cons: 1. In grown-ups the thymus becomes atrophied and is replaced by fatty tissue.

2. Thymectomy brings about no complications, at least, during the first months after operation.

"Who was thymectomised?" Burnet asked. "Grown-ups were," smiled Miller and added: "To test the thymic version thymectomy should be done on newborns."

Both of them understood the implications. This way of testing ensued directly from Burnet's immunity theory. According to this theory immunity maturates only after one's birth. Perhaps, this organ is so big in newborns because it starts the entire system?

A week later Miller left for London.

The very first experiments on thymectomizing newborn mice confirmed "the thymus version". After this operation the infant mice remained immunologically defective until their death which came in two or three months. They were slow in their growth and had constant skin inflammations, diarrhea, and increased sensitivity to infections. Antibody production was very poor, and there were almost no lymphocytes in the blood. The immunity was suppressed so that alien skin grafted from other mice and even from rats was not rejected.

If a thymus is transplanted to these immunodeficient mice, or thymic cells are introduced into their blood, their functioning becomes normal. This means that the thymus is absolutely necessary to trigger the work of the entire immune system.

In 1961 the journal *Lancet* published Miller's first paper on the thymus, called "The Immunological Function of the Thymus".

This is how the central immunity organ, the thymus, known to anatomists long before was discovered. Only nobody had known before what the thymus was doing in the body. Now it is known to trigger the lymphocytes inhabiting all lymph nodes and spleen, and circulating in blood to recognize and kill the alien cells.

The Search for Moby Dick

Melvill's hero, captain Ahab, dare-devil and fanatic, who furrowed the seas in the search for Fate —the white whale Moby Dick, once came out on deck and nailed a golden dublon onto the mainsail.

"This dublon will be given to the one who first sees Moby Dick."

And the Captain himself spent days and nights in the barrel fastened on top of the mast, trying to locate the White Whale.

The secret through which the thymus triggers the immune system became Miller's Moby Dick.

What is the mechanism?

There are at least three possibilities, giving rise to three hypotheses. The humoral, or liquid, hypothesis, suggests that this substance provides

for lymphoid tissue maturation in the organism. The settling out hypothesis says that lymphocytes leave the thymus and filter throughout the body. And, finally, the education hypothesis holds that the thymus constantly receives the cells incompetent in immune affairs, and releases competent ones. This is why they are called immunocompetent lymphocytes in scientific literature.

So, Miller, and many other scientists after him started the search for Moby Dick. Now there is an argument over who was the first to see him, and this will be related later. The golden dublon is still there, maybe waiting for you, my dear reader.

It should be mentioned that the searchers for Moby Dick very soon paid attention to a certain fact and remembered another fact in connection with it.

Thymectomy of infant mice caused the main cells, lymphocytes, to disappear from the animals' lymph nodes and spleens. Immunity lost its voice. Though, this voice was not equally missing in all the forms of immune response.

Alien grafts were not rejected. As we already know, their rejection depends on the accumulation of activated lymphocytes which destroy the invadors. In other words, activated cells perform their work themselves. We have already mentioned this form of immunity, cell-mediated immunity. Its soldiers are killer lymphocytes. Thymectomized animals lack this type of immunity completely.

Another form of response to alien substances, the production of antibodies, is called, as you remember, humoral immunity. This form of im-

munity is not completely silent. The organism stops producing antibodies to some of the strange antigens, while to others the antibodies are still produced.

Maybe the thymus is only a part of the thing?

There and then another fact was recalled. This fact had been known 5-6 years before Miller's experiments; it was described by a group of veterinary doctors headed by Dr. Chang in 1956. They worked in Wisconsin, USA, studying the development of chickens with removed Bursa Fabricius.

The bursa described by Fabricius in the 18th century is something like the human appendix, the blind branch of the intestine. The only difference is that the appendix is located in the middle of the intestine, while Bursa Fabricius is at its terminal point. This organ is found only in birds.

Chang's research group showed that the removal of bursa in newly-hatched chickens renders them incapable of producing antibodies.

This was what the immunological Ahab remembered. Why is the immune response in these chickens suppressed if their thymus is intact? So researchers started experiments on chickens.

In 1963 both Australians and Americans published a number of works in the Proceedings of the Conference on the thymus. This conference was organized by Robert Good, a well-known pediatrician and immunologist in the United States. I mention this because Good will be not once mentioned later in our story.

So, the chickens' thymus was all right, while their humoral immunity did not work. What was

the matter? Maybe, these were "cockroaches' ears"? Or was it not the thymus responsible for immunity in birds? Maybe, the conclusion on switching off the immunity in bursa-ectomized chickens was not quite correct? The researchers at Wisconsin described only the suppression of antibody production, which does not take care of the immunity as a whole. This is only humoral immune response. And what about the cell-mediated form?

After removing the bursa the experimental chickens were grafted with pieces of alien skin. The rejection of the alien tissue is a function of the cell-mediated, not humoral, immunity. Antibodies are not involved in this process. If cell immunity is also cancelled, skin scrap will not be rejected. In these experiments the grafted skin was rejected in 12 days. In terms of this form of immune response bursa-ectomized chickens behaved as normal ones.

Chickens of another group were thymectomized and showed the opposite picture: alien skin was not rejected and the ability to produce antibodies was preserved, though at a markedly decreased level.

The conclusion was unambiguous: the thymus controls the development of the lymphoid system in its part responsible for the cell type immune response; and Bursa Fabricius controls the activities of the other component of the immune system, that is the humoral part which manifests itself by antibody production.

This is the case with birds. What about mammals and humans? They have no Bursa Fabricius but maybe a similar organ is hidden some-

where? Or, maybe, mammals do without an organ that controls the development of humoral response system? Maybe, the entire system is triggered by the thymus and we do not need to look for an organ that performs the same function as the birds' bursa?

Yes, we do! There is such an organ, though no one knows where it is. This was proved by Robert Good.

At the same time the pediatrician and immunologist Good studied and tried to cure children with congenital deficiencies of the immune system. He saw that there are some congenital defects that corresponded to the absence of the thymus. Cell immunity did not work, foreign grafts were not rejected, and antibodies were still produced, though to a markedly lower extent. These children were athymic or had underdeveloped thymus. Fortunately, these deficiencies are generally very rare, 1 case per 100 thousand newborns.

The other type of immune system deficiency is identical to that of bursa-ectomized chickens. Cell immunity is all normal, but no antibodies are produced by these children. Not even proteins which could have served as protective antibodies were produced. Good called this disease agammaglobulinemia, that is the absence of gamma-globulins in blood.

The main point of this is that children suffering from agammaglobulinemia have normal thymus. They have some other organ that is underdeveloped or completely lacking, an organ similar in its function to Bursa Fabricius in birds. But nobody knows what is the name of this or-

gan. It clearly generates the cells that filter down through the lymph nodes and spleen to produce antibodies. But where is this organ?

The analogue of Bursa Fabricius is still to be discovered. When? Perhaps, tomorrow.

The Art of Discovery

So, one organ, the thymus, is responsible for producing lymphocytes that are capable of turning into killers, and the second one, Bursa Fabricius or its yet undiscovered analogue, is in charge of generating lymphocytes capable of producing antibodies. But if the thymus provides for the cell-mediated type of immune response, why does its removal disturb antibody production? Why does the production of antibodies decrease and they become defective?

This was the question that had been troubling immunologists for a number of years. It has prevented them from finally dividing the immune system into two subsystems. It was clear that they exist and are somehow related. But how?

Science is often compared with art. "Science and Art"—this was the title of the report presented by one of the most prominent immunologists, Jean Dausset, at a congress of transplant specialists in Hague in 1969.

Indeed, these two streams of human culture have much in common. Like art, there can be classical and applied science. Both fields of activity call for sacrifice and complete dedication. Both require inspiration to find a new solution for yet unsolved problems. In both cases much

depends on the method. When an entirely new method is developed, both require figurative thinking, art more than science.

Science needs accuracy. This is the main difference between science and art; accuracy and reproducibility. Anything created by a researcher anywhere on Earth must be reproducible in any other place using the description of the method and materials used. This is impossible in art. Images are irreproducible. Leonardo da Vinci himself could not have reproduced his "Gioconda"; "Lilies" by Claude Monet and Degas' "Blue Dancers" are equally irreproducible.

In 1970 UNESCO initiated an experiment carried out by art experts in Toronto, Canada. Several ten thousand people each was given ten beautifully manufactured big coloured charts. Each of these charts contained ten reproductions of pictures by well-known and lesser known artists of all times and trends. There were Renaissance pictures, Dutch school classics, cubists, surrealists, etc. Impressionism and abstractionism were also presented. Each chart contained pictures belonging to all these trends. The artists' names and the names of the pictures were not indicated to avoid hypnotizing the audience with well-known masterpieces.

Each of those who have received the charts was to point out the picture he most liked from every chart. Each pointed out ten pictures he considered the best. So several hundred thousand answers were obtained, more than sufficient for statistical analysis. Which trend do you think won first place? Impressionism: Monet, Degas, Renoir, Matisse, Van Gogh, and others.

The use of imagery, the main sense focused in one concentrated effect, so typical of impressionism, is not an unusual quality in the best scientific experiments, generalizations, and theories.

Tayashi Makinodan, an American scientist of Japanese extraction, worked in the biological department of Oak-Ridge National Laboratory. It was there that he, together with his colleagues, developed a method of research that proved extremely useful for immunology. It was called the *in vivo* cell culture. *In vivo* is Latin for "in living body". Before Makinodan the *in vitro* meaning "in glass", method of cell culturing was well known and widely used, and is still quite popular. Cells from the blood, connective tissue, kidney, or cancer cells can be placed into a nutrient solution that is poured into special glass tubes. These cells live, function, and multiply in the *in vitro* culture.

There are certain cells, however, which cannot live in a tube. Nutrient solutions, even the most perfect ones, are not good enough for them. No tube can reproduce all the conditions they enjoyed in the blood-washed tissues of the integral body. These cells include, among others, lymphoid immunocompetent cells.

How could they be cultured? How could their life be studied? Some special method was needed. Without such a method, the regularities of their lives could not be learned, the potencies of the cells from various tissues, such as the spleen, lymph nodes, thymus, and bone marrow, could not be compared.

Makinodan developed a method to do this. He used a mouse as a test-tube, a living body with

all its ability to support cell life, provided for by an integral organism. In order to switch off the body's own cells, so that they should stop their work and would not interfere with studying the activities of the cells placed into such a "test-tube", Makinodan irradiated the mouse with X-rays. The body's own cells were killed, and those cultured (now in the *in vivo* culture) lived, functioned, and multiplied.

The activity of these cells could be studied in isolation. Only they lived and worked, and no other interfered with the study.

During ten years of experiments Makinodan and his colleagues seemed to have done everything possible. They showed the specifics of functioning for the immunocompetent cells capable of antibody production. They found out that spleen cells were most active as antibody producers, the second most effective were lymph node cells; thymic cells produced antibodies to a much lesser extent, and bone marrow cells were absolutely incapable of synthesizing antibodies.

Cells from newborn animals were taken and the peculiar features of their function were determined. Then the same was done for the cells of old people suffering from cancer. The researchers determined the number of immunocompetent cells in the spleen alone and in the mouse as a whole, and then observed how various chemicals and physical factors affected them, analysing their rate of multiplication and many other things.

They seemed to "wring" everything out of their method, having staged every possible version of experiment they could think of over ten

years. And still the most interesting thing was missed; that which Jack Miller and Graham Mitchell did in 1968, in Australia, with the help of their method.

It was the same Jack Miller who first thymectomized newborn animals and discovered the central role of the thymus. Continually thinking over the role of the thymus and Bursa Fabricius and the two types of lymphocytes, he appeared to be more prepared than Makinodan for staging the decisive experiment.

Indeed, it is difficult to realize why Makinodan did not perform the experiment that Miller staged in Australia. Apparently, he was carried away by studying the work of each cell type separately. It never occurred to him to mix different cells.

Makinodan worked in the truly classic style, while Miller worked in the best impressionistic style. Together with his Australian colleagues, he did the following experiment; 10 million thymic cells were placed in the *in vivo* culture and the number of accumulated antibody-producing cells were counted. The experimentors knew the modest abilities of thymocytes in this respect and were not surprised to find only 45 antibody producers. In parallel they placed 10 million bone marrow cells, which cannot work at all, into the same culture. Only 22 antibody producers were formed. The third (main) experimental group contained a mixture of thymic and bone marrow cells, 10 million cells of each type. The *in vivo* culture should have accumulated 67 antibody producers; 45 at the expense of thymocytes and 22 at the expense of bone marrow cells.

In the end, 1250 antibody producers accumulated twenty times higher than that expected!

The trick was clear; these cells can work only in cooperation, in close contact. This was called cellular cooperation in the immune response. They either work together or some cells make the others work.

This question was solved by the Australians themselves. Their next published paper said that antibody producers originate from the bone marrow cells. Thymocytes are just helpers, whose direct assistance is needed to trigger the work of bone marrow cells.

T-, B-Cooperation

A year passed after the papers by Mitchell and Miller were published, and several dozen more publications appeared during this time. The entire immune system was visualized as two separately located but cooperatively working cell systems. They were designated by the letters T and B.

These symbols were introduced by the well-known British professor Ivan Roitt, the Chairman of the Committee on Immunological Education of the International Immunological Society. In 1969 he wrote a scientific review on the work dealing with cell cooperation in the immune response. For convenience, the cumbersome words "thymus-dependent" were denoted by the letter T, and "bursa-dependent", by the symbol B. The symbols took root and are now used by everybody. T-cells, or T-lymphocytes, originate from the thymus. B-cells, or B-lymphocytes, are inde-

pendent of the thymus. They appear and live in the bone marrow where there are no T-cells. There are no B-cells in the thymus, only T-cells, and only B-cells in the bone marrow. All the rest of the lymphoid organs (lymph nodes, spleen, and blood) contain both populations. It is there, in the peripheral lymphoid organs where they meet, cooperate, and work together. So, if a disease-stricken immunity is to be restored, both cell systems, T- and B-lymphocytes, must be taken care of.

There are a number of molecular-biology problems in immunology, including the structure of the immunoglobulin molecules, decoding its basis (the structure of active "recognizing" antibody centres is now being successfully decoded), and the structure of the receptors by which lymphocytes recognize alien antigens and through which they cooperate with each other. The last problem is especially exciting.

How do T- and B-lymphocytes cooperate? What does their interaction mean? Do they transmit something to each other? Are these events known? These points of cellular cooperation in the immune system are of interest not only to immunologists. This is a mystery of general biology, and a breakthrough made by immunologists in this area made the new science popular and attractive for specialists from various fields, and for medics and biologists specialized in the subjects other than immunology. Problems such as preserving the body's genetic uniqueness, grafting organs, treating cancer, and cellular interaction are also of interest for the general public.

T- and B-lymphocytes start active cooperation

when alien cells or substances (antigens) enter the body. These alien substances act as a signal that triggers the entire immune response, which is ended when B-lymphocytes are transformed into plasmatic cells producing antibodies. Cellular interaction is the first link in the whole sequence of events.

Interestingly, full-fledged T-B cooperation is initiated only in the presence of a third cell, long known as the Mechnikov macrophage, which had been earlier thought only to devour foreign particles. The theory by Ilya Mechnikov stood out in a new way. Antibody production appeared to be impossible without macrophages. They are indispensable for triggering the entire process of T-B-lymphocyte cooperation.

Antigen molecules greatly vary, but all have several common features. They are always big molecules, hence they are called macromolecules. Indeed, a small molecule cannot bear the signs of activities of an alien genetic system. Apart from their size, antigen molecules differ in the specific groups of atoms they bear, the so-called hapten groups. The antibodies' active centres are aimed against these hapten groups. The carrier part of the molecule is the framework that bears the hapten groups. Our discussion will centre on the carrier and hapten parts of the antigen molecule. The following is one of the hypotheses that explains the molecular mechanism of cellular cooperation.

T-lymphocytes bind one part of the antigen molecule, let it be its carrier part. The antigen binding with T-lymphocyte receptors is the first step in the process of cellular cooperation. When

there are many antigen molecules, many "torn-off", "floating" receptors with antigens attached to them are developed.

These floating constructions are made up of a receptor similar to immunoglobulin, the "boy's slingshot" described earlier, which is bound with a lymphocyte at its handle. Antigens are attached to the two ends of the slingshot with their carrier parts. The hapten parts stick out. These "torn-off" slingshots with the hapten parts of alien molecules stuck out are floating around.

Here macrophage comes into play. It has certain sites on its surface with a special affinity to the torn-off end of the slingshot. The slingshots bind to this site and draw up as soldiers bristling with hapten parts of the antigen molecules.

B-lymphocyte receptors cannot attach to the carrier part of an antigen molecule. They have an affinity only to their hapten parts. So the bristle of slingshots drawn up on the macrophage clenches up with B-lymphocyte receptors. Every receptor binds one hapten group. A kind of "short circuit" occurs simultaneously through a dozen or hundred contacts. This shock is the signal that triggers B-lymphocytes, who multiply and generate antibodies with a specificity to their own receptors, that is antibodies against the alien agent.

However, the B-lymphocyte would not work without the T-lymphocyte with torn-off receptors next to it. Otherwise the signal will not be transmitted. The B-lymphocyte needs the confirmation which is given by the T-lymphocyte. The chemical nature of this confirmation is not yet finally decoded. It is only known to be a protein

bound with carbohydrate, a glycoproteid. It is called the immunopoiesis inductor, the substance that stimulates the immune response in the B-lymphocyte, or the second signal needed to start antibody production. These are molecular events that take place between T- and B-lymphocytes with the participation of the macrophage, the events needed to trigger the entire process of antibody production.

B-lymphocytes and macrophages are not needed to trigger the cell response, which is expressed, as you remember, by accumulating killer lymphocytes. T-lymphocyte interaction and the one signal coming from the attachment of the alien antigen are quite sufficient. T-lymphocytes start multiplying, growing in number. Their ability to recognize the foreigners, to bind and destroy them is enhanced. Here comes the army of effector lymphocytes, or killer lymphocytes.

Helpers and Suppressors

Any car, if it is in order and able to function, should have at least four control systems; switching on, acceleration, braking, and switching off. We start a car with a starter, increase speed by means of the accelerator, slow it with brakes, and stop the engine with a key. There is no "switching off" system for biological mechanisms. Switching off means death. The acceleration and braking systems are necessary. These are the nerves which increase the frequency of heart contractions, and other nerves that decrease it. Eye pupils are dilated in darkness and constricted in

bright light. This is the general law of the organism.

Immune response is triggered by alien antigens. Three cells work in cooperation. The B-lymphocyte receives the triggering signal and gets ready for work. The macrophage passes the antigen over to the T-lymphocyte, which starts helping the B-lymphocyte to enhance antibody synthesis. Hence its name, T-helper. T-helper lymphocytes are the accelerators of the immune response. They accelerate the immunity machine and draw the immune response to its maximal level due to multiplication of B-lymphocytes and their maturation into plasmatic cells which work as antibody factories. Once the speed is gained, the factory starts mass production. The more factories, the more production...

For many years immunologists thought that there was no braking mechanism for the immune system. They thought that antibody producing cells were triggered to multiplication and maturation. Then they accumulated, matured, aged and died. The result was the start of antibody production, followed by their accumulation and disappearance. It remained obscure, though, why cell multiplication was not endless. What stopped the process? Why, when a high quantity of alien antigens were used, was the immune response dramatically inhibited? Perhaps, there is a certain, yet unknown, braking system?

These "braking" cells were discovered in 1972. They also appeared to be T-lymphocytes. However, in contrast to T-helpers, they inhibited the immune response, hence their name, T-suppressors. Their basic mode of action was blocking,

cancelling, or inhibiting the activity of helper cells. T-suppressors have a special device for recognizing T-helpers. Having recognized them, they neutralize their activity. Since B-lymphocyte cannot work without assistance, the immune response is inhibited or stopped.

In normal conditions suppressor cells are accumulated several days after helper cells, braking the immune response in due time, and preventing its endless "acceleration" In cases of immunity disturbances T-suppressors can bring a lot of trouble, but this will be explained later.

One more point to conclude: immune response is performed not by a trio, but by a cellular quartet, macrophage, B-lymphocyte, T-helper, and T-suppressor. Their concerted activity gives rise to a fifth, plasmatic cell, which actually produces antibodies. A macrophage delivers an antigen, B-lymphocyte generates plasmatic cells, which manufacture protective proteins, and T-lymphocytes control their performance. They enhance or inhibit it regulating the whole process. Therefore, in scientific literature lymphocytes of thymic origin (T-lymphocytes) are called regulatory cells. And the thymus itself. In 1974, at the 3rd International Congress of Immunologists in Brighton, one of the discoverers of T-suppressors, the American professor Gershon called the thymus "the conductor of the immune orchestra"

Double Recognition

They work together, B-lymphocyte and T-lymphocyte. The first recognizes microbial, viral, or any other alien particle, to produce antibodies

against it. T-lymphocyte also recognizes an alien particle to help B-lymphocyte start its work, to trigger its multiplication, hence its name—T-help er. Macrophage, the third member of the team, presents this foreign particle to them, nice and ready, on a silver plate. That is why in scientific literature the macrophage is referred to as the "antigen-presenting cell". It presents an antigen particle, while T- and B-lymphocytes recognize it to be foreign. Once this done, they start their work, developing what is called the immune response. For many years it was thought that both T- and B-lymphocytes recognize the alien particle *per se*, recognize something *alien.* Then Rolf Cinkernagel, an immunologist from West Germany, found this to be wrong. He took mice and infected them with a virus.

A virus locates itself in cells and lymphocytes. To kill the viruses, it is necessary to find the infected cells, make sure that they contain viral antigens, and kill them together with the viruses. Lymphocytes recognize where is the virus, that is recognize the foreign particles.

The researcher mixed infected cells with T-lymphocytes and saw that lymphocytes recognize viral particles only if the viruses are fixed on cells of the same organism. If the virus-infected cells belong to another organism, the viruses remain unrecognized, and, consequently, alive. Thus, it was shown that foreign antigen particles given to T-lymphocytes on a silver plate are recognized as foreign only if a "plate" is one's own. The lymphocyte does not want to take the particle from a strange plate. So, T-lymphocytes do not see something "foreign", but something

as "one's own" defiled with "foreign" They recognize their own altered stuff. They look into the way it is altered, and develop an immune response against the insolent agent who had dared to effect this alteration.

This way of T-lymphocyte action was named double recognition, the recognition of the complex consisting of two components, one's own molecule and a strange one. It recognizes the "me" plus "not me" complex. "Me" or, as it has been put before, "the plate" is a complex molecule consisting of proteins and sugars called glycoproteids. They underlie the tissue incompatibility in transplants, which I'll speak of in detail later in this book. And now let us repeat: when a macrophage presents a foreign particle on a glycoproteid plate to a T-helper lymphocyte, the lymphocyte ensures that the plate belongs to the organism; the particle is presented by the organism's own macrophage. Applications from strangers are not admitted. Cooperating cells must belong to one organism, must be genetically identical. Otherwise cooperation does not take place or is dramatically restricted. Therefore double recognition is also called genetic restriction.

The Dictatorship of the Lymphocyte

Stem Cell—What Is It?

The blood of humans and all other mammals is a solution of proteins which contains cells of three main groups, that is red blood cells or erythrocytes, white blood cells or leucocytes, and

thrombocytes. The first, red coloured cells, transport oxygen. Leucocytes or white cells capture and destroy the alien particles, including microorganisms that penetrate the blood. Thrombocytes are the cells of thromb, or blood clot, which appear at cuts or scratches, cause blood to coagulate at the wound and stop the bleeding. All these cells are produced in the bone marrow. They appear due to the multiplication of precursor cells, as if from "seeds" One such seed gives rise to thousands of erythrocytes, leucocytes, and thrombocytes.

What is the number of such precursor seeds? Does every sort of cells have its own special seeds, as many scientists thought? Or is there a single common precursor for all the cells, as it was argued early in this century by the Russian histologist Alexander Maksimov? There were no answers to these questions until 1961, when Canadian researchers Till and Geoffrey MacCoulach developed a technique that made it possible to count these "seeds", and to look at which one gives rise to which cells. To put it scientifically, this technique enabled the scientists to see which of three differentiation routes, that is erythroid (the development of erythrocytes), myeloid (the development of leucocytes), or megacaryocytal (the development of thrombocytes) was taken by the cells.

In order to count the number of these "seeds" and see how they transform, lethally irradiated mice were intravenously treated with bone marrow cells. Colonies of blood cells visible with a naked eye grew in their spleens. The number of "seeds" was the same as the number of colo-

nies. 60 per cent of the colonies accounted for erythroid cells, 30 per cent were myeloid cells, and 5 per cent were megacaryocytal cells. The remaining 5 per cent were hardly discernable. And the remarkable thing was that an erythroid colony, or the "seeds" from which erythrocytes grow, administered intravenously to irradiated mice, gave rise to all three types of colonies in the same proportions, that is 60 : 30 : 5. This means that any type of blood cells can be generated from one precursor. Maksimov was right; there is a single universal starting cell for all types of blood cells. By common consent, it was called the hemopoietic stem cell. All branches of hemopoiesis come from this stem. Everything originates from it.

But who tells this cell what it should transform into, which way it should take? Does the formula 60 30 : 5 always hold, or, depending on the organism's needs, is some other formula applied? Who determines the fate of this stem cell? And what happens if the fate is not properly determined? Will not leucaemia, or blood cancer, be among the consequences?

Lymphocyte Versus Stem Cell

In 1966 a post-graduate of the Laboratory of Immunology at the Institute of Biophysics of the USSR Ministry of Public Health, Liya Seslavina performed experiments that showed the results of interaction between stem cells and lymphocytes. Before these experiments it never occurred to anyone to bring together the lymphocyte, the central figure of the immune system, and the

stem cell, the central figure of the haemopoietic system. The great boom in cellular interaction research had not yet started. It began after 1968, when it was shown that B-lymphocytes cannot start their work of "manufacturing" antibodies without "having talked" to T-lymphocytes. T-B-interaction began one of most popular research areas. The investigators were so carried away with the interaction between the two types of lymphocytes that they never thought of the possibility of lymphocytes interacting with stem hemopoietic cells.

In our first experiments, we mixed spleen cells from the mice of two species, a million cells from each one, in a test-tube. The spleen is a very special organ in that it is both lymphoid and hemopoietic, containing both lymphocytes and stem cells. One population of spleen cells contained 20, and the other, 15 stem cells. The mixture was to contain 35 cells, but we found only 12 of them. What happened to the others? Who found them in this two-million-strong hurly-burly? And, having found them, what did they do with them: were they killed or was their life route changed by ordering not to multiply, not to form hemopoietic colonies by the formula 60 : 30 : 5?

Having suggested that this had to do with lymphocytes, we immediately staged a new series of trials. We made two cell mixtures, one containing only lymphocytes, the other stem cells with a minimal quantity of lymphocytes. To do this, we mixed lymph node cells containing no stem cells (100 per cent lymphocytes) with bone marrow cells of another genotype, that is taken from the animals of another strain. All bone mar-

row stem cells were detected by lymphocytes and deactivated, or destroyed to the last cell!

The effect was very strong. One tenth of the lymphocytes were needed to detect and destroy stem elements among a million bone marrow cells. The targets were spotted amazingly quickly. A worker in our laboratory Vladimir Man'ko treated the mice with a mixture of lymphocytes and alien hemopoietic cells and immediately after that gave them antilymphocytal serum (ALS), the preparation which destroys all lymphocytes. Stem cells remained alive and formed colonies in the spleen. However, if ALS was introduced one hour after the cell mixture injection, it was already too late. One hour was enough for the lymphocytes to accomplish the main part of their job, causing stem cells not to multiply and not to give rise to the colonies.

In 1969 Rakhim Khaitov posed another question. What happens if the bone marrow from two different donors is mixed? Bone marrow contains lymphocytes, though much less of them than the spleen or lymph nodes. Maybe the stem cells in both mixed cell suspensions will kill each other?

For the first time our laboratory struck on a problem of great practical importance. Bone marrow grafts from various donors are in clinical treatment of various forms of anaemia, leucosis, or radiation sickness. This treatment is useless if stem cells in the mixture destroy each other. Bone marrow graft is done for the sake of stem cells which are then assimilated and start to multiply thus restoring the disturbed hemopoiesis in the patient.

Using chromosome analysis to accurately iden-

tify when and which multiplying cells are inactivated. Khaitov answered the question unambiguously. Transplanted bone marrow should not be taken from two or more donors, since the hemopoietic stem cells of the graft will kill each other.

So, inactivation of foreign stem cells by lymphocytes is a manifestation of tissue incompatibility. In contrast to previously known types of incompatibility, it proceeds quite rapidly (12-14 days are needed to reject foreign skin) and is aimed against the most essential part of the tissue, that is the cells producing all the rest of this tissue. The tissue has been transplanted and is still functioning, but its roots, hemopoietic cells, are already hemmed in and all the tissue is doomed.

Which genetic systems control this incompatibility? Other investigators, Eduard Panteleev and Illaria Dishkant, joined in the experimentation.

All previously encountered effects of tissue incompatibility mainly depended on differences in the so-called major genetic system of tissue incompatibility. In humans this is called HL-A, and in mice H-2. For several years our group performed hundreds of experiments. All possible genetic combinations of lymphocytes and stem tissues were tried. In 1976, at the International Congress of Transplant Specialists in New York we were able to report that our phenomenon is not controlled by the H-2 compatibility system, it has its own controlling genetic system. This was a final proof of the fact that here we faced an entirely new phenomenon.

This many years-long genetic Odyssey revealed another significant fact. Lymphocytes were shown to destroy stem cells from mutant animal strains; they recognize, find, and destroy stem cells which differ by a single gene altered by mutation.

This meant that our phenomenon was related to the uppermost function of the immune system, immunological supervision, which provides for everyday protection against cancer. There are 10^{13} cells in the human organism. The mutation frequency for various cells varies from 10^{-6} to 10^{-7}. Hence at any one moment our body contains no less than a million mutant (genetically altered) cells; these include cancer cells. Somebody has to detect them, identify them as traitors, and destroy them. This is what lymphocytes do.

A second question of practical value was posed, as the first one, in 1969. During our experiments we observed that when we administered bone marrow stem cells into the vein of irradiated mice, they give rise to colonies growing in the spleen. If lymphocytes were administered together with the bone marrow, no colonies grew. Two processes occur in one and the same organism; stem cells tend to multiply and generate colonies, while the lymphocytes hurry to kill them, since they are alien. Lymphocytes win, and no colonies are formed.

And what if some substance, like previously known medicine or newly synthesized preparation, is introduced into this system? If the substance affects neither stem cells nor lymphocytes, everything will happen as usual, and no colonies

will grow. If the substance kills both types of the cells, the colonies will also fail to grow. If it is toxic for hemopoietic cells, again, the colonies will not grow. In only one version, if the tested substance kills selectively lymphocytes causing no harm to hemopoiesis, will a researcher see the growth of colonies.

This reasoning allowed us to find a rapid and convenient method for selecting immunodepressive and lymphotropic preparations. Immunodepressive preparations are needed to suppress the immunity for organ transplants and autoimmune diseases, and lymphotropicones are required for treating lymphoid system tumours, such as lymphomas, lympholeucoses, and others. We examined a dozen preparations ourselves, and our method is now applied in many institutes.

Changing the Formula 60 : 30 : 5

In 1969 another important event took place. I mean the 12th International Congress of Hematology and Blood Transfusion. At this congress professor D. Barnes, on behalf of four well-known British cytologists presented a paper called "Inactivation of Stem Cells in Mixed Cultures of Spleen Cells, Studied Using the Chromosome Marker" Concluding his paper he said, "Summerizing the above it can be said that we have confirmed the inactivation of colony-forming cells in the spleen cell mixtures."

The phenomenon first described by researchers in our laboratory was being checked, while we were already moving further. The same day, in the same hall of the congress we presented our

paper first reporting another effect of the interaction between lymphocytes and stem hemopoietic cells.

Everything we have mentioned refers to the interaction between lymphocytes and genetically different, alien stem cells. But do they react with syngenetic, that is genetically identical cells, with the cells living in the same body? The first positive answer to this question was obtained by our team in 1968. Yes, they do, although not killing the cells they change the direction of differentiation. Remember that stem cells develop into approximately 60 per cent erythroid, 30 per cent myeloid, and 5 per cent megacaryocytal colonies. If these cells, however, are brought together with lymphocytes that at that moment are "excited" by genetically alien cells, differentiation takes another course. Lymphocytes "order" almost all stem cells to develop in the myelocytal direction. The formula 60 : 30 5 is changed to 0 : 90 : 5. This means that there are no red blood cells among the offspring of stem cells, leucocytes account for most of the offspring.

What is the purpose for this lymphocyte behaviour? Perhaps, it is the need for a more vigorous fight with the strangers who have triggered their activity? Remember that lymphocytes are phagocyting cells, devouring the strangers that invade the body.

In 1970 the fact that in some cases lymphocytes altered the direction of bone marrow stem cell differentiation was confirmed by the work performed abroad. The Japanese researcher Kitamura and his colleagues accurately reproduced our experimental scheme. Syngenetic lymphocytes

changed the direction of stem cell differentiation towards myelopoiesis.

This phenomenon naturally entailed a host of questions. The most interesting of them was answered in 1975 by our post-graduate Natasha Aleinikova. If lymphocytes order stem cells to be differentiated into leucocytes, what will happen if the body lacks lymphocytes, if it is lymphocyte-deficient?

Lymphocytes are mainly produced in the thymus, the central organ of immunity. If the thymus is removed, the body becomes deficient in the main bulk of lymphocytes, in T-lymphocytes.

The thymus was removed from mice to observe what effect this could have. Several weeks passed; the mice became T-deficient. The investigators tested the capacities of stem cells from the bone marrow of these mice. These cells were shown to have lost the ability to produce myeloid colonies. Our formula became 90 : 5 3. The hemopoiesis was almost exclusively of erythroid nature, which gave rise to an excess of red blood cells. So, myeloid hemopoiesis really does need the influence of lymphocytes.

Now the time is ripe to tell about another interesting feature of stem cells that is important for the balanced work of the entire hemopoietic system. They constantly migrate from bone marrow to the blood to settle in the spleen and other places of the bone marrow, if these places become deficient in these cells. Microorganisms or any other alien agents penetrating the body make this migration more intense. In T-deficient mice, this migration is stopped almost completely, but intravenous injection of lymphocytes from lymph

nodes instantly brings the situation back to normal. Stem cells come out of the bone marrow into blood, and the formula of their development also becomes normal, that is 60 30 5.

We do not yet know the practical implications of this part of our studies. Perhaps, some kind of malignant blood diseases, such as erythroblastosis, the excessive multiplication of red blood cells, results from a defect or a failure of the T-immunity system? Perhaps, certain viruses disturb the lymphocyte ability to give orders to stem cells, or make them give wrong orders, which lead to leucosis? Only one thing is clear, the interaction between lymphocytes and hemopoietic stem cells makes a mechanism of hemopoiesis regulation.

I have spoken about lymphocyte's dictatorship in such detail because this phenomenon was recorded December 1, 1977 as a discovery in the USSR State list of Discoveries under No. 192 with the priority from April 15, 1967. The discovery certificate states: "Previously unknown phenomenon of the interaction between lymphocytes and hemopoietic stem cells has been established, as a result of which alien stem cells are inactivated, and genetically identical ones change their differentiation path. Authors: citizens of the USSR Rem Petrov and Liya Seslavina."

A discovery in science is not a routine thing. The number in the list is the quantity of discoveries registered in our country since 1947, in all fields, not only by medics, but also by geneticists, physicists, chemists, engineers, all those dealing with objective phenomena and regulari-

ties of nature. The total result of their activity is 192 discoveries in 35 years, which is about 5-6 discoveries a year.

The Molecular Messengers of Immunity

Structure and Function

Most biologists and medical researchers work in two immense scientific fields, physiology and morphology, so they are divided into physiologists and morphologists. Physiologists study the functions of the organs and systems, such as the heart and the entire cardio-vascular system, and the nervous system, the cerebral or spinal brain. But there is no function without structure. Any function is performed by somebody (or something). The heart is contracted by the contraction of muscular cells. Muscular cells contract due to the contraction of special structures contained in these cells—myofibrils, which is the Latin for "muscle fibres"

In the last century morphologists described what they saw with their eyes. Then they started to examine structures under the microscope, and then—under the electron microscope. They discovered the cell structure of living matter, they saw the intracellular structures, that is the cell nucleus, its membrane, mitochondrias, and microsomes. Physiologists discovered functions. They discovered that the nucleus contains the heredity apparatus and controls the cell's life, mitochondrias provide energy resources for all cellular functions, microsomes serve protein synthesis.

In each of these cases the morphologists pose the problem for physiologists. "Here is the organ", they say "Please, determine its function." However, there are dozens of other examples. Physiologists discovered the conditional reflex, and morphologists decoded the structures of its links. Physiologists studied the laws of memory, but no one knows what structures are responsible for them. At the same time, a lot of structures have been described whose functions are known quite superficially, if at all.

Morphologists and physiologists do not argue who is more important. They are not competing, but cooperating. They know that there is no function without a structure and no structure without a function. They are united by the following simple formula: "Structure—function"

The Century of Mediators

If a biochemist who studies the chemistry of life processes reads the previous pages, he will inevitably ask: "Isn't this too simple? Which are the means for a structure to perform its function?" Imagine a nervous terminal, the nervous fibre which is attached, say, to a muscular cell. There is a structure—the nervous terminal, and another structure, the muscular cell. The nerve's function is to give an order "to contract" to the muscular cell. The order is given, the muscle has contracted. The structure has performed its function. How was the order conveyed? Who played the role of a messenger transmitting the order?

It appears that the nervous terminal (or, simply, the terminal of a nervous cell) has discharged

a special substance, as though sent a molecular messenger. This substance was received by the muscular cell membrane, it triggered a chain of chemical reactions which shortened the molecules of a special protein, myosine, constituting muscle fibres, myofibrils. The muscle contracted. These substances, acting as molecular messengers, help to initiate the functions of the structures and transmit them from one structure to another. They are called mediators, or go-betweens. The hormones adrenaline and noradrenaline are the best known among the mediators of the nervous terminals that activate the function of the receiving structures. They are responsible for our moods, they help us to endure the hard moments of stress.

Various cell structures in the body perform their functions with the help of mediators. The pancreas controls the sugar blood level by secreting the hormone insulin. It makes liver cells transform blood sugar into glycogen and deposit it in the liver. If there is much sugar in the blood, insulin is discharged, and sugar goes into the liver. If there is little sugar, insulin production is inhibited and the liver's glycogen turns into blood sugar. Thus the mediator, the hormone insulin, helps to sustain the normal sugar level in the blood. It is now clear that the formula "structure—function" should be made more complex, that is "structure—mediator—function"

The mediator is always a specific molecule, sometimes quite simple, more often complex. For instance, adrenalin has a molecular weight of about 300 Dalton (the unit indicates how many times heavier a given molecule is than a hydro-

gen molecule; further in the text we shall omit the denotation of this unit, as is common in scientific publications). The molecular weight of insulin is about 3 thousand. But it is always a molecular structure. Morphologists do not see it. It can be "seen" by biochemists and molecular biologists.

Hormones are the most striking of the mediators discovered in the 20th century. They include growth hormones, sex hormones, insulin, and other amazing molecules that mediate the functions of the internal secretion glands which control the work of numerous body systems. Even more astonishing mediators have been discovered recently. Neuropeptides, small molecules produced by nervous cells, became a sensation. For example, encephalines, whose molecules consist only of 5 amino acids, have hundreds of times stronger anaesthetizing effect than morphine, the strongest known anaesthetic (they are also called the endogenic morphines, or endorphines). Biochemists have already isolated these amazing substances, decoded their structure, synthesized them in a laboratory, and have started to use them in medicine.

Delta-peptide of sleep, a molecule a little bigger than that of endorphine, containing 9 amino acids, is sleep mediator. It causes deep sleep when given in a dose of 10^{-5} g per kilogram of animal's weight. Mediators stimulating the ability to study and accelerating memorization have been discovered. One of them, nonapeptide-lysyl-vasopressine, has become especially well known.

Mediators of the Immune System

A cellular orchestra consisting of macrophages, B-lymphocytes which turn into plasmatic cells, T-helpers, T-suppressors, and T-killers performs in the immune system. These cells emerge in various organs (the bone marrow, the thymus, connective tissue, and others) from a common precursor, the hemopoietic stem cell, which will be mentioned not only once later in the text. All "the orchestra players" circulate in the body, gather together in the spleen or in lymph nodes, and work in ensemble. It was already said that when they first meet, they recognize each other with the help of receptors. But they also use the "language" of chemical signals to transmit information at a distance. They can invite each other to act, give "orders" and exchange information. Molecular messengers work tirelessly within the immune system.

Until now, researchers have identified about 30 soluble substances, the so-called humoral factors differing in their chemical composition, and acting as mediators—intercellular messengers which trigger immune reactions. Thus, having detected an alien cell, the target of its action, the T-lymphocyte secretes a mediator called MIF, that is the factor inhibiting macrophage migration, or Migration Inhibiting Factor.

Why does a lymphocyte secrete this mediator? The purpose is to stop the passing-by macrophage, the cell which performs the function of the main body's sanitarian. The secreted mediator will not allow the macrophage—the devourer of dead cells—to rush by the target. The "strang-

er" will be hit by a T-lymphocyte and absorbed by the macrophage.

Another mediator, the so-called helper factor, is secreted by T-lymphocytes to make B-lymphocytes synthesize antibodies against foreign agents, such as microorganisms, viruses or alien protein substances.

There are mediators which enhance the functional activity of macrophages and lymphocytes, and those that stimulate cell multiplication (this mediator is called the mitogen factor).

Take, for instance, the transfer factor. This mediator is capable of transferring "knowledge" from already "trained" lymphocytes to isolated ones, which have not yet made contact with the body's enemy. Mediators that curb the cell activity include the factor inhibiting cell multiplication, called lymphotoxin. Lymphotoxins assist T-killers in destroying the target.

Chemotaxis is the phenomenon of the active motion of cells to and from a chemical stimulus. T-lymphocytes can secrete mediators (chemotaxic factors) providing for positive or negative chemotaxis in other cells of the body.

Thus, with the help of various mediators T-lymphocytes perform their conductor's function. They can enhance macrophages' activity or inhibit it, they can draw additional cell detachments to the battle-field, or stop their supply, trigger B-lymphocytes into producing protective antibodies or give the command "stop, that will do". The latter is done by T-suppressor lymphocytes by secreting, whenever necessary, the suppressing factors, the antagonists of helper factors.

It should be stressed that a number of research-

ers all over the world are now working on the problem of the immune system mediators produced by T-lymphocytes. They try to isolate these substances in pure form and study their nature and their chemical and physical properties. The problem is that most of them are similar in their molecular weights and chemical and physical characteristics. Therefore it is not always possible to see clearly whether these functions are performed by a separate chemical mediator or whether one mediator behaves in different ways.

Most lymphocytal mediators, the so-called lymphokines, have a protein nature. They are stable when heated to 56 °C, stable to enzymes breaking the nucleic acids, DNA and RNA, but sensitive to those that decompose proteins. Their molecular weight varies from 10 thousand to 80 thousand.

Lymphokines provide for the interaction between T-lymphocytes, B-lymphocytes, macrophages, and other cells, and some of them take a direct part in destroying the invaders by lymphocytes.

The special group of mediators sustaining multiplication of lymphocytes were given the name of interleucines.

However, the problem of the molecular messengers of immunity does not boil down to identifying lymphokines and interleukines. The principal messengers are the hormones of the thymus and bone marrow, which stimulate maturation and normal functioning of T-and B-lymphocytes. The fact that they exist proves that it is molecular messengers acting at different levels of the hemopoietic and immune systems that provide

for the normal functioning of these systems. Any malfunction in their activity is more often than not due to the lack of a certain mediator.

Thymosine, AFT-6

Hormones of the thymus attracted the researchers' attention long before the discovery of T- and B-lymphocytes and their role in immune reactions. It was noticed that thymic extracts exhibit various kinds of biological activity, in particular, they stimulate immune reactions. However, until the thymus was proved to be the central organ of immunity these studies had an intermittent character.

In 1961, the Australian Jack Miller removed the thymus in newborn mice. They developed the so-called wasting syndrome: undergrowth, baldness, intestinal disorders, blood changes, and, most importantly, immunity disorders—the transplanted alien cells and tissues were not rejected, and microbial invasions appeared to be lethal.

Thus the central role of the thymus in immunity was discovered. Soon after that the investigators found that thymic extracts given to the animals, considerably mitigated the wasting syndrome, if not cancelled it completely.

Then a special species of athymic mice was bred.

They have an underdeveloped thymus, lack T-lymphocytes, and show disturbances in their entire immune system. T-deficiency in these mice can be compensated for by hormonal preparations isolated from the thymus.

Unexpectedly, thymic preparations were shown

to exhibit an anti-tumour effect. The basic mechanism of their action was revealed. They cause T-lymphocyte to mature from precursor cells. This caused a boom in research on thymic hormones. Many laboratories initiated work on isolating and studying these active components of immunity. The results were not long in coming.

Quite recently, the American immunologist Alan Bach isolated a thymic factor from blood serum. He called it serum HT-factor of the thymus and completely decoded the amino acid composition of the molecule, containing only 9 amino acids.

Another American investigator, Allan Goldstein, isolated a thymus preparation called thymosine and used it to treat children with congenital underdevelopments of the thymus. These are rare diseases, when T-system of immunity is partially or completely missing, as in athymic mice. The children suffer greatly, are markedly underdeveloped, and die either of infectious complications or of various forms of cancer. It is still too early to speak of the results of this study.

Researchers at the chair of immunology of the N. I. Pirogov 2nd Moscow Medical Institute, jointly with the Central Research Laboratory, the Center of Cancer Research of the USSR Academy of Medical Sciences, and Clinical Hospital No. 55 under the general supervision of the Academician of the USSR Academy of Medical Science Yuri Lopukhin have undertaken a vast study of the nature, biological action, and clinical use of another active fraction of the thymus, AFT-6.

This preparation was obtained in the laborato-

ry headed by the Candidate of biological sciences Vitali Arion. In terms of its chemical and physical properties AFT-6 has certain advantages over other thymic preparations, including Goldstein's thymosine. To begin with, it is much purer. This is shown by the markedly lower molecular weight of AFT-6 that accounts for the basic preparation's activity.

The principal biological effect of AFT-6 is its ability to restore the T-system of immunity. This has been shown in experiments on thymectomized animals. In identical tests, an AFT-6 preparation is active at a dose of 1 microgram per 3 million lymphocytes, while Goldstein's thymosine requires a dose of 9-12 micrograms per 3 million lymphocytes. AFT-6 acts on T-lymphocyte precursors, enhances killer activity, and increases the number of T-lymphocytes both in cell cultures and in patients suffering from their deficiency. It was called T-activine.

The preparation was successfully used to treat certain diseases of the lymph system in man, in particular, lymphogranulomatosis. There are grounds to suggest that T-activine will prove useful in a broader way, as an effective means for rehabilitating the T-system of immunity.

A Stimulant of Antibody Producers—SAP

Until recently we knew nothing of the hormones or mediators that provide for the normal maturation of the B-system of immunity. We can't accurately locate the organ for B-lymphocyte maturation in mammals, though we assume it to be the bone-marrow. A recently described class of

mediators produced by bone-marrow cells is very interesting in this regard.

The first mediator of this type was found in our laboratory, at the Institute of Biophysics of the USSR Ministry of Public Health, by a team headed by the doctor of biological sciences Avgusta Mikhailova. The first observations were made in 1968, and the study is currently under way.

Mikhailova treated mice with an alien protein —antigen. Four days later the animals' lymph nodes showed accumulations of plasmatic cells producing antibodies. Lymph node cells were extracted and placed into the feeder medium—*in vitro* (that is in glass, in a glass plate). After 18 hours the number of antibody producers and the number of antibodies produced were counted.

The same cells together with equal quantities of bone marrow cells from normal non-immunized mice were placed in other tubes. The suspension of bone marrow cells contained no antibody producers, all the more since by themselves they can not produce antibodies. The cells were counted 18 hours later; the number of antibody producers and antibodies in the mixture grew threefold.

The obvious question was, what were these cells from—the lymph nodes or the bone marrow? In other words, whether the immune cells of lymph nodes make non-immune bone marrow cells join the work, or bone marrow cells intensify the work of previously triggered and the now mature population of antibody producers.

The cultured cells were divided by a membrane impermeable for these cells but permeable

for the soluble components produced by them. The immune cells of lymph nodes were placed on one side of the membrane, and bone marrow cells on the other side. The number of antibody producers was counted after 18 hours of culturing. Antibody producers did not appear in the bone marrow suspension, while in the lymphoid one they grew threefold. Hence, it was bone marrow cells that produced the soluble factor, the mediator providing for the threefold increase in the number of mature antibody producers. This happens as soon as in three hours, without cell division. Consequently, the mediator activates the cells ready to produce antibodies but "silent" for a time being.

The next stage of the study was to isolate this mediator from the feeder medium containing the bone marrow. The researchers first made sure that this medium really accumulated the stimulator. For this purpose the lymphoid cell suspensions were mixed not with the bone marrow, but only with a cultural liquid in which bone marrow cells had been living for 18 hours. The effect was reproduced.

After this the liquid was divided into fractions by using column chromatography (this method makes use of the differences in molecular weights and sizes of protein molecules). Of 40 isolated fractions only those with molecular weights of about 13 thousand proved to be active. This is quite a small molecule, since molecules of antibodies sometimes reach the weight of 900 thousand.

The bone marrow stimulating factor was called

SAP, which stands for stimulator of antibody producers. The SAP molecule consists of two components, a protein and a ribonucleic acid. One million bone marrow cells placed into the culture produce 10 milligrams of SAP. When administered to an organism, the preparation's activity appeared to be even more pronounced than for cells cultured *in vitro*; the lymph nodes of an immunized mice accumulated 4-5 times more antibody producers than the *in vitro* cultures.

The last thing to be clarified was whether or not this stimulator has any prospects for practical application. The experiments were staged on cells from lymph nodes and the bone marrow of experimental mice. These cells were genetically identical, taken from the animals of the same genotype. All the individuals within one pure strain are identical to each other, like identical, monozygotic twins. In these conditions, cells interact with each other, and SAP, the mediator of this interaction, is active. Would it work with the cells of different genotypes or different species?

This was not an idle question. For example, the interaction between T- and B-immunity systems takes place only when T- and B-cells are genetically identical. An alien T-lymphocyte will never trigger a B-cell. For this reason the practical application of this phenomenon is rather questionable. In most cases diseased people do not have identical twins. And no other T-lymphocytes and mediators secreted by them can trigger the work of B-lymphocytes.

Here, too, SAP demonstrated its advantages.

Bone marrow cells from rats, chickens, pigs, and calfs proved to be suitable as sources of the stimulator. This makes it quite promising for pharmacology. Mikhailova observes that "in treating a number of infectious diseases a patient is given prepared antibodies—gammaglobulins, to neutralize the microbial toxins and eliminate intoxication. But it would be much more effective to mobilize the protective capacities of the organism by increasing the quantity of antibodies with the help of SAP. This preparation can also be used for enhancing the effects of vaccines, for treating chronical infections and other illnesses connected with the defects of the B-system of immunity." I can add that SAP might be an effective anti-allergic agent, since one of the basic reasons for allergies is that the body fails to produce protective antibodies against allergens.

Non-Infectious Immunology and Tissue Compatibility

Non-Infectious Immunology

Ask your biologist and medic friends, "What is immunity?" I first took this opinion poll in 1968. In nine cases out of ten I got the answer: "It is non-susceptibility to infectious diseases."

Time passed and interest in immunology became greater. An increasing number of people learned that immunology deals not only with preventing infectious diseases, but embraces a

host of most important non-infectious problems. This year I repeated the experiment with the opinion poll. Only a half of my "experimental friends" gave the same answer as before. The other half decided that immunology is an important science dealing with the mechanisms that sustain good health. Explaining this answer they used non-infectious examples, such as cancer, allergy, pregnancy, blood transfusion, organ transplantation, and aging.

The term "non-infectious immunology" was very significant for broadening the boundaries of immunology. It became quite popular in the last twenty years. Despite the fact that there is no special non-infectious branch of immunology, there is the universal science of immunity. Although this term is a temporary one, it played a great, positive role in overcoming the obsolete notions of immunology which had taken shape back in Pasteur's times. For many years the term "non-infectious immunology" focused attention on immunity problems which have nothing to do with infectious diseases. These include blood transfusion and tissue incompatibility in transplants, immune mechanisms for complications at pregnancy and protection against cancer, the causes of certain blood diseases, rheumatoid arthritis, asthma, and other allergies. The term "non-infectious immunology" implicitly declared that this science studies not only how to protect man against infectious diseases, it is much broader; immunology has the keys to many non-infectious problems. The term fought the inertia of scientific thinking.

Francis Bacon once wrote that pondering over

what is possible, people use examples from the past; they anticipate the future with an imagination occupied by the past. This way of reasoning is often erroneous, since rivers flowing from the strings of nature do not always fit old river beds.

The inertia of scientific thinking is both good and bad. It is good since it gives support for a researcher studying nature ever further and deeper. Inertia is responsible for the critical attitude towards anything new and unfamiliar, calling for self-evident proofs. It is the inertia of scientific thinking which helps to break ungrounded scientific speculations, sometimes huge and harmful. This inertia played an important role in bringing to ashes the theory that rejected genes as the carriers of heredity, and a number of speculative medical theories and treatment methods, such as treating the microbial illness dysentery with sleep.

The inertia of thinking can also blind a scientist, deprive him of objectiveness, and make him disprove the new, in spite of everything. Perhaps, this is the greatest evil of the inertia of scientific thought. If I am asked, "Is inertia more good or evil", I would have answered, "There is more evil in it."

A scientist is guided by past tracks, but he should not follow these tracks blindly and recklessly. A scientist proceeds in a certain scientific way, but he should not consider all others to be fruitless. A scientist respects and even honors the authorities of the past, but he should not consider their opinion to be the absolute for today. Due to the inertia in thinking surgeons, despite the brilliant results of a Vienna obstetrician

Ignaz Semmelweis, continued to wash hands not before an operation, but after it—for further 20-30 years. Due to inertia, cybernetics was denounced as an idealistic obscurantism. Due to inertia many scientists still stick to the dogmatic approach that discards an unexpected experimental result which at first glance seems to be absurd or contradicts common sense.

Progress often calls for rejecting a customary concept or applying it to unusual, new phenomena. This is when inertia becomes the worst enemy of scientific progress. It blocks the channels of our thought which would have finally led to the long-sought answer. Thought cannot follow this channel, since its entrance is barred by a customary "impossible" or "It was great Pasteur who first showed ..."

The late 19th and early 20th centuries saw the triumph of young microbiology and young immunology. These were the years of "The Chasers for Microorganisms", as they were called by Paul de Kruif, the author of the book of the same title. These were the years when immunity, as magic "Open sesame!" showed new facets of the kindness towards people. The scientists have already learnt to vaccinate against rabies and anthrax; they are preparing the vaccines against cholera and tuberculosis, they save children from dyphtheria by giving them antidyphtheria immune serum. The word "immunity" sounds like salvation. Immunity is non-susceptibility to infectious diseases. Immunity is the protection against microorganisms. Immunity is cells devouring pathogens, and antibodies which appear in

blood to destroy these pathogens and their poisons.

Among the triumphs of discovering new ways to develop immunity against microorganisms several scientists who refused to keep pace with the others remained unnoticed. They discerned the second face of immunity. They saw that immunity is not always a friend, that it can also be an enemy.

At that time their voices were hardly heard. Facts gained by them were only comprehended later, in our time. At that time the inertia of thinking carried the researchers along tracks of creating immunity against infectious diseases. And they were right. At that time the infections remained the most terrible scurge of humanity. But despite all this, several scientists managed to overcome the inertia and, even then, unmasked the other—the non-infectious—face of immunity.

Today it is an unforgivable inertia of thinking to interpret immunity as a means for protecting the organism against the pathogens of infectious diseases. And not an innocent one. If this is an attitude of a layman, then it is just a delusion. If it is repeated by a researcher, it is pure ignorance. By saying this, he aggravates the inertia of scientific thinking, blocking the productive channels of his readers' thoughts. This is unforgivable, since these channels were first discovered more than 80 years ago by a Belgian Jules Bordet and a Russian Nikolai Chistovich. This happened very late in the last century. Both scientists were then young and worked in Paris,

in the Pasteur Institute, in Mechnikov's laboratory. The honour had befallen them to overcome the inertia of thinking.

Jules Bordet and Nikolai Chistovich

At the turn of the century most researchers were carried away by studying immunity to microorganisms. They discovered pathogens of new diseases and studied the mechanisms of non-susceptibility to them. They developed vaccines.

And then, among this thrilling flood of studies, 28-year-old Bordet gave his thoughts to the problems of immunology with no special pertinence to microorganisms and non-susceptibility to infectious diseases. Bordet posed the question in defiance of the inertia of scientific thinking.

The question was whether antibodies are produced only in response to the introduction of bacteria and bacterial toxins, or are they also generated by the presence of non-microbial cells, such as alien red blood cells—erythrocytes.

In a previous chapter the experiment of introducing cholera vibrion to a rabbit was described. In response the animal produced antibodies which stuck the cholera vibrions together and then dissolved them. These antibodies did not interact with any other microorganisms.

In 1898 Bordet staged the identical experiment, only a rabbit was given red blood cells from sheep instead of microbial cells. After some days the rabbit's blood serum started sticking together and dissolving the sheep red blood cells. Destroyed were the sheep's and only sheep's cells, while the erythrocytes from other animals,

including human ones, worked perfectly well in the immune rabbit serum. It contained strictly anti-sheep antibodies. If a rabbit is given human red blood cells, his blood will produce antibodies which glue together and dissolve only human red blood cells, and no one else's. It was the same specificity as that towards a microorganism.

At the same time, Chistovich described antibody production in the animals' blood after subcutaneous or intravenous administration of non-microbial and even non-cellular alien protein substances like the proteins of blood serum. His experimental animals developed antibodies against the administered serum. These antibodies, added to the alien serum, caused the aggregation of protein molecules and stuck them together, so that the transparent serum became turbid.

The phenomenon was called precipitation, and the antibodies got the name—precipitins. They are also strictly specific. A rabbit treated with human serum produces precipitins reacting only with this serum, one treated with murine serum generates antimurine precipitins.

Back in the late 19th century it was shown that immunity is not only a fight with microorganisms. It is a fight against various, or, more exactly, any agents of biologically alien origin. The organism fights, it produces the weapons against anything alien entering its inner medium. In the final analysis, the organism does not care whether this agent bears cholera, typhus, influenza, or alien blood, alien tissue, or alien protein substances, even though they caused no illness. The body fights anything foreign getting into it, and the means of its fight are always the

same. They underlie immunity, both infectional and non-infectional, which is the subject of our primary concern.

Bordet, Chistovich, and their teacher Mechnikov founded non-infectional immunology, an important component of the modern science of immunology.

Tissue incompatibility encountered in transplants is a direct consequence of the observations by Bordet and Chistovich. Of course, it is easy for us to speculate on this 85 years later. Everything seems simple and logical. We are more clever, with the benefit of hindsight. However, it took 45 years for science to comprehend the immune nature of rejection. This is the period that elapsed from the inception of non-infectional immunology to the moment when Peter Medawar plotted tissue incompatibility of transplants on the map of immunology. No one before him realized the reason for the failures of all the attempts to graft a foreign organ, though some researchers came very close to the solution of this problem.

Alexis Carrel and Emmerich Ulman

Nothing in science appears suddenly. Each discovery has its own "prehistory" Experiments are performed based on those already done. Every scientist has precursors that prepared the soil for his work. Pasteur would have never developed the vaccination principle if the microbiological concept of infectious diseases have not been formulated. Alexis Carrel, who proved the biological nature of tissue incompatibility in

transplantation, was induced by Emmerich Ulman, the first surgeon to carry out kidney grafting.

On January 24, 1902, at a session of the Vienna Surgical Society, a senior lecturer Emmerich Ulman presented a paper entitled "Renal Transplants". He explained experiments on transplanting a dog's kidney from its normal place to the dog's neck and he exhibited the dog.

On January 24, 1902, at a session of the Vienna Surgical Society, a senior lecturer Emmerich Ulman called "Experimental Renal Transplants". It was not an ordinary peripheral publication. No, the Vienna magazine was at that time the leading medical journal of Europe. Ulman wrote: "It has been considered impossible to transplant such a big organ as a kidney. However, it was done, and the viability of the transplanted kidney was retained along with its physiological function... Further experiments will show whether or not kidneys can be transplanted from one dog to another,... and, finally, whether or not (though it seems hardly possible) transplanted kidneys can take over the burden of complete blood purification. In other words, it will be seen if the animal will survive when its own kidneys are removed and only the grafted ones are left to function."

On June 27, 1902, at the next session of Surgical Society Doctor Ulman was speaking again. "At first I failed to transplant kidneys from one animal to another, but today I can demonstrate to you, such an outstanding audience, a goat which has the kidney of a dog grafted in its neck. You can see that the kidney is functioning

quite normally, and the urine is dropping from the end of the ureter drawn outside. I must sincerely admit that the success of the experiment amazed even myself. Though it was long known that the kidney removed from the body, perfused by alien blood, starts its secretion, I never suggested it possible in a living body. This viewpoint was shared by all the experts."

So, Emmerich Ulman effected all three types of transplant, that is auto-, homo- and heterotransplantation. Apparently, he never distinguished between them. He did not report in the press the fate of the transplanted kidneys and never published any data on organ transplants, although he continued to work fruitfully as a surgeon. In 1902 he was 41; he stopped his academic career in the 1920s. No one knows why he was carried away with the transplants or why he got disappointed in them.

Alexis Carrel started his experiments influenced by Emmerich Ulman. He wrote about it himself. Today it is commonly agreed that Carrel initiated the scientific era of organ transplantation. And this is true. He substantiated and formulated biological (not the surgical!) nature of incompatibility. He is the discoverer of this phenomenon, and nobody argues it. And yet, there was someone before him. The senior lecturer Emmerich Ulman. But, surely, he, too, was induced by someone else....

Alexis Carrel, who had finished Lion University, was a good student of the history of medicine and an expert on the history of surgery. He collected all the reliable descriptions of tissue and organ transplantation. In the 10th century

B.C. Hindu priests were successful in reproducing injuried ears, noses, and lips by using scraps of skin from other parts of the body of the same patient...

In 1503, a Sicilian doctor Branca tried to transplant a slave's skin to restore the nose of his master. But Branca was less successful than his ancient Hindu colleagues.

There is much information on the transplants. Some of these accounts are reliable, others are quite unlikely. Descriptions of successful transplants can be found, but the convincing proofs of the failures are much more abundant. It is quite clear that the doctors have never been able to transplant tissues from one man to another. They could not do it, as they cannot do it now. The Hindu colleagues of Branca were no more lucky; the point is that they transplanted the tissues of the same man, while Branca tried to graft skin from one man to another. And even such a powerful factor as "a slave's skin" was of no help.

But this escaped Carrel's attention.

Both doctors and patients have become accustomed to the thought that surgery has unlimited possibilities. Carrel was a surgeon. And, as every surgeon, he considered the lack of mastery, the imperfection of surgical techniques to be the reason for the failures. Others did not doubt this, either. This was the thought everyone had become used to.

And, indeed, why should they think otherwise? Why should not the grafted tissue fuse? The tissue is the same. Skin, for instance, is similar in all people. Even in the slave and the master.

Even in the vanquished and the victor. And even... in the white and the coloured. A bit more pigment in the skin which is otherwise quite the same. And if we take kidneys or liver, there are even less differences to be seen. Hence, if the vessels (which are, incidentally, similar, too) are well sutured, and blood (the same in everyone) flows along these vessels to feed this tissue or organ, then everything will be OK. Be it tissue or organ—they should fuse anyway. This was what Carrel thought and this was what everybody thought.

Carrel's reasoning was quite natural. In the near future surgery will reach utmost perfection in its technique. But its basic method—the removal of an injured organ—is terribly limited. It should not be like this. Forced, destructive surgery should be replaced by creative, replacing, reconstructive surgery. An injured organ should be removed and replaced by a healthy one.

This is what is needed.

This is a major task, worth devoting one's life to. The medics of the past and the surgeons of our day have not learned to do it. They just have not reached perfection in operating. They have not yet learned to stitch the vessels. The key to the problem is the surgical technique. Alien tissue should be accurately adjusted. A surgeon should thoroughly stitch layer to layer, vessel to vessel, nerve to nerve. The operating technique should be improved to perfection.

This was what Carrel thought, neglecting the fact that, when ancient Hindu doctors cut the patch from the same patient, they succeeded.

When Branca, the Italian, "borrowed" a piece of tissue from someone else, he failed.

Carrel devoted his life to the technique of organ and tissue grafts. Confidence in success, confidence in surgical mastery never left Carrel. The inertia of thinking summoned him to action. The major need, he thought, was to provide normal nutrition to the grafted organ. In other words, normal blood inflow and outflow were most important. The major thing was to stitch the vessels together as accurately as possible.

Carrel graduated from the medical faculty in 1896 and became a well-known experimental surgeon several years after. He developed a vascular suture. Two years were spent to elaborate this fine surgical technique. Vessels were sutured layer to layer, wall to wall. The creator of the vascular suture became known not only in France, since nobody in the world was then able to suture the vessels. In 1900 Carrel received the degree of Ph.D. in medicine. At that time he was 27.

When he was 31, the young surgeon was invited to work at Chicago University.

At 32 he created a miracle. It was in 1905.

There were two tables in the operation room. A dog covered with sterile cloth was lying on one of them. An anaesthesiologist controlled its pulse and breathing. The dog's leg, also in sterile cloth, was on the second table. It had just been amputated. Carrel examined the cut tissues looking for arteries and veins. The limb was to be sutured back to its old place. Success was awaiting! The bones and muscles were already connected, the vessels and nerves were sutured,

layer to layer, wall to wall. The skin was stitched.

A day there passed, then a week, a month, a year.

There was no doubt! Mastery won.

Alexis Carrel, the first surgeon in the history of medicine, reattached a limb that was completely parted from the body. The leg was whole forever. The dog used it almost as easily as before the operation. The same year Karrel repeated the miracle with a kidney. The removed organ was replaced anew into the same dog. It worked forever. These operations made Carrel even more famous.

When he was 33 he was invited to the Rockfeller Institute, New York.

Carrel saw that he was following the way of the Hindu priests. The amputated leg was reattached to a dog, not the leg of some other dog, but the one from which it had been cut off. He had not yet started Branca's way. Years of work were ahead of him, but his plan and his goal were clear; the tasks were set.

Carrel reported about his work, gave interviews to journalists. He considered that these attempts were only the beginning, only the affirmation of the surgical technique. The scientist declared that in the near future alien organs will be grafted. He was sure in his methods, which were perfect. The main experimental model was the renal graft.

The first "renal" experiment published by Carrel together with Goutry dealt with transplanting this organ from its normal place to the neck. The kidney fused and functioned well. A year later

they published the results of the experiment which was to be repeated a thousand times by hundreds upon hundreds of surgeons. This experimental model for studying the problems of organ transplants is widely used even today. The new paper was called "Successful Transplantation of Both Kidneys from One Dog to Another with the Removal of the Two Normal Kidneys from the Latter".

Observe Carrel's belief in the experiments success. He calls transplantation "successful". In his paper he mentions that the dog was running and jumping by the eighth day after the operation, but says nothing of the fact that on the ninth day the dog started vomiting. The operation was repeated, the kidney stopped working, and the dog died. He thought that this was not worth mentioning: if one survived for eight days, another will last for eight years.

Carrel continued his work. The scientist was searching and this meant that he was to go through years of testing his fortitude. This was the beginning of this test. All the accomplishments, when he fused the amputated organs, were behind him. As soon as he tried to graft another, though quite similar organ, taken from another dog, he invariably failed.

The same vascular suture, the same brilliant surgical technique. The same success... but only during the first days of the operation. 10-20 days pass.. Then the alien organ is rejected. The experiments follow one another. Either a suture breaks, or a vessel is obstructed, or else a dog develops cardial deficiency.

But how can occasional failures shake the be-

lief in the omnipotence of surgical technique? The experiments continued, dozens, hundreds of experiments.

The experiments were performed not only on dogs, but also on cats. The new method of transplanting two kidneys together as a single complex including a section of aorta and vena cava above and under the kidneys was elaborated. Some cats survived for as long as 16 days.

Years passed, bringing no case of complete success. Not a single one!

The technique for each case was perfected and became quite artistic. Not a single spare motion was allowed, not a single unjustified injury of the transplanted organ. Not a spare second was spent. As it often happens, even in science, the reasons are looked for in something familiar, in something already known. This was the time when microorganisms were considered to be the reasons for all illness. In surgery every formation of pus was ascribed to microorganisms.

Every rejection was accompanied by some disturbances at the very locus of the operation. The experimentors always blamed microorganisms and tried to perfect the methods of sterilization, but to no avail: this did not bring a single success.

The organ was grafted instantly after taking it from the donor—the rejection followed.

The organ was preserved in nutrient solutions before grafting—the rejection followed.

The organ and the locus of grafting were specially treated with antimicrobial solutions, antiseptics—the rejection followed.

Every possible trick was tried, but none of

them gave good results—tissues and organs taken from another organism never fused.

Carrel developed a method of preserving the organs in nutrient media. He discovered a way of tissue culturing in test-tubes. In 1912, when Carrel was 39, he was awarded the Nobel Prize for developing the vascular suture and creating a method for culturing organs and tissues.

But the original idea was not confirmed. The inertia of thinking, the belief in the boundless possibilities of surgery inspired the researcher for a number of years. This belief gave him energy to perfom hundreds of experiments. Yet, the inertia of thinking was still to be overcome.

A vigorous researcher and brilliant surgeon, Carrel was to recognize: transplanting of organs and tissues between two seemingly identical organisms (but only seemingly) is impossible. The reason for this impossibility lies beyond the surgical mastery. Had he only decided that the operation technique was imperfect and he would have faced long years of senseless work. The courage of the scientist told in that he realized: the task was not just beyond his powers, but outside the possibilities of surgery as a whole. All-mighty surgery was not quite all-mighty.

In 1910, in the paper "Long-Term Results of Kidney and Spleen Grafts" Carrel wrote: "Since an organ taken from an animal and replanted back into this very animal by way of certain technique continues its normal functioning, and since this organ stops functioning if transplanted to another animal by way of the same technique, physiological disorders cannot be the consequences of surgical factors. The changes to which the

organ is subjected might be caused by the influence of the host, that is by biological factors."

Carrel knew nothing of these biological factors. At that time it was impossible for him, the surgeon, to comprehend the reason for the incompatibility. Even the immunologists knew too little of immunity, and immunologists, too, suffered from ponderous inertia in their thinking. Immunity was thought of only as a force that protected the body from microorganisms. A number of years were to pass before it became clear that the immunological army starts a battle not only with microorganisms, but with any other alien cells, tissues, and organs.

Alexis Carrel was a surgeon who was engaged in transplanting not by chance, but quite consciously and deliberately. He was the first surgeon who smashed his dreams against the barrier of incompatibility. He was the first surgeon who understood that this was not a problem to be solved by a surgeon.

It is interesting to note that his "senseless" work brought about the vascular suture and the methods of tissue culturing.

But these were not the foremost successes of his "unsuccessful" work. The main accomplishment was to overcome the inertia of thinking and to realize that surgery alone, even with superhuman mastery, could not solve the problem of organ transplantation. Second, the tissues of one individual were shown to always differ from those of another. The future was to find the material substrate of these differences, and, as we already know, it eventually did. This was not a

general answer, but the solution so accurate and specific that these differences can even be used in the science of crime detection.

Peter Medawar and Emil Holman

Sir Peter Medawar is a prominent British scientist, a Nobel Prize winner who received his Lord's title for his scientific achievements. He will be mentioned more than once later in these chapters. The only important thing now is that he took the next step after Carrel; he showed that biological nature of rejection falls into the category of immunological phenomena. Alexis Carrel was his precursor, but not only Carrel. The story about Medawar starts with Holman.

In 1923 a young Viennese surgeon Emil Holman was engaged in skin transplants for curing skin injuries in children. He grafted 150-170 small pieces of skin taken from donors onto injured surfaces. The grafts fused for some time and promoted healing. But in some cases strange phenomena developed. Several days after repeated grafting the children felt bad and showed rash all over their bodies. Doctor Holman recalled that repeated administration of alien proteins can cause immunization, and in these cases he decided to remove previously grafted skin scraps.

Having made this observation, Holman started to graft skin pieces not from random donors, but from those chosen deliberately, so that in the first graft a child should get the pieces from two different donors, and in repeated grafts from the first two and from the third whose skin was not grafted to the child before.

Holman discovered a surprising fact. If the skin for the repeated graft was taken from the same donor as that for the first one, the injured skin grafts were rejected twice as soon as at first transplantation. If skin from a new donor was used for the repeated operation, no accelerated rejection took place.

Emil Holman made a strikingly accurate assumption about what might underlay the nature of tissue incompatibility. He wrote, "It is reasonable to suggest that each groop of grafts triggers the formation of their own antibodies which are responsible for further disappearance of transplanted skin."

He only made the assumption but never developed the problem any further, never continued the research.

That is why the honour of the discovery and validation of the immunological nature underlying the rejection of incompatible tissues belongs to the British scientist Peter Medawar, though he started working 20 years later. It was he who plotted tissue incompatibility reactions on the map of immunology.

During the Second World War Doctor Medawar together with the surgeon Thomas Gibson were engaged in perfecting methods for skin transplants which were so needed in war time. He started from repeating Holman's experiments and verified that the transplant taken from the same donor for the second time is rejected much faster than the first one, thus showing the role of immunizing the organism with the primary graft.

In contrast to Holman, Medawar did not con-

fine himself to the assumption. He performed hundreds of experiments on animals, examined the microscopic pattern of rejection and determined the immunization specificity, which gave him basic proofs of the immune nature of rejection. In 1944 Medawar published the paper called "The Behaviour and Fate of Skin Grafts in Rabbits". In this work he demonstrated that the mechanism of alien skin rejection falls into the category of immune reactions.

If you open any textbook, if you ask anyone who is the discoverer of the immune nature of tissue incompatibility, you will receive the answer which would be quite correct: it is Sir Peter Medawar, a Nobel Prize winner. Still, much was done before him. In 1910 Alexis Carrel said: "Look for the nature of incompatibility not in surgical failures, but among the biological reasons." In 1924 Emil Holman suspected immune reaction, and in 1944 Peter Medawar discovered this reaction, or, rather, proved this to the world.

Interestingly, Holman never argued for the honour of the discovery. In 1975, in his final years, recalling his early works and his unproved assumption, he wrote in one of his publications: "What a brilliant possibility it was that we missed!"

Blood Transfusion and Pregnancy

A Stamp in a Passport

In the beginning of this book I mentioned the millions of human lives saved by immunology owing to artificial immunization, or vaccination,

against smallpox, poliomyelitis, measles, and many other infectious diseases. In this chapter I will tell about two more practical achievements which are even greater in terms of the scale of their application. What I mean is blood transfusion and the so-called rhesus of the mother and her foetus, her future child.

Every blood transfusion made by a surgeon, therapist, obstetrician, oncologist or traumatologist is always done jointly with an immunologist. The doctor who does the blood transfusion holds a syringe in his hands, while the fate of the patient is held by immunology. There are millions of transfusions, thus millions of lives.

Fifteen per cent of all women are doomed to rhesus-incompatibility with their future children. The fate of these children is determined by modern immunology. Again millions of fates! Nowadays these fates are resolved so easily, just as a short entry. There is a stamp in a passport. In my passport, for instance, this entry looks like this: "Group B(III), Rh+"

Blood transfusion is in most cases a procedure of urgent, or emergency help. There is no time to determine the blood group of either the victim, or the donor who is giving his blood after an automobile accident or some other injury accompanied with blood loss, while to look it up in a passport is a matter of minutes.

Karl Landsteiner

Karl Landsteiner received a very broad education. This was not only the credit of medical faculty of Vienna University. This deep scientific

erudition was the result of the researcher's own restless inquisitiveness.

Official education always makes but a foundation of one's knowledge, one's broad range of interests. The rest is developed by discarding unnecessary things, and, most importantly, by looking for additional knowledge.

"Auditor et altera pars"—"Listen to the other side" To avoid a lop-sided approach, Landsteiner not only listened to his teachers, but also attended the lectures of their opponents. He never believed one scientist without getting acquainted with the opposite viewpoint.

As a medical student, Karl Landsteiner not only liked chemistry but also got involved in immunology. The combination of these two disciplines enabled him to become an immunologist of an entirely new type.

Landsteiner graduated from the University in 1891. He worked in the University clinics, in the Institute of Hygiene, and then in the Institute of Pathology in Vienna. Here Landsteiner initiated his immunological research which was quite original for that time. He published five to ten papers every year and each of his further works contributed to a full and clear portrait of his scientific individuality. At the same time he was creating a new, previously unknown aspect of immunology.

Chemical thinking has always been drawing biology closer to the exact sciences. At that time, at the dawn of exact biology, chemical thinking had divided the general process of immunity into two parts. One was the body's response to alien, foreign particles or substances, microorganisms

or proteins entering blood or tissues. The other concerned the nature of the substances that triggered the body's immunological responses. By that time these triggering substances received the general name of antigens. For example, microorganisms or sheep red blood cells cause the response in a rabbit's body, in particular, trigger antibody production. This is the role of antigens.

To name something does not necessarily mean to understand it. There is a term, but what is the reality standing behind it? The effect of the antigens was clear but that was not enough. No one knew either the structure of these substances, or the number and the nature of antigens in various alien cells and proteins. All this was still to be determined.

To solve these tasks meant to make biology a more exact science. There was no immediate practical interest in these problems. They were purely theoretical and Karl Landsteiner devoted all his life to solving these problems.

Now he is considered to be one of the greatest immunologists. In 1930 he was awarded the Nobel Prize, while in 1900 he was only 32, he was just a young researcher. His interests went far beyond any practical need of medicine. So, it was no wonder that an early result of Landsteiner published in 1901, for some time remained an interesting but quite "inexpedient" observation.

Landsteiner found two antigens in human red blood cells. One of them was called "A", and the other, naturally, was referred to as "B" In the course of his abstract studies Landsteiner found an interesting thing. Not every red blood cell of a person contained both antigens. Some

had only the "A" antigen in their red blood cells and the others had only "B" Some had neither "A" nor "B".

Moreover, those whose red blood cells had A antigens contained anti-B antibodies in their sera, and those with neither A nor B showed antibodies against both antigens.

Karl Landsteiner mentioned these interesting results in his paper under a modest title, "On the Agglutinative Properties of Normal Human Blood" He never thought of the practical implications that this might entail. He published his paper and went on with his abstract studies of these substances. It was not until some years later that Landsteiner's discovery found practical, clinical applications.

In 1914 the First World War broke out.

Pirogov called the war "the traumatic epidemy", and it was then that such an epidemy started. There was a lot of injuries, some of them quite severe and unusual. This war was characterized by new kinds of weapons which caused new kinds of injuries.

The problem of blood transfusion acquired new meaning. In previous centuries doctors did not once attempt to transfuse blood during various illnesses and injuries, which was, naturally, especially important in cases of great blood losses. However, all attempts to make blood transfusion a routine practical measure of everyday health care led nowhere. The results were commonly known and gave no reasons for hope. Every third or fourth patient undergoing blood transfusion developed most severe complications, which were often lethal.

Blood transfusion resulted in a patient's death too often. It was a tremendous risk to employ this procedure, which justified itself too seldom.

In certain countries blood transfusion was legally forbidden. In the XVII century in France, Doctor Denis, a professor of Sorbonna University, together with a surgeon Emerits transfused blood to a patient whose case was hopeless. They were at first reluctant to do it but the wife insisted and they agreed. The patient died and the wife brought an action against the doctors.

To the credit of the time, the suit was carried out by the French Academy of Sciences. To the credit of the French Academy it did not condemn the doctors. The French Academy forbade blood transfusion but did it in a wise way. It decided to allow transfusion after special approval of an authoritative commission and called for detailed discussion of the result after each operation. The results accumulated were to the same effect: blood transfusion was dangerous, and, in some cases, lethal.

In 1914 practicing doctors gave attention to the observation by Landsteiner which had seemingly nothing to do with practice. Based on his studies, they started to use not just any blood but only that whose red blood cells did not stick together in a patient's serum.

In practical terms the procedure boiled down to determining A and B antigens in the red blood cells of the donor who was giving the blood and the patient accepting the blood. Donor's blood was mixed with patient's serum. If the red blood cells were stuck into clots, then it followed that the patient's blood contained substances in-

compatible with the donor's antigens. That meant that the blood did not go. Only blood compatible in group antigens could be transfused.

More than 60 years have elapsed since then. Two World Wars have stormed away. During this time hundreds thousands of diseased and wounded were saved by blood transfusion. This method of treatment is used in all hospitals throughout the world. Blood is transfused not only in cases of heavy blood loss but also at certain diseases other than injuries and in some complicated surgical operations. Notice that it all started with the "modest" abstract observation of a young immunologist.

Landsteiner's work divided all mankind into the four groups, based on their blood properties, or, rather, based on their A and B antigens. There is the first group, or, in other words, the zero group since it has neither A nor B antigen, but instead, contains both anti-A and anti-B antibodies. "Antigen-free" blood of this first group can be transfused into any other blood, since it lacks the substances triggering the immune mechanisms. These red blood cells are "antigen-free", and will not stick together in serum.

But, on the other hand, blood containing A or B antigens cannot be transfused into this blood. The serum of this first (zero) group has antibodies both for A and for B. It means that only the same, antigen-free, zero (first) group blood can be transfused into this blood.

Applying the same reasoning for the other groups we can easily arrange the pattern of blood transfusion: which groups of blood can be transfused to which people. Suppose we have the

fourth group, called AB, which indicates the existence of both antigens in the red blood cells, i.e. the absence of these antigens in the serum. This means that this blood cannot be transfused to any other blood groups—all of them contain antibodies to either A- or B-antigen. On the other hand, any blood can be transfused to this blood group patients, they make universal recipients. The first (zero) group is, so to say, more altruistic: they get less for themselves than they give to others. The fourth is more selfish: they get more for themselves than they are able to give to others.

The same reasoning can be spread to the other two groups. The second is A group, lacking B antigen, but containing anti-B antibody. The third is B, where there is no A antigen, but there is an antibody against it.

This reasoning can be done also by a reader himself. First, he will find out whether he has got it or not, and, second, he will spare the author a tiresome work of repeating all these A's, B's and anti's.

Female Rh—, Male Rh+

After practicing doctors had comprehended and triumphantly applied the discovery of blood groups, which received the name of AB0 (a-b-zero) system, the researchers started to look for other antigens in red blood cells. In 1927, an unsatisfied Karl Landsteiner together with Philip Levin discovered four more antigens. Two of them were called M and N, and comprised the MN system. The two others were called P and p. Thus, there appeared to be three red blood cell antigen

systems that united the seven various antigens.

The antigens M, N, P, and p appeared to be unimportant for blood transfusion. Nevertheless (again in an abstract way) the scientists developed techniques for their analysis and established the percentage of people carrying this or that antigen. For instance, 42 per cent of the British have the antigen A; B, 8 per cent; AB, 3 per cent; and 47 per cent fall into the zero group. As for Russians, 36 per cent have A blood group; B, 23 per cent; AB, 8 per cent; and 33 per cent fall into the zero group. Based on the system MN mankind is divided in the following way: 30 per cent carry M antigen, 20 per cent contain the antigen N, and 50 per cent have both antigens in their red blood cells.

Not all antigens found in human red blood cells are mentioned here. More than seventy of them are known, and this number is constantly growing. They can be found in many different combinations. Antigen structure, the relationships between antigens in human red blood cells are as unique as fingerprints.

In 1940 Karl Landsteiner together with Alexander Wiener started studies to compare the antigen properties of human and monkey's blood cells. They administered red blood cells of macaque-rhesus monkeys to rabbits and obtained an immune serum against the red blood cells of these monkeys. It then turned out that the serum against monkey red blood cells sticks together the red blood cells of most people. Hence the cells of most people contain some antigen present in macaque-rhesus red blood cells. This antigen got the name of rhesus-factor.

The researchers described the technique of determining rhesus-factor in human blood. It appeared to be present in red blood cells of 85 per cent of Americans and lacking in the rest 15 per cent. The ratio between rhesus-positive and rhesus-negative people in other countries is about the same. Only in Japan and some other Far East countries there are very few rhesus-negative people, not more than 1 per cent. Further detailed studies have shown that there are six basic varieties of this antigen which build up the antigen "Rhesus" system. These antigens are denoted with the letters C, D, E, c, d, and e. People are considered to be rhesus-positive if their blood cells contain the major system antigen, that is the antigen D.

At first this discovery also seemed practically irrelevant. But as soon as a year afterwards a very interesting coincidence was noticed.

If a rhesus-positive man marries a rhesus-negative woman, their new-born children will quite often have jaundice. Red blood cells are destroyed and the cell pigment enters the serum, coloring all the tissues. This destruction of red blood cells is called "hemolysis", and this jaundice in newborn babies is called hemolytical jaundice. Sometimes this disease is very severe, and the children die. Some of the babies die before delivery, during the last months of pregnancy.

If both mother and father are rhesus-positive or both are rhesus-negative, so to say, rhesus-similar, there is no such complication. Everything is all right also when mothers are rhesus-positive, no matter what is the rhesus-factor of the father. Numerous observations brought about the

conclusion: hemolytical jaundice of newborns is caused by rhesus-incompatibility of mother and child, not yet a baby, just a foetus.

A child always inherits half of his features from his mother and a half from his father. If the father has rhesus-factor in his cells then his child can also have it, that is to be positive in this feature. Still, this child is developing in the body of his mother who can also be rhesus-negative. In other words, a foetus with the father's heredity produces the rhesus-antigen lacking in his mother's body, an antigen which is alien to the mother's organism. If this rhesus-antigen penetrates the mother's blood it will cause the formation of anti-rhesus antibodies. From the mother's body the antibodies get into the blood of her would-be child, still a foetus. They glue and destroy red blood cells. The foetus either dies before delivery or the newborn baby develops hemolytical jaundice—a severe, often lethal disease in newborn if only one is produced in time and alive.

When the mechanism of this disease was understood, the tremendous practical value of the discovery by Landsteiner and Wiener became evident. Once the possible complication could be foreseen, scientists started to look for the means of its prevention and treatment.

A Disease Eradicated by Immunology

Immunization of the mother with rhesus-antigens of the developing foetus does not take place in the beginning of the pregnancy, nor even in the middle of the term, but at the end, more exactly, during delivery. At the initial terms of its

development the embryo does not have any circulation system—no heart, vessels or blood. There are no red blood cells, as well. Then, once all the organs and tissues appear and red blood cells start their circulation in the body of the foetus, they do not yet carry rhesus-antigens on their surface. At the last, these antigens appear.

However, the mother is not yet immunized by these antigens, since the blood and red blood cells of the developing child do not get into the mother's organism. The foetus's and mother's circulation systems are still separate. A special organ, the placenta, separates the circulation systems of these two organisms. Placenta is a biological membrane. On the one side is the mother's blood, and on the other there is the blood of the child. All nutrients and oxygen do penetrate through this membrane, but it remains impenetrable for all cells, including red blood cells.

Of course, there are some minor disturbances, such as the break of a small blood vessel, or a slight infectious disease which disturbs the circulation. If such accidents are accumulated, then by the end of the pregnancy mother's blood has antibodies against the rhesus-positive red blood cells of her own child.

There are not so many of them as to do harm to the child, but not enough to bind the newly emerging red blood cells and to prevent further immunization. This is why, during the delivery, which is always accompanied by hard vascular disturbances of placenta, a lot of rhesus-antigens gets into the mother's circulation. This causes strong immunization with the production of a large quantity of antigens. The next child, during

the second pregnancy, develops in much tougher conditions. He is constantly under the impact of the destructive anti-rhesus antibodies. Therefore hemolytical jaundice of the newborns most often accompanies not the first, but the second delivery.

The immunological method for preventing hemolytical jaundice in newborns (it is the only existing method) is as follows. If the mother is rhesus-negative and the father is rhesus-positive, then by the end of her first pregnancy she should go to the clinics several days before the usual term. Just before the delivery or straight after it she will be given an immune serum with high quantity of anti-rhesus antibodies. They will cause no harm to the child but by having bound the antigens which penetrated the mother's blood during the delivery, they cancel the immunization process. Serum-born antibodies will disappear from the mother's blood in 2-3 weeks, and her own ones will not be produced. The second child will be beyond danger.

This method has been approved throughout the world. Its effectiveness, assessed by the World Health Organization, is 98 per cent. This means that in 98 of 100 cases the hemolytical disease in the future child is cancelled. Effectiveness this high is not so often found in medicine. That is why professor Alvin Viskurski, summing up the achievements in preventing hemolytical disease in newborns, called his paper "The Disease Eradicated by Immunology" Let us back it up with a simple arythmetical calculation. If there are 200 million people living in a country, 100 million of them are women. 15 million of them are rhesus-

negative. During their life most of them, let it be 10 million, want to have a second child. So, only during the lifetime of one generation immunology gives health to these 10 million babies.

If, for some reason, anti-rhesus globulin was not administered, and severe hemolytic jaundice appeared, then the doctors would have to resort to very complex and, so far, not as effective measures. The newborns undergo blood transfusion: the baby's blood is completely replaced by a compatible donor's blood. All the antibodies against rhesus-antigen are removed from the baby's body, and the red blood cells stop to be destroyed.

Individuality and Crime Detection

Antigen Kaleidoscopes

Now it is time to recall the title of the book "Me or Not Me" To distinguish between one's own organism, own cells or own proteins and an alien organism, alien cells or alien proteins—this is the principal mission of immunity. To do this, all organisms (every one!) must be different from each other in features recognizable by the immune system. Now the question is: how many features are there—ten, a hundred, or a thousand?

Let us answer this question with a simple example. A microorganism penetrates the body, triggering the formation of antibodies. The antibodies are strictly specific. When the bacteria of typhoid fever enters the body, antibodies appear only against these bacteria, and when there

are cholera microorganisms there are the antibodies against cholera vibrio. Anti-typhoid fever antibodies do not affect the cholera pathogens, and, vice versa, anti-cholera immune sera fight only with cholera microorganisms, but not with typhoid fever bacilli.

It follows that the antibodies of typhoid fever pathogens differ from those of cholera. The antigens of other bacteria, such as those of plague, dysentery, anthrax, diphtheria, tularemia, and others, are also different. All microorganisms differ from each other in a range of features, and, primarily, in their antigens. Please do not think that every one has the only one antigen. Each microorganism has a whole set of antigens.

Let's take typhoid fever bacteria. It is a microscopical bacillus, 1-2 microns long, with a number of thin pedicles, or flagella. This microorganism contains a dozen antigens. The principal ones are H-antigen in the flagella, and O- and Vi-antigens in the body. The latter accounts for the aggressive qualities of the microorganism.

If some alien substances, other than microbial antigens such as human blood cells, are administered to that animal blood, they cause the formation of antibodies interacting only with these human cells, that is gluing these cells together. Antigens appear also if animals are treated not with cells but with cell-free proteins such as the blood serum of some other person. These antigens interact only with human proteins, not affecting the animal proteins.

If even microorganisms have several antigens each, imagine how many of them there should be in human blood and tissues. There are cer-

tainly many more than a dozen. There are about thirty of them in blood serum alone.

It was most graphically demonstrated by a French scientist of Russian extraction, Peter Grabar. We have already mentioned the ties between chemistry and immunology. Let us now talk about physicochemical methods. Grabar immunized a rabbit with human serum and had every reason to expect that every serum antigen would trigger the formation of its own antibody. No doubt, he was right. After that he placed human serum into agar-agar gel and transmitted electric current through it. Various antigen proteins were distributed in the electric field in different ways, since they all were different in their molecular sizes and electric charges.

Grabar treated the gel with rabbit serum containing antibodies, and each antibody bound its antigen, bringing about multiple precipitation. (Transparent serum grew turbid). 19 precipitation arcs appeared. It was surprisingly simple, therefore tremendously beautiful. Then this method was improved. As a result human serum showed the presence of up to 25-30 various antigens. This is the result of today, and who knows what will be discovered tomorrow?!

Apparently, the same is true for every other type of human cell. The most detailed study in this respect was undertaken for red blood cells. Some people have antigen A in their red blood cells, others have B, some have both A and B, and some, neither A, nor B. This is the antigen system AB0 (a-b-zero), of which we already know; then the antigens MN were discovered followed by Rhesus (Rh) system, consisting of

eight antigens, along with the antigen systems of Duffy and Kell-Cellano. By now 14 systems are studied in detail, which contain more than 70 various antigens composing the unique antigen pattern of red blood cells.

In terms of basic red blood antigens this pattern in a man might look like this: 00, MN, Ss, DD, Cc, ee, Le^{aa}, Kk, Fy^{bb}, Lu^{ab}, Pb, Jk^{aa}, and some other man could have it like this: AB, MM, Ss, Dd, Cc, Ee, Le^{ab}, Kk, Fy^{ab}, Lu^{aa}, Pp, Jk^{bb}.

When one looks at these signs, this symbolysm of protein individuality, one thinks of some visiting card of every living creature. You have one antigen card, I have another, your cat has some third one, your neighbour has a fourth, and so on. The number of antigen kaleidoscopes is as much as the number of living beings on the planet.

This reminds me of a "visiting card" of the Earth transmitted as radiosignals on November 16, 1974 by a Puerto Rican radiotelescope towards the star accumulation Messier-13. This is an accumulation of 30 thousand stars. If at least 3-4 planets are rotating around each of these stars, then there are not less than 100 thousand of them in the accumulation. Imagine how great the possibility is that life and creatures of reason do exist at least on one planet.

Imagine that these creatures of reason will receive this transmitted series of signals composed by a group at Cornwell University headed by Drake and Oliver. Will it be difficult for them to decode this series of 1679 transmitted signs? How long will it take them to guess that the

number of 1679 is not an ordinary one, that it can be obtained only by multiplying two simple figures 79 and 23, which cannot be divided into any figure? And if they guess it, will they contrive to arrange the signals as 79 lines, 23 signals in each line? If they do, they will get something like a page from a check-paged notebook. Since there are the signals of only two types, built on the principle "yes or no", or, if you wish, crosses and nills, they will give a special pattern on a page.

The inhabitants of another planet will hold "the visiting card" of the Earth. They will see the figures from 1 to 10 demonstrating our scale of notation. They will see the coil, the symbol of nucleic acid, underlying all life on our planet. In the centre—a conventionalized figure of Man, the host of the Earth. To the left is the number of 4 billion, the population of our planet. To the right, the figure 14, the average height of a man, measured by a wavelength on which the signals are transmitted (its length is 12.6 cm). The representatives of a distant civilization will see much more on this card. But wouldn't it look senseless for them? How long will it take to decode and understand this information?

"Antigen kaleidoscopes", unique for every living being, are the living messages of nature to human reason, to the scientists: decode them and you will get to know many important things. And the scientists are trying to do it. It's just that no one yet knows at what stage of their way they are now. Did they contrive to divide all the messages into 79 lines, 23 signs in each, and are

they now decoding the general pattern? Or have they not yet grown even to this first stage? Who knows?

Antigen patterns are born not only by red blood cells. The studies of other cells and tissues have shown that these, in terms of their antigens, repeat the pattern of red blood cells, as a mirror of a kaleidoscope ornament. But, besides, other cells have also their own antigens, which are absent from red blood cells.

This point is very important for us.

The main antigens, which account for the rejection of alien tissues in transplantations, are found not in red blood cells. These antigens are called transplant antigens or the antigens of tissue incompatibility. Human red blood cells lack most graft antigens. All tissues and organs which can be grafted contain these antigens but red blood cells do not.

Fortunately, blood contains not only red blood cells, or erythrocytes, but also white cells, or leucocytes. It is these white cells that have the antigens of tissue compatibility. Hence, once a blood sample is taken from man, virtually the whole antigen set can be determined; Grabar's method gives the antigens of blood serum, red blood cells account for blood groups, and leucocytes show special transplant antigens.

What are these antigens?

The various scientists who discovered these antigens gave them different names. A pioneer in this area, the well-known French immunologist Jean Dausset, who received the Nobel prize for this discovery, called the leucocytal antigens HU—1, 2, 7, 12, etc. The first two letters "HU"

are taken from the word "human". An immunologist from Leiden, Ion Van Rood, who found a number of transplant antigens, designated them as 4a, 4b, 5a, 5b, 6a, 6b, 7a, 7b and 7c. He tried to stress the genetic connection between various antigen groups. The American researcher Paul Terasaki used the combination of three letters, that is HL-A1, HL-A2, HL-A3, etc. This stands for: Human-Leucocyte-Antigen 1, 2, 3. Other authors used different denotation, different symbols for the same antigens. Then these denotations were compared, and, at a session of WHO Special Expert Committee, uniform nomenclature was adopted.

Today we know of more than 60 leucocytal antigens. The whole set of transplant antigens were named HLA, that is Human Leucocytal Antigens. They are divided into 4 groups: HLA-A, HLA-B, HLA-C, and HLA-D. Individual antigens in each group are denoted by figures. For instance, my antigen kaleidoscope is this: HLA-A2,9, HLA-B5,12, HLA-C5, HLA-D3.

Man is no exception. The antigen structure of animals is not less complex. And each animal species has its own antigens, and antigen kaleidoscopes differing from human ones. Each animal has its own pattern of antigen kaleidoscope.

Immunologists Follow After Holmes (A Modest Imitation)

Mr Leslie Brent, a well-known private detective, puffed at his time-fossilized, palm-polished pipe and sank into a deep armchair. Boundless savanna, violet Australian grassland, the cradle of

countless cattle herds and wild kangaroos, stretched behind the window. This small town, just as the two previously visited by him, had almost no industry besides a slaughter house and meat-processing factory.

Brent again recalled the evening when his cosy Sydney apartment was visited by the president of the largest meat-and-milk company. He was very much distressed and told Brent that some companies had already gone bankrupt, his own already suffers millions in losses. This was due to the activities of a gangster organization, and his company could not look to the state authorities for help.

"Why not?" the detective asked.

"If the police are involved in the matter, we'll be charged with violating the principle of free trade."

"For a year someone has been flooding the market with fabulously cheap beef. It is sold at a price far lower than its cost, which means that those who are selling it spend no money for getting it."

"Perhaps it's smuggling?" asked Brent.

"No, smuggling is out of question. We checked with the frontier-guards. They guarantee that during the last ten years not even a dozen head of cattle were imported into Australia legally or illegally. The Commissar General of the Customs laughed at me, saying that it is easier to hide a hundred pearls from the customs than a hundred cows.

"Then, perhaps, larceny?"

"Definitely not. We have established strict qualitative and quantitative control. The quantity of

meat products manufactured by the factories exactly corresponds to meat intake. The basic goods, sausages, contain an accurately established amount of beef. Thefts at meat factories are out of the question."

"This means that someone has learnt a cheap way to produce beef from the sun and the wind."

"Dear Mr Brent, you are joking, and the firm will crash in several months."

"No, I'm not joking, I'm just pleased with the coming trip. I haven't seen real sun and haven't breathed dry step air for ages. I find your case interesting enough to entertain me during my vocation. I start tomorrow. See you later, sir."

No more than half an hour after the departure of the excited president, the telephone rang.

"Hello!"

"Hi, good old Lesly! Busy as always, I'm sure."

This was Dr. Nossal, a childhood friend and a favourite opponent in debates and discussions.

"I'm free for two weeks" the doctor was saying, "What would you say about having a trip to an ocean coast?"

"No, only to the savanna."

"That's great! Frankly speaking, I love the savanna too. I never figured out how to distract you from your business and tried to entice you with a trip to the coast. When do we start?"

"Tomorrow morning."

The violet expanse over the window became darker. The pipe turned cold. The famous detective seemed to fall asleep. Dr Nassal came in.

Say, Leslie," he said, "it seems to me you are

solving this meat-and-milk problem of yours instead of having rest."

"A man always solves problems. But sometimes he just observes. Sit next to me and look at the window. Do you see the gates of the slaughter-house?"

"It's getting dark, the gates will be open in a minute, a herd of cows will be brought out and sent further into the step."

"What a remarkable discovery", the Doctor laughed, "Every little boy knows it. The cattle which remains unslaughted at the end of the day is brought to a pasture."

"But, for some reason, they bring out the same number as they brought in in the morning. "

They heard the squeak of heavy gates from the window. For ten minutes or so the friends observed silently as a large herd of cows was brought out of the gates.

"Are you sure," the Doctor asked, "that they bring out the same number as they have brought in in the morning?"

"Yes, now I am, and I'm sleepy. Tomorrow at dawn we are going to a spot not yet destroyed by slaughters, meat-processing factories and people."

... The morning step was lilac-coloured.

A herd of kangaroos crosses the road. The driver of the "Jip" was waiting calmly with the engine switched off. This was not a big herd, no more than a hundred heads.

"They disappear more and more, our beauties," the driver sighed, "only two years ago, if you could get here during kangaroo's morning 'exercise' you would lose more than half an hour

on the highway. And now the big herds are scattered and the small ones are all slaughtered".

"And who kills them?" Brent asked briskly.

"I don't know, some people with excellent carabines, good automobiles, and licences for unlimited trap and fire for kangaroos."

"It seems to me that we did not waist our time by going to the step," said the detective to Dr. Nossal, "I have a feeling that I'm going to catch the end of this meat-and-milk tangle."

"Are you expecting to meet a clairvoyant in the step?"

"I have already met him," Brent retorted, "He is sitting at the wheel of our automobile. Would I have known that he would say, we could have stayed at home instead of going to the step. We went to the step to look for the beginning of the tangle, and the step gave it to us. I'm only afraid that it will not be easy to untangle it."

"Where is this thread?" the Doctor asked.

"Here it is," the detective pointed to the dust stirred up by a kangaroo running away.

"But the supercheap meat at the market is excellent beef, not kangaroo meat," Dr. Nossal remarked.

"Exactly! Kangaroo meat goes to sausages, then the beef can be sold cheaper to undermine the competitors."

"I wonder," the Doctor said pensively, "If your suggestion is correct, why do you think it will be difficult to unwind this tangle?"

"Because it is impossible to prove the kangaroo origin of the sausages. Not even the most keen taster and most experienced chemist can tell it."

Doctor Nossal burst out laughing, genially

looking at his friend. The detective knew that the Doctor was laughing so amiably when his medical knowledge can be of a decisive help.

"Nothing is simpler. Give me one sausage, and my colleagues at the Institute will determine not only the animal whose meat this sausage is made of, but even the breed of this animal. If ten animals were used for making this sausage, I'll enumerate all the ten of them."

"How will you do it?"

"Using immune sera. Just as doctors determine the blood groups in man. We'll prepare the sera for all species of these animals and we'll be able to detect their proteins. They are all different. There are no identical ones, even if they are turned into perfectly identical sausages."

Four weeks later the Attorney General of Australia charged two big and prosperous companies of breaking the Australian law forbidding the use of kangaroo meat for producing sausages.

In fact, there is really a law concerning sausage meat in Australia, and the most prominent Australian immunologist, Frank Burnet, in his book "The Integrity of the Body and the Immunity" jokingly suggests the use of immunology to control this law.

Ruling Out the Paternity

When, in 1930 Karl Landsteiner got his Nobel Prize, he delivered a special lecture. In this lecture he said that the discovery of new antigens in human tissue cells will be endless, until it becomes clear that there are no two people iden-

tical in respect of antigens. This prediction of his has been confirmed, and is now not only of a theoretical interest. It received, among others, practical application in forensic medicine.

Imagine a situation like this: there is a blood stain, and it is necessary to determine its origin. Whose blood is this, an animal's or a human's? There is no need to explain that this situation would most often have to do with crime detection, and that solving this problem often means answering the principal question of the investigation. This question can be answered only by means of immune sera. There are no other indicators to distinguish, say, between human and dog's blood. Here microscopic or biochemical methods of research are powerless.

Forensic medics have quite a set of immune sera for various specificities at their disposal: those against the proteins of man, horse, hen, dog, cow, cat, and others. The blood stain to be investigated is washed off, the solution is cleared from the specks of dust and the particles of the thing on which the stain was found, and precipitation reactions are conducted using the complete set of the immune sera. If a certain animal or human serum makes the solution turbid, meaning that it has caused precipitation, then the blood stain belongs to this animal or man.

Let's say that the forensic expert concludes: "The knife is stained with human blood." The one suspected in the murder says: "Yes, but this is my blood: I have recently cut my finger with this knife." Then the examination goes on. Antisera against the blood groups appear on the expert's table. And, again, immunology gives an

accurate answer: the blood belongs to group AB and contains M factor, which is rhesus-negative. The situation becomes quite clear. The characteristic obtained is identical to the antigen characteristic of the suspect's blood, meaning that he had really told the truth, it is his blood.

To conclude, let us imagine another situation with important moral implications. Suppose that war or some calamity has separated parents and children. The children lost their names. Is it possible to find your own child among others? Antigens are inherited. If the father and mother do not have M factor, then it cannot be found in the child. And, conversely, if both parents belong to group A, the child cannot have B or AB blood group.

Yes, this is actually true. The only accurate and objective method for establishing the paternity (the mother is usually known) is the immunological one. In some countries, such as the United Kingdom the questions of determining the paternity are especially tender spots. But there it is most often not related to war. Strict laws on the paternity are underlied by strict rules on the heirs and the rights for inheriting capitals, titles, and privileges.

Imagine some lord who declares his heir a boy whose mother is not the lord's wife. Then it is necessary to prove that the boy is his son. Or some gentleman suddenly appears who claims to be an illegitimate son of a millionaire and hence his heir. It might be true, but, on the other hand, this gentleman might be a swindler. The question is solved by analysing the parents' and children's antigens.

Let us consider the rules of inheritance on the example of several antigen systems. The table below shows the "antigen maps" of the hypothetical father and mother. In our case, in AB0 system, the father belongs to the zero group, and the mother, to AB group. One feature is always inherited from the father, the other from the mother. Their child can only have the group A0 or B0. If he turns out to have AB group, he should look for a different father, and if it's zero group, he has a different mother.

Regularities of Inheriting Blood Groups

Antigen system	AB0	MN	Rhesus		
Antigens in the father's red blood cells	00	MN	dd	CC	ee
Antigens in the mother's red blood cells	AB	MM	dd	Cc	Ee
Possible antigen combinations in the child	A0 B0	MM MN	dd dd	CC Cc	Ee ee
Impossible variants	00 AB	NN	DD Dd	cc	EE

If the assumed father or mother is not related to the child, then the negative answer is most often obtained by an analysis based on the AB0 system. In some cases other antigen factors, such as MN, and rhesus, are also analyzed.

In our example, in the system M the father has both M and N types of antigens, while the mother has only one of them, and her character-

istics in this system is MM. Their child must contain M factor, so if his characteristics is NN it means that he is not their child.

Sometimes the discrepancy between the child and one of his parents is revealed only after examining a large number of systems. But if the child is really born to these parents, the correspondence of inheritance regularities is always absolute in all the antigens.

Unfortunately, it is very difficult to try all the antigens. Therefore it is juridically more difficult to claim the paternity. An argument is always ready: "You haven't tried all possible antigens, at least since not all of them are yet discovered." The denial of paternity is always absolute. A legal expert is sure: these people are not the father and the son!

Individuality—Above All: All Alien is Alien

Once Again Remembering Carrel

Tissue or organ transplantation from one place to another within the same animal is a success. Attempts to transplant an organ or tissue from another individual from the same species, for instance, from one dog to another, even if these dogs are of the same breed, always resulted in the grafted piece of tissue or organ being rejected.

A section of the organism, be it skin or organ grafted to a different place of the same body, or to another representative of the same species, or

to an individual of quite a different animal species, is called a graft.

Over and over the surgeons repeated the attempts of Holman and Medawar. They staged special experiments on themselves and volunteers. A piece of skin was cut from a man, and a similar piece from another man was sutured in its place (of course, using anaesthetic), sutured soundly and in sterile conditions.

This, however, was of no help. The skin scrap was alien, and triggered the immune mechanisms which promoted reactions against the antigens of the grafted skin. The immunological war began. The body produced antibodies, and the cells (the soldiers of our army, our defenders) surrounded the graft. The entire organism of the recipient built up a barrier of these cells to isolate itself from the alien donor tissue.

The immunological reaction to a graft is unusually intense. The sutured skin scrap seems to fuse for the first two or three days. The edges of the grafted piece merge with the surrounding skin. The vascular network is restored and starts its work, the blood of a new host flows along the vessels of the graft feeding it. But by the 5th to 7th day the circulation is disturbed. The bordering hem of the host's cells grows bigger. Antibodies appear, and by the 10th to 16th day the graft is rejected.

When a skin scrap is grafted again from the same donor, the recipient is already immune to it, and the graft is rejected twice as fast as before. Skin from a different donor is rejected with the same speed as at first, that is in 10 to 16 days. This proves that the main enemy is im-

munity and that, like the antimicrobial immunity, this immunity is specific.

The immunity guards individuality. Only your own tissues with their individual set of antigens, with their own unique pattern of antigen kaleidoscope can exist in your body. This is the barrier to surgeons endeavouring to graft skin, bone marrow, kidney, or anything else to an injuried or diseased patient. The army of immunity prevents them from doing it. The maxima: "Individuality is above all; all alien is alien" is unquestionable for it.

Now you understand why immunity, which saves us from death in the struggle with microorganisms at other times, becomes our enemy, of course, an enemy, relatively speaking. Putting it more mildly, sometimes immunity interferes. It guards the consistency of the inner medium, it vigilantly watches the body's biological individuality. Should we consider it an enemy if sometimes it blindly continues to do its job when we do not need it? Still, it is more useful than harmful.

So, although immunity is no enemy to us, sometimes it would be better if it does not exist at all.

As soon as cells or tissues differing in at least one antigen get into the body, they trigger antibody production. Lymphocytes go for the alien tissue and doom it to destruction.

If a surgeon tries to graft the alien skin to a wound or burn, this skin will be rejected no matter how skilfully it has been sutured. If a doctor attempts to transplant some internal organ or a part of this organ and if there is no room

for it to be rejected into, it will with certainty be dissolved. Phagocytes will devour it in small parts, slowly and ruthlessly. Even a bone, if it is alien, will be dissolved, destroyed by the microscopic devouring cells.

Surgery, which has gained amazing mastery, was stuck in front of its most cherished dream: not to be confined only to removing a diseased organ, but also being able to replace it by a healthy one. The immunological army put the barrier of tissue incompatibility in the way of this dream. Today the surgeons' mastery is great enough, they are not intimidated by the technical problems of grafting some others' hands, legs, kidneys, lungs, and hearts. Yet, even the simplest operation, such as bone marrow transplantation, is impossible if the donor is incompatible with the recipient.

The Institute headed by Dirk Van Bekkum, placed in Rijswijk, a small town near the Hague, deals with bone marrow transplants for treating radiation sickness, blood diseases, and congenital immune system defects. The basic problem in this area is tissue incompatibility in transplants. One would think that there is nothing simpler than a bone marrow transplant. It is neither kidney, nor heart, not even skin. Nothing has to be cut or sutured. There are only two injections with special syringes. The first one is to a donor, to get the bone marrow cells from his ilium, and the second one to a recipient, to administer these cells to his ulnar flexion. That's all, and it seems to be quite simple.

Still, bone marrow grafts are one of the biggest problems in transplants. In contrast to many

other organs the bone marrow contains a high number of lymphoid cells, which, as all the cells of immune system, recognize only the orders of their own genes. Once they get into a different body, they instantly start to develop the immune response against this body. It is alien for these cells, and the lymphocytes are activated, multiply, and start to "gnaw at" their new host from inside. This phenomenon has been called the "Graft versus host reaction".

When a doctor grafts a kidney, he has only one concern: to suppress the patient's immunity, so that his organism would not reject this kidney. Bone marrow transplants pose another problem: how to prevent the grafted cells from killing the patient. This happens in cases of bone marrow incompatibility. But in practice the bone marrow is almost always incompatible. A perfectly suitable donor, compatible with a patient in all his numerous antigens, is a very rare event. The probability of finding such a donor is between 1 in 7000 to 1 in 20 000.

In the summer of 1969 I had an interesting discussion with Van Bekkum. We were walking from Rijswijk to Delft, and the evening was very special. We had been just watching TV broadcasting the "Apollo-11" landing on the Moon. Edwin Aldrin descended the ladder of the moon module and stepped onto the Moon surface. He took some devices and arranged them at some distance from the apparatus.

Under this impression we could not help thinking how scarce were our achievements in immunology in general, and in bone marrow grafts in particular. We talked of how much medicine is

lagging behind as compared with technology. Then we decided that this lagging behind was quite relative. Owing to medicine mankind was spared of most infectious diseases, the average life span grew from 40 to 70 years during the last century.

True, during the same time owing to the achievements of technology people learned to drive automobiles and fly planes. And now they are walking on the Moon.

And still, the point here is not the lagging behind, but the extremely complex character of organ and tissue transplant problems. Van Bekkum stopped, looked at the water of the channel we were passing, and said: "If man walks on the Moon, but is not able to graft bone marrow, it only means that it is easier to walk on the Moon than to graft bone marrow; otherwise we would have solved this problem long before."

It is amazing how precisely he grasped the situation. These two problems seem uncomparable in their scale: on the one hand, the tremendous problem of Moon landing, and on the other, only the need to overcome tissue incompatibility in transplants. But this second task is much more difficult for mankind. To solve it would mean to spare people of many blood diseases, including leucosis (blood cancer), radiation sickness, and immune disturbances. Organ transplants will become a routine surgical operation.

And then immunologists will be able to engrave proud words on a corrosion-resistant plate, the words immortalizing a greatest achievement of mankind. A plate like the one left on the Moon by the crew of the "Apollo-11":

"Here the people from the planet of Earth first stepped on the Moon. July 1969. We came with peace for all mankind."

The level of modern surgery would enable the doctors to graft any organ in any place of the body. Today there are no inaccessible places for surgeons. The problem is that the results of grafting depend not only on the level of surgery and the degree of surgical skill. The point is that nothing alien can be fused due to antigen differences. The immunological army is always faithful to its principle: it prevents the fusing of an alien organ, bone marrow, or skin.

All alien is alien!

A graft is always rejected, if only a grafted organ is not taken from a twin, and not just any twin, but only an identical one.

Identical, or monozygotic, twins are those developed from one ovicell. They are identical in all respects as two drops of water. Of course, there are also twins which are quite different from each other. There are twins of different sexes, the brothers and sisters from different ovicells. These are hetero-zygotic twins.

The similarity between monozygotic twins is sometimes so strong that even the parents cannot distinguish between their twin children. Similarly, the immunological army of each of the twins "gets confused", not in the twins themselves, but in the antigen composition of their tissues which are identical as two drops of water. The immunity army of each of the twins takes the other's tissues for his own, does not produce antibodies against them and does not try to reject them.

Even this is not quite true. In fact, they have

the same tissue, though there are two different people.

There is one fertilized cell, which starts to develop and first divides into two cells, then into 4, 8, etc. in geometrical progression. At a certain point, say, at the stage of 8 cells, this aggregate could be divided into two parts, four cells in each, and these will go on developing independently. This is followed by tissue differentiation and the formation of organs, giving rise to the two foetuses and then two children.

But these two children have one tissue, emerging from one cell, with the same genes, and the same antigens. It is natural, therefore, that a transplant from one monozygotic twin to another will be successful. The immune system will not consider the graft to be alien, and will be silent. Today there is a number of successful skin, kidney, and bone marrow grafts from one identical twin to the other. The organs fuse and function normally.

Unfortunately, not all people are twins, and not all twins are monozygotic. This means that the success of human-to-human grafts is an exception from the general rule, namely: "Grafted organs are doomed to death"

Medical Watergate

Experimenters often asked themselves the following question: Is it possible to wash off the antigens from a grafted tissue, skin, for instance, or to treat this tissue so that the antigens will be neutralized, will dissolve and disappear? Of

course, the cells should be left viable, otherwise the tissue will stop functioning and die.

All the scientific data available testified to the negative answer. If the cells retain all their capacities, if they live and multiply in a normal way, they will reproduce all their antigens no matter how much we try to remove them. The genes located in the cell nuclea will give accurate commands. Unless the genes are changed the commands will remain the same. And we are yet incapable of changing genes in the desired direction, even less so in a complex tissue or organ.

However, until quite recently there was a belief among certain researchers that there should be some ways of treating an alien graft so that it will become compatible and fuse. Some of them tried long washing of an organ or tissue to be grafted with nutrient solutions. Another placed the grafts into deep freezers, to freeze them to —20, —70, or —190 °C, and expected antigen simplification. Others treated the skin scraps with weak Formalin solution. Formalin is known to condense proteins and render them insoluble. They thought that the antigens would fail to detach from the grafted scrap and stimulate immunity.

No arguments could stop these enthusiasts. They did not want to wait until gene engineering learns to change the cell gene sets in desired directions. They wanted to succeed in a stroke, overnight, despite the elementary biological rules. The attempts of disputing with fanatics always failed.

Once in a while scientific magazine published the papers reporting the successful fusing of an

alien skin scrap owing to a certain physical or chemical treatment prior to transplant. And it was always difficult to decide whether the author is frankly deluded or he deliberately tries to delude others.

A scientist has a right to be deluded, but he never has a right to forge the experimental data. It is quite senseless in science for two reasons. First, because the very essence of research consists in multiple checking suggestions until the truth is elicited. Second, forged data will be by all means refuted by others. Forgery in science can never be concealed.

Doctor William Sammerlin was the head of skin diseases clinics in Stanford University, California. It was there that, in 1970 he claimed to be able to take skin from one man, and, having kept it for two weeks in a special nutrient medium, graft it to another. The skin was said to fuse with no rejection reactions.

With this sensation (and a sensation it was, since it promised, after all these years, to finally bring a solution to the problem of tissue incompatibility in transplants) Sammerlin came to Minnesota in 1971 to complete his work for Ph.D. degree. There he applied to Robert Good, a well known immunologist, whose name has been already mentioned in this book. Good allowed Sammerlin to work in his laboratory, to find out whether the sensation was true or groundless.

Sammerlin's cheating would have probably been revealed earlier, but in 1972 Good was invited to head the United States' largest Memorial in Sloan-Kettering Cancer Centre near New York. This was not only recognition of his personal

achievements. It was a recognition of a major role of immunological problems in cancer diagnostics, prevention and treatment.

So Professor Good moved to New York.

Sammerlin's sensation remained uncovered. However, something prevented Good from completely believing this young experimenter. Maintaining of the doctor's thesis was postponed. As for Sammerlin himself, he was invited by Good to work in Sloan-Kettering.

The experiments were striking. Skin scraps from black mice fused on white ones. Black hair was growing on these scraps. Sammerlin became a Professor of the Memorial Cancer Center.

Professor Good charged his two post-graduates to check Sammerlin's experiments. No fusing. Black skin scraps are rejected.

Sir Peter Medawar, a British scientist, who had received the Nobel prize for work in skin grafts, also tried to reproduce Sammerlin's experiments. In his interview with "New York Times" reporter he declared: "I gave up this work, having become disappointed in it."

But there were Sammerlin's white mice with their fused skin scraps and black hair growing on them.

Nobody knows for how long this miracle would have lasted, if it were not for the event which took place early in the morning of March 30, 1974. Vivarium assistant caught Sammerlin near the cages with the experimental animals. Sammerlin was painting the hair of the white mice with black marker.

On the same day Professor Good set up a special committee. Two weeks later the fake was

made public. On April 18, 1974, "New York Times" published a report by a well-known journalist Jean Brody "Charge of Faking Experimental Results Worked up Sloan-Kettering Cancer Researchers". He called this "Medical Watergate". The thirty-five year-old professor was expelled from his job. He turned out to be incompatible with science.

So the immune reactions of rejection failed to be overcome by means of a special nutrient solution and a marker. This problem was closed also by other fanatics, both the ones sincerely deluded and those who tried to delude others.

"How come?", a reader would ask, "It is well known that surgeons transplant skin, bones and even vessels from one man to another. For instance in cases of extensive burns the skin is taken from volunteers and grafted to the victims. You can read this even in newspapers."

Don't think that this skin is taken for nothing. It is transplanted, and during the first days it serves the burnt hand and foot. It manages to help. First, it covers the wound. It is later rejected, but it serves as a framework for one's own new skin. On its place there remains an isle of newly emerging skin. The young tissue spreads from this isle, so these transplantations are quite useful.

So it is not for a purpose that the doctors look for volunteers to share their skin in favour of a burnt. Grafted bones and vessels will be dissolved, but they will serve as the framework for new and young bone or vascular tissue of one's own.

Today these grafts are widely used in surgery. Special services collect skin scraps, vessels, and

bones, preserve them, and, if need be, supply them to the surgical departments. These services are called tissue banks; there can be skin, vessel, or bone banks. The first tissue bank was set up in the USA in 1950.

What Does It Mean—Killer?

To attain any purpose certain means and ways are needed. The immune system has its purpose—to guard the body's genetic consistency. The body should not contain any genetically foreign cell. Whether an alien cell penetrates the body, or its own one is changed, the purpose remains the same: to destroy it.

If there is a purpose, there should be a means. The major means is T-lymphocytes emerging in the thymus. They perform the basic functions of the immune system, recognizing the genetical newcomers or genetical traitors (mutants). They handle the ways of destroying, or, at least, hindering the activities of genetically different cells.

This does not mean that all the finest details of these ways are studied and known to science. The opposite is rather more likely. We do not yet know all the details. But there are the phenomena, hence, there are the possibilities to study them. The finest details will become known in the nearest future.

The very first phenomenon of this series was discovered in 1960 and was named the lymphocyte cytopathogenic effect. These immunological functions of the lymphoid system are performed directly by lymphocytes. Here directly means by way of direct contact.

In this case the lymphocytes themselves attack the antigens of the alien cells. They attack the antigens that have already been dealt with at these pages, those called graft, or incompatibility antigens. These lymphocytes were called sensitized lymphocytes, i.e. those with increased sensitivity to certain antigens. Sensitized lymphocytes are accumulated in the body after it is affected by the graft antigens. For instance, if a piece of skin from man "X" is grafted to man "Y", the greater number of antigen-sensitized X-cells will be accumulated among "Y" lymphocytes.

In 1960 two scientific papers were published in the USA. One was by Andre Gowaerts and the other by Rosenau and Henry Moon. Both of them demonstrated the same phenomenon, namely, the cytopathogenic effect of sensitized lymphocytes towards the alien cells. New terms appeared after these publications. The cells against which the sensitized lymphocytes were acting were called target cells. The lymphocytes themselves were called killers.

To show the lymphocyte cytopathogenic action, target cells of mice, rats, dogs, or humans are grown in a nutrient medium. When added to the nutrient medium, sensitized lymphocytes are attached to the cultured cells in several hours, form aggregates around these cells and destroy them. This phenomenon is immunologically specific: only those cells are destroyed against which killer lymphocytes are sensitized. This phenomenon is reproduced on lymphocytes from lymph nodes, spleen or peripheral blood; it does not need antibodies, does not depend on antibody formation.

T-lymphocytes are fighting alone. They fight

for dear life, and once destroying the target, they die themselves. They are like bees defending their swarm, who sting, though after that they die themselves.

The killer lymphocyte bears special structures on its surface. They are called receptors. Using these receptors, it recognizes an alien cell and attaches to it so strongly that its cellular wall breaks in the point of attachment. The substances pernicious for the target are discharged to destroy it. These substances fall into the category of toxins or enzymes dissolving cellular membranes. Calcium ions play as yet an unknown, but very important role. In the presence of these ions, killer lymphocytes are especially effective in breaching the cellular wall of the target cells. If one lymphocyte fails to destroy the target, two, three, four, ten or a hundred of them will come to help.

Self-Reproduction of Killers

Imagine the following situation. Day and night the thymus is producing T-lymphocytes. A host of these cells contains all the possible variants for recognizing various alien newcomers, various target cells. Every variant, or, as it is said in immunology, every clone recognizes a certain target. There are tens of thousands of specialized clones. But there are not so many cells in each clone; and if all of them fall upon the corresponding target cells, start to kill them and die themselves, the thymus can fail to cope with the job. The point is that the thymus is producing all possible clones for all the possible battle

fields, not just a single "precise" one. This means that every clone, having faced its target, should be able to increase the number of soldiers. It should be able to reproduce itself. This makes another means for attaining the major goal: the destruction of one's alien, the destruction of the "not me".

A Canadian researcher Barbara Byne was looking for a way of infallibly distinguishing between the leucosis cells and normal leucocytes.

Leucosis, also known as blood cancer or leucaemia, steals up insensibly. An abnormally high quantity of white blood cells, or leucocytes, accumulates in a patient's blood. Treatment is not very effective, and the predictions are sad. It would help to learn to timely distinguish between the leucosis and normal cells, to make use of this difference and slay the malignant cells, leaving only normal healthy ones.

Once Barbara Byne decided to mix the leucocytes from a leucosis patient with those from the blood of a healthy donor. She placed the cell mixture into a vial, added a nutrient medium and put it into a thermostat at 37 °C. She got a mixed cell culture and day after day she studied it under the microscope.

Several days passed. There was no doubt, mature lymphocytes in the mixed cell culture were transformed into blasts, young cell forms, and multiplied. There was no such blast-transformation in a culture of normal lymphocytes. Leucosis cells alone, without the normal ones added to them, also stayed quiet. Blast-transformation took place only in the mixture of leucocytes from a diseased and a healthy person.

Everything is accurate! These data are to be published immediately. They are of importance for many scientists studying leucosis.

The paper was sent to a magazine, but the studies went on. The researcher did not cease to ask new questions. "Perhaps, the ability to be transformed is characteristic not only of leucosis cells?", she was thinking. "Indeed, I mixed the leucocytes from a diseased person with those from a healthy one and got a certain effect. But what happens if I mix the leucocytes from two healthy people, without leucosis. Maybe, the ability to transform is inherent not only in leucosis lymphocytes? Maybe, I have discovered a more general property of lymphocytes to recognize alien cells to respond to them? Maybe, leucosis has nothing to do with it?"

No, leucosis has nothing to do with it. In 1964, Byne discovered a new fundamental property of white blood cells. She mixed lymphocytes from two healthy people and got blast-transformation. She tried several more pairs of people, and the result was always the same. Only when the lymphocytes to be mixed belonged to brothers or sisters—identical twins, no transformation was observed. In all the other cases, when the cells alien to each other were mixed, there was transformation. Interestingly, the more alien the cells were to each other, the stronger was blast-transformation, the more intense was the response to strangers, the faster was the multiplication, or growing in number.

Later on it became clear that it is T-lymphocytes that have this ability to transform into blasts, then multiply and grow in number. This

results in accumulating a great number of sensitized killer lymphocytes capable of killing the alien targets.

Allogenic Inhibition, or the Atmosphere of Hostility

It would seem that until the body accumulates a sufficient number of killer lymphocytes, grafted cells should sustain normal functioning. However, this is not exactly so. Newcomers feel bad from the first instant, just because they are surrounded by strangers. Indeed, not until the immune rejection reactions are developed, are they threatened by a straightforward murder. But life for them becomes quite difficult. Lymphocytes have a gift for creating some microclimate favourable for their fellow cells and hostile to strangers.

In 1964 a Swedish researcher Karl Hellström introduced a new notion, and, hence, a new term: syngeneic preference. Mind it, I say "introduced a new notion", not "discovered a new phenomenon" Still, it was Hellström who discovered it.

It is always like this in science, it is more important to comprehend than to notice. The role of pioneer in science is always conventional. Tissue incompatibility in transplants was observed by many researchers, while the biological (non-surgical) reason for incompatibility was formulated by Carrel. The immune nature of rejection was observed by Holman, while it was Medawar who discovered it. Hellström was not the first to see the preference discovered by him, but he was the first to identify it.

Even before 1964, an American immunologist

and geneticist George Snell had noticed an oddity. He grafted cancer tumours from one mouse to another. Cancer cells fused, and the tumour grew. However, the further fate of grafted tumours and animals was different and obeyed strict regularities. Snell was working on the pure strain animals (incidentally, he bred them himself), and this helped him understand the oddities and formulate the laws. This is not a literary hyperbole; this is how Snell's rules are called: "Genetic Laws of Transplants"

According to these laws, the fate of the experimental animals goes like this:

(1) The tumour grows and turns out to be lethal if a mouse it is taken from is genetically identical to the one it is grafted to (for instance, both mice belong to A strain).

(2) The tumour does not grow if the transplant is carried out on the mice of different breeds (the tumour from the strain A mice does not grow on a B mice).

(3) A tumour of one breed (A) grows on the offsprings of this breed, no matter who is the second parent (B, C, or others). In other words, a tumour A grows on the animals AB, AC, etc.

(4) On the contrary, a tumour from the hybrid AB or AC offsprings fuses neither on A nor on B or C mice; AB grows only on AB, and AC, only on AC offsprings.

These laws are immunologically explainable. Only the tissues containing no additional antigens fuse and grow. Therefore the tumour A fails to fuse on B mice. The tumour AB fails to fuse on A mice, hindered by the antigens of B breed; neither it fuses on B mice, prevented by A an-

tigens. Alien antigens trigger immune responses, and bring about the accumulation of lymphocytes aggressive against the cells with alien antigens. The alien tissue is killed.

If "A" cells are grafted to the organism A, they grow and multiply. The same happens when cells A get into the organism AB; these cells contain nothing strange or alien for this organism, they have only the antigens of A genotype, contained also in this organism, which is AB. No immune response is developed: there are no alien elements. It is here, in the third law, that the oddity is observed. The tumour A grows both in A and in AB organisms, but much slower in the second organism. No immune response is developed, but the growth is hindered. What is the reason?

Based on this oddity, Hellström made a conclusion on syngeneic preference. He showed it not to be specific only for tumours: he showed it to be a general phenomenon, to be a law. In all cases genetically identical (syngeneic) tissue always fuses, grows and multiplies preferably to a non-identical (or non-syngeneic) one. It is so even when no immune response against it can be triggered, as with hybrid offsprings, irradiated recipients, or under the influence of immune suppressing preparations. The life is difficult for a grafted alien tissue even in the absence of immunity in its classical sense. It is difficult for it to grow and multiply in the alien environment.

For more than 10 years the mechanisms of syngeneic preference phenomenon remained obscure. Naturally, even now not everything is

clear. But, by the mid-70s some points became clear owing to work by Gustav Kudkovich.

It appeared that the atmosphere of hostility created by lymphocytes can be considered as a certain variety of immune response. It was only just that Snell and the researchers who followed him knew nothing of the antigens that are exhibited on the parental, but not on the hybrid cells. This requires the functioning of two identical genes (two similar alleles, as geneticists say), while in hybrids gene pairs are different, composed of two different alleles.

Kudkovich called these bone marrow antibodies Hh, that is hemopoietic histocompatibility—tissue compatibility antigens of hemopoietic tissues. Graft immunology was enriched with the discovery of immunological response against special antigens exhibited only in homozygous situation, that is in the case when both alleles of the genes controlling these antigens are identical.

Lymphocytes "Hit the Core"

One graphic manifestation of lymphocytes' killing action on alien cells was shown at the Institute of Biophysics in Moscow. This was mentioned in the chapter "The Dictatorship of the Lymphocyte", but, I think, now it is worthwhile to speak of it in detail.

In 1965 a young researcher came to our laboratory and applied for post-graduate study. She had been working at a different institute before and was engaged in freezing and storing bone marrow for transplants. Yet, she was attracted

to other problems dealing with the reasons and mechanisms of tissue incompatibility, the problems of graft immunity.

There were formalities and examinations, and the laboratory was reinforced with a new worker. It was really a full-fledged worker, not just a post-graduate, since Liya Seslavina could do a lot of things in the laboratory. There was no need to teach her the ABCs of experimental techniques. Straight off she started the research. Her task was to take quantitative account of cytopathogenic effect of immune lymphocytes and to see how this effect is suppressed under radiation sickness. Some quantitative assessment was needed to be able to count cells in a test-tube directly.

Rosenau technique did not work.

Then David's procedure was rejected.

The Freedman's method was discarded, which, to begin with, turned out to be a phoney: lymphocytes killed no cells in his system, so it never reached a point of calculations.

We spent hours inventing shapes for vessels most convenient for bringing together immune lymphocytes and target cells, that is the cells to be killed by these lymphocytes. We spent hours thinking of how to contrive and calculate the killed cells, to calculate in a most accurate way.

Weeks and months passed. A year elapsed. Everybody felt sorry for Seslavina. Time was passing but she was getting no further in her work.

We decided to give it the last try: to mix the spleen cells from the immune mice with the spleen target cells, and then calculate the num-

ber of stem cells there. Perhaps, these cells will be the first to be hit out. If this does not go either, then we'll have to work in the old fashion.

Remember, the stem cells are the cells underlying the life of the entire tissue, such as the spleen or the bone marrow; these give rise to all the rest of the cells. That is why they are called stem cells. They are like a stem of a tree from which all the branches, leaves and fruits grow. Would these cells be beaten, all the tissue will die.

In ten days we were discussing the results of the first experiment. There were 20 stem cells in one spleen, and 15 of them in the other. So, the mixture should have contained 35 cells. Yet, only 12 cells were counted, meaning that more than 60 per cent of them disappeared. Stem cells have disappeared. So, they are the ones exposed to the attack of immune lymphocytes. These lymphocytes "hit the core".

Attention! It seems like we have come across something worth studying.

Liya Seslavina staged seven trials running, and seven times she observed stem cell inactivation... And then she nearly repeated the case with Barbara Byne.

The Canadian researcher took the leucocytes from leucosis patients and drew a conclusion to the effect that leucosis cells are inactivated under the influence of alien leucocytes. This was the way she published her data. Seslavina took immune lymphoid cells and came to a conclusion that it is the immune lymphocytes that kill alien stem cells.

But, perhaps, non-immune ones do the same?

Perhaps, normal lymphocytes also beat down these cells? Seslavina staged seven more experiments, and it was then that she made a discovery. It was known that immune lymphocytes can destroy the cells. It was not measured in a precise quantitative way, but it was known to happen. But it was not known that normal lymphocytes have the same property.

Seslavina got a positive answer. Having first seen alien stem cells, normal lympocytes inactivate them. This was how this phenomenon was called: "Inactivation of Non-Syngeneic (that is alien) Stem Cells".

Classical immunology has got accustomed to the two basic reactions aimed at rejecting the alien: the production of antibodies and the emergence of specifically sensitized cells carrying the active structures on their surface. Both of them appear 3-7 days after the intrusion of an alien newcomer, and get accumulated even later.

As is now known, grafted tissue appears to be in a difficult situation long before the production of antibodies and accumulation of specifically armed cells. First, its future killers, lymphocytes, are stimulated to multiplication. Second, it is very difficult for this tissue to propagate and grow in a new, "alien" surrounding. Third, the most important, stem cells, which determine the growth, propagation, and the whole life of this tissue, are the first to be beaten out. The tissue is grafted, it is functioning, and, with more or less success fulfilling its basic tasks, but it is already doomed, its "core" is hit, its "roots" are hemmed from the very first days.

The Nobel Prize of 1980

The Nobel Prize of 1980 was awarded to two American researchers, that is Snell and Benacerraf, and a French scientist, Dausset, for their outstanding works in immunogenetics. Before these three scientists became leading specialists in this branch, they had represented three different disciplines, namely mouse genetics (Snell), clinical immunohematology (Dausset), and experimental immunology (Benacerraf). Geneticist, physician, and immunologist—this union embodies a many-sided significance for discoveries in immunogenetics, the discoveries of primary importance for biology as a whole, for theoretical immunology and practical medicine. Snell, Dausset, and Benacerraf laid the foundation and, to a great degree, provided for erecting the very edifice of modern immunogenetics and the genetics of immune response. For this they were awarded the Nobel Prize.

At that time George Snell was 80. He was born on December 19, 1903, in Bredford, Massachusetts, USA. He received a bachelor degree at Dartmund college, and a Ph.D. in medicine at Harward University in 1930. From 1935 to 1969 he worked in Jackson laboratory in Bar-Harbor. He is now a resigned professor emeritus of this laboratory.

The essence of the laws formulated by Snell is very simple (from the modern viewpoint): the slightest genetic differences between the donor of the transplanted tissue and the recipient is enough to cause the rejection of alien material. Snell also completed the generalization stating

that the origin of incompatibility reaction lies in the group of genes localized in the so-called H-systems (histocompatibility systems) of the mice.

Among these genetic systems (there are about fourteen of them) there is a so-called H-2, which plays the leading role in the rejection of alien tissue. Detailed studies of this system revealed its most complex genetic organization. Suffice it to say that there are about 500 genes in the H-2 system. Some of them not only control tissue fusion or rejection, but, as it was found out later, regulate various immune reactions of defence.

A series of studies on the major histocompatibility system in mice became a precursor for the search of a similar system in man. This research was stimulated mainly by the need to develop the immunogenetic principles of grafting human material from one individual to another.

Ten years after Snell, in 1958, Dausset wrote: "The study of about 20 immune antileucocytal sera of the patients subjected to numerous blood transfusions performed using 12 leucocyte varieties showed that 4 varieties of leucocytes reacted in a similar way with 5 sera. Apparently, this suggests that these leucocyte varieties lacked a certain antigen, which is present in all the rest of the leucocytes. The studies of this antigen are presently under way. It is present in about 60 per cent of French population examined. It is inherited according to the common rules. Finally, a woman patient whose leucocytes did not contain this antigen received blood exclusively from a donor whose leucocytes contained this antigen. As a result, she developed antibodies that did not affect the leucocytes lacking this an-

tigen; thus, we managed to obtain pure anti-Mac antibodies."

In fact, this modest abstract describes the discovery of the first antigen and, hence, the first gene of the major histocompatibility system in humans.

Jean Dausset was born on October 19, 1916 in Toulouse. After finishing the Mishley lycee in Vouviere he studied at the Medical faculty of Paris University. Between 1937 and 1959 he worked as a doctor in various Paris clinics, and, starting from 1963, in the Immunological Institute of the University clinics San-Louis in Paris. He is the author of pioneer works on transplantation in France. Dausset was the first President of the French Society of Transplantologists; he is a full and honorary member of a number of scientific societies and academies. Now he is the President of French Immunological Society.

In 1958, examining the sera from the patients after repeated blood transfusions, Dausset found that these sera have the capacity for detecting alien substances (antigens) on the leucocytes of certain randomly taken people. This method of searching for antigen differences is not a new one. It underlied the detection of blood group antigens in humans. Still, Dausset's service was that he was the first to discover a new system of leucocyte-connected antigens, establish its genetical diversity, and determine the dominating role of this system's genes in developing the incompatibility conflict in transplants. Subsequently the leucocytal antigens were united into an integral system called HLA (Human Leucocytes-Antigens).

Detailed HLA genetic maps underlied the choice donors and recipients, which allowed establishing the degree of antigen similarity between them and underlied the possibility of organ and tissue transplants. Later on the studies were joined by scientists of Great Britain, USA, the Netherlands, and the Soviet Union.

Today the HLA system is studied almost as profoundly as the mouse H-2 system. It is widely used for solving the problems of population genetics, for choosing donors for grafting the kidneys, bone marrow, and other tissues. Specific HLA antigens were found to be connected with certain diseases.

Still, the mouse H-2 complex remains a pilot in this problem. The HLA system was shown to be the analogue of the mouse H-2 system. The similarity between the two systems concerns both genetic organization and the diversity of antigen and immunological functions controlled by these genes. This fact is of principal importance. It serves as a bridge between the experimental and clinical transplantology, providing the researchers with "a mouse model" for studying genetics, biochemistry and physiology of incompatibility phenomena, the basis of practical utilization of the experimental data.

In 1972 Benacerraf together with Mac-David published a paper called "Immune Response Genes Linked with Histocompatibility System" Analyzing their own and literature data, the authors came to the conclusion that a new class of genes was identified, the genes controlling the immune response.

"Thorough study of the immune response car-

ried out during the last three years on mice carrying the known recombinant H-2 alleles with respect to branched antigens (T, G)-A-L, (H-G)-A-L, and (Φ, G)-A-L, controlled by I_R-I-gene(s), showed that the I_R-L locus is located in the middle of the H-2 region, to the right of the Ss locus and to the left of the K region."

Baruch Benacerraf was born on October 19, 1920 in Caracas (Venezuela). He finished Janson lycee and studied in Colombia. In 1939 he moved to USA. Beginning in 1970 he worked as a Professor of pathology at the medical faculty of Harvard University (Boston, Massachusetts). In July 1980 he was elected the President of the International Union of Immunological Societies.

The late 60s saw a series of papers by Benacerraf and his colleagues on the genetic control of the intensity of the immune response. The fact that the intensity of the immune response to alien material is a genetically underlied property has been known before. However, in the 40s and 50s there was no way to perform the detailed genetical analysis of this phenomenon due to the absence of adequate experimental models.

By the early 60s immunologists already had at their disposal (due to the efforts of Snell and a big group of geneticists associated with him) a number of pure animal strains (mice, guinea pigs, and rabbits). Moreover, by that time immunochemists had already synthesized protein antigens with a limited range of specificity. All these important experimental achievements enabled the scientists to trace a specific gene providing for controlling the immune response to a certain antigen in strictly controlled conditions.

It appeared that immune response genes are localized within the major histocompatibility system. Each individual has its own unique gene set. This fact of principal importance, since it follows that the intensity of immune response to a bacterial, viral or graft antigen is not a general property of all humans or the animals of a certain species. On the contrary, the intensity of immune response is always specific—a certain individual responds to a certain antigen with a certain immune response. This entails a conclusion of some practical importance: depending on the original immune responsiveness to a vaccine one should use the patterns of preventive vaccination most effective for a given individual.

Later on both Benacerraf with his colleagues and a big army of immunologists and immunogenetics managed to elicit the functional uniqueness of H-2 and HLA-systems in developing the immune response. It is the genes of these systems that "conduct" the immunological situation in the body, be it the rejection of alien grafted material, development of autoimmune disorders, effective vaccination, cancer pathology, or immune deficient state.

The chromosome region where the immune response genes are mapped was subsequently called the I-region of the Major Histocompatibility Complex. By 1975 the antigen molecules, the Ia antigens coded by the I-region genes were discovered. I-control of macrophage-T-lymphocyte and T- and B-lymphocyte interactions was demonstrated (these are cell interactions critical for immune response). In 1977 it was shown that the functional activity of various T-lymphocyte

classes is coded by different genes of the Major Histocompatibility Complex. Cytolytic T-cells respond to previously known H-2 antigens, that is SD antigens. The functions of T-helpers and T-effectors are connected with the products coded by the I-region. The I-region appeared to be a complex one; it comprises 5 subregions: A, B, J. E, and C.

In the recent years it has been shown that T-lymphocytes recognize alien substances only when these substances form a complex with histocompatibility antigens or with la molecules. The latter was proved by Benacerraf.

To be sure, all these achievements are connected with the names of dozens, or even hundreds of researchers. However, the basic facts and generalizations were presented by Snell, Dausset, and Benacerraf. The significance of modern immunogenetics for biology and medicine cannot be overestimated. This area of knowledge includes such problems as organ and tissue transplants, treatment of autoimmune diseases, immunotherapy and immunoprevention of cancer, new principles for developing vaccines against the infections which are not yet overcome, and many others.

The Age of Organ Transplantation

Milan Hašek

If immunity is so ruthless to alien cells, then organ and tissue transplantations are quite senseless. This was considered to be a firm rule until 1953, and the very idea of replacing a diseased

organ by a healthy one seemed to have no prospects whatsoever for most researchers. In 1953 Peter Medawar and Milan Hašek, two scientists in two different countries, independently of each other, turned this idea into a most promising and exciting one.

In summer 1952, Milan Hašek, a young lab assistant at the Institute of Experimental Biology of the Czechoslovakian Academy of Sciences in Prague, went to a poultry farm. This was how everything started, or so Milan Hašek himself asserts.

The laboratory launched an interesting research project. It was not quite clear, or, rather, it was quite unclear what happens if, during the period of embryonic development two embryos are given one common circulatory system, so that before the two independent organisms emerge, blood of one of them would pass through the vessels of the other, and vice versa. The main point here was common blood, rather than a common circulatory system. The circulatory systems are different, but they are connected at one point, and blood becomes common for the two organisms.

It was not clear whether or not this model could be created. It was not clear whether or not this model would be viable. In case it would, it was unclear whether or not this operation would affect the life span. It was unclear in what way this mutual influence of two embryos from two different species would affect their further independent life (provided there would be any further life).

It seemed impossible to stage this experiment

on rabbits, dogs or any other mammals, since mammalian embryos develop in the womb of the mother's body. But how could the circulatory systems of two embryos developing in different mothers' bodies be connected experimentally?

It seemed to be impossible.

Birds' embryos are much more accessible. They develop separately from their mothers. They can be grown without mothers at all. Birds' embryos are partitioned off from the world by just a thin egg shell. A network of blood vessels connected with the circulatory system of the embryo's body develops under the shell on an external membrane.

This membrane develops by about the 8th day of eggs' incubation at 37 °C. It is called the "chorioallantoic membrane" These membranes can be connected if, after the 8th day, small windows are cut out in the shells of the two eggs.

In short, the first thing to be done was to go to a poultry farm and arrange the delivery of eggs from various hen species to the Institute. Second: an incubator had to be set up in a laboratory, to grow chicken embryos connected via chorioallantoic membranes. No mother-hen, even the most delicate one, coud be entrusted to hatch something as delicate as these fused eggs. After the chickens have hatched, they can be studied to determine the influence of the embryonic connection, or, as it was called, embryonic parabiosis.

Parabiosis means "near life". The word "parabiosis" had several meanings. For instance, "near life" can be understood as something between life and death (half-dead). But in this case,

when we say "parabionts" we mean something different: two lives developing next to each other.

Vexingly short days of experimenting were followed by endless weeks of waiting for the results. These thin and subtle membranes were not so easy to connect. Surgical sutures were of no help here. It took some time to find the right solution. The chorioallantoic membranes of two ten days-old chicken embryos fused perfectly only when the embryonic tissue from a third, younger embryo was placed between them. The membranes fused and extended their blood vessels into one another.

A test experiment was performed: a dye injected into the blood of one embryo appeared in the blood of the other.

So, this experimental technique was refined and mastered. It was possible to start the consistent examination of chickens, former parabionts. But before doing this, tens and hundreds of chicken embryos passed through the laboratory, operating-room, and incubator.

The results finally obtained were most convincing. Hašek had the objects of the basic experiments to work with: the chickens hatched from the linked eggs. (The chickens themselves were not linked; their fused shells were left stuck together, and the chickens pecked it apart and got out.) In what way, if at all, will they generate antibodies to each other's antigens? New experiments beckoned.

The chickens' age was 107 days. These were young white leghorn hens. In the embryonic period of their life they had been parabionts. The vascular membranes of the would-be chickens

had been linked on the 10th day of the eggs' incubation in a thermostat. As expected, on the 21st day the chickens were hatched and got their numbers: 516 and 517.

Milan Hašek is preparing a syringe to start immunization of the two chickens. He takes blood from both of them. The labels on the test-tubes read: "Blood of Parabiont No. 516" and "Blood of Parabiont No. 517". The next thing is to immunize the chicken No. 516 with the blood of No. 517, and, vice versa, the chicken No. 517 with that of 516. In response to introducing the blood from other chickens, normal, non-parabiont leghorns were known to produce antibodies which bind together the red blood cells of the administered blood; this was also confirmed once again in Hašek's experiments.

The researcher immunizes his little charges Nos. 516 and 517, time and again.. There are no antibodies, the chickens are inert.

Hašek repeats immunization four weeks later, receiving the same results.

Former parabionts produce no antibodies against each other's red blood cells. Otherwise, their immunity remains completely the same. They did not lose the ability to generate antibodies in general. When the red blood cells from other, nonparabiotic, chickens get into their blood, these chickens with their numerical names respond quite normally.

Later on it appeared that the skin from former parabionts can be grafted to their unusual partners. And this skin fuses, while a skin scrap from any other chicken is rejected in its usual term, which is 8-12 days for chickens.

Something new, previously unknown to science, was discovered. It was a phenomenon opposite to immunity.

Once in contact with an antigen, an adult animal stimulates its immunity and produces antibodies. But if this contact with alien antigens of another body was in an embryonic state, the adult animal builds up a resistance to these antigens. With respect to these antigens, the immunity is switched off for the rest of the organism's life.

The immunity, the guard of the body's individuality, surrendered. The fortress that had seemed to be impregnable had been penetrated. A chink was found in the armour of biological uniqueness of an individuum.

Nothing has yet penetrated through this chink, but it did appear. It becomes clear that some controlled action can, if only to a certain extent, deprive an individuum of his uniqueness.

Hašek published his discovery in 1953. But the name he gave it, "vegetative hybridization in birds" was not the most appropriate one. This word combination had been previously compromised by the works of Lysenko, who tried to replace genetics with vegetative hybridization. Therefore, Hašek's publication was met quite sceptically at first.

Again Peter Medawar

During the same year of 1952, a British researcher, Sir Peter Medawar, together with his young colleagues, was engaged in skin grafting on calves. They were especially interested in finding

out what happens to the skin grafted from one twin calf to the other.

Don't think that all is absolutely clear with twins. One could have thought that the skin just fuses in monozygotic twins and is rejected in heterozygotic ones. But everything is much more complex.

If the twins are identical, if they had developed from one ovicell (monozygotic twins), then the skin always fuses. If they are genetically non-identical, if they emerge from different ovicells (heterozygotic twins), the skin from one should not fuse on the other. This is true in theory, but not always in practice.

It appeared that in certain, clearly non-identical twins, skin grafts fused forever, as their own skin, while the skin from any other calf was rejected. It was clear that this amazing tolerance of twin calves to each other's skin resulted from a certain natural process. Medawar remembered the works by the scientists from the USA and Australia.

In 1945 an American scientist from California, Ray Owen, discovered an interesting thing. When two calves develop simultaneously inside the womb, their circulatory systems come in very close contact and they exchange blood. Owen showed that newborn twin calves have each other's red blood cells circulating in their blood.

Owen found what Hašek was to simulate eight years later. He ought then to have tested these calves' immunity mechanism towards each other. It was not, though, that he failed to have guessed, it was that the time was not yet ripe. The scientific community needed these several inter-

vening years between Owen and Hašek to take a step forward. This step was taken in 1949, when Burnet and Fenner made public their immunity theory. The phenomenon noticed by Owen became clear in the framework of this theory. Burnet and Fenner formulated a general statement, that is: if, during the period of embryonic development, a body has contact with certain antigens (in this case, with the tissues of a different body), it will remain immunologically inert to these antigens for the rest of its life. This prediction was to be verified.

Whatever the authors of intricate experiments have in mind, whichever objectives they set for their experiments, it is life and practice that finally set the records straight. Hašek's experiment, and, especially, a correct explanation of his results would have been impossible prior to these works. It was not because these works were that indispensable, but because they gradually, step by step, led the scientific community to these works. Immunology was maturing and during course of its development it could not have missed the milestone represented by these experiments. This is why the brilliant experiments by Hašek cannot be neglected; they should be taken into account in all subsequent works and reasonings, by all immunologists to come.

In 1953, Peter Medawar, then professor of London University, together with his colleagues Rupert Billingham and Leslie Brent, published remarkable experiments. They were designed to reproduce the rare natural phenomenon described by Owen. By simulating this phenomenon, the forecast by Burnet and Fenner could be checked.

Hypothesis: the embryo's encounter with alien cells is to result in a tolerance to the corresponding antigens in future life.

The experimental object: pure-strain of mice of two species, gray CBA and white A.

Experiment: the artificial introduction of A mice cells to CBA foetuses. The result expected: the newborn CBA mice develop a tolerance to the cells and tissues of the A species.

Given below is the description of an experiment from this series, published in *Nature*, October 3, 1953. This was experiment No. 73.

At the 15th to 16th day of gestation, a CBA female was anesthetized and its abdomen was opened along the medianal line. The foetuses were seen through the stretched uterine wall. This wall was pricked with a thin needle, and 10 milligrams of cell suspension prepared from the spleen and kidneys of A strain mice were administered to each embryo. These cells were viable and, according to the theory, they were to fuse in the embryos. (This is a new premise for us, which has not been mentioned yet: just like many other things, the immunity in the embryo is not yet developed. Grafts fuse well instead of being rejected: the embryo has no immunity.) Then the abdomen was sutured. Four days later, in due time, the mouse delivered four little babies. They were all quite normal in appearance.

In eight weeks, just as they ought to, the mice grew up to weigh about 21 grams each. Each of them received skin grafts from the A mice, which consisted of tissue of the same nature, of the same antigen structure as the cells introduced to the embryos.

Eleven days later the grafted skin was examined. This was not an arbitrary term. As was shown by the preliminary experiments, the skin of A strain grafted to CBA mice is rejected in eleven days. The grafts were rejected in two experimental mice, and in three others the grafted skin was perfectly all right. It fused like their own tissue. It was only its colour that gave away its alien origin: the white spot caught the eye against the background of gray CBA hair. The white hair typical of A mice was of normal density and coarseness.

Fifty days later the skin from the same A strain was once again grafted to one of the three mice. Since that day the mouse has borne two alien skin grafts.

Something new, previously unknown to science, was discovered, something opposite to immunity.

The contact of an adult animal with antigens results in the stimulation of immunity. The triggering of immunity by artificial stimulation has been long called actively acquired immunity. However, if the body's first encounter with antigens, in particular, with alien cells, takes place in the embryonic period, the opposite effect appears: the immunity to these antigens is switched off for the rest of the organism's life. Medawar called this phenomenon, which is similar to acquired immunity but has the opposite sign, actively acquired tolerance.

This triggered a flow of experiments in Medawar's laboratory. The mice in the cages bore the hair of different colours from different species. These were the non-rejected skin grafts, the glar-

ing evidences of the fact that the incompatibility barrier can well be overcome.

The papers and lectures by Medawar added fuel to the fire. His works became so popular that, when in 1960 he delivered his lectures at Harvard, the largest lecture-hall in the University failed to seat all those who were eager to listen to him. An additional hall was needed, where the lectures were transmitted by radio.

Thus, in 1953, Hašek in Czechoslovakia and Medawar in Great Britain, independently of each other, and without knowing each other, described a new immunological phenomenon. Naturally, each learned of the works of the other from periodicals.

"At the time when my works were published," Hašek said, "Medawar's paper came out. I saw it to be the proof of my results, and presently tried his intraembryo injection method to induce tolerance in chicken and mice. In his turn, Medawar tried our technique. We first met at the Embryological Congress in Brussels in 1955, where we had the first chance to talk and to share our experiences."

This is the way for the real scientists. The joy of cognition, the joy of surprise, in the first place. They do not argue as to who was the first to hit upon some thought, and by exactly how many hours or days earlier than the other. They repeat the experiments of their far-away fellow researchers. Enthusiastically and sincerely, they shake each other's hands.

The Successes of Kidney Transplants

The discovery of tolerance freed the problem of transplantation from the deadlock, despite the fact that it was still impossible to get a patient in need of a renal, skin or bone marrow graft, back into his embryonic state, impossible to develop anew graft tolerance as discovered by Medawar and Hašek. But there was a breakthrough in that now it was believed that incompatibility could perhaps be overcome. If it cannot be done experimentally—well, then, some clinically suitable methods can be found. As Daniel Granin aptly said: "Why did not ancient explorers discover the North Pole together with the South one? Were they not courageous enough, or, perhaps, there was no need for these discoveries? No, neither of these is true! To begin with, they had to have known that these Poles existed somewhere." After 1953 it became clear that means for overcoming the immunological incompatibility barrier exist somewhere.

Vigorous work started. Immunologists and surgeons all over the world concentrated on transplantation (this was what they called a new border-line science dealing with organ and tissue grafts). The next dozen years brought about really striking results. In 1966 the International Society of Transplantologists was set up. In 1967 the first congress of this society was held in Paris. The first results were summed up. This can be very briefly put in two phrases. First, immunology found ways to choose the most suitable donors. Second, it found the means for sup-

pressing the immunity system or response, and passed these means over to the surgeons.

Now, the selection of a donor.... Remember that the first tissue compatibility antigen was discovered by Jean Dausset in 1958. By now, more than 80 of them have been found. The tissues of every individual contain about 4 to 8 of these 80 antigens. This means that the number of likely combinations is not less than several tens of thousands. In other words, it is far from being simple to select a donor who has antigens identical to those of a given patient. I should even say it is next to impossible. What is then the point in this screening?

It appears that there is something to it, and not so little. The point is that the significance of various differences and the "strength" of various antigens are different, and the graft antigens contained in a kidney, heart or any other grafted organ can be found on the leucocytes, or white blood cells. These are used for typing the would-be recipients and donors. Thus the lesser of two evils is chosen, that is a donor-recipient pair with the fewest possible differences. The combination here is based on a well-known immunological rule: the less are the differences in compatibility antigens, the weaker the tissue rejection, and the easier it is to overcome it.

To make this screening more effective, as many would-be donors and recipients as possible should be typed. Only if the selection range is wide enough, can a sufficiently high degree of compatibility be achieved. That is why the specialized international organizations of "Eurotransplant" were formed in Leiden, Rome, Paris, and Oslo.

They perform this screening in several countries simultaneously.

How is this typing carried out? To begin with, a set of special sera is needed. These have been developed during the last decade by several teams of immunologists working simultaneously in various countries. Step by step, by endless trials and comparisons, they discovered one leucocytal antigen after another, creating new typing sera. As has already been mentioned, the one to start this work was a well-known French immunologist, Jean Dausset. Then he was joined by the laboratories of Terasaki (USA), Van Rood (Holland), Batchelor (Great Britain), Ceppellini (Italy), Ivani (Czechoslovakia), Zaretskaya and Zotikova (USSR), and other scientists in other countries. Today various laboratories have sera sets typing scores of tissue compatibility antigens.

Blood is taken from a patient, and white blood cells are isolated from it. They are washed and, in separate test-tubes, mixed with each typing sera. Then some non-toxic dye, such as methylene blue, is added to these mixtures. If the white blood cells contain a certain antigen, they will absorb the dye in the respective tubes. This is due to the fact that the antibodies impair the functioning of the white blood cell membrane, and the dye easily penetrates inside. Thus, the antigen pattern of human white blood cells is determined within several minutes. The only thing now is to compare it with similar patterns in other individuals and to select a pair with the fewest possible differences.

This is what immunologists gave to surgeons.

First, they taught them to select the donors optimal for a given transplantation. Of course, it does not totally evade the incompatibility problem, but its degree can be minimized. It is at this point that immunodepressive therapy, immunology's second gift to surgeons, can be used most effectively.

Modern chemotherapy has a range of remarkable preparations which hit the very site of the body that the doctor needs this very moment. We will not discuss here antibiotics which selectively affect microorganisms, nor hormones, having mentioned only insulin which makes the liver turn the blood sugar, glucose, into the liver stocks of glycogen. Remember ether and other anaesthetics with their unique property to switch off the consciousness, or lobelin, the respiratory center stimulator. At doctors' disposal are the chemicals capable of lowering and raising the body's temperature, stimulating cardiac activity, and many others.

Are there any chemical agents capable of cancelling the immune response? Today the answer is yes. There are such chemicals, though very toxic ones. To inhibit the immune reactions of rejection, these agents must be administered in near lethal doses.

There are no strictly specific agents which only cancel immunity, while not affecting any other important functions, that is, non-toxic ones, precisely because the mechanisms of immune response are still quite obscure. Nobody knows the way in which the cell, having contacted with an alien protein, builds up the molecule of the antibody directed against this protein. But the

time will come, and chemotherapy will enrich its arsenals with substances selectively stopping the production of antibodies. Then the incompatibility barrier will be overcome completely.

But even now we can boast of some successes. These successes show that certain chemicals can induce a state of prolonged immunological nonresponsiveness and help achieve long-term fusing of alien cells, tissues, and organs. Unfortunately, they suppress the whole of immunity, including antimicrobial protection. The danger of post-operation infectious complications is dramatically aggravated.

The means used today for overcoming immunological tissue incompatibility are but the very first steps. Still, the progress is beyond any doubt. It can best be illustrated by renal transplantation between humans.

Before various physical, chemical or biological means of suppressing immunity were used in practice, that is before the late 50s, all clinical attempts at renal grafts ended in the organ's rejection within several weeks. During 1960-1962 came the first reports on the use of immunodepressors, such as X-rays, 6-mercaptopurine, cortisone, and others. The grafted kidneys were now sometimes (but not more often than in 20 per cent of the cases) functioning for up to 6-9 months.

New chemicals were developed, and their application methods were improved, which markedly increased the number of longer-living grafts. According to the International Renal Center, in 1965 the percentage of kidneys grafted from nonrelated donors and functioning for more than a

year grew to 30 per cent. The research in this area is now in progress. According to the data of the same center, based on analysing over 20 thousand transplantation cases, in 1975 this figure reached 60 per cent. When the kidneys from the related donors were used, this figure was as high as 87 per cent. 65 per cent of all grafted kidneys are functioning for more than two years.

The immunodepressive effect of antilymphocytal sera (ALS), that is the sera against lymphocytes, the cells producing antibodies and triggering the rejection of the grafted organ, is worth special mentioning. ALS is obtained by immunizing horses or other animals with human lymphocytes. These horses become immune to human lymphocytes, and the sera isolated from their blood are capable of killing human lymphocytes. These sera, called ALS, found broad application in clinical practice.

The biological effect of ALS has been studied since the time of the prominent Russian scientists Ilya Mechnikov and Alexander Bogomol'tsev; however, it was not before late 1962 that this preparation was looked upon as an agent for suppressing graft rejection. The first surgeon to use ALS in renal grafting was Professor Thomas Starzl, Colorado, in 1966. The problem of today is to develop ALS of narrow (monoclonal) specificity, to attack T-killer lymphocytes alone. Strong hopes in this respect are pinned upon hybridoma technology described in the chapter "Immune Biotechnology"

Keen interest in ALS is due to the fact that it suppresses graft immunity to a higher degree than antimicrobial immunity. The grafted organ

is not rejected, yet the body retains its ability to fight infections.

Still, the immunity suppressing preparations known today are broad range poisons, which damage a number of the body's systems. An overdose of a preparation causes death, while an underdose results in restoring immunity and rejecting the organ. So the patient balances between life and death as a tight rope walker. The better the preparation, the stronger is this rope. But even ALS markedly suppresses hemopoiesis and dramatically undermines the body's protective forces against microorganisms.

The fundamentals for producing depressors of direct action, which only cancel the rejection but do not damage the rest of the body, are still to be developed.

Immunology provided surgery with criteria for choosing the optimal donor and with the means to suppress immunity. Which of these gifts is to be improved first? What is more important? It is difficult to say.

The choice of the donor alone does not solve the problem completely, since it is next to impossible to select the one identical with a patient in all his tissue antigens. However, the fewer are the differences, the easier it is to combat rejection. This is why even very prominent surgeons, recognized experts in their field, fail to attempt organ transplantation until they receive the help of immunology, and try to select a donor compatible in his tissue antigens.

Of course, the time will come when the preparations for suppressing immunity and preventing rejection become specific and perfect.

Then, perhaps, there will be no need to type and select donors and recipients. But today the immunodepressive treatment is quite toxic and dangerous. The higher the degree of incompatibility of the grafted tissue, the graver is the danger.

Therefore typing is absolutely indispensable. But, the question arises, how can a suitable kidney donor be found, if the likelihood of two people being identical in their compatibility antigens is 1 : 10 000 or even lower? Could a doctor screen a thousand possible donors before he finds a suitable one? Of course, he could not, this being impossible. There can never be such a number of donors "on hand" neither among healthy volunteers, nor among the victims of accidents, when healthy people die, say, in an automobile crash. The selection of a donor-recipient pair is accomplished in a special way. It is all done in a roundabout way: it is not choosing a donor for a recipient; it is selecting a recipient for a donor.

Eurotransplant

Without knocking on the door, Davy Shippers entered a spacious professor's study, one entire wall of which was a window.

"Dr Van Rood! There is an urgent telegram, through the governmental channel from Upsala, Sweden."

Professor Van Rood, the director of the regional Eurotransplant department in Leiden, pushed aside the manuscript he had been reading. His hand sharply reached the tumbler switch of the internal radio network. His head, with rich and

boyishly fluffy hair, turned towards his young colleague who had just entered.

"Read this!"

Running his fingers through the telegraph tape, the young man read hastily:

"At 2.37 p.m. a 40 year old man was brought to a clinic. Multiple breaks of the spine and cranium base; he never regained his consciousness, and his state is hopeless. The commission certified the death of the brain. Artificial circulation is being maintained. The accident took place at 2.10 p.m. The abdominal viscera are not affected. Can be used as a renal donor. Blood group A, MN. Rhesus CDe, Duffy—a, Lutheran—b, Kell—Kk. Leucocytal antigens: HLA-Al, 2, B-5, 7, C-3, D-1.

"Please, sit down, Dave," said Van Rood to the young man and looked at his wrist-watch, which showed 14.05 p.m. He switched on the radio set.

"Attention of the computation department! Please code the donor's biological identity: A, MN, CDe, Duffy—a, Lutheran—b, Kell—Kk, HLA-A1, 2, B-5, 7, C-3, D-1.

Will you repeat, please? Thank you. Waiting for your answer."

The Professor turned to his young colleague.

"Please, fill in the chart of isoantigen identity for this man and find out his birthplace. Put the chart into the group of Scandinavian countries. Incidentally, prepare the information on the incidence of HL—A-15 antigen among the residents of Sweden and Norway. I suppose this new antigen is much more widespread there than among Englishmen and Frenchmen."

A green light flashed, Van Rood switched on the tumbler.

"Yes?" "A suitable, virtually identical recipient is in London, in the British National Renal Centre. A man of 37, two-sided hydronephrosis. He's been in the clinic for a month and a half on the artificial kidney; waiting for a transplantation. His name is Evans."

"Thank you." The light went off. The time was 4.15 p.m.

"Davy, please, urgently contact the London Renal Centre. Inform them of the donor suitable for Evans. Call for the urgent confirmation of the offer."

Once again Van Rood became absorbed in his papers. But, as usual, not for a long time. At 4.40 p.m. Davy came in.

"They've called up from London. A British military aircraft has started for Upsala to pick up the kidney."

"Why military?"

"Well, they have a contract. So to say, peaceful use of military aircraft. I've informed Upsala. The kidney in a special container will be waiting at the airport. It will be delivered to London in three hours."

"That's the way to do it, Davy. I think, in four hours it will already be serving for another man."

All this is neither a joke nor a fantasy. This international organization, Eurotransplant, really exists, and an immunologist from the Dutch city of Leiden, Professor Van Rood, is the head of one of its department. In August 1969 he was in Moscow, at the XIIth International Congress of

Transfusionists. He told us how this international organization was set up. And the scene I've just described, was told by him almost exactly as it stands here, though it refers to the initial period of Eurotransplant's activities. Now the performance has become even smoother.

What purposes does this European centre serve?

Imagine that you need a man with certain characteristics: fair-haired, aquiline-nosed, 179 centimetres high, with the weight of 75 kilograms, the lung volume 5.8 litres, foot size No. 41, 30 years old, married, has two children at the age of... and so on, for three dozens more indices. The question is: is it easy to find such, or at least a similar man? It is virtually impossible, even more so if we wish him to be a donor of a kidney or any other organ to be grafted to our patient.

Here I have enumerated external indicators. The donors for transplantation are selected based on different qualities: the specific features of his cell and tissue structure, or tissue antigens. But the task is the same. An identical one cannot be found anywhere on the globe. The probability of such a chance is negligible. But there can be someone remotely resembling, and someone very similar. The greater the similarity, the more successful is the transplantation.

So the problem arose as to how to find these similar people. We can put a patient into a hospital, determine his tissue characteristics and start to search for a donor, such as a man who dies in an accident, perhaps an automobile crash, and whose organs can be used for transplantation.

The accident victim does not meet the requirements and the donor is lost. Another accident occurs but again no match. The likelihood of the similarity is very slim. One or two hundred donors can be screened, and to no avail whatsoever. So the idea appeared to do it the other way round. Not to select a donor for a patient, but to look for a patient waiting for an operation, to match an accident victim who is a potential donor.

Indeed, there are hundreds of patients in dozens of world hospitals, say, with kidney insufficiency. They sustain their life only because several times a day they are connected to the "artificial kidney" apparatus. They are waiting for a suitable donor. Their tissues can be typed beforehand, and all the data can be sent to an integrated centre. A computer will memorize several hundred or thousand versions of individual human patterns, with all three, four, or more than a dozen features. An adequate recipient can be found for any random donor. The main thing here is only to arrange the proper communication and prompt delivery of an organ for transplant before it dies.

This is how Eurotransplant was initiated. Dr Van Rood said at the Moscow congress: "The functions of this organization are as follows: the data as to the patients in need of renal graft, their leucocyte and blood groups, as well as any other relevant information is printed on special cards and stored in a computer memory. Every month this computer makes a photoreprint with all these recipients listed in special tables according to their white blood cell groups. These photo-

reprints are sent to various centres affiliated with Eurotransplant. If one of these centres has a potential donor, it telephones the closest most suitable recipient. The doctor in charge of the donor should contact the doctor supervising the patient. 67 patients have already obtained kidneys through the card-indices of Eurotransplant, the organs to be grafted were on the average 2-5 times more suitable than those chosen without Eurotransplant."

Of course, it is difficult to find a kidney, to say nothing about a heart. But a patch of skin to cover a burnt surface, bone marrow for treating leucosis or radiation sickness can be supplied by virtually any healthy person. Any of us can be a donor of bone marrow, as well as a blood donor. In these cases the success is altogether based on overall typing. Today many countries have started typing antigens vis-a-vis compatibility among big groups of people. In the not so distant future, I think, apart from the stamp of blood groups B/III/Rh$^+$, I'll have the mark HLA-A2, 9, B-5, 12 in my passport. These are my four basic tissue compatibility antigens. At any moment my bone marrow can save a person with the same characteristics, that is B/III/Rh$^+$, HLA—A2, 9; B-5, 12, from, say radiation sickness. Also, in case I'm in need of a donor, it will be fairly easy to find a suitable one through the special card-indices or computers.

In treating radiation sickness by means of bone marrow transplantation, Professor Good from Sloan-Kettering Institute, New-York, uses a card-index containing the data of 20 thousand typed donors. Radiation sickness appears because

leucosis, or blood cancer, can be treated only by irradiating a patient with X- or Gamma-rays. The leucosis disappears, but radiation sickness develops. It can be cured only by grafting a compatible bone marrow. The only thing is that it must be compatible in all known antigens.

Naturally, each of us can give a part of his bone marrow to another person. Therefore, having screened several thousand donors, we can find a compatible one. There is thus no need to suppress the immunological responses with medicines toxic to the entire body. But what is to be done for heart transplantation? A compatible donor for this operation cannot be found even through Eurotransplant. A heart for transplantation can be taken only from a patient who is dying in a most sophisticatedly equipped hospital, dying, for instance, of a cranial-cerebral trauma. The brain is already dead, while breathing and heart-beat are sustained artificially. Transplantation should be effected immediately. To get a donor under all these conditions is an extremely rare event. There is about the same chance of this happening as there is of guessing all six figures in a lottery. Therefore all the hopes for a successful heart transplantation are pinned on immunodepression, which is still far from being perfect.

Heart Transplantation—Myth or Reality?

Once upon a time there was a kingdom, one like many others. It was thriving, getting welthier every day, and was never afraid of anything. King Cerebrus ruled there, and he never thought

of any enemies or invaders, since he was a lucky king. He inherited his luck from his father, who, in his turn, had got it from his father. And nobody knew for sure how this luck had first appeared in their kin.

This luck lived in the king's palace, and its name was Lymphus. This Lymphus was a spellbound Gin. Ancient wizards, Cerebrus's forbears, created this Gin and endowed him with the ability to generate countless hordes of staunch worriers, lymphoids, from his body, and entrusted them with a duty—to guard the kingdom from foreign invaders. There was a great oath that Lymphus gave to his creators; to listen to nobody's pleads, to obey no one's orders. Even if the king himself asks him to spare some strangers—not to obey the king himself. This was the incantation bound on the Gin settled in the kingdom, which provided carefree and peaceful life for the country for many years.

Taciturn Lymphus has sent out his warriers to all quarters and corners of the kingdom. He sits in the Palace, not intervening into others' affairs, not asking for special privileges for himself! Neither does he impose his opinions on others, nor obeys anyone's orders. His own cares also bother no one but himself.

Would one of his faithful lymphoids come tearing along from the South or North, would he report that in some place some bandits had violated the border, or seemingly peaceful-looking foreigners had gotten into the kingdom, the Gin would get up. He would drink his magic beverage given to the highest grandees in the palace, generate a hundred thousand armed lymphoids

and send them to the strangers. All of them would be hit by the faithful guards, even if the enemy were scattered in the cities and hidden among the natives. Not one of them would be left alive. Cerebrus does not even trouble himself to protect his kingdom against the raids of the invaders, whose hordes are living all around and bursting to get into the country to make good at its riches.

The kingdom of Cerebrus is wisely arranged. Every city has its own craft. Skilled workmen never get distracted from their job. One city makes footware for the entire kingdom, another weaves canvas, the third makes clothes. The capital, the city of Cor, is in the centre of the country. This city supplies the entire country with food. The workers of this city work day and night and dispatch food into all the other cities. Should the work be stopped in Cor, all the entire kingdom would perish. But who can stop it? Ubiquitous lymphoids look closely at every creature: whether it is the country's citizen or a stranger (a stranger must die).

The kingdom of Cerebrus enjoyed its life for a time, but then a fire broke out in Cor. All the workmen died, and no other city could manage to do the work of Cor.

The kingdom was starting to die.

The Cerebrus applied to his brother, Cerebrus-the-Older, who was governing his own, equally beautiful kingdom and he said:

"Have you heard of the country that stood on the sea-side, where a flood destroyed all the cities, and only the capital survived since it had been very well protected?"

"Yes, I have heard of it."

"I am going to take this city and to move it, together with all its workmen, into the place of my Cor. Let them work, let them save me and go on living themselves. They will die all the same without their kingdom."

"This is hopeless," the older brother said, "Lymphus will destroy all the foreigners. He has the order of his ancestors. And none of the wizards have ever decoded the incantation which bound the spell upon the Gin. Nobody can give him order: hit these strangers, but leave those alone since the kingdom needs them. If they are strangers, he kills all of them."

"I'll kill Lymphus!" Cerebrus exclaimed.

"Then you will die instantly. Remember Cerebrus the Junior. His Gin died, and the hordes of barbarians from across the oceans, forests, and steppes invaded his kingdom by land and by air and crushed it in three days."

"Then give me a poison for Lymphus. I will add it in small portions into his magic beverage. which he takes before producing lymphoids. He will get weaker and will not be able to generate so many warriers. Perhaps, I will find a happy medium when there are still enough lymphoids to protect the country against the barbarians, but too few to kill the workmen of the city moved to our kingdom from a foreign one."

"Do try," said Cerebrus the Older. "You have no other way out. But remember; this poison is a very strong one. Even its fumes are dangerous for all the craftsmen and for you yourself. Even the breath of Lymphus will become perilous. Poi-

sonous exhalations will spread all over the kingdom."

So Cerebrus took the poison, and he poured it in small bits into the cup of Lymphus in the morning and in the evening. Three days passed, and the Gin became weak. He was not so attentive to the messages of lymphoids and did not generate new troops. Then Cerebrus moved the capital from the destroyed kingdom to his own. All the rest of the cities came back to life, the kingdom was revived. But the countless hordes of enemies started to creep into the country, killing the craftsmen and ruining the cities.

Cerebrus got scared and stopped giving poison to Lymphus. The Gin came back to himself and generated a swarm of lymphoids. They slaughtered all the invaders, and, at the same time, made short work of the foreign craftsmen in the city of Cor. The life in Cor became feverish, the normal rhythm was broken, threatening to stop altogether at any moment. What could Cerebrus do? Again he poured some poison to Lymphus's cup. But already he was very weak on his legs himself.

This was the end of the carefree life of the kingdom. It was balancing between life and death as on a blade's edge. Too much poison—Lymphus goes to sleep, the city of Cor comes back to life, but the barbarians crawl from across the border. Too little poison—lymphoids appear, they kill the barbarians, but have no mercy for the skillful workmen of Cor. There is no more joy in the kingdom. All the craftsmen, and the city governers, and the ministers, and the king him-

self are poisoned with the deadly stuff and loosing their vigour.

So Cerebrus issued a call to all the kingdoms, "Who will discover the secret of Lymphus the Gin? He, who will decipher his incantation, who will teach him to beat the enemies as before, sparing the foreign workmen—he will get all the riches and royal honours and glory for ever and ever."

I am not sure, though, that such a wizard will turn up. Or what if the Kingdom of Cerebrus dies before the secret is discovered? And how many more kingdoms will die?

And this is the end of the tale.

And now think: isn't a man with a grafted heart like the Kingdom of Cerebrus? The doctors suppress his immune system (lymphoid tissue) with poisonous preparations to prevent heart rejection. These preparations are poisonous not only for lymphoid tissue, but also for many others. The immune defence is suppressed not only against the grafted heart, but also against the microorganisms, the pathogens of infectious diseases. An overdose of such a preparation will bring some relief for the heart, but the patient can die of infection at any moment. An underdose of the preparation can any moment result in heart rejection.

Therefore heart transplantation today is an experiment only within the capabilities of big hospitals, desperately courageous surgeons, and desperate patients. There is a fight for every day, week or month of these patients' life. Every day they are under their doctor's control. Any minute they are prepared to take from the stock of

medicines and other means sustaining their unstable balance between life and death.

And now you tell me: heart transplantation—myth or reality?

Today it is not a myth; it is a real fact practised by many surgeons. But it is not a reality either. Immunologists must still work a lot to discover the secret of Lymphus the Gin, to teach him not only to distinguish between "his own" and "someone else's", but also, among these "someone else's" to distinguish between friends and enemies.

The first heart transplantation was done by a Cape-Town surgeon, Christian Barnard, on December 3, 1967. Three days later a similar operation was performed by an American surgeon Andrian Kontrowiz. In 27 more days, on January 2, 1968, Barnard grafted a heart to another patient, despite the fact that the first one died 18 days after the operation due to immunological rejection of the grafted organ.

This is the dynamic account of the monthly number of heart transplantations performed in 1968, after the first operation by Barnard: 4 in January-February, 1 in March-April, 16 in May-June, 14 in July-August, 34 in September-October, and 32 in November-December.

66 operations in 4 months is a record-breaking figure. This marked the climax of the enthusiasm about this kind of operation. There was a rivalry between the world surgeons as to who would graft more hearts. Denton Kooly, Houston, very soon became the winner in this contest. All in all, he grafted 22 hearts. He conducted his first operation on May 3, 1968, and the last one, in April

1969. All the patients died, either in several days or in several months. Norman Shamway, one of the most prominent American surgeons, working in Stanford University, was even more prolific: he performed 70 operations, and worked on them for a longer time than the others. According to his data, if a patient survives the first four months, then, in a half of the cases, he is likely to live for a year.

The poor results of the operations made surgeons give it up. As early as 1969, the enthusiasm about it was on the decline. This is the dynamic record of the number of grafts performed in the world in 1969: 14 in January and February, 14 in March and April, 5 in May and June, 3 in July and August, 4 in September and October, and 2 in November and December.

In 1972 British Parliament issued a law banning heart transplantations in humans. Some other countries followed suit.

The enthusiasm had obviously vanished. The sensation was over. The first assault was replaced by a long-term seige of a stronghold called incompatibility. Denton Kooly put it this way: "I'm now looking back at heart transplantation as one of the operations we tried to perform, and, for the time being, gave up."

The Prospects for Grafting Various Organs

The future of transplantology depends on future successes in overcoming immunological tissue incompatibility. One should not think, however, that all the difficulties will pass assuming the incompatibility barrier is overcome.

Most of the researchers are sure that ways to overcome incompatibility will be found during the next 10-15 years, so the following description can serve as a kind of prediction for the future.

Kidney. Kidney transplantation today has occupied its due place in surgical practice as a means for saving patients with irreversible renal lesions. A good deal of success can be credited to a remarkable apparatus, the artificial kidney. The patients can be connected to this apparatus, and, for several days, weeks or even months, they can live with their own or grafted kidneys not functioning at all. This time can be used for preparing for the operation, getting the grafted kidney pass the crisis, should rejection have already started, bringing a patient out of a grave state, or grafting a second, or even a third kidney. Even now dozens of men and women became fathers and mothers after a renal graft. The International Renal Centre gives special information on these cases to illustrate the utmost effectiveness of curing a mortally sick person.

In the future this operation will serve for effective treatment of certain congenital renal diseases, traumas, tumours and inflammatory diseases, or nephritis, in many clinics, if no other ways of their therapeutical treatment are found. The difficulties likely to arise here are due to the following two problems.

The first is the conservation of kidneys and their long-term preservation in organ banks. When this problem is solved, it will do away with one of the biggest organizational difficulties of today, dealing with obtaining the kidney and the need for its immediate transplantation.

The second problem is the innervation of the grafted organ. It can be argued that world practice has already shown this organ to be quite autonomous, despite the fact that all the nerves running into the kidney are cut off during the operation. Nevertheless, the innervation of the organ is very important, and the nerve fibres restore slowly and imperfectly. Therefore the problem of nerve regeneration is a crucial one in transplantology, not only in the field of renal grafts. The kidney can still function without innervation, unlike many other organs, such as the eye or the hand.

Heart. In heart transplantation the problem of long-term organ preservation is of even greater importance. Now the grafts are made with a still warm heart, removed from the chest of a man who has only just died. This creates not only technical, but also grave moral problems. What should be considered the moment of death? Is it the heart stoppage or the death of the brain tissue? We know that a halting heart can be revived, and, if the brain has not perished through the time of its idleness, the entire body will come back to life. But if the brain has been changed irreversibly while the heart is still beating, the human being with his personality is lost. Even though, can he then be considered dead?

I'm not going to exaggerate the moral problems. Humankind has faced even more intricate problems of social ethics. This one will also be solved in due time. Now, it is not this we should concentrate upon, but the means for overcoming incompatibility and the art of conserving the viable heart for long periods. It is only after this

that heart transplantation can become more or less a commonly accepted method for treating hopeless heart troubles, progressing stenocardia, severe infarcts, traumas, and some grave incurable illnesses. In contrast to renal grafts, the number of heart transplantations today is smaller. The initial enthusiasm has faded away. To a certain extent this is due to the lack of a reliable apparatus which could substitute for a heart. "The artificial kidney" can replace the functioning organ for weeks if it becomes weaker or comes to a halt. But artificial circulation devices can replace the heart for only several hours, and even this only with the lanced chest. So the doctor is in fact inable of alleviating the work of the grafted heart, even less so to replace it with another one if it has stopped.

The proceedings of a special meeting on heart transplantation held in the National Heart Institute, USA in 1969, give an interesting calculation. Having assessed the number of potential recipients in need of heart transplantation, in other words, the number of patients who can be cured in this way, the expert group came to important conclusions. In 1968 100 heart transplantations were conducted, or, more precisely, 101. After the main obstacle, incompatibility, is overcome, this figure will grow more than 100 times. 10 thousand transplantations annually will be performed in world hospitals. When proper circulation-assisting equipment is developed, this figure will be twice that.

Liver. The core of the matter here is also the problem of conservation and developing an artificial organ. The liver is an especially big and

fragile organ. The methods for freezing it or any other ways of conservation would have to be even more sophisticated. Several operations were performed in the clinics of the Soviet Union on temporarily hooking the liver into a patient's circulation. This is not a true transplantation, but rather the use of a live donor's organ for temporarily replacing the function of an affected organ. True liver transplantations in the future can be useful for extremely severe liver diseases, tumours, and rare congenital troubles, when a child is born with a liver with no bile ducts.

Endocrine glands. Transplantation of the thyroid and parathyroid glands, the insular apparatus of the pancreas, adrenal glands and sex glands are not likely to pose any special difficulties, and will become commonplace operations for treating diseases of these vitally important regulators of the body's activity.

Brain. The problem of brain transplantation is especially complex, and here I can't be too optimistic. Two problems are uppermost here. One of them is nerve regeneration.

It was so arranged by Nature, that the nerve cells of an adult are not capable of dividing, and the nerve fibres regenerate to a very limited extent. If ways to overcome this obstacle are not found, the transplanted brain will turn out to be isolated from the rest of the body, and no information will get through.

The other point of concern here is that a brain transplantation in actual fact implies the transplantation of the personality, when a person is put into the body of another person. And who will serve as the brain donor? This is nonsense

altogether. If a human personality is determined by the brain, then the brain cannot be "a donor's one", since it defines the person itself. It is a body that will be the donor's. Hence, there is no problem of brain transplantation. It automatically turns into the problem of attaching the whole body of one person to the brain of another. So, perhaps it is more fair to speak about transplanting a body to the brain (or to the head). But this is already beyond the framework of our book, dealing with organ transplantation, incompatibility barrier and the associated problems and prospects, not with transplanting personality from one body to another. This, of course, is another, extremely exciting psychological problem. Interestingly enough, it can also be, like this book, formulated as "Me or not me."

Congenital Defects and Old Age

Primary Immunodeficiencies

The immune system, as any other system of the body, can get sick. The story about its sicknesses should be started from the congenital, or, as are also called, primary defects of immunity.

A child was born absolutely healthy and quite normal. A most thorough medical examination showed no deviations from the norm. The child grows up and develops properly, enters school and studies well; he falls ill as much as all others, he goes in for sports. Everything is all right. He is a big boy already, his friends drive motorcycles, and he, too, wants to have a motorcycle.

He goes to medical commission survey. The

conclusion of a surgeon: "OK" The conclusion of a therapist: "OK". Blood examination: "OK" X-Ray study: "OK" The last doctor to pass is an expert in eye diseases. What can be simpler? He has perfect vision. He is a first-class rifle-shot. And, suddenly, the conclusion of the oculist "not fit for driving transport vehicles"

Why so? How come? There are many drivers working with bad vision. They wear glasses to drive cars. But glasses will be of no help to this boy. He has a congenital vision failure that has just been revealed. He cannot tell green light from red. This failure is called daltonism, because a well-known physicist Dalton suffered from this failure and described it with the exactness of a scientist dealing with the physics of light.

Another example: congenital heart failure. The child is quite normal. Everything is all right with him. He is growing up, smiling, crying, and prattling his first sounds, and nobody knows anything. But then the time comes for the child to walk. There comes the first stress in his life that needs the enhanced work of the heart. But the heart has a defect. The child suffocates quickly, he feels short of breath, the heart does not cope with pumping oxygen-enriched blood from the lungs to the rest of the body. Oxygen deficiency results.

The older he grows, the more difficult it is for his heart. He starts to lag behind his fellow-kids in everything. Naturally, the parents turn to the doctor. The doctor diagnoses: "Congenital heart failure."

The third example is the immunological defect. The newborn child, as in the previously

mentioned two cases, is in all respects as normal as all the other babies. He seems nice and healthy during the first weeks of his life, when the antibodies obtained in his mother's womb and taken with his first mother's milk are still circulating in his blood. But very soon his latent trouble shows. Endless infections start, like pneumonia, skin pustules, antritis, otitis, and pneumonia again. And this goes on for years on end.

These children fall especially far behind in their development. They are weakened, they often cannot walk, and, in most cases, they are intellectually inferior. This is because they are constantly ill, balancing between life and death. Before antibiotics were introduced into practice, these children more often than not died right during the first year of their life. Now the infectious complications are treated, and there are sometimes 10-15-year-old children in the clinics of immunodeficiencies (the defects of the immune system).

Congenital immunity defects make the body defenceless towards microorganisms, even those normally present on man's skin, in his mouth or intestines which cause no adverse effects whatsoever. With congenital defects of the immune system, these microorganisms cause constant infections which finally result in death. Antibiotics somewhat help the body but do not cure it. It is the immune system that should be cured, just like the state of rest or an oxygen blanket alleviate the state of a patient with congenital heart failure, but do not cure it. In one case it is the heart failure that should be done away with, and in the other it is the failure of immunity.

The first is done by surgeons, the second, by immunologists. Sometimes they do it alone, and sometimes together with surgeons.

Congenital immunodeficiencies are more correctly called primary ones. More correctly because this word stresses the primary immunological character of all the events. In other words, the disease, retardation in growth and development, and, finally, death are all consequences of one primary reason: the congenital defectiveness of immunity.

Secondary immune defects are those acquired as a result of some impact, like the effect of ionizing irradiation when radiation sickness is developed. The immunity is dramatically reduced, since the lymphoid tissue, the organ of immunity, is destroyed by the irradiation. Secondary defects usually accompany severe starvations, and poisonings with cytotoxic agents. These poisons affect the cells' ability to divide, to reproduce. And the work of the immune system totally depends on the ability of lymphoid cells to divide.

That is why so many anti-cancer preparations are dangerous: the core of their action is a cytostatic effect. While killing cancer cells, they most often also destroy the cells of the immune system. Secondary immunodeficiency appears. The patient treated against cancer should be constantly protected against or saved from the infectious complications.

The word 'primary' elicits one more facet of the problem. I mean its genetic nature, its hereditary character. Congenital immunodeficiencies fall into the category of hereditary diseases, like hemophilia, some kinds of deafness or dwarf state.

Hemophilia is also called royalty's disease. The first case registered in dynastic chronicles was that of a son of the famous Queen Victoria. Since all the tsars and kings married only tsars' and kings' daughters, this hereditary illness spread among the reigning families of Europe. The son of Nikolai II also suffered from hemophilia.

This disease never affected females. Only boys got it. The basic sign was that blood failed to coagulate, and any little scratch could lead to a fatal hemorrhage. Some kinds of immunodeficiency are quite similar to hemophilia in their inheritance pattern. In these cases, also, it is only boys who suffer from the illness.

In one of her X-chromosomes a woman bears a "defective" gene or a set of genes responsible for the deficiency. But the other chromosome contains the duplicate genes. They provide for the normality of the function they control.

If this woman gives birth to a girl, the baby will be healthy, no matter which of the chromosomes, intact or defective, is inherited from her mother. This is because her second X-chromosome comes from the father who is free from this defect. (Should he have it, he would have never lived to a father's age.)

When this woman gives birth to a boy, he has a fifty-fifty chance of having a defective chromosome. Since he is a boy, he has only one X-chromosome. The second one from this pair is called Y in man, and bears no duplicating genes. This is why the blemish borne in X-chromosome is manifested only in boys, or, rather, in half of all the sons produced by these mothers.

All the girls are healthy, and half of the boys are sick. Half of all the girls from these mothers bear a defective X-chromosome, of which they are unaware until they produce a son with an immunity defect.

Not all the immunodeficiencies are linked with the X-chromosome, or, as we say, are sex-linked. Most of them are inherited in a different fashion. The only rule that is true in all the cases is this: the "defective" genes are of the recessive character, that is, they never manifest themselves if their duplicating genes are in good order. You might remember that all the genes contained in any chromosome, the repository of genes, have duplicates contained in the second pair chromosome.

There are 23 chromosome pairs in man. Twenty two of them are called autosomes, and the 23rd one (XY), the sex chromosomes. Only X-chromosome genes in men are not duplicated, since its pair Y-chromosome is much shorter than the X one. The recessive character of inheritance means that the defect is manifested only if both genes are defective. There is virtually no duplicate. And it so happens that both mother and father are healthy, though some of their chromosome pairs (say, the ninth one) has a "defective" gene.

Let us denote a normal gene controlling a certain link of the immune system with a capital G, a "defective" gene with g, the father's chromosomes with Roman figures, and the mother's with Arabic figures. Then, the genetic formula of the 9th chromosome in the father can be presented as 1XG-1Xg, and that in the mother,

9G-9g. Both father and mother bear the recessive genes g, but both are healthy owing to the duplicates G.

Their children inherit half the mother's and half the father's chromosomes. In their ninth chromosome, there can be 1XG-9G, 1XG-9g, 1Xg-9G, and 1Xg-9g. The children who inherited one of the first three combinations will be healthy. Those having the fourth combination (1Xg-9g) get a pair of "defective" genes. There is no duplication. The congenital deficiency appears.

Nobody knows which of the newborn babies will have this combination. This is how fate would have it. But, as is always the case in genetics, provided there are sufficient statistics, precise figures are reigning: 25 per cent of the children produced by the parents bearing the recessive gene, have the effect of this gene manifested. Here Mendel's law is working, that is: the frequency of recessive gene manifestation in the second generation is 1 3.

Types of Immune System Defects

In order to discuss various types of immunodeficiencies, we should recall the agents and mechanisms of immunity. The immune response of the body to any alien intrusion consists of two components. They are known as cell-mediated immune response and humoral immune response. The active factor of the first form, or its effector is a sensitized T-lymphocyte. The active factors of the humoral response are antibodies, that is, the proteins belonging to the class of immunoglobulins. The antibodies are produced by plas-

matic cells, which in turn come from B-lymphocytes.

The entire immune army of the body, which provides the specific responses to the alien antigens, consists of two lymphoid cell systems, T and B. The thymus and bursa of Fabricius are the two central organs of immunity.

The thymus in mammals is located behind the sternum. It is characterized by a very big size in newborns and a very small one in adults. The size and the weight of the thymus gradually decrease throughout the life of the body. Once this was the reason for considering that the thymus functions only during the first months of one's life, and then gradually atrophies.

This appeared to be false.

The thymus functions throughout the whole life of the body, teaching lymphocytes the immunological ABCs, or, as it is termed in scientific papers, immunocompetence. It generates immunocompetent lymphocytes, the ones called T-lymphocytes, meaning thymus-dependent.

These cells cannot emerge without the thymus; the body is incapable of generating sensitized lymphocytes in response to alien antigens without it. If this were not so, the immunity against many kinds of viruses would never form, grafted organs and tissues would not be rejected, alien cells, including cancer ones, would not be destroyed. If the thymus is replaced, the animal becomes immunologically defective, it falls ill and dies, though many antigens are still generated. This is because the main role in antibody production is played by B-lymphocytes.

The bursa of Fabricius is a specific organ. It

is absent in mammals, and only birds have it. This aggregation of lymphoid tissue is located near the colon. If the bursa of Fabricius in chickens is surgically removed, the immunodeficiency develops that is different from the one typical of the thymus-less animals. Only antibody formation is affected in these chickens. There are no antibodies, no matter how many times the birds are immunized.

Still, the cell-mediated forms of immune response develop, antiviral immunity appears and the alien tissues are rejected. The bursa of Fabricius is in charge of only those lymphocytes which generate antibody-producing plasmatic cells. In contrast to T-lymphocytes, these were called B-lymphocytes (from the word Bursa), which means Bursa-dependent.

True, neither this organ nor even an analogue are as yet found in mammals or in humans. It is assumed that the function of producing B-lymphocytes is performed by Peyer's patches, small aggregations of lymphoid tissue spread over the whole of the intestines; or, even more likely, by the bone marrow. There are no direct evidences of this, however. Still, the terms "the immunity B-system" and "B-lymphocytes" became commonly accepted and now concern not only birds, but all other animals, as well as humans.

There are 30-40 billion lymphocytes circulating in human blood. From 50 to 60 per cent of them are T-cells, 20-30 per cent, B-cells, and 10-20 per cent of lymphocytes fall into neither of these categories. These were called zero cells. What is their mission is still unknown. The ratio between T- and B-lymphocytes in the spleen is

almost the same as that in the blood. In lymph nodes, however, T-cells are more abundant, accounting for 80 per cent of the lymphocytes.

To be sure, T-cells in the thymus and B-cells in the bursa of Fabricius in birds or in its analogue in mammals do not appear from nothing. They have their own precursor, common for all blood cells. It is called the hemopoietic stem cell, from the word "stem", similar to the stem of the tree, from which all the branches spring. Stem cells are generated in the bone marrow and, through circulation, arrive in the central lymph organs mentioned above, wherein they give rise to T- and B-lymphocytes.

The general scheme for developing T- and B-systems responsible for the cell- and humoral immunity, respectively, is as follows. Every day, or hour, or minute the bone marrow produces and discharges stem cells. Part of them is drawn by the blood into the thymus. There they start to multiply, at the same time turning into T-lymphocytes. Another part of stem cells is blood-drawn into the bursa of Fabricius or into its, not yet discovered, analogue. There they produce the swarm of B-lymphocytes.

T- and B-lymphocytes from these two central organs flow out into the circulation, settle down in the spleen and lymph nodes, penetrate every part of the body. Ubiquitous, they attend to every tiny corner of our organism; at any moment they are ready to become active and destroy any strange newcomers.

This is the scheme. The only thing now is to point out the junctions, or the stages that can be defective because of genetic problems. There are

four basic forms of primary immunodeficiencies, or congenital abnormalities of the immune system: a genetic block which inhibits the development of stem cells, a block inhibiting the development of T-cells, a block inhibiting the development of B-cells, and combined defects.

The example of the disease with the isolated B-system defect is the one given above, affecting only boys. It is called the infantile, sex-linked agammaglobulinemia. This is the latin for "the total absence of gamma-globulins", or antibodies, in the body. An example of pure T-deficiency is the hypoplasia (or underdevelopment) of the thymus, or Di George syndrome. A combined defect, when both T- and B-systems are affected, is typical of the disease called ataxia-teleangiectasy, or Louis Bard syndrome. Mixed deficiencies are especially severe and hardly subject to treatment.

In 1977 a well-known pediatrician and immunologist, Robert Good, whose name has already been mentioned in this book, presented me the book "Primary Immunodeficiencies" edited by him. The reason I recalled this now is the very interesting dedicatory inscription he wrote in this sizable book: "We are to study immunity deeply, to the benefit of humankind, and our patients with congenital immunodeficiencies are our best teachers."

This is really so. Primary immunodeficiencies are examples of specific links of the immunosystem selectively switched off by the Nature itself. What are their manifestations, how are they to be diagnosed and treated? Patients with congenital immunodeficiencies provide the pos-

sibilities for answering these questions. They teach medicine to assess the impairments in the specific links of the immunity machine not only in genetic diseases, but also in all other cases, in most varied ailments.

The cases when immunity disturbances are not congenital are called secondary immunodeficiencies. The work of the immune system is assessed by the same links as in the "teacher-patients" Healing of disturbances, treatment, or, as it is now often said, immunocorrection are based on the same principles.

One more set of very important events occurred in 1981-1984. The word "achievements" is not really appropriate for describing these events, though they are connected with a discovery in immunology followed by another discovery in virology. In 1981 in the USA a new disease was described, the so-called Acquired Immune Deficiency Syndrome, or AIDS. The disease was discovered due to new clinical methods for assessing the immune status of patients. The immunodeficiency described in the USA was different from those previously known in that the victims developed a sharp decrease in one of the lymphocyte varieties, the so-called T-helpers. This resulted in a wide range of consequences: some had pneumonia, others, tumours, intestinal disorders or pustulous lesions. The rate of fatality was extremely high. Half of those who had fallen ill died within two years. The disease spread in the USA among homosexuals, hemophiliacs, and drug-addicts. It aroused a lot of problems in science and public health care. Clinical immunology became a demand for all big hospi-

tals. In 1983-1984 the pathogen of this disease was discovered. This was a previously unknown virus that was called HTLV-III. It penetrates the body through blood and by sexual routes, settles down in T-helpers and destroys them. Thus appears the many-faced immune deficiency —AIDS. The task for immunology in the nearest future is to find ways of treating AIDS and develop a vaccine for its prevention.

Diagnosis of Immunity Disturbances

The principle objective of science is practice. Immunology, as well as other fields of medical biology, studies the inborn defects not only to understand the work of the immune system and the genetic control of this work, but also to treat them. To treat not only primary immunodeficiencies, but also those which might appear in the adult due to illnesses or any other reasons.

But what is the treatment, and what is to be treated? Apparently, the treatment should be different in each specific case. It is one thing when the T-system is affected, quite another when it is the B-system or stem cells. So, before treatment in each case, the immunological responsiveness should be assessed separately: are there T-cells and how do they work? Are there B-cells and how do they function? For this purpose diagnostic tests for T- and B-cells were developed and introduced into clinical practice.

To assess the functional activity of the B-system, at least three parameters are to be evaluated. First, the number of B-lymphocytes circulating in the blood should be counted. Second, the

blood should be tested for the presence of immunoglobulins, since antibodies mean immunoglobulins. The third is the direct analysis of antibodies against a number of antigens, either appearing after special immunization or entering the body at the expense of normal microflora.

If there are no immunoglobulins in the blood, it means that the body fails to synthesize them, and, consequently, is incapable of producing any antibodies at all. If the blood level of immunoglobulins is reduced, then it should be clear which class of these proteins has mostly contributed to this reduction. All blood immunoglobulins, that is, all circulating antibodies, fall into five categories: immunoglobulins M (IgM), immunoglobulins G (IgG), immunoglobulins A (IgA), immunoglobulins E (IgE), and immunoglobulins D (IgD). The first three groups account for the main bulk of immunoglobulins and play the most important role in the protection against infections. Their blood level in an adult is normally in the range of 500 to 600 mg-per cent for IgM, from 1000 to 2000 for IgG, and from 100 to 200 for IgA. For a five-year-old child, these levels are lower and amount to 200-300, 500-1000, and 50-100 mg-per cent, respectively. The analysis of the blood levels of these three immunoglobulins is absolutely indispensable for assessing the functional activity of the immune response of the B-system in patients.

There are some known types of primary immunodeficiencies, when the synthesis of only one class of immunoglobulins is affected, namely IgA. These immunoglobulins are especially important due to their remarkable specific feature.

They are resistant to the digestive action of many enzymes, they can leave the circulation, penetrating the saliva, the mouth, bronchial and intestinal lumens, to face certain microorganisms which are just about to invade the inner medium of the body, its blood and tissues. Thus, class A immunoglobulins serve as an outpost combatting the first strikes of an aggressor. No antigens of this type—no forefront. Infectious complications of the mucous membranes of the mouth, the nose, the bronchi, and inflammations of the intestines are especially frequent and severe.

In other forms of immunodeficiencies, both A and G class antibodies are lacking. This is the main bulk of especially active antibodies.

Any artificial immunization or natural infection of the body triggers antibody production. First, class A antibodies are produced. These are the antibodies of primary response, that is the very first ones, still "cumbersome" and often "unskillful". Only after several days is antibody synthesis switched over to producing class G immunoglobulins. These are already the antibodies of secondary response. Their molecular weight is not 900 thousand, as with IgM, but as little as 160 thousand, they are abundant and highly active.

If the defect of the immune system lies in blocking IgG synthesis, the only weapon of the diseased body is the primary type of antibodies falling into the M class. These antibodies are not completely stable to a number of bacterial species.

Finally, total agammaglobulinemia means that no antibodies at all are produced.

The reader will understand, of course, that a failure in the body is not always a matter of a simple alternative: there is or there is not a particular thing. There are various degrees of manifestation, there are hypogammaglobulinemias differing in their quantitative indicators. This is why, apart from analysing immunoglobulins of various classes, it is necessary to test the body for its ability to generate not the immunoglobulins at large, but specific antibodies.

To this end, the titres of antibodies against widespread microorganisms, such as *Escherichia coli* and *staphylococcus,* are determined, or a patient is immunized with some special agent. However, live vaccines cannot be used, since they can cause an infectious process in an immunologically defective organism. The experts of WHO (World Health Organization) recommend the whooping cough-diphtheria-tetanus vaccine, dead antipoliomyelitis vaccine, and some others.

Different methods are used to assess the functional activity of the immunity T-system. Again, the starting point is the calculation of T-lymphocytes circulating in the blood.

It has been mentioned above that, after antigen stimulation, T-lymphocytes multiply and turn into sensitized lymphocytes that head for the place of antigen dwelling. Both these processes can be observed and measured.

The first process is called T-lymphocyte blast-transformation. This is conducted in a test-tube under the impact of a special stimulant, phytohemoagglutinin. This polysaccharide substance is isolated from beans. Blood is taken from a patient's vein; lymphocytes are isolated, put into a

flask with a nutrient and phytohemoagglutinin, cultured for three days. Then the cells are collected to count lymphocyte transformations into blast, or young, multiplying cells.

This count can be taken on a radiometric counter. Lymphocyte cultures are pretreated with radioactively labelled thymidine, and its incorporation into the cells is measured. The higher the level of blast-transformation, the more intensive the incorporation, hence, the more active is the T-cell system.

The ability of sensitized lymphocytes to approach the antigen, triggering specific immune response, the so-called delayed type hypersensitivity reaction, is assessed by means of various skin tests. These tests make use of a well-known agent—tuberculin, the one applied onto children's forearm to find out whether or not a child suffers from tuberculosis. Remember your school years when all of you went for tuberculin tests. But the most informative is a skin test with dinitrochlorobenzene. This substance is applied onto the patient's skin, and then it is done again in 14-21 days. If skin reactions fail to develop, then the function of the thymus, T-lymphocytes and the entire T-system of the immune response is defective.

Developments in immunology bring about progress in the methods of diagnosing the disorders in T- and B-lymphocyte system. Clinical immunologists have learned to measure the blood level with the help of an E-rosetting reaction. It appeared that T-lymphocytes can attach to sheep red blood cells. If these cells are added to lymphocytes which have been isolated from human

blood, the cells surrounded by the rosettes of sheep red blood cells are clearly seen under the microscope, and their percentage can be counted. The normal level of T-lymphocytes in the blood is 60-70 per cent. B-lymphocytes form the rosettes with red blood cells loaded with a blood protein complement. These rosettes are called complementary. The normal percentage of B-lymphocytes in the blood is 15-25.

In recent years ways have been found to count and assess the functional activity of T-helpers and T-suppressors separately. Methods for evaluating the condition of phagocyting cells were improved, as well as many other methods and techniques.

Immune Engineering

There is a world-known American Space Center in Houston, where, since 1974, there lives an austronaut unknown to the world. Only five seconds in his life did he breath the air all the other human beings on Earth are breathing. In the first years the newspapers wrote that this boy, David by name, did not know how flowers smell; he did not know what Mother's kiss or Father's embrace were like. He never walked holding hands with other children. He never even touched the skin of any other person.

For five years he never left an air-tight plastic chamber. When he was six years old, the scientists and engineers designed for him a movable isolated system, a special suit with a survival hand cart. Getting from the plastic chamber into the air-tight suit by means of sluicing, Da-

vid could expand his world: he could work, he could go into the parks and other places. But no microorganism could get into the chamber or the suit. If the air-tightness is disturbed and the microorganisms do penetrate, the boy will die.

There is sterile air, sterile water and food, sterile toys, handkerchiefs and clothes supplied into the chamber and the suit. It is all called "maintaining microorganism-free conditions" David suffers from severe combined immunodeficiency. He is defenceless in the face of microorganisms. He lives under this bell-glass, and the doctors are waiting: perhaps his immune system will start working? Perhaps it was underdeveloped and will very soon catch up? If not, they will have to resort to immune engineering. Not to the engineering of sterile chambers and suits, but to healing engineering.

The life of a child with an inborn immunodeficiency can be sustained for some time by antibiotics preventing infectious complications. Before the advent of antibiotics it was just impossible to observe the immunodeficient children. They died at very early ages. This means that antibiotics therapy and other ways of treating the infections can serve as a means for curing these patients. But in practice it is hardly possible.

Prevention of infectious diseases can also be achieved by actively immunizing the patients against the most widespread infections. Though not as effective as in normal bodies, it sometimes leads to a certain degree of nonsusceptibility. However, all these procedures can only but prolong a child's life. They are not truly heal-

ing measures, since they do not cure the basic defect, they do not eliminate the first cause, such as the failure of B- or T-systems of immunity.

The truly healing measures against B-system deficiency which is manifested in a reduced ability or complete inability to synthesize immunoglobulins, lie either in substituting the lacking immunoglobulins or replacing the lacking B-cells. The first way is a constant administration of immunoglobulins (gamma-globulins) isolated from the blood of healthy people. An effective dose is 25-50 milligrams of pure immunoglobulins per kilogram of a patient's weight per week.

This therapy is quite effective for partial B-system defects, when immunoglobulin production is reduced, but not entirely blocked. Constant administration of gamma-globulins provides for normal functioning of the people afflicted, who, owing to this treatment, survive to an adult age.

The replacement of lacking B-cells is only possible by their transplantation from tissue-compatible donors. Since the bone marrow is the basic source and receptacle of B-cells in the human body, its transplantation means the transplantation of B-cells. The replacement of lacking immunoglobulins or B-cells with new ones—this is already immune engineering.

I first heard the word combination "immune engineering" from Academician Yuri Lopukhin. This combination stresses the idea that treating immune system defects does not mean just the isolation of immunoglobulins or the bone marrow, or even thymus transplantation. Each specific case of immunodeficiency calls for its own, special engineering solution.

Immune engineering today is the only way of truly eliminating the cause for immunodeficiencies, since its objective is to replace the defective parts of the immune system by normal ones. This does not mean, of course, that the problem of immunodeficiencies is now solved completely because the transplantations of bone marrow cells, lymph nodes, spleen, or thymus have been introduced into clinical practice.

This would have been a solution to the problem, if tissue and cell transplantation in itself were not fraught with the consequences of tissue incompatibility. However, there are effective techniques to reduce the incompatibility problems. The first is the thorough selection of donor and recipient in their tissue compatibility antigens, or typing of donors and recipients. The second is the use of immunodepressive preparations weakening the rejection of grafted organs, tissues or cells. The treatment of immunodeficiencies using transplantation methods also requires typing, and, in some cases, the use of immunodepressors.

Compensating for a defective T-system of immunity is only possible by transplanting T-lymphocytes or the thymus. Humoral factors, transforming hemopoietic stem cells into T-cells, are not yet isolated, so a defective T-system is more difficult to compensate for than a defective B-system. It has already been mentioned that B-system defects can be combatted by systematical immunoglobulin treatment. In treating T-defects, only transplantation is effective. This way is more promising, since, at the same time, it can also be used to restore the B-system.

There are eight types of transplantations used

for treating various immunity defects. Seven of them were applied in different world clinics. The eighth method was developed by Yuri Morozov and Yuri Lopukhin in the 2nd Moscow Medical Institute. These types of transplantations are briefly outlined below.

1. Transplantation of either bone marrow, spleen and lymph node cells or blood lymphocytes from adult donors.

2. Transplantation of the thymus from an incompatible embryo or from an adult donor.

3. Combined liver and thymus transplantation from the same incompatible embryo donor (embryo liver is a blood-producing organ supplying hemopoietic stem cells).

4. Transplantation of the whole bone marrow from a donor compatible with a recipient in tissue compatibility antigens.

5. Transplantation of stem cells isolated from the bone marrow of an immunologically mature (adult) compatible donor. This can be combined with thymus graft.

6. Transplantation of isolated stem cells or the whole bone marrow from the parents preceded by introducing antibodies against the tissue compatibility antigens of the patient. This is done to reduce incompatibility.

7. Transplantation of stem cell fractions isolated from the bone marrow of the parent, along with immunodepressive therapy.

8. The essence of the eighth method is grafting two organs at the same time, in one block: the thymus and the sternum taken from a newborn (still-born) donor.

The advantage of this transplantation is not only in the "anatomical convenience" of the operation, though this is not the least important factor. Indeed, the thymus is placed straight beyond the sternum, which is one of the main receptacles for the bone marrow. Blood flows of these two organs are closely connected. Therefore, when arterial and venous blood alvei from this block are connected with any artery and vein of a recipient, excellent blood circulation of both organs is established.

The "immunological phylosophy" of grafting this block is that all the components of an immunity T-system are transplanted at once. Normal bone marrow discharges healthy stem cells, which, through the blood circulation, reach their own healthy (donor's) thymus and turn into T-lymphocytes. Furthermore, the bone marrow in man is a reservoir of B-cells. In other words, this graft would normalize the B-system at the same time. This is why the transplantation of the thymus-sternum block is one of the most effective means for treating combined immunodeficiencies, when both T- and B-systems are defective, the most promising method of immune engineering.

What Happens in Old Age?

We have discussed the diseases of the immune system. Inborn or acquired, they affect various links of the system and have their own peculiarities. They all need to be cured. But defects in the functioning of various organs can also appear in old age. All the systems get worn out, the

heart starts to malfunction, muscles, vision, and memory become weaker. But what about the immune system, this "unblinking eye" guarding the body against any stranger coming from outside or a genetical traitor appearing inside it? What happens if it gives way? Isn't this the reason why cancer occurs so often in old age?

On August 20, 1971 the 1st International Congress of Immunologists was opened in Washington, D.C. Thus, this day became a kind of Independence Day for immunology. Immunology acquired independence, not only in essence but also formally. Before that day it had been represented only at certain meetings or sections at microbiological, physiological, or genetic congresses. Now, the independent international society was set up, which brought together immunologists from all over the world; the first immunological congress was held, attended by over three thousand researchers from various countries.

The Congress was held in one of the largest hotels in the outskirts of Washington, in Sheraton Park Hotel. The delegates lived there, and all the sessions were held in the spacious halls of the hotel. They were arranged in such a way that the participants could listen to the talks of most prominent scientists and engage in free and relaxed discussions.

Famous immunologists gave their talks in the mornings during plenary sessions. The discussions were in the afternoon, at the so-called workshops. By now the combination of sessions and workshops had become the most popular form of scientific congresses.

All the contributions to be presented at the

sessions are requested in advance, from the scientists known for their most fruitful work. At the sessions there are no questions, no arguments or objections, there are no discussions whatever. People only listen to the speakers. At the workshops everything is different: there are no talks or lectures, there are only questions, discussions, and arguments, not on the talks given at the previous session, but on any chosen problem in general.

Every workshop has a chairman, appointed by the Organizing Committee from the people most familiar with the given subject. For instance, Robert Good, whose name has been mentioned more than once in this book, was the chairman of the workshop on the congenital defects of the immune system. The organizer of the workshop on the immunology of aging, and the chairman of this workshop was Professor Roy Walford.

The people who were interested in this question assembled. The chairman came up to the blackboard and wrote down two questions:

1. What happens to the immune system with aging?

2. Isn't aging a consequence of the failures in the work of the immune system?

Then he sat down at the chairman's table and declared the floor open for discussion.

As for the first question, the members of the workshop held more or less the same opinion. As all other body's systems, the immune system gains strength in youth, from 16 to 30-40 works at its peak, and then deteriorates more and more with every decade.

Tayashi Makinodan from Oak-Ridge suggested

a very convenient criterion, which was adopted right then. He called it Relative Immunological Potential (RIP). He suggested that RIP would mean the immunological work performed by a million lymph cells taken from the bodies of different ages. So, if a number of antibodies produced by 1 million spleen cells in a youth in his prime is taken for 100 per cent, the RIP of a newborn is only 8, and that of an old man, from 20 to 30. In a newborn, however, this low level leaps up to very high figures in just several weeks, while in an old man it only reduces with time.

The second question written on the blackboard was the question on the cause and effect. Does immunity become weaker as old age arrives, or does old age come because the defence system against outer and inner enemies becomes weaker? There was a long dispute, in which there were no absolute winners. The arguments were too intricate.

Walford himself put forward the following hypothesis.

Over the course of long years of life, abnormal variants get accumulated among the cells of the immune system, the lymphocytes. Was there some adverse agent that acted once, or twice, or three times? Or the cell material in multiplication got divided in a wrong way, and a bad lymphocyte was generated? Or the mutations, the gene changes in lymph cells, accumulated? Who knows? What is important is that as a result, there appeared abnormal lymphocytes carrying out the function of supervision, something like mad policemen.

So, instead of doing their service in good faith, catching enemies or traitors (abnormal body cells), these "crazy policemen" go for "loyal citizens" (good working cells), destroy them, prevent them from performing their functions, produce antibodies against them, and so on. This results in failures in the hemopoiesis system, disturbances in the heart muscle, decrepitude of skin, and other troubles coming with old age. Putting it in medical terms, autoimmune reactions develop, and an abnormal immune system starts aggression against the normal body cells.

There really are some autoimmune diseases, such as rheumatism, lupus, or some forms of anemia. But these will be spoken about later.

Walford's idea is not commonly recognized, but it has a number of proponents. At the congress of 1971 no common opinion was arrived at. Perhaps, it will not be arrived at even in 1991. But at the time of that immunological congress, many facts on the work of the immune system in old age had not been yet obtained. Immunologists were already aware of the existence of T- and B-immunity systems. They knew that T-lymphocytes carried out the police functions of surveilance. But in what way they change in old age, and how many of them circulate in old people's blood was not yet known.

These data appeared a year later; they were discussed at another International Congress in Kiev. In July 1972, the IX World Congress of Gerontologists, the experts in the science on aging, gerontology, was held there. A special symposium dealt with the immunology of aging. Interesting contributions were made by Vladimir

Kozlov (Novosibirsk), Rakhim Khaitov (Samarkand), and Viktor Man'ko (Moscow). Our friend Tayashi Makinodan also came to the congress, this time from Baltimore. There, not far from Washington, he headed a newly established US Gerontological Center. But still he remained an immunologist, as he was. The principal scientific problem of the Center was the immunology of aging.

At this congress the data on various cell immunity systems were first arranged in good order: on the stem cells giving rise to T- and B-lymphocytes, on T- and B-lymphocytes, their interaction, activity, and other aspects.

Kozlov demonstrated that the number of stem cells in an aging body steadily decreases. And it is these cells that flow out of the bone marrow, and, arriving in the thymus, serve as a "sowing material" giving rise to the army of T-lymphocytes.

Khaitov showed that, indeed, with every year of life there are less stem cells leaving the bone marrow, and less of them coming to the thymus. The absolute number of T-lymphocytes falls several times with years. Mind you, this concerns T-lymphocytes, the cells responsible for the immunological surveillance. This was later shown both in mice and in humans.

For instance, Konstantin Lebedev counted the blood from 400 people of various ages: newborns, 5-year-olds, 16-year-olds, 40-, 70-, and 80-year-olds. The absolute number of T-lymphocytes in the unit of blood volume only falls throughout one's life. The number of B-lymphocytes is about the same, and that of T-cells steadily decreases.

Victor Man'ko together with Liya Seslavina demonstrated the decline of T-lymphocytes' "potency" in old age. They are not as active as they used to be in their cooperative work with B-cells. There are not enough of them to kill genetically alien cells with their old vigour.

Tayashi Makinodan formulated the basic problem of the immune system in an aging body. In his lecture, which was meant not to be a popular one, but a purely scientific one, he said: "...With years the police function of the immune system is affected." T-lymphocytes come to function poorly, they grow short-sighted. They see patent incendiaries, strangers speaking a foreign language, but fail to discern inner saboteurs and traitors.

This is why the body's protection against microorganisms in old age is not so greatly affected. What is really weakened is the protection against cancer, that is against vicious malignant cells formed in the body itself. Various forms of cancer appearing in old age are really due to the defectiveness of the immune system. And vice versa, we are all safe thanks to the immune system that faithfully guards our body against cancer.

Immunity Against Cancer

Lev Zilber. Cancer Antigens and Oncogenes

Immunity can only play a role in anti-cancer protection provided cancer cells have antigens alien to the body. This is evident, because other-

wise there would be nothing for the immune system to destroy, since it destroys only alien substances. Therefore, the works in cancer immunology started with the search for cancer antigens. Soviet immunologist Lev Zilber was a pioneer in this area.

Back in 1949, Zilber developed a method demonstrating antigen differences between cancer and normal cells. The initial reaction towards his results and their interpretation was quite sceptical, if not hostile.

The sceptical reaction was quite natural. One proof is never enough in science. Further evidence is needed, corroboration by different tests and different researchers. The hostility was because Zilber's report was taken as an attempt to prove the viral nature of cancer. But it was exactly what Zilber was inclined to think: as long as alien proteins were found in the tumour, they could as well be viruses.

Many researchers and doctors refused to accept this viewpoint. They were all well aware of the fact that cancer was not an infectious disease, and it was inconceivable that a virus, cancer pathogen, could be of the same kind as those of smallpox, measles or influenza, all the more so since cancer was known to be caused by some external impacts sometimes, like lip cancer in smokers.

Fifteen years later, when Zilber elaborated his virogenetic theory of tumours, the debate on cancer antigens was already nearing its completion. The existence of these antigens was no longer questioned, nor was the theory itself, even in its most primitive interpretation: pathogen viruses

had already been discovered for some of the tumours.

The viruses causing tumour development were called oncogenic, or tumour-generating, viruses. The situation, though, appeared more complex than just a virus infecting a normal cell. The virus penetrates the nucleus and sets its nucleic acid (that is, its genes) amidst the nucleic acid (the genes) of this cell. This changes genetic code, alters the orders given to the cell, and obeying these wrong orders, guarded by wrong schemes, the invader starts to construct its proteins, to build its body.

An interesting point in all this is that, for a number of viruses, known beyond any doubt to cause tumours, these "cancer genes" were proved not to belong to the virus. They had been previously borrowed from human or animal cells. Oncogenes are a kind of "wandering" genes which travel in Nature attached to viruses. But if this oncogene attached to a virus reaches a human or animal cell and builds into a suitable site for its genome, this cell turns into a cancerous one. This astray gene-oncogene gives the order, and a cancer protein is constructed. Since the roving gene has come from elsewhere, the protein is an alien one.

Both virus antigens and the cell's own antigens built on the altered orders were revealed in cancer cells. This confirmed the viro-genetic theory, which suggests not a simple viral infection, but rather a combination of a viral infection with inborn or acquired conditions allowing for the virus to build inside the cell's holy of holies, its genetic apparatus. The cell itself

becomes genetically alien. The cell, not the virus!

But, as we know, once there is something genetically alien, the immunity should come onto the stage. Anything not one's own is alien: this cell is to be destroyed.

This does not mean that the viral nature of cancer is recognized by everyone, and immunologists do not insist that this is so. In fact, they "do not care" why the cells are altered. What is important is the fact that a cancer cell bears the features of genetic alienness in the form of the so-called cancer antigens. All the more that there are some cancer antigens of a non-viral nature.

When an oncogenic virus inserts its genetic code (nucleic acid) into the hereditary apparatus of a cell, this cell starts to synthesize a new protein which is odd for it. It produces this protein "at the bidding" of the viral nucleic acid. As a result, all the cells form the same cancer protein.

Under the impact of the virus, a tumour can appear in various parts of the body, in various individuals of the same species, and even in various animal species; the cancer antigen will be the same: the antigen produced "at the bidding" of the virus genes.

A totally different situation prevails for the tumours induced by certain chemical agents. These agents are called "carcinogens", from the words "cancer" and "generate" Examples of these substances are methylcholanthrene, benzopyrene, and many, many others. Physical carcinogens include all kinds of ionizing radiation.

If a dozen totally identical individuals, like the mice of the same pure strain, come in contact with the same carcinogen, all twelve result-

ing tumours will have their own cancer antigens. In other words, the same chemical agent causes different genetic changes in different cells.

An oncogenic virus forces one and the same programme upon all the cells. A carcinogen hits at random, causing different changes in different cells. Geneticists and oncologists study the mechanisms of this phenomenon. The crux of the problem for immunologists is that cancer cells always bear the features of alienness: cancer antigens.

This Double-Faced Anticancer Protection

It was back in 1942, when Gorer, a researcher from Great Britain, found antibodies against tumour cells in the blood of tumour-bearing animals. Of course, this fact by itself could not be taken as a proof of immune protection against possible tumours. All the more that in 1952 Nathan Kallis, a young scientist from Bar-Harbor, together with his teacher George Snell, the one who bred many pure-strain species of mice so much needed for studying tumours, demonstrated a paradoxical regularity. Indeed, the animals with grafted tumours appeared to have antitumour antibodies in their blood. But if this blood is injected to any other animal, it is then easier to graft a tumour to this animal, and this tumour grows faster. Instead of inhibiting, antibodies enhance tumour growth. This phenomenon was called immunological enhancement.

How can we call it, antitumour protection?

This all leads to a rather strange situation. On the one hand, the immune response to the tumour cells was demonstrated. On the other, this

response gives no protection against the tumour, on the contrary, promotes its growth. Some researchers lost all interest in the antitumour immunity. Others remained in doubt: as long as there is immune response, there should also be protection. So they went on searching for protective immune response to tumour grafting.

Americans Richmond Prehn and Joan Maine got results that looked rather convincing. They induced tumours in mice by means of a chemical carcinogen, took small pieces of this tumour and grafted it to a group of mice of the same genetically pure strain, i.e. identical to the first ones in all their antigens. The tumours started to grow.

Mice of another group belonging to the same strain were inoculated with the killed tumour. In a week pieces of this tumour dissolved. After that the mice were treated with live cells of the same tumour. They also dissolved. There was no cancer, meaning that there *was* some immunity, and this immunity was directed against tumour antigens, because all the other antigens in the cells of the animals belonging to one pure strain were identical.

The problem began to shape up. Thousands of researchers joined in the studies. And the next exciting story was told to the world by Swedish immunologists Karl and Ingegard Hellström. They developed a method of inhibiting the growth of tumour cells by lymphocytes *in vitro,* or in a test-tube.

The essence of the method is as follows. A piece of tumour is taken from an animal bearing the tumour, and disintegrated into a cell suspen-

sion. These tumour cells can be placed into a nutrient solution in a test-tube or in a special flask (a glass dish with a flat bottom). Microscopic cells settle down on the bottom and start to multiply. Several days later one can see the colonies of cancer cells, which grow so abundant that they fuse together and cover the bottom of the dish with a continuous layer, like pond scum. Only it consists of cancer cells instead of innocent algae....

The Hellströms mixed these cancer cells with lymphocytes of healthy animals. This leads to nothing special. Lymphocytes displayed no immune activity. Cancer cells multiplied and grew as usual. Then the researchers decided to test the lymphocytes from a tumour-bearing animal. If the immune system resists the tumour growth, the lymphocytes must have some killer capability.

The Hellströms were in a more advantageous position than all the previous researchers. Their studies were conducted in 1969-1971, when it was already known that it was T-lymphocytes that are able to kill alien cells after immunization. The Hellströms treated mice with a carcinogenic chemical, methylcholanthrene, which causes sarcoma, one of the most malignant forms of cancer. They cultured these sarcoma cells in the dishes with a nutrient medium. Then the lymphocytes from normal and cancer-bearing mice were added to this culture. The lymphocytes from the affected animals turned out to be immune: they displayed anticancer activity; the growth of tumour cells was markedly inhibited.

After that the Hellströms performed a series of experiments with skin cancer in rabbits. The

peculiar feature of this tumour is that in most animals it actively grows (persists), turns into very malignant carcinoma, and kills the tumour bearer, but in some of them it gets reduced (regresses) all by itself and finally disappears. The first group of rabbits was called persistors, and the other—regressors. It appeared that the lymphocytes from the animals of both groups were equally active against cancer cells, inhibiting their growth. However, if, along with lymphocytes, blood serum is added to the same culture, the results are different. The serum from the persistor animals cancelled the inhibiting effect of the lymphocytes, while that from regressors did not.

The scientists showed that the serum factor interfering with the work of lymphocytes is due to antitumour antibodies. The Hellströms called them blocking antibodies and formulated a very popular hypothesis of blocking antibodies. According to them, the production of antibodies due to B-system and cell-mediated immune response governed by T-cells are in a way competing processes. Immune lymphocytes recognize tumour cells and destroy them. On the other hand, antibodies cause no harm to cancer cells, on the contrary: attaching to these cells, they shield or block them and thus protect them from being destroyed by immune lymphocytes. According to this hypothesis, the fate of the tumour and a tumour-bearer depends on the ratio between the production and accumulation of immune lymphocytes. If the first factor wins over, the tumour will grow, if the second dominates, it will be destroyed.

The system of T-lymphocytes is a central sys-

tem of anticancer defence. The thymus, the central organ of the T-system, is the headquarters of antitumour immunity. This was the conclusion shared by almost all researchers, but not quite all of them. Still, for a long time the arguments of those in doubt were very weak. And then a special strain of mice was bred.

These were defective mice, bearing a faulty gene preventing the development of the thymus. If a male and a female of this strain are mated, then, as Mendel's law would predict, 25 per cent of the offspring would be abnormal: they would lack the thymus. The breeders managed to endow these mice with one more genetic feature, the gene of hairlessness. So, 25 per cent of thymusless baby mice are bald at the same time. They are easily distinguished from the others. These thymusless mice are called the "nude mice" in the literature. Of course, they are not long for this world. In several days or weeks they die of infections. Their immunity is so weak that they do not even reject grafted skin. To prolong the life of these athymic mice, they are to be kept under some special, preferably sterile conditions.

So, those who did not support the theory of T-lymphocyte anticancer protection staked their claim on nude mice. These mice were kept under sterile conditions until their natural old age. If there was no anticancer protection without T-lymphocytes, all the athymic mice would have died of cancer. However, this was not the case. The percentage of the tumours among nude mice was the same as that in normal ones. So, the sceptics concluded: it was not lymphocytes that pre-

vented tumour development; at best, they join the fight later, once the tumour has already formed.

So the question arose in immunology: what are the cells that prevent tumour development? Some said these were surely some cells other than lymphocytes; others only maintained that these were some cells other than T-lymphocytes.

NK—Natural Killers

It is interesting to trace how ideas develop. In 1967 in our laboratory the interaction between lymphocytes and stem hemopoietic cells was discovered. This was described in the chapter "The Dictatorship of the Lymphocyte". The essence of the discovery was that lymphocytes, interacting with a proliferating stem cell, determine the course of this cell development if it is the body's own cell, and kill it if it is not. They kill it at once, at the very first contact, with no further notice, with no preliminary immunization.

This is the natural forefront of the defence against all alien, including cancer, cells. It is the line of defence that is there even before the classical forms of immune response with the accumulation of immune T-lymphocytes or antibody-producing B-cells.

So, immune lymphocytes accumulated in the body after immunization and capable of killing all strange cells, including cancer ones, emerge in the body upon a special signal, some time after the first contact with alien antigens. Apart from these, there are original killers of strange cells. They need not be accumulated, they exist

naturally under normal conditions, and their function is to kill a proliferating cancer cell upon very first contact, before the immune response is developed and T-killer lymphocytes are accumulated. These pre-existing, natural lymphocytes, the killers of cancer cells, were found in the thymusless mice in very high quantities. They were called "Natural Killers" They build up the front defence line against cancer. The second line of defence consists of T-lymphocytes.

In terms of their characteristics, NK-lymphocytes can be grouped with neither T-, nor B-cells. They do not come from the thymus. The athymic mice lack T-lymphocytes, but have Natural Killers in plenty instead. There is no second line of anticancer defence, but it is made up for by the reinforced first line.

Later, NK-cells were found not only in mice, but in other animals, and in humans. Their level in patients is assessed to hint at the effectiveness of the therapy. The researchers look for ways to stimulate these cells.

This is how an abstract idea that appeared in 1967 was transformed into a clear-cut mechanism of anti-cancer immunity in ten years.

Why Is Immunity Ineffective Against Cancer?

This standard question is not quite fair, since most of us are nice and healthy, suffering from no tumours owing to daily effective, and I stress it, effective work of the immune system that kills all the altered cells. So, the ineffectiveness is the exception rather than the rule, but an exception that costs some of us very dear.

What are the reasons for these fatal exception?

The generation and growth of a tumour (an aggregate of cells differing in their antigens from a host organism) present a real immunological puzzle. The crux of this puzzle is: why the tissue with alien antigens is not rejected? The situation is quite opposite to that pertaining to grafting alien organs or tissues. We all know that the slightest genetic difference in a grafted skin or kidney is enough for the immune system to detect it as foreign and to reject or destroy it.

The challenge of immunology in organ grafting is either to cancel or to inhibit the system of immunological surveillance. The situation should be achieved when the tissue with alien antigens is not rejected due to incomplete immunological surveillance.

The challenge for immunology in cancer treatment is quite the opposite: to restore or reinforce the system of immunological surveillance. It is quite possible that these both tasks have a common basis and will be resolved simultaneously in the future. It is not so important where the solution will come from: graft immunology or cancer immunology.

What causes the failures of immune response against a growing tumour?

To be quite frank, nobody knows. There are only certain suggestions, more or less plausible hypotheses. Here are some of them.

The hypothesis of immunological tolerance. This concerns the tumours of proved viral origin. It is suggested that the viral particles constantly subsist in the cells of the animal to be affected

in a latent, dormant form. It should be stressed that these particles are in the very "heart" of the cell, among its hereditary material, its genes, and divide together with chromosomes during cell reproduction. This is how they get into the sex cells, and are inherited by the emerging foetus, the kernel of a new body.

As long as the alien substances entering the body during the period of its embryo development lead to tolerance, or immunological non-responsiveness, the newborn organism will not respond to these viruses. So, if these viruses get activated under the impact of some factors, leave the dormant state and start to turn normal cells into cancer ones, the immune system takes no notice of it. This system is tolerant.

The hypothesis of the immunodepressive effect of the tumour. It is suggested that cancer cells secrete some unknown substanses suppressing the immune response. This suggestion has no serious experimental proofs. It is known, however, that cancer antigens can suppress the lymphocyte's activity by blocking lymphocyte receptors, somehow blinding them. The lymphocyte surrounded by antigens cannot find a cancer cell.

A very popular hypothesis is that of a *disbalance between the development of the immune response and tumour growth.* According to this hypothesis, the tumour tissue grows more vigorously than the lymph cells that emerge and multiply in response to it. The lymphocytes capable of combatting the tumour get depleted, and the body becomes immunologically defenceless against this tumour.

One more hypothesis is based on the regulari-

ties of *genetic control of the immune response.* Each organism has its own set of immune response genes. We've mentioned them earlier in this book. These genes are called IR-genes, from the words Immune Response. The genes are denoted by the figures IR-1, IR-2, etc.

No one knows for sure how many of them are there. Each of them controls the body's ability to respond to a specific antigen. If someone has a "strong" gene IR-1, it means that he has a very good response to a certain antigen X. But if he has this gene in a "weak" form, he will not be able to respond to this antigen X. At the same time, he can have a "strong" IR-2 gene, and, despite his "weakness" towards antigen X, he will cope very well with an antigen Y.

There are a lot of genes, and most of them are "strong", so we are not afraid of microorganisms bearing several antigens. Let's say a microorganism bears the antigens X, Y, and Z. A certain person, for example, has a "weak" IR-1 gene and cannot respond to the antigen X. His lymphocytes will recognize an intruder and kill it at the expense of responding to the antigens Y and Z.

But what if there is an alien cell with only one strange antigen? What will happen to our man? His immune system will miss the stranger and will not prevent him from living and multiplying.

Now we are all right. Our IR-gene set is in good shape. But each of us has several genes that fall into the category of "weak" ones. This is such a trifle that we do not even notice it. Microorganisms and viruses penetrate the body and

get destroyed. Mutants appear among the cells of our body. Changed cells are also destroyed, until it comes to a mutation leading to an antigen invisible for the immune system of a certain individual. It is invisible because his IR-gene providing for the response to this (and only this) antigen, appears to be "weak". Then the emerging cancer cell is not destroyed, it proliferates and gives rise to a tumour.

This is why we all have different cancer antigens. This is why a great challenge for immunology is to learn to turn genetically low-responder individuals into high responder ones.

How Can Anticancer Immunity Be Stimulated?

When we say the word "history", we usually recall the things that happened centuries or at least decades ago. Take the history of the struggle with smallpox. We recall ancient Chinese physicians who triturate the scabs from the sores of the diseased people in their mortars to blow the powder into the noses of healthy people. Then Edward Jenner, who 200 years ago prepared a vaccine for people using a cow pox. The decrees of 1918 on the obligatory vaccination of the entire population. The history was crowned by the victory. There is a long history, and there is no more smallpox.

The immunotherapy of cancer is now taking its very first steps. Today there are more hopes than meaningful achievements. But the hopes are strong.

The initial logical step is supported by the

proof of the fact that anticancer protection is provided by T-lymphocytes and Natural Killers. It means that for treatment it is necessary to stimulate the cellular reactions of immunity.

What is the way to do this?

At first it seemed very simple. There are some microorganisms that stimulate the T-system, there is Interferon that stimulates NK-cells. The microorganisms stimulating T-cells include the pathogens of tuberculosis. Of course, nobody would infect a cancer patient with tuberculosis. But there is the tuberculosis vaccine, the famous BCG, which consists of weakened tuberculosis bacilli and is quite safely administered to all the newborns in maternity wards. This vaccine can be administered to people of various ages. There will be no tuberculosis, but T-lymphocytes will be stimulated.

Many present-day schemes of cancer treatment include multiple injections of BCG or other stimulants of the T-system. Several of these schemes are now jointly tested by American and Soviet oncologists in the All-Union Centre for Cancer Research of the USSR Academy of Medical Sciences There is a special Soviet-American agreement providing a course of research that excludes any error. The best scheme is to prove itself in practice on both continents.

The second way seemed more realistic in the beginning. All lymphocytes should be extracted from the blood, which is not so difficult given the modern state of the art. There are special blood separators. They are based on the same principle as those separating whole milk into cream and the defatted fraction. These apparatus

separate blood into serum, red blood cells, white blood cells and lymphocytes.

With the help of special equipment, blood can be channelled through the apparatus and then flow back into the patients't body free from lymphocytes. After some time, all lymphocytes will be gathered in one glass vessel. They can be mixed with a stimulant activating T-cells. One long-known stimulant is phytohaemoagglutinin, a chemical extracted from beans. Then the stimulated lymphocytes can be brought back to the patient's blood alveus, and, wipped up, these cells will go after the tumour cells.

This technique seemed sure to be successful. But no, in its simplest modification that I've just described, it led to nothing. All T-lymphocytes were extracted and wipped up, but the curing effect was quite doubtful.

This work, though it gave no method of treating cancer, turned out to be very important. It demonstrated that in humans, as well as in other animals, lymphocytes are cloned. They are, so to say, divided into different arms of the service, and each detachment or clone, is aimed at different enemies.

There are thousands of clones among billions of lymphocytes. One clone cannot substitute for another. If the clone against the tumour is small, weakened, or underdeveloped due to weak IR-gene, or else there is no such clone at all, then there is nothing to stimulate. All the clones are stimulated against everything on Earth, but this very one is lacking. Or, probably, this defective clone becomes completely exhausted under the influence of too strong a stimulation.

Phytohaemoagglutinin is indeed a very strong stimulant: it drives all lymphocytes very intensely, but in an unknown direction.

There are also some problems with interferon, a stimulant of Natural Killers. Interferon is a protein produced by lymphocytes and certain other cells in response to the stimulation by various substances, the so-called interferon inducers. These can be viruses, alien antigens, the above-mentioned phytohaemoagglutinin, alien nucleic acids, a number of artificial polyelectrolytes, and other agents. Interferon enhances the anticancer activity of Natural Killers. But the trouble is that murine interferon helps only murine cells, and the swine one, only pig's cells. Human NK-lymphocytes can be stimulated only by human interferon. Another problem is that a curing anticancer effect is achieved only by using very high doses of interferon. Where should it be taken from?

Researchers learned to culture human blood cells in a nutrient medium for producing interferon. But there can never be enough donors to accumulate the quantities of the substance sufficient for treatment. All the hopes are pinned on immune and gene-engineering biotechnology. The hopes are great. Both biotechnological trends are about to lead to high outputs of the preparation. The gene of interferon has already been isolated from the producer cells; it was synthesized anew and introduced into intestinal bacilli, *Escherichia coli*. Now this bacillus will produce the substance. This microorganism is capable of unlimited proliferation; so the only thing left is to isolate the preparation and purify it.

Vaccines Against Cancer

Apart from all this, there also remains a classical approach: the development of an anticancer vaccine that would prevent the body from tumour development.

The idea of vaccination with the tumour antigen is one of the oldest in the young history of cancer immunotherapy. The strategy is as follows. A tumour is found in a patient. This, of course, can be removed surgically. But the insidiousness of cancer is that it is metastasic: it grows into the neighbouring tissues, settles down in other organs. This colonization happens even before the operation, but it sometimes occurs after it, because cancer cells had already circulated in blood and precipitated elsewhere. Several months after the operation, the metastases are found in the lungs, in the liver, and other organs.

Many researchers hope to prepare a vaccine from the ectomized tumour to administer after the operation. Vaccine-stimulated lymphocytes will do away with metastases. The questions are: How to prepare this vaccine? Which antigens should be extracted? What is the fast and accurate way to see whether or not the patient's lymphocytes respond to this individually prepared vaccine?

One such approach to evaluating the lymphocytes' response has been proposed by the Cherchicks from Birmingham. The path which led to their final technique, published in 1974, is worth special mentioning.

Some ten years before that the Cherchicks had

not even dreamed of oncology. They were botanists and studied the geotropism of plant roots, or the tendency of roots to turn towards Earth. If a dug-out plant is laid or even turned roots up, all the thin roots will again turn towards Earth. The cells will multiply in such a way that each daughter cell will be aligned towards Earth's centre. It is as if some extra weight rolls down to the lower part of the cell and points to the right direction for the division.

This was what the researchers suggested: before the division cycle, when the cell body is destructured, special granules are displaced downwards. If this is true, then the impacts contributing to the destructuring should enhance root geotropism. This was confirmed. X-Ray irradiation of roots, ruining many cell structures, enhanced geotropism.

To register the degree of cell destructuring the Cherchicks used an optical device measuring the dispersion of polarized light. Having started the method going, they asked themselves the question: Are all the cells destructed before division? The answer was yes, both for animal and plant cells. And what about humans?

To answer this question, cells capable of division were to be taken from a subject. Lymphocytes can well serve the purpose if stimulated with phytohaemoagglutinin. The Cherchicks added a stimulant to the lymphocytes of a healthy subject and put the cells into their device. Twenty minutes later destructuring was registered. It was clear that the lymphocytes had received the signal and in 20 minutes prepared for multiplication.

So the last question arose: how would the lymphocytes of cancer patients behave? It appeared that there was no destructuring of the lymphocytes' body within 20 minutes of action of phytohaemoagglutinin, a strong, but nonspecific stimulant. But this phenomenon was observed in "cancer" lymphocytes under the impact of proteins isolated from tumours.

Be it this, or any other method, but the way will be found. Oncologists will learn to stimulate T-lymphocytes specifically against the tumour. This way is quite promising, but not the only one. Some other approaches are being tried. Thymosine, thymarine, T-activine, and other thymic hormones providing for the normal functioning of the thymus are now being tested in clinics. Other mediators of the immune system are also being tried. The efforts are made to combine the methods of immune therapy with ray and chemotherapy.

In many cases, immunological methods help detect cancer and made a correct diagnosis.

"Liver cancer" or "intestine cancer"—these diagnoses are not only frightful to utter, but also difficult to make. It is not so easy to detect a tumour hidden inside the body. And successful treatment or surgical operation is only possible if this tumour is revealed during its early stages, when it is not too big, before it has colonized, as metastases, in all the body's organs. Ideally, it must be revealed at the very beginning of its development. Here immunological methods are beyond any competition.

The most convincing example of immunodiagnostics concerns the primary cancer of liver.

Soviet scientists Harry Abelev and Yuri Tatarinov have demonstrated that the cells of liver cancer produce a special antigen belonging to embryonic proteins. It was called alfa-phetoprotein. Detected in blood with the help of a special immune serum, this protein undoubtedly means liver cancer.

The production of embryonic antigens turned out to be an indispensable feature of most cancer cells. A special embryonic antigen called Intestine Embryonic Antigen, is found in the blood of intestine cancer patients. Another antigen is characteristic of gastric cancer. Immunodiagnostics of the tumours of the kidneys and the nervous system is also important.

The data of lymphocyte studies are also of some diagnostic value. For instance, with neuroblastoma (the tumour of the nervous system), lymphocytes in children become capable of destroying the nerve cells. This can be revealed by the Hellström methods mentioned above. Tumour immunodiagnostics is improved with every passing year.

When I am asked whether I believe that immunologists will learn not only to diagnose cancer but also to treat it, I firmly answer: yes, I do believe in it. I think this will be their common success together with oncologists, surgeons, biochemists and geneticists.

Allergy and Other Slips of Immunity

Prevention Upside Down

In 1902 the French scientist and traveller, chemist and pharmacologist Richet set out on a small expedition around the Mediterranean. The objective of the expedition was to try and find new poisons previously unknown to pharmacology, that could be isolated from simple sea animals. On the yacht there was a vivarium with various laboratory animals. There were also dogs among them. The sponges, mollusks, and other sea animals taken out of the sea were ground up, water-soluble substances were extracted from them and given to the experimental animals in different doses. The scientist looked for strong poisons.

Once sea anemones were tried, small animals, as bright as flowers. An aqueous extract containing anemone proteins was injected into dogs' blood. Nothing happened. Several weeks later the extract from the same animals was again injected into the blood of the same dogs. The extract was non-toxic, but the dogs had a terrible response: weakness, diarrhea, and convulsions. Richet called this phenomenon anaphylaxis.

All of you surely know the word "prevention" or "prophylaxis". When we say that "the car is in for preventative maintenance", it means that the mechanics examine the car, fix it and do everything to prevent the car and the driver from having an accident. The departments of labour protection at factories deal with the prevention of labour traumas. To prevent rickets, children are given cod-liver oil rich in vitamin D.

"Prophylaxis" in Greek means "for protection". If another Greek prefix, "ana", is added, it will produce a different word: anaphylaxis. This prefix changes the meaning of the word to the opposite. For instance, anachronism means a wrong, reverted notion of time; anarchy is something opposite to order.

The word "anaphylaxis" means something opposite to prophylaxis: anti-protection, the development of susceptibility, the increase in sensitivity, instead of enhancing the stability. The smallpox vaccine, say, reinforces the immunity, makes the body unsusceptible to smallpox. It provides for smallpox prevention, or "prophylaxis". But, as Richet showed, there are also cases when alien antigens administered to the body increase its sensitivity towards these antigens, instead of protecting against them; this is what is called anaphylaxis.

Theobald Smyth did not know about Richet's observations and discovered anaphylaxis anew. He illustrated even more vividly that immunity is not always a friend, that sometimes it can cause death. The year was 1904. Smyth was analysing the antitoxic strength of horse antidiphtheria serum. For this, horse serum was administered intravenously to guinea pigs. The experiments needed a lot of these quite expensive animals, and the experimenter tried to save them. He decided to use the guinea pigs who, several weeks before, had already been treated with horse serum.

The saved guinea pigs looked quite healthy, and they were healthy indeed. Detailed clinical examination would have revealed no deviations

from the norm whatsoever. So Smyth took a syringe and confidently injected the studied serum into one of the animals. In no longer than a minute the guinea pig became terribly restless, started to run round the cage, breathe feverishly, sit on its hind paws, and rub its nose with the front ones, as if trying to get rid of something hindering its breathing. It was obviously suffocating. Half a minute later sneezing and sharp bark-like coughing began. Death came in 2-3 minutes.

What was the matter? Perhaps an air bubble got into the vein upon the injection, and clogged some important brain vessel?

The experimenter took several more animals. The effect was the same, the shock came invariably. When however, he took a fresh guinea pig, previously untreated with horse serum, the injection caused no adverse effects. So, the previous injection made the animals supersensitive to subsequent treatment with the same serum. The same, mind you! This phenomenon, just like the generation of antibodies, is characterized by utmost specificity.

Immunization upon primary injection of an alien serum is of quite a special type. Instead of developing a stability to repeated administration, as is the case with antimicrobial response, the body becomes oversensitive. The state of oversensitivity received the name of anaphylaxis (from the words "ana"—against, "phylaxis"—to protect), and death under these conditions was called the anaphylactic shock.

Mind you: no microorganisms, no poisons, nothing harmful. Just a repeated administration of

the alien serum—and death. Only the serum must be the same. If the first time horse serum was administered, the second time it should also be horse serum. Rabbit serum will cause no anaphylactic shock. A second administration of rabbit serum will cause shock only provided the primary injection was of rabbit serum. It later appeared that anaphylactic shock can be reproduced not only in guinea pigs, but also in other animals.

It also turned out that anaphylaxis is not just an interesting consequence of a special experiment. This is a frequent and very troublesome complication in clinics. Repeated administration of an alien serum to man can also cause anaphylactic shock with a lethal outcome. But serum administration is an important medical procedure. For instance, antitetanus serum is always injected into wounded patients; sometimes antigangrenous serum is also used. Antidiphtheritic serum is given to those suffering from diphtheria. And in almost all cases, these sera are prepared by immunizing horses with the appropriate toxins.

If shock does not come (and it can easily be avoided by administering a fractional preparation in small doses), then, in some cases, a lingering complication develops, the so-called serum disease with fever, vascular disorders, and pruritic skin rashes.

I have told you about the experiments by Theobald Smyth which were published in 1904. A year later the journal *Russian Doctor* published the observations of Sakharov, who also described serum anaphylaxis in guinea pigs. He performed

his experiments without knowing about Richet's anemones or the "recycled guinea pigs" of Theobald Smyth. A year passed, and Otto, a student of Paul Ehrlich, studied this phenomenon in great detail: he was already aware of the observations made earlier by his colleagues.

The interest in anaphylaxis grew. It became clear that it was an immunological reaction, one of the aspects of the second face of immunity: that immunity can be not only a friend, but sometimes an enemy. A series of papers on the dangers of the repeated administration of non-microbial proteins appeared.

Class E Antibodies

Every spring for ten years Sergey Vasileiskii left Moscow at the time when poplar was "blooming". He went to the south where poplar blooming had already finished, or to the north, where it was not yet started. This year he stayed in Moscow, and nothing happened. Not only did he escape hospital, where he had spent some time previously during this blooming time; he was not even sneezing.

The trouble is that Vasileiskii suffers from an allergy. Starting from the age of 16, the beginning of summer for him was accompanied by fits of suffocation, bad cough, and heart disorders—every year. All this came as a bolt from the blue, sometimes in the middle of June, sometimes in the beginning of July. His condition gets worse in several days. He is taken to a hospital. He is "pumped full" of calcium chloride, Dimedrol and other anti-allergy drugs, and in two weeks or so

the affliction passed to come back at the same time the next year.

He was afraid of the arrival of summer, thinking that his ailment came along with hot weather, until one day he noticed that it always started exactly on the day when poplar fuzz appeared in the air, and passed when the streets were cleaned from white fluff.

We are all annoyed by poplar fuzz. It litters our rooms, thrusts into our eyes, hinders breathing. In dry years it often causes fires. Some people grumble: "Why on Earth do they keep these trees in the cities?" Vasileiskii would have best of all liked to hew them all out, but it was not possible, and so he chose to leave Moscow for these periods instead. This was the only way to escape the disease.

June comes, and Sergei is walking around examining poplar aments. His friends meet him and ask: "Why are you still in Moscow?" "The summer is cool this year," he answers, "poplar will bloom later than usual."

Once, five years ago, Sergei came to me and said: "Believe it or not, but it has nothing to do with poplar!"

"What do you mean?"

"My allergy."

"And what does it have to do with?"

"It's a grass called timothy. It always blooms together with poplar, and its pollen lingers at the same time as poplar fuzz."

And Vasileiskii told me that he had applied to one of the allergological consultation laboratories set up by Academician Andrey Ado. These laboratories examine and treat people suffering

from allergies, an increased sensitivity to various agents.

There are the most unexpected forms of allergies one can imagine. Some people cannot eat eggs, or strawberries, or crabs. Should this "forbidden fruit" get into food, the man would hardly manage to leave the table before he feels an itching rash on his skin and heart weakness. Cases when an ambulance is needed are not uncommon.

One is lucky if he knows what causes his rash or suffocation. But more often than not the enemy is unknown. The patient suffers for years but can do nothing about it.

There are allergies to perfumes, cosmetic creams, milk, house dust, sheep wool, the pollen of camomile, timothy or other grasses, to streptocid, penicillin and other medicines, to dyes, to some kinds of soap and thousand of other agents. Nobody knows why it hits some people and spares others, but its mechanism is the same as that of anaphylaxis. Antibodies against a certain allergen appear in the body (allergen is an agent causing allergies). Once it gets onto the skin, into food, or inhaled with air, the allergen causes an attack.

In the allergological laboratory, Sergey Vasileiskii was examined for his sensitivity to a score of allergens, in spite of the fact that he was convinced of the "guilt" of the poplar. During the first examination the solutions of ten different allergens were injected into ten points of his forearm by means of a very thin syringe needle. These solutions included the extract of poplar fuzz. None of the points reddened. The

first ten solutions did not contain his allergen. Then ten extracts from the pollens of various grasses were introduced. One point reddened and swelled, and an itching blister appeared. The allergen for this point was prepared from timothy pollen.

This is how the poplar's good name was re-established. Still, Sergei was to leave Moscow for the north or south, as before. As before, he watched the poplar, no longer as an enemy, but rather as a friend warning of the time when timothy was starting to bloom.

In June-July this year Vasileiskii did not leave Moscow at all. His allergy was done away with. He underwent a course of special immunotherapy cancelling the increased sensitivity to the guilty allergen, in his case the pollen of timothy.

Treatment of allergies became possible when in 1970 class E immunoglobulins were discovered. Remember, all antibodies belong to the group of proteins called immunoglobulins. There are three main classes of immunoglobulins: M, G, and A.

These are all "loyal" and "respectable" antibodies. There are plenty of them in blood. They protect us against microorganisms and viruses. Once a microorganism or strange protein penetrates the body, the antibodies of these three classes appear and bind the invador, block it and prevent it from entering any inner tissues. The antibodies of class G are most abundant, and they do most of the blocking.

If, in response to some strange agent, class E antibodies (reagins) are produced—then it's too bad. These antibodies virtually do not circulate

in blood. They withdraw to the tissues and attach to the cells. Allergen penetrates blood, and there is no one to block it. It goes further, to the tissues. Here, at the territory of the cells, the allergen joins antibodies. The resulting complex is far from being harmless for the cells and tissues, and this, of course, leads to an illness.

The essence of the treatment is that a vaccine is prepared from the guilty allergen, and the patient is immunized until the "respectable" blocking antibodies of G class appear in his blood. The allergy is gone. These antibodies prevent the allergen from penetrating the tissues, blocking it straight in the blood. Since no allergen reaches the tissues, no complexes with reagins are formed. One no longer has to flee either north or south.

This is the case history of Sergey Vasileiskii and thousands of other allergy patients. Many of them were suffering for years. They would have been suffering for the rest of their lives if it were not for the immunologists who discovered the secret of reagins, a special, previously unknown class of E immunoglobulins.

It is often asked: what did the Nature create this class of antibodies for if their only function is to cause trouble? Nobody knows for sure. But since all animals and humans contain this class of proteins, though in very small quantities, it must be needed for some reason or other. It is suggested that these antibodies provide protection against certain parasites, such as intestinal worms, tapeworms, and others. Usual antibodies can cause them no harm, while those attached

to the cells can, if not destroy it, at least arrange a cell wall around the parasite, to fence it off from vital tissues and organs.

Crazy Immunity

Mice, not patients, were brought to the Institute of Rheumatism of the USSR Academy of Medical Sciences. There were no such mice in the Soviet Union. This strain of mice had been bred by Australian experimenters, and the foremother of the strain was a black mouse caught in New Zealand. This is why this mouse strain is called New Zealand Black.

The Director of the Institute of Rheumatism, Valentina Nasonova, got these mice from the USA, from her foreign colleagues, in accordance with a Soviet-American joint programme on rheumatism and so-called collagenoses (the diseases of the connective tissue), including especially systemic red lupus.

Why did they need mice in a clinical institute engaged in medical treatment? Why mice, and all the more, why those of some special breed?

A corresponding member of the USSR Academy of Sciences, Nasonova is one of the leading experts in diagnostics and treatment of rheumathoid inflammation of joints and systemic red lupus. She knows better than anyone else that these illnesses fall into the category of the so-called autoimmune diseases.

"Autoimmune aggression" in simple words means something like "the immune system attacks its own host body" In other words, autoimmune diseases appear when the immune

system "goes crazy" and starts destroying normal body cells.

This is not just a failure in the work of the immune system, not a weakening of its protective functions, but a distortion, a betrayal. Instead of police guarding loyal citizens, there is the "fifth column" which destroys these citizens. And if before it was thought that it is the joint that is guilty in rheumatoid disease of the joint, now it is known: it is the immune system that is affected. If before red lupus was considered to be an illness of the blood and skin, now it is known that this is not so. It is the immune system, not a joint or skin, that needs to be treated.

These diseases are caused by the antibodies that destroy blood cells, the cells covering the surface of vessels and joints, and the like. These antibodies are so aggressive that not a single cell can resist them; the reason is that they are directed against the main nuclear substrate of all the cells, against DNA. To remind you, DNA is the material composing the cells' hereditary apparatus, their genes. So, the sick immune system produces antibodies against the most essential part of the cells, their genetic apparatus.

Another kind of autoantibodies present in rheumatoid diseases are the antibodies against antibodies. A certain part of the immune system indeed "goes crazy". Normal lymphocytes produce normal antibodies against microorganisms, viruses, and other enemies. At the same time, crazy lymphocytes produce antibodies against these normal antibodies: antiantibodies. Antigen-antibody complexes are deposited in joints, kid-

neys, and other organs and tissues, hindering their normal work.

The Institute of Rheumatism works on improving the methods for treating autoimmune diseases. An understanding of their real nature yielded new methods based on suppressing the immunity. The suppression of immunity during kidney transplantation prevents the immune system from ruining the grafted organ, and, in a similar way, suppression of immunity in autoimmune diseases cancels the aggression of crazy lymphocytes.

To be sure, the suppression of immunity with the help of chemical agents is far from a purposeful treatment of illness of the immune system. It is not the entire system that went crazy! Some part of it, perhaps quite a big one, works all right, faithfully fulfilling its police function. Only one group, one "platoon" or "company", has turned traitors. But we suppress the entire army. Unfortunately, there is no better method yet. We are not yet able to detect and neutralize the traitors inside the police system. This is why there were mice brought to the clinical institute instead of patients.

When New-Zealand Blacks reach six-months old, which corresponds to about 25 years of a human life, they inevitably develop the diseases similar to rheumatoid ailments in humans. There are the same antibodies against DNA, the same tissue affections, the same "craziness" of some detachments of the immunological army. These mice are indispensable experimental models for studying autoimmune diseases and finding new methods for their treatment.

Using the New Zealand Blacks it was shown

that the core of autoimmune diseases lies not in changing the proteins of affected tissues (joints or skin), but in changing lymphocytes, who become aggressive against normal tissues. It was with these mice that it was first shown that the reason for the illness lies in the immune system, not in the organ that aches.

The proofs were self-evident. The spleen and lymph nodes were taken out of the aging mice with autoimmune disorders. Lymphocytes were isolated from these tissues and administered to young two-month-old mice. At this age the mice are quite healthy. They do not have any autoantibodies. There are no traitors in their immunological army.

Lymphocytes extracted from the aging mice and administered into blood of young animals have traitors among them. And very soon all the signs of the disease appear. Young mice have a detachment, or, as they say in immunology, a clone of "crazy" lymphocytes in their blood.

Frank Burnet suggested we call these lymphocytes "forbidden clones". Whenever they appear, they cause autoimmune disease. These can be either the ones mentioned above, or autoimmune affections of the thyroid gland, kidneys, and other organs. Nobody knows why these forbidden clones appear. It is very difficult to remove this very clone without disturbing all other, normal and "sane" lymphocytes. A challenge for immunology is to perfect ways for removing these clones. This will give the doctors a reliable means for treating autoimmune diseases.

Immune Biotechnology

A Recognition Machine

The word "industry" brings to mind pictures of factories, mines, and big machines. The word "technology" reminds us of apparatus, precision equipment, physical and chemical processes. Technology is a method of production on an industrial scale.

The word "biology" generates images of animals, plants, all kinds of infusoria, microorganisms and cells... But in our days, new and unexpected word combinations appeared and attracted the attention of theoretical and practical scientists. These are "biological industry" and "biological technology". What do they mean?

To begin with, it is not something entirely new for humankind. Wine-making is a biological industry, since wine results from the fermentation of grape juice with wine yeast. The technology of wine production is biotechnology. The production of bread is also biotechnology based on flour and baking yeast. Fodder ensilage, biological purification of sewage, the production of cheese, yoghurt and clabber—all of these are biotechnological processes making use of microorganisms in industry. What, then, are the new aspects?

A new, and a very important feature is that people learned to use not only yeast and bacteria common in nature, but also artificial microbial and non-microbial cells. Think about this for a minute. It means an artificial live cell doing

something that people need, and, what is even more striking, on an industrial scale.

Of course, an artificial microorganism is not the Homunculus of Doctor Faustus created in a test-tube from inorganic matter with the assistance of some mysterious forces. According to modern science, it is some previously known microorganism, say, *Escherichia coli,* with some strange genes artificially introduced into its genetic apparatus. Because of these genes, *Escherichia coli* produces some new substance needed by people, for instance, insulin, a hormone necessary for treating diabetes. A human gene is introduced into *Escherichia coli,* so it produces human insulin. Before there was no way to be treated with human insulin. There was nowhere to get it from because it was produced by the pancreas in humans. Now the production of this biotechnological insulin is under way. Artificial *Escherichia coli* of a required type is cultured in a nutrient medium and generates the required product.

Gene engineering received its "citizenship rights" in microbiological industry for producing a number of proteins and enzymes, superfast-working yeast, antibiotic-generating microorganisms, and others.

An equally exciting method was developed by immunology: the method of cell engineering, the method for creating artificial cells of animal origin, or, more exactly, artificial immunocompetent cells working outside an animal's or mans' body. These artificial cells were called hybridomas. Hybridoma technology is now in the forefront of immune biotechnology, that is the technology of

producing diagnostical and medicinal immune sera, as well as protective substances, including the mediators generated by the cells of the immune system. What kind of production by immunocompetent cells is of greatest interest for medicine, microbiology, virology, agriculture, and even chemistry? Of course, antibodies.

Remember that the main principle underlying the work of the immune system is that, in response to the alien substances entering the body, be it a microorganism, alien protein, polysaccharide or anything else, it produces the antisubstances of high specificity. These antisubstances are the proteins of a certain type (immunoglobulins) called antibodies. Each antibody molecule has recognition centres that discern and attach themselves to the antigen, the substance that had stimulated the formation of this antibody. This antigen is recognized with unprecedented precision. The antibodies against X antigen recognize only X, and those against Y recognize only Y, even if X and Y differ, say, in only one amino acid, in a surface chemical group, or even in this group's configuration.

In fact, the immune system is a machine, unique in its universality, that recognizes bioorganic substances and generates agents of absolute specificity, antibodies, against them.

A Technological Chain

The technological chain in immune biotechnology is composed of the following three links. The input is the substance against which the antibodies are produced (these antibodies are further

used as specific reagents or as medicines).

For decades living beings, be it rabbits, horses or healthy humans (donors) have been acting as a producing link. Animals or humans were immunized, their blood was taken, the serum was isolated from this blood, and antibodies were extracted from this serum. The main disadvantage of this technique is not only that big farms of animals and big groups of donors are needed; it is also that antibodies produced by a whole body are not totally identical to each other, or, as immunologists would say, are not monoclonal.

This technological link has recently been dramatically improved due to the hybridomas that I have already mentioned: these are cell lines producing antibodies and important mediators of immunity outside the body, in test-tubes. flasks, and cell reactors. Biological products thus produced are monoclonal, which means that they are standard and reproducible.

The output of the biotechnological chain is characterized by strikingly sensitive immunochemical methods for detecting bio-organic compounds with the help of antibodies, as well as the use of antibodies for purifying the substance by means of immune sorption, when the needed substance is trapped by antibodies. In the chapter "Molecular Messengers of Immunity" I mentioned Interferon, an antiviral agent produced in the body and in cell culture. So, a single passage of the column with antiinterferon antibodies through a culture liquid containing interferon gives an agent five thousand times more purified, removing all the admixtures of cellular and cultured origin.

Highest Precision Reagents

To distinguish between swine and bovine insulin by chemical methods, one needs to have both preparations purified and in sufficient quantities, in order to analyse the amino acid sequence of the polypeptide chain and to find out that the 8th threonine residue in bovine insulin is substituted by alanine. You can easily imagine the problems inherent in such an analysis, the time it will take and the skill of operators capable of performing it. With the help of antibodies, the identification and quantitative analysis of these substances are made with the highest sensitivity by a lab assistant within several minutes. For this the analysed substances need not be purified; they can be mixed or be a part of complex multicomponent systems, such as blood serum, culture liquid used for growing microorganisms, or a mixture at the output of complex biochemical reactions.

For instance, immunoelectrophoresis of human blood proteins allows the instant qualitative and quantitative analysis of up to 30 proteins: albumin, glycoprotein, lipoproteins, transferrine, and others. The identification of all these individual proteins and their combinations is impossible without immunological methods, just as without antibodies it is impossible to determine blood groups in man, select a donor for an organ graft, determine the quantity of this or that hormone in blood, detect a single needed cell, and the like.

Immune biotechnology can provide for the production of reagents needed not only for medicine and immunology itself, but also for all scien-

tific and applied fields dealing with the indication of *any* biological substances, viruses, bacteria, and cells. The accuracy and sensitivity of immunological methods are beyond any comparison.

That is why immune biotechnology is indispensable not only for medicine, but also for microbiology, virology, molecular biology, and bio-organic chemistry; it is necessary for the production of hormones, proteins, enzymes, toxins, vaccines, for developing indicator methods for detecting single microorganisms, cells or separate microorganism and cell clones, which is a vital need in gene engineering and in various branches of microbiological, food, and drug industries.

Owing to the achievements of immune biotechnology, antibody reagents became very simple and convenient to use. There are special gel cassettes produced on a commercial scale, where gels contain certain antibodies. A drop of a studied liquid applied onto the gel is enough to induce the formation of the so-called precipitation rings (precipitation of antigen-antibody complexes), if the liquid contains the analysed antigen. Judging by the diameter of the ring, the antigen concentration can be determined. There are special devices that automatically record antigen-antibody precipitation in the flow of the liquid passing through the capillary tubes. The device detects milligram quantities of antigens. Most sensitive and subtle methods make use of labelled antibodies and antigens.

In 1955 an American immunologist, Albert Coons, managed to attach fluorescent dye to antibodies. These fluorescent antibodies made visi-

ble the analysed substances in the cells. This is how immunoglobulin synthesizing cells and cellular structures of immunoglobulin origin were discovered.

Using the method of fluorescent antibodies, a single microorganism can be found and identified among thousands of other bacteria, directly in smears, without preliminary seeding into a nutrient medium. This method does not need antibodies labelled against all analysed bacteria. What is needed is just a panel of common rabbit antibodies against our microorganism and one fluorescein-labelled antiserum against rabbit immunoglobulins of IgG class. It will dye only the bacteria attached to specific rabbit antibodies.

Industrial immunobiotechnological laboratories produce special kits with all reagents needed for the rapid examination of any mixture and substrate, in order to reveal a desired antigen, for instance, an agent of sausage poisoning, botulinic toxin (BT).

Anti-BT-antibodies are fixed in the wells of plastic panels available in these kits. The liquids analysed for botulin are placed into these wells for several minutes. If poison gets into any of the wells, it will attach to the antibodies. After that, enzyme-labelled anti-BT-antiserum is added to the wells (most often this enzyme is peroxidase). In the wells where botulin has attached, peroxidase-labelled antibodies bind to the previously formed complex. If hydrogen peroxide and chromogen (dying substance) are added to this complex, the hydrogen peroxide is decomposed by peroxidase and the chromogen will change its colour.

This immunoenzyme method can also be used for analysing the substances at a concentration as low as one to ten billion grams per litre.

Another widely used test is that for allergy diagnostics. Its essence is that the allergen is identified with the help of iodine isotope-labelled antibodies. Remember that an allergy is caused by the excess of IgE immunoglobulins against allergens which have penetrated the body, such as pollen, home dust, food allergens, and others.

Antibodies are attached to the particles on which the allergen is sitting, and then are precipitated with labelled anti-IgE-immunoglobulins. If the precipitate is radioactive, it means that it contains allergic antibodies (immunoglobulins) against our allergen, so an allergy really does exist. If not, there is no allergy. To find the allergen means to find the key for treating the disease. Like the immunoenzyme method, the radioallergosorbent test is highly sensitive and does not require sample intracutaneous allergen injections, as is routine in allergy diagnostics.

The competition method between the analysed and radioactive antigens is widespread in various fields of science. It is used for the indication and quantitative assessment of bio-organic compounds with a sensitivity of up to 10^{-12} gram per litre.

The Cancer Cell for Peaceful Purposes

For the methods of immune biotechnology mentioned above animals are required, to extract certain proteins, immunoglobulins, and antibodies from their blood. There are special farms for breeding small (rabbits, mice, rats, guinea pigs)

and big (goats, donkeys, horses) animals. It should be borne in mind, however, that the antibodies against the same substance produced by different animals of the same breed, and sometimes even by one and the same individual, are far from being totally identical.

This is not only due to the individual characteristics of animals and varying features of the immunizing material, but also to the ability of immune system cells to produce a number of different clones (a clone is the progeny of one cell; the number of clones is equal to the number of proliferating cells). Each lymph clone synthesizes its own version of a specific antibody. The serum of immunized animals always contains the product of work by many clones, where the antibodies make up a "family" of very similar, but not identical antibodies.

Immune reagents obtained in different laboratories, or in the same laboratory but at different times, are not quite identical. Therefore, notwithstanding their high degree of specificity, they are not perfect reagents. To attain their absolute specificity, one needs to resort to complex technological operations on antibody extraction from the sera. Recently, immunology has solved this problem, which, at the same time, reduced its demand in animals.

In 1975 British scientists George Köler and Caesar Mielstain worked out a method for obtaining cell hybrids, or hybridomas. These hybridomas are formed by fusing lymphocytes taken from immunized animals, with myeloma cells extracted from the bone marrow and cultured in a nutrient medium.

Myeloma is a form of blood cancer. Like other malignant cancer cells, myeloma cells are capable of unabated proliferation. For reasons yet unknown, they emerge in the bone marrow, multiply faster than any normal cells, flood the body, and destroy it. Extracted from the body and placed into the nutrient medium, they do not lose this malicious trait to multiply endlessly and unrestrainedly. A culture of these cells is "immortal", it can be grown by tons on end. But what for?

In contrast to it, lymphocytes, like other "noble" cells, multiply exactly to the extent required by the body. Placed into the most perfect nutrient medium, they die very quickly. So here we face a biotechnological paradox. The cells unable of producing the required antibodies in a culture are "immortal", while those capable of it fail to live in a nutrient medium.

Hybridoma is the utilization of the cancer cell for industrial purposes, not as a killer, but as a peaceful partner. Lymphocyte endows hybridoma with the ability to synthesize needed antibodies, while myeloma renders it capable of surviving and endlessly proliferating in an artificial medium. Therefore the hybridoma-synthesized antibodies can be produced in unlimited quantities. These antibodies are identical to each other in all parameters and interact with only one antigen.

Thus, the test-tube-obtained preparation can serve as an ideal reagent to various organic substances, ideal diagnostical or curative agents. The range of specific reagents to be produced in this way is virtually unlimited. It can include antibodies against blood and tissue proteins, antigens

of various organs, cancer and normal cells, viruses, bacteria, parasites, certain chemicals, and many other agents.

In recent years the interest in hybridoma technology has been growing more like an explosion, both in its theoretical and practical aspects. Hundreds of researchers from different countries joined in the work. The nearest future will obviously see companies and factories producing monoclonal antibodies as unique reagents, diagnostical and medicinal preparations.

Of course, it is not so simple to obtain lymphocytal hybrids. It is done in several steps. A hybrid clone is accumulated whether in a test-tube or in an animal body. At all the stages of this accumulation, cell samples are to be kept in liquid nitrogen so that at any moment a researcher can come back to any stage and to preserve needed clones for the future.

Monoclonal antibodies have already contributed greatly to science. With their help the structure and genetics of immunoglobulins were analysed, the lymphocyte receptors, with which they recognize "their only" antigen, were discovered and studied, the reagents of the cancer centre were obtained, experimental treatment of blood cancer was performed in animals, monospecific antibodies against certain microorganisms were prepared, and many other vital problems were solved.

Fruitful cooperation between gene engineering and immune biotechnology is graphically illustrated by a well-known anti-viral and anti-tumour preparation called Interferon. Its industrial production became possible with the help of mi-

croorganisms into whose hereditary apparatus the genes coding for Interferon synthesis were inserted. However, it was still quite difficult to isolate the preparation from the culture medium and purify it. This challenge was met by hybridoma synthesizing antibodies against Interferon; recently, with the help of monoclonal antibodies, Interferon free from any admixtures was obtained in experimental quantities.

Vaccines of the Future

Principles Underlying Artificial Vaccines

For several years the newspaper "Trud" ran a column entitled "Medicine and Life". This column contained popular science articles on the achievements of medicine and medical biology. In August 1978 the editor of the science section asked me to speak about the most interesting results obtained in our scientific department. This was the time when Rakhim Khaitov and myself obtained the first but doubtless results that confirmed the previously formulated principle underlying vaccine preparations of an entirely new type. Since Rakhim was not just a common worker of the department, but also my "comrade-in-arms" in experimental verification of this principle, I wanted the story about our joint results to be published and signed by both of us.

We discussed the size of the article, basic reasoning, logics of narration, main sections and conclusions. After that each of us separately took up the pen and wrote a complete text of the ar-

ticle. Several days later both texts were compared and combined. The integrated text included the best pieces, best logical god-sends, most convincing reasoning and most clear-cut conclusions.

So we got an article of the required size, but optimized in its contents.

It is impossible to work like this with an outsider, but it is the best way of doing things with one's student and friend. This is how Rakhim Khaitov and myself wrote the first popular article on the vaccines of the future, followed by two other ones for "Science and Life" magazine and the "Future of Science" collection.

Now these three articles are in front of me on the table, and, preserving their average size, I'll try to optimize the text once again, for you, dear readers.

A hundred years ago, in 1881, the great Pasteur substantiated the basic principle of vaccines, protective inoculations providing for non-susceptibility to the pathogens of infectious diseases. A human or an animal is given attenuated or killed microorganisms. The body copes with them quite easily; the immune proteins, the antibodies capable of destroying not only attenuated, but even full-fledged pathogens, and capable of neutralizing their toxins, appear in the blood. All the substances contained in the microbial cell, including the toxic ones, against which antibodies are produced, were called antigens.

This is how it was done in immunology ever since: to create non-susceptibility, the most important antigens are to be isolated from a microorganism and administered into the body to immunize it. The best possible vaccine would con-

tain a mixture of the most crucial microbial antigens. But the antigens isolated from a microorganism and duly purified for some reason or other were not as "useful" as live attenuated microorganisms. But this was not all. There were certain infections against which it was impossible to obtain vaccines even from live attenuated microorganisms.

It looks like the immune system is kind of incapable of developing the resistance against a number of antigens and microorganisms. There are still no good vaccines against influenza, dysentery, malaria, VD (gonorrhea, syphilis), and many others. A vaccine against cancer seems to be a very distant dream.

New approaches and principles were needed. Science is always searching for more effective and promising ways. But are there any prospects today for creating and developing vaccines of a new type?

Large teams of researchers are now deeply engaged in developing new and effective vaccines. I'll tell you about two trends of research which are based on absolutely unique and non-traditional approaches to developing vaccines of a fundamentally new type.

One such trend is headed by Michael Sela. It consists in synthesizing polyamino acid structures simulating natural antigens. I'll speak on these interesting studies in more detail somewhat later.

The other trend lies in developing fully artificial antigens that have no analogue in nature. The principle underlying these antigens has been elaborated in our department. It consists in build-

ing complex "non-natural" macromolecules based on synthetic polymers that trigger a strong immune response to the antigens attached to these polymers.

The gist of the problem is to obtain artificial vaccines that would consist not only of crucial antigens and their fragments. Macromolecules of artificial vaccines should also contain a structure providing for the intense production of antibodies against various antigens, irrespective of their nature, and, what is worth special emphasis, irrespective of specific genetic features of the immunized body.

Genotype, or the body's hereditary structure, takes an important part in forming antibodies. The same body can be "strong" (high-responder) to one antigen and weak (low-responder) to others. The production of antibodies (immunogenesis) is controlled by the genes of immune response (IR-genes). And if a gene providing for the ability to generate immune response to a given antigen is lacking in an individual, no vaccination would lead to antigen production.

This poses another most complex task: artificial vaccines should stimulate the formation of antibodies to various antigens in any organism, even in a genetically low-responder or non-responder one.

The principle we have arrived at makes use of complex macromolecules that consist of a required antigen determinant (a most crucial antigen) and certain desired artificial part that makes the entire macromolecule independent of whether the genetic control of immunogenesis and other im-

munobiological specifics of the body is weak or strong.

The immunity-stimulating substances have been desperately sought for and thoroughly studied for a number of years. In previous years, the effect of immunity stimulants was assessed only based on the quantity of antibodies produced, that is, judging by the final stage of immunogenesis. Now the studies have reached an entirely new stage. We are looking for the immunostimulating substances by examining the mechanism of their action at the level of individual cell events, and the separate immunogenesis stages that add up to form the immune response as a whole.

Synthetic Polyelectrolytes Perform the Functions of T-Helpers

One of the most intriguing facts was obtained from the experiments on the so-called T-deficient mice, the mice whose thymus had been removed, the organ responsible for producing T-lymphocytes. These animals serve as models for the underdevelopment or complete absence of the thymus in humans. Children born without the thymus very soon die of infections and tumours, since they do not have T-cells and show no immunity even when immunized with strongest vaccines. It appeared that synthetic polyelectrolytes administered to T-deficient animals increased their ability to produce antibodies 20-50-fold. In other words, polyelectrolytes can perform the function of T-helpers!

Old age is always accompanied by quite a sub-

stantial T-deficiency due to age atrophy of the thymus. It is the lack of T-lymphocytes that accounts for a lot of diseases in old age. As soon as polyelectrolytes were shown to replace T-lymphocytes, it would have been quite reasonable to try and use them for correcting age-related immunity deficiency as well.

A single injection of poly-4-vinylpyridine administered to old animals whose ability to produce antibodies is reduced to one tenth of their initial level, turned out to restore the immune response completely. It was something like the rejuvenation of an old immune system. These are still only experiments, but they promise to provide valuable results for clinical immunology.

These data are of great biological meaning. I have already spoken about the genetic control of immune response. The action of immune response genes was found to be mediated mainly through T-lymphocytes. In other words, the products of IR-genes are "expressed" on T-cells to a much greater extent than on other cells taking part in immunogenesis. It was illustrated by the experiments in which the individuals incapable of immune response, for instance, to antigen A, for genetic reasons, respond to this antigen after T-cells from an individual responder to this antigen A are transplanted to them. A non-responder individual turns into a responder one. This phenomenon was called phenotypic correction.

So, an immunizing effect in low-responder organisms (turning low-antigen substances into strong antigens) can only be achieved by means of phenotypic correction, and this is the way to "evade" genetic control. The genes of immune

response, or IR-genes, act through the system of T-lymphocytes that either trigger B-lymphocytes or inhibit their performance in antibody genesis. Hence, one of the ways to evade genetic control lies in promoting T-independent immune response to our antigen.

Apart from IR-gene-related immunological "non-responsiveness", there are a number of inborn and acquired immunodeficiencies when the defects in the lymph system's development are localized at the level of T-cells. So, the use of polycations and polyanions as agents providing for T-independent immune response opens new possibilities for both the stimulation of T-deficient immune response in case of immunodeficiencies, and for artificial correction of genetic failures in antibody production (phenotypic correction).

Nude mice born without the thymus serve as an experimental model of congenital T-deficiency. Their genes controlling the thymus's development are linked with the genes providing for the hair growth. So the absence of hair is a sign of inborn underdevelopment or the complete absence of the thymus. "Treatment" of nude mice with polyelectrolytes restores their immune response ability to a very considerable extent.

To achieve a phenotypic correction of immune response, the mice of various pure strains were used. All the mice of one certain strain are complete genetic copies of one another. The genetic system containing the genes of immune response is localized in the 17th chromosome of these mice. The genetic map of this chromosome section is amply studied, so the strains of mice can be selected in such a way that, for instance,

all the individuals from one strain will readily respond to antigen A (high responsiveness), but will develop no immune response to antigen B (low responsiveness), and vice versa.

Poly-4-vinylpyridine administered to low-responder mice appeared to turn them into high-responder ones. In other words, these mice acquired phenotypic (external, not-genotype-controlled) ability to respond to immunization with the same intensity as high responder individuals.

As was already mentioned, immune response independent of the thymus means that we have overcome genetically predetermined or acquired immunological "non-responsiveness" localized at the level of T-cells. The same is true of synthetic thymus-independent antigens.

All these studies lead to a formulation of the principle underlying the new type of immunizing preparations. These preparations consist of macromolecules composed of a required antigen determinant and artificial polyelectrolyte providing for the stimulation of immunogenesis, and, consequently, for T- and IR-independence of the entire molecule.

What is so special about these substances, poly-4-vinylpyridine and polyacrylic acid? The prefix "poly" means that these polymers are chemicals of high molecular weight. Their molecules consist of many identical groupings, monomeric links. The molecular weight of these chains can reach 10 thousand, 100 thousand or 1 million, which means that they are 10 thousand to 1 million times heavier than a hydrogen atom. Let me remind you that big proteins have molecular weights from 100 to 900 thousand. Giant polymer mole-

cules are obtained by the successive binding of low molecular compounds (monomers) to the active centre at the end of the growing chain.

Polymerization was discovered back in the middle of the last century. It was then that the first polymerizable monomers, such as styrene, acrylic acid, and others, were isolated. Poly-4-vinylpyridine and polyacrylic acid are polyelectrolytes, i.e. the polymers that become multiply charged in solutions. A dissolved macromolecule acquires a lot of periodically repeated charges, corresponding to the links which make up the giant molecule. Depending on what charges (anions or cations) appear on the polymer chain, all polyelectrolytes are divided into polyanions, polycations and polyampholytes. The latter are characterized by the presence of both groups in one molecule.

Polyelectrolytes include most important natural biopolymers, proteins and nucleic acids. In protein solutions, carboxy groups become anions (—COOH), and aminogroups turn into cations ($—NH_2$). Some amino acids, such as lysine, belong to cations, and others, such as aspartic and glutamic acids, to anions. Protein molecules are polyampholytes. Blood plasma is a complex solution of electrolytes that are common in nature. Synthetic polyelectrolytes used in our studies, polyacrylic acid and poly-4-vinylpyridine, are totally artificial and have no analogues in nature.

An important and interesting property of synthetic non-natural polyelectrolytes is their ability to form complexes with proteins and polysaccharides. But it is proteins and polysaccharides that form the antigens of infectious diseases. Tumour antigens are also proteins. What if we

obtain an artificial polyelectrolyte-protein complex? What biological properties will it display, taking into account a possible stimulating effect of the polyelectrolyte on immunity? Jumping ahead, I can say that the research in this direction led to the synthesis of artificial macromolecules with striking properties.

The difficulty of these studies was in that the complexes of synthetic polybases and polyacids with the proteins, which can be obtained under experimental conditions *in vitro,* turned out to be quite unstable in the face of physiological pH values and ionic strength, and, naturally, were disintegrated upon entering the body. Therefore the chemists (Kabanov and Mustafayev) set themselves a goal to synthesize the polymers which would form stable complexes with proteins, withstanding the conditions of a live body, and, at the same time, would display all the immunostimulating properties of the polyelectrolytes described above. This was done in the following way. The chains of poly-4-vinylpyridine were loaded with side radicals which provided for strong bonds with protein macromolecules. These radicals were represented by hydrocarbon groups.

An interesting regularity was observed. If the number of carbon atoms in these radicals was less than 10, they formed complexes with proteins at the expense of electrostatic bonds. These are weak bonds broken under conditions of the body. Strong enough hydrophobic (water-repellent) bonds with protein globules provide the radicals with a number of carbon atoms equal to or more than 10. Protein complexes with polyelectrolytes bearing hydrophobic radicals are fairly

stable and do not disintegrate upon entering the body. It should be noted that the more carbon atoms in the side radicals, the stronger is the interaction between the polyelectrolyte and hydrophobic sites of protein molecules.

Assembling of Antigens on Polyelectrolytes

So, "non-natural" polyelectrolytes have a strong influence on immunity. Purely synthetic poly anion or polycation agents administered to animals enhance certain stages of immunogenesis. In the final analysis, these substances intensify immune response. Naturally enough, the thought appeared that has already been mentioned above: if a weak antigen is attached to a macromolecule of a polymer stimulating antibody production and providing for thymus independence, this "assembled" molecule would be a perfect combination of both antigen specificity and stimulating properties. Who knows, maybe it would lead to "superantigens"?

There was an attempt to make such an artificial synthetic antigen based on poly-4-vinylpyridine macromolecule.

A simple chemical compound, trinitrophenyl grouping, was used as an antigen determinant. The substances of the trinitrophenyl type are called haptenes. By themselves, they cannot stimulate the immune response, i.e. antibody production, until they attach to a protein or any other natural macromolecule. We bound trinitrophenyl grouping to a "non-natural" poly-4-vinylpyridine to obtain an antigen with remarkable properties.

It was very easy to induce antibody production against a haptene attached to a very simple polymer.

Antibodies to a haptene on natural carriers are usually formed up on additional stimulation by special substances (the so-called adjuvants). When the immunization is effected with a haptene incorporated into poly-4-vinylpyridine molecule, no such stimulation is needed. And, finally, antibody production triggered by this antigen appeared to be thymus-independent, not requiring any T-cells.

T-deficient animals immunized with a synthetic antigen produce the same or even greater quantity of antibodies than healthy animals. When immunized with the same haptene injected not in poly-4-vinylpyridine, but in the form of a protein molecule, T-deficient animals produce no antibodies unless additionally stimulated with adjuvants.

In our further works we used bovine serum albumin (BSA) as a simulating antigen. BSA is a fairly weak antigen that induces immune response only after multiple immunization with additional stimulation with adjuvants. Attached to polyelectrolyte molecules, BSA appeared to induce an elevated immune response to the protein determinant of this artificial antigen. Artificial antigens were prepared as BSA-polycation complexes formed either at the expense of weak electrostatic or stronger hydrophobic bonds, or as a so-called conjugate, where BSA is covalently bound with polycation (these bonds are stronger than the former two ones).

The copolymer of 4-vinyl-N-ethylpyridine and

4-vinyl-N-cetylpyridinebromides was chosen to be the complex-forming polymer.

This was chosen because at a neutral pH it is capable of forming the complex with BSA by means of electrostatic salt bonds at the expense of both ethyl (C_2H_5) radicals and by hydrophobic interactions between cetyl ($C_{16}H_{33}$) radicals and non-polar sites of protein globules. So in this case we could expect especially strong protein-polyelectrolyte complexes that do not dissociate at physiological value of ionic strength. In the particles of the complex each polycation (a thousand links-long) binds two protein molecules in aqueous solution.

While studying the synthesis of highly immunogenic antigens, we tried to assess the effectiveness of covalent bonds. This assessment was important for both the theory of antigen action and, from a practical standpoint, for developing maximally stable compounds.

When we immunized animals with our artificial antigens, they induced immune response 50-100 times higher than that induced by BSA. This extraordinarily enhanced immunogenicity of an artificial antigen obtained by attaching protein macromolecules to a polyelectrolyte cannot be explained by the conventional immunostimulant effect. Protein and polyelectrolyte injected separately, or unstable complexes, disintegrating in the body, also enhance immune response. But this enhancement is incomparable with the immune response induced by a protein-polyelectrolyte complex where the antigen is attached at the expense of hydrophobic or covalent bonds.

So, synthesized complex macromolecules can

be considered enormously strong artificial antigens assembled from a protein and synthetic polyelectrolyte, each of them separately being either non-immunogenic (polycation) or weakly immunogenic (albumin). The necessary condition for inducing the immunostimulating effect of polyelectrolytes bound with the antigen molecule is the sufficient stability of this complex in the body.

There is every reason to suggest that these principles can be extended to viral and microbial antigens.

Loop on the Carrier

A novel interesting approach to synthetic vaccines was offered by Michael Sela. His idea is to perform an artificial synthesis of unique molecular structures composed of crucial antigens (antigen determinants), each of them typical of a certain pathogen of an infectious disease. According to Sela, several such fragments would build up a macromolecule that would serve as a synthetic analogue of a polyvalent vaccine immunizing against several diseases at once.

Is this realistic? The answer is yes. But only for the antigens whose immunizing ability is not reduced in dead vaccines. For all other antigens the problem is still unsolved.

A characteristic feature of immune response is that vaccination against various infectious pathogens induces antibodies against specific antigen fragments located on the pathogen surface. In other words, the immune system produces antibodies not against the whole virus or microor-

ganism, but against specific and unique molecular structures. This was taken into account.

Sela started his research with elegant molecular-immunological experiments. Egg white contains an antigen lysozyme, which is very convenient to work with. Its primary and spatial structures are well known. Sela isolated the so-called "loop", a fragment of lyzosyme molecule that consists of 24 amino acid residues successively attached to one another. This isolated loop attached to a synthetic macromolecular carrier and administered to a body induces the antibodies against lysozyme. Polyalanine and polylysine, the artificial analogues of a protein molecule, were used as carriers (natural proteins are the chains of various amino acids, while polyalanine and polylysine are the chains where all the links are identical, composed of the amino acid alanine or lysine, respectively).

Now the most interesting thing. Since the amino acid sequence of the loop was known, it was possible to synthesize its several parts. Immunization with these individual synthetic products attached to carrier showed what part was the most crucial one, which fragment triggers the production of antibodies identical to those produced in response to a natural product.

Sela vividly demonstrated the possibility of using artificial analogues of natural antigens to generate antibodies capable of reacting with natural antigens.

Clearly, similar results could be obtained for various proteins with known amino acid sequences. Progress in this field will depend on the achievements in studying the structure of pro-

tein membranes in various viruses and microorganisms. The first attempt to synthesize an antiviral vaccine was a success. A fragment of the protein membrane of coliphage MS-2, the virus affecting *Escherichia coli,* was synthesized. This fragment was attached to a polyamino acid carrier, polyalanine.

Rabbits immunized with the resulting antigen upon additional adjuvant stimulation formed virus-neutralizing antibodies identical to those produced in response to virus vaccination.

Vaccines Free from Ballast

An important advantage of the new principles for creating vaccines is that the resulting products contain no ballast substances contaminating absolutely all present-day vaccines. Indeed, dead microorganisms (or proteins, polysaccharides, and other compounds isolated from them) include hundreds of antigens. These vaccines usually contain less than one per cent of crucial antigens. It means that, upon vaccination, more than 99 per cent of the immune system "runs idle", producing antibodies against the ballast antigens. It is these unneeded antibodies that cause all sorts of complications (like allergies and others) with vaccinations.

Another advantage is the possibility of designing some preset, specifically needed vaccinating molecules. Say, from five to ten determinants of various diseases are attached to one synthetic macromolecule and used for immunization. All these determinants would replace modern vaccines with a very small number of properly designed

macromolecules. Thus, the methods of molecular engineering seem to bring about different kinds of polyvalent synthetic vaccines. This "seems like a dream today", said Sela. Will this dream come true?

It should be mentioned that the synthesis of individual molecular structures of crucial antigens and their assembly on one macromolecule do not give a final solution to the problem. Sela suggests that the "patterns" for synthetic vaccines should be borrowed from nature. In his talk at the III International Congress of Immunologists in Sydney in 1977, he said: "We should copy Nature not in whole, but in parts." Natural molecular structures are to be copied by the methods of molecular engineering. But whether or not this principle is suitable for preparing synthetic vaccines against all microorganisms and viruses, nobody knows. Bearing in mind that microorganism-isolated antigens (these are the antigens whose fragments Sela is going to copy) are not as effective for immunization as live attenuated microorganisms; this regularity is quite likely to apply also to the "artificial assembly" vaccines. Moreover, there are still many infectious diseases against which even vaccines of live attenuated microorganisms do not work. In the future, I think, model antigens will be replaced by real-life microbial ones.

These were the first steps towards designing synthetic antigens of a new type. If certain infectious antigens (in response to which there is a weak or no immune response), or their determinants attached to polyelectrolyte macromolecules are shown to trigger effective immune protec-

tion, our principle for constructing the vaccinating molecules would provide an approach for designing a wide variety of future synthetic vaccines. It is now difficult to say which principle for designing vaccines, "imitations of Nature", or the search for "non-natural molecules" with the desired features, would prove more effective. Only one thing is clear: these principles will be found. Then it will be possible to assemble antigen determinants of different microorganisms and viruses on the same polymer chain. This chain is to provide a strong immune response to all the antigens used. It is known that cancer antigens are too weak to cause an effective immune response against a tumour, but a combination of cancer antigens and stimulant polymer molecules may well be conceived as the basis for an anti-cancer vaccine.

The possibilities of would-be synthetic vaccines are virtually boundless. It should be borne in mind, however, that this is all now at the stage of scientific search, and the studies have not yet left the laboratories. Further serious work is needed to that the principles for developing synthetic antigens may become a reality of life.

Theory and Learning

Burnet Versus Burnet

We all seem to have gotten used to the thought that a scientist's courage is nourished by his belief in his ideas. A scientist's courage that is a

selfless work to prove his ideas, the stake he is ready to be burnt at to vindicate it. But there is also a different kind of courage: to recognize that you are wrong, that your theory is incorrect and should not be supported. This is the courage of a loser, but perhaps this is not the right word. It is the courage of unbiased objectivity in evaluating one's own ideas. The courage to say: "I was wrong."

These pages have already given some examples of courage inevitably accompanying objectivity. At the dawn of the science on immunity, when its first theories were created, in the times of great immunological discussion, the rivalling fellow-scientists disproved each other and themselves and openly recognized their mistakes. They displayed courage, they were marching ahead.

Indeed, this is the norm of behavior rather than an outstanding event in the scientific community. Not so long ago academician Yakov Zeldovich argued against his own theory of the Universe and put forward a viewpoint much differing from his previous one. Scientists have no moral right to say, like Cronin's Brodie, that they do not change their opinions since, at any given moment, they do not consider themselves to be wiser than before.

If a scientist sees that he was wrong, he says: "I was wrong," and proves this with his deeds.

The Director of the Medical Research Institute in Melbourne and PhD of London University, the author of the most popular and most plausible theory of immunity, Frank Burnet, was preparing his talk.

His theory that most convincingly explained

all previously unknown aspects of immunity, that successfully predicted a previously unknown phenomenon, the theory that had existed for about eight years, since 1949, no longer withstood the attack of new experimental data. Many facts remained unexplained, some aspects were based on the assumptions discarded by modern genetics.

Burnet, the future Nobel Prize winner, was preparing the talk disproving his own theory. The theory supported by many scientists who gave new evidences in favour of it. And now he, the creator of this theory, is going to speak against it, display its weakest spots, since who knows them better than he himself?

He recalled his first experience in immunology. He was a student of Melbourne University, and it was more than 40 years ago. Burnet became one of the greatest world immunologists, and his theory explaining immunity was one of the most widely recognized. And this theory did not satisfy him any longer.

What was missing in this theory that seemed to foresee everything? It failed to explain the main point: how does the body discern an alien intruder, how does it distinguish between his own and something foreign? It also failed to account for tolerance, to explain what happens when the body stops to recognize strange antigens. The problem of recognizing "its own" and "something foreign", the central problem of immunology, was overshadowed. None of the previous theories tried to explain how the immunological army identifies alien cells, tissues or proteins. Burnet's theory did not answer this question either.

It was a long-nursed decision to speak against

his own theory. But he did not want to sound unfounded. He had to work, to find and put forward something new and more perfect. Now it was finally possible. The hypothesis of the mechanism of distinguishing between "one's own" and "not one's own" has already been formulated, and all the rest of immunity's aspects were explained in this way even better.

In two weeks Burnet was to leave for London. A fundamentally new theory of immunity was to be submitted to the verdict of world science. The history of science would have one more example of the courage of objectivity. Burnet would not only disprove his old theory, but would also display the most vulnerable spots in his new one, and the ways for its experimental proof or refute. And even if this new theory proved to be wrong, it would stimulate scientists for new research. The important thing was to make investigators stage the experiments that would refute it if it were wrong.

What breakthroughs in biology made the previous theory assailable? What should not be dismissed when the new one is designed? The first thing is that the information flow in any cell goes from gene to protein. In other words, the genes in a cell nucleus serve as a material substrate bearing the information, or the "plans" according to which the cell lives and builds its proteins. The chemical structure of a gene is desoxyribonucleic acid (DNA). It acts as a matrix for the ribonucleic acid (RNA) specific for this gene and is built with great precision. Specific proteins are built based on ribonucleic matrices. This is the scheme: DNA-RNA-protein.

Modern genetics and biochemistry have proved that the protein structure is determined by the structure of RNA, which in turn depends on the specific structure of the corresponding DNA section. There is only one way to make a cell synthesize a new protein, and that is to change the DNA structure. And this is what in fact happens. The changes in DNA are incidental and usually do not correspond to the impacts of the current environment.

This does not mean that the alterations in DNA cannot be caused by external impacts. They can, but DNA changes are not equal to these changes. One and the same impact can bring about various DNA changes (mutations), and the opposite is true: different impacts can cause the same mutations.

But the bad thing is that the alien antigen forces cells to produce antibody proteins at its own discretion. An antibody is a molecule of a specialized immunoglobulin protein matching the antigen. It has been previously considered that, upon entering the cell, the antigen itself becomes a matrix for immunoglobulin synthesis. Genetics and biochemistry proved this impossible. Protein obeys only one matrix, and that is its own RNA. Perhaps antigen alters RNA? Impossible again, since RNA obeys only one matrix, its own DNA. But the alien antigen protein cannot purposefully affect DNA: this is a law.

A theory should not contradict the fundamentals of modern genetics. The new theory by Burnet borrows the main idea from the teaching on evolution, the teaching on the development and improvement of life on Earth.

The theory of evolution explains the improvement of forms of living organisms by constant natural selection. From tens and thousands of various forms, the conditions of external life select the most adapted ones. These most adjusted organisms have more advantages, better chances for survival, to leave progeny.

But where do these thousands of different individuals from which the selection is made come from? Who or what serves as a supplier of the forms for selection? This is provided by mutations, random haphazard gene changes already mentioned.

Mutations do not seem to be too frequent, an average of one mutation per million individuals. But there are many many genes. Every organism contains at least several million genes controlling several millions corresponding traits. So it appears that in any sufficiently big community of organisms belonging to one species, or, as they say, in one population, there are always different versions of organisms differing in various traits.

Once they have appeared, mutations are passed from generation to generation, so finally every population accumulates a great number of different versions of mutated genes, and, correspondingly, different versions of traits controlled by these genes. Thousands of somewhat differing individuals, or forms for selection, accumulate in every population of organisms.

Immunological Meadow

Imagine a meadow with hundreds of thousands of flowers growing on it. As a result of mutations these flowers vary in the forms of their calyces. Let us denote the predominant forms as A, B, C, and D.

Insects, like very tiny flies, are always flying over the meadow and can get into any calyx and transfer pollen from one flower to any other. All flowers are pollinated, and all of them have equal chances to leave seeds, to leave progeny. This goes on from year to year. All the flowers, A, B, C, and D, are blooming in the meadow.

Now imagine that our meadow is colonized and dominated by other much bigger insects. So big that they can only get into the calyx B for nectar. The flowers with these calyces immediately gain an advantage over the rest of them. Now mainly B flowers are pollinated, they leave progeny more frequently than the others. Selection is working. In two or three generations most of the flowers in our hypothetical meadow will have B form calyces.

This, of course, is a much simplified scheme, but without it, it would be difficult to explain Burnet's theory.

Immunoglobulins are produced by lymph cells. There are very many of them. The size of the population (that is, the total quantity) of lymph cells in a human body is 10^{12}. It is not millions and not even billions. It is trillions! Imagine how many mutant cells, differing from one another, there are in such a big population.

The formulas of immunoglobulin molecules

synthesized by various cells are also different. And even if there is only one mutant gene per million, the 10^{12}-strong population of lymph cells should contain 10^6, that is, a million cells which produce different forms of immunoglobulin molecules. Among the million versions of immunoglobulins there can be most varied ones. And no matter which antigen gets there, it will find a suitable molecule, like a key to a lock. Each form of cells together with its progeny makes up a family called a clone. So, all the lymph tissue consists of cell clones. It is non-uniform from the very moment of its origin, cloned from the very beginning.

Let us come back to our meadow. But there are no longer flowers on it. Now imagine the meadow as a population of lymph cells: cells producing immunoglobulins are there instead of flowers. They differ not in the forms of their calyces, but in the forms of immunoglobulins they produce. Let us denote them with the same letters: A, B, C, and D.

Suppose that an antigen *b* has entered the body. There is no need for it to interfere with the flow of genetic information DNA $\longrightarrow$ RNA $\longrightarrow$ $\longrightarrow$ protein, a cell's emergency stock. The molecules of the *b* antigen are circulating throughout the body and meet the cells that, in line with their genetic nature, generate immunoglobulins appropriate for this antigen. Antigen *b* is bound with such a cell and becomes a stimulant for it. So it starts rampant multiplication to produce the globulin antibodies corresponding to this antigen, and later on these attach to this antigen and neutralize it.

Each division yields two cells from the original one, these two each produce two more, and so on. The cells of the B clone become abundant. And if the same antigen enters the body again, the antibodies are produced faster and in higher quantities than the first time.

Thus, the antigen became the determining factor for choice, the factor for selection of a certain cell clone. This is why Burnet's theory was called the clonal-selection theory of immunity, or the theory of clone selection.

This theory holds that the immune system recognizes alien cells and proteins because it contains lymph clones against any alien newcomers. There are no clones against the body's own cells and proteins. Such clones cannot be accumulated because the generation and accumulation of clones take place during the embryonic period, when the lymph system is still very weak.

As soon as a mutation results in a cell capable of responding to normal antigens of the host body, this cell starts, so to say, "closing in" and tries to start an attack. But it is too young and immature, it cannot respond by multiplication, and it dies: no clone is accumulated. So, a newborn organism has no cell clones against its own antigens. Hence, the matter is not that lymph tissue can in one way or another recognize "its own"; it just does not have cells capable of producing antibodies against the body's own antigens.

We now know that there are such cells, but they keep quiet. They are quiet because the thymus, the central organ of immunity, produces special cells that forbid all the lymphocytes to

work against "one's own" As you know by now, these lymphocytes received the name of T-suppressors, which means thymus suppressors.

Burnet's theory gave rise to thousands of experiments and ideas on its verification, support, or disproval. These works revealed new facts and regularities in immunology. The theory has been improved. The idea of clones proved to be quite true, and the mechanisms of a cell's work are being refined. Researchers are trying to find out which pathological disorders result in the generation and accumulation of "banned" clones, aggressive against normal body cells. There was evidence that autoimmune diseases are caused not by the emergence of forbidden clones, but by the disappearance of suppressor clones.

In his critical analysis of weak spots in his theory, Burnet always stressed that the positive effect of the theory was also in stimulating a new flow of research to prove or disprove it. Burnet's reasoning is in keeping with John Lilly:

"If it turns out that I am all wrong I will be consoled by knowing that, in truly scientific research not a single experiment can be considered a waste: even experimental attempts to disprove a theory reveal new and valuable data."

Twenty five years of scientific development showed that Burnet's main point was right. The lymph system really consists of tens of thousands of clones. Each of them is aimed against a certain antigen. Science divided lymphocytes into T and B ones, discovered helpers and suppressors. But cloning was not refuted. It also concerns these lymphocytes. True, the mechanism of clone generation, the reasons for the broad va-

riety of clones that explains an unusually wide "repertoir" of the immune system, do not fit into the interpretation of Burnet's theory. The entire "immunological vocabulary" cannot be explained by the spontaneous mutations which occur during the course of embryonic development. The mechanism seems to be more complex.

It turned out that the assembly of an immunoglobulin molecule in the cell is controlled by two pairs of genes located on different chromosomes. One supervises the construction of the heavy polypeptide chain of the molecule, and the other, of the light one. Remember that each chain is assembled by two genes. Before this immunological decoding was done, there was a rule in molecular biology: one gene—one polypeptide. But here two genes are working on the construction of one polypeptide! One gene codes for the basic, or constant, part of the chain—it is called a C-gene; and the other, the most crucial, codes for the variable part of an antibody. It is with this part that the antibody recognizes an alien substance. These parts are different, or variable, in all antibodies. The gene is called a V-gene.

It is these V-genes that are so abundant: V_1, V_2, V_3, ..., V_n. Different varieties of these genes appear due to mutations during the course of the body's development, but only partly. Many of these varieties are already there and transferred from generation to generation through sex cells. Do not be alarmed, not so many genes are needed to supply, say, a million antibody varieties. The recognizing part of an antibody is composed of two chains, the heavy and the light ones, meaning that two V-genes, like V_1 and V_{40}, or V_1 and

V_{572}, or V_{87} and V_{10}, etc., have worked to build this molecule. Since there are two genes involved, only about thousand varieties of V-genes are enough for building a million specificities, since $1000 \times 1000 = \text{million}$.

In any event, lymph clones pre-exist in the body, and each of them can generate one antibody. Taken together, they provide for protecting against any antigen intrusion.

Immunological Mobiles

Have you ever happened to see mobiles, those movable modern sculptures invented by Alexander Calder? In contrast to motionless "stabiles" they are in constant motion. A minor impact, be it a slight wind, sun becoming bit warmer, a bird that lands on the construction or an onlooker that touches one of its parts—and it is all set in motion. It goes out of equilibrium and starts to live and stir before it calms down in a new state of equilibrium. Now the whole construction has quite a different appearance. This appearance stays as it is until a new impact moves the mobile out of this state of equilibrium and sets it moving to reach a new balance.

The transfer from one combination of parts to another is not so fast, and the would-be positions of the parts are virtually unpredictable. And since the disturbing impacts are constant, a good mobile is in constant life, changing the levels of its components, acquiring different forms within the variety of possibilities open to it.

I had the pleasure of visiting the exhibition of Calder's mobiles in 1968 in Amsterdam. Before

that I had seen them only in pictures, and also in primitive versions in homes of my acquaintances. Some of them did not even know that these constantly swinging little fishes or birds dangling from the ceiling on a system of beams are called mobiles. Real mobiles, of course, look much more impressive. There are dozens of beams slightly curved or as straight as arrows, but invariably exquisite, attached to each other but by far not at their centres of gravity. There are beams with one arm not more than a centimetre and the other of about a metre and a half. The short arm has an odd-looking weight of a dozen kilograms balanced by a small sphere or a picturesque petal weighing not more than several grams. Touch the big weight, move it one millimetre, and the small one will rock, glide or dance, making much longer voyages, while the shifts of the big weight are virtually invisible. But this intricate beam is not alone. It hangs on the end of another one, that is balanced with a weight or another beam. And that one supports more and more beams... There are different shapes of weights and beams, arms of different lengths. These fantastic constructions are attached to base beams, by hanging a string from a hall ceiling, to a special stand or arch in a garden. All of them are of different shapes, all are living and moving. These are mobiles.

In 1968, when I was enjoying the collection of these moving sculptures, T-lymphocytes were discovered. The transformation of B-lymphocytes into antibody-producing plasmatic cells was found to depend on T-lymphocytes. They were called T-helpers. The more T-helpers, the more intense

is antibody production. In 1972 T-lymphocytes of the opposite action, or T-suppressors, were discovered. They suppress the activity of T-helpers. The more T-suppressors, the fewer T-helpers, the lower is B-lymphocytes' response to an alien antigen. Later it turned out that this entire system of immune response does not work if the macrophages are lacking or non-existent. It is these macrophages that catch an alien agent and present it to lymphocytes.

I knew nothing of this when, in 1968, I was examining "breathing" mobiles. But now, thinking of how the immune system works, I invariably recall mobiles with their constantly changing balances. The macrophages' impact decreased, the beam with B-lymphocytes lowered. The quantity of T-helpers grew, the arm with their weight lowered, while a "small ball' of B-lymphocytes shot up, even higher than the entire beam would have lifted if there were a bit more macrophages.

But it all hangs together with suppressors. If there are many of these, the whole half of the immunological mobile slides down. Macrophages present an antigen, T-helpers push up the B-end of the beam with all their might, but, to no avail. The aggregate of responding beams goes down, and the pulled-up B-ball will be below any average norm. There is no immune response, no antibody production.

The situation can be improved only by countersuppressors. They balance the beam against suppressor cells. The balance changes. Suppressors stop their pressure. The beam with B-lymphocytes flies up. Immune response gets back to normal. Incidentally, countersuppressors were

discovered simultaneously by two researchers, Spartak Gambarov in Moscow, USSR and Gershon in the USA. Gambarov described them even before Gershon, in 1980.

There are thousands of clones in the body. Each has its own helpers, suppressors, counter-suppressors. There are thousands of mobiles, thousands of beams. Thousands of disturbing antigen and non-antigen impacts constantly change mobiles' balances. I am haunted by this symbolic image of the immune system. It is not because the immunological mobile looks like Calder's constructions, but rather because this image enabled me to comprehend what normal indices of the immune system are.

What we have become accustomed to is this: a normal condition is pulse 72 beats per minute, arterial pressure 70/120 mm Hg, the blood level of red blood cells 5 million, gastric juice acidity 40 units, etc. We are trying to introduce a set of normal indices also for the immune system: B-lymphocytes, 15 per cent, T-lymphocytes, 60 per cent, including 20 per cent helpers, 12 per cent suppressors, etc.

Believe me, the future will prove this wrong. The normal level of the B-ball in the immunological mobile can be provided by entirely different combinations of the indices pertaining to other weights. An elevated level of helpers means nothing if the system is short of macrophages. But if their weight is enough, everything can be cancelled by suppressors. On the contrary, a low number of helpers might be ideal if there are no suppressors. Clinical immunology will have to determine some integral indices, perhaps for-

mulas or monogrammes, that will help assess the immunity's norm or pathology, health or illness.

The image of the immunological mobile is also useful for understanding one of the latest theories of immunity created by Nils Jerne.

The network theory, or the theory of networks, includes one more correlation in the clonal lymphocyte system, in all the relations between precursors, helpers, and suppressors. This is the correlation between idiotype and antiidiotype, or receptor and antireceptor.

Remember, when we discussed the structure of antibodies, the main protective proteins, we mentioned that these proteins belong to immunoglobulins. There are five classes of immunoglobulins, A, G, M, E, and D. This reflects antigen differences between the molecules' heavy chains. Furthermore, immunoglobulins in different individuals differ like blood groups. These intraspecies differences are called allotypes. There are over 20 forms of allotypes.

Now imagine two antibodies in one and the same body of the same class, of the same allotype. One antibody is against the antigen X, another, against Y. Everything is the same, apart from the recognizing part of the molecule. These two molecules will differ, being called idiotypes.

So, there are five classes of antibodies, over 20 allotypes and endless numbers of idiotypes. Each clone of B-lymphocytes produces its own unique antibody molecules, its own idiotype. Receptors, i.e. structures used by lymphocytes to recognize an alien antigen, have the same idiotype.

The wide variety of idiotypes would have been no more than a curious example of the unique-

ness of complex structures in Nature. But what Jerne suggested and then proved is that the body produces antibodies against each of his own idiotypes, and, consequently, there are antireceptors against any receptor. He called these antibodies antiidiotypes. The relations between idiotypes and antiidiotypes form the basic mechanisms of immune response regulation.

Now the antigen X has entered the body. The corresponding clone of anti-X lymphocytes, or idiotype X, has started to multiply and accumulate. But who will stop it? According to the network theory, it will be stopped by the clone producing antiidiotype X, that is, by antibodies against anti-X antibodies, so to say, anti-antibodies. This clone will start multiplying as soon as the first clone is accumulated. But who will stop this second wave? It will be anti-anticlone, that is antiidiotype against antiidiotype. In other words, antiidiotype is always a suppressor. Some proponents of the network theory maintain that it is antiidiotypes that are born by suppressor cells and used as receptors.

The network theory by Nils Jerne has a promising future. It very well explains autoimmune diseases as hyperproduction of antiidiotypes. It gives a new interpretation of leucosis, or blood cancer, as a deficiency of the appropriate idiotype inhibiting a certain clone. It suggests new ways for stimulating and suppressing the immune response not by treating the body with antigens, but by exposing it to antiidiotypic antibodies. Perhaps, active immunization is possible without vaccines?

But then our immunological mobile becomes

even more complex. It acquires additional impacts, additional beams. Each clone has an anticlone. Each mobile has an antimobile. A further understanding of the development of immune processes is impossible without mathematical models.

Mathematical Models

I know of several dozens of publications in quite serious international magazines dealing with mathematical modelling in immunology. But there is only one monograph on this subject. It was published in December 1980, by Nauka Publishers in Moscow, and was then translated in the USA. I was honoured to write an introduction to it. The book is called "Mathemacial Models in Immunology", written by a prominent Soviet mathematician and academician, Guri Marchuk.

The fact that the monograph on a medical-biological subject is written by a mathematician is an event in itself. If mathematicians start to work in what seems to be a narrow field, it means that this "narrow" field is really of great humanitarian importance.

The book gives an effective tool for analysing multicomponent process of the body's response to an antigen, and a number of promising practical recommendations on the diagnostics, prediction, and treatment of infectious and non-infectious processes, in which immune system responses play a determining role.

Mathematical models by Marchuk are principally different from those suggested by other authors for describing immune responses. Most previous mathematical works of this kind sought to achieve the most accurate mathematical descrip-

tion of various procedural elements, the dynamics of cell interactions or other immunological phenomena. Intentionally or unintentionally, they were guided by the principle: immunology for mathematics. The equations by Marchuk, with all their mathematical logic, rest on a different principle: mathematics for immunology. The interaction between the body and an alien proliferating antigen, be it bacteria, viruses or any genetically different cells, is made up of four basic parameters.

1. The alien cells, generally denoted as antigen or virus (V), which have penetrated into the body reproduce. The change in the number of viruses in the body depends on their proliferation rate within a given period of time, minus their number neutralized within the same time by previously existing or newly emerging antibodies. The notion of antibody is also generalized to include both immunoglobulins and cell structures neutralizing a given antigen, such as T-lymphocyte receptors.

2. The immune system responds to the antigen intrusion by accumulating immunocompetent antibody-forming cells (plasmocells, according to Marchuk). The VT complex, i.e. the complex of antigen V with the receptor of a recognizing T-lymphocyte, serves as a triggering substrate. The number of accumulating plasma cells depends on the number of triggered B-cells and their proliferation rate, minus their depletion through aging.

3. The number of antibodies present within a certain time interval depends on the rate of their production minus the quantity bound by an an-

tigen and the quantity excreted at the expense of their natural catabolism.

4. The work of lymph, or, what is the same thing, the immune system of the body depends on the normal functioning of other systems and organs. Naturally enough, a virus affects some system (or organ), not necessarily the lymph system. It can be the liver, the lungs, etc. In any case, the lesion can be so grave that it affects the work of the immune system. In other words, in simulating immune response, one should take into account organ disturbance caused by a virus. Marchuk introduces a generalized notion of "the mass of the affected organ" It depends on the destructive ability of the virus, which is different for different diseases, minus the restoring part of the cells.

These four parameters represented by four equations completely describe the interaction between the immune system, or, more exactly, the body, and the virus. Introducing the fourth parameter, the weight of the affected organ, into the system of equations gives everything required for the logical identification of a given immune response with an infectious disease as a whole.

The simplest model of the body's immunological response to a virus is at the same time the model of the infectious disease. The most captious critics would fail to find here processes unaccounted for, if the basic processes are meant. Neither could they prove that any of the four parameters are unnecessary for further solutions. And it is further solutions that form the essence of the book.

A group of mathematicians headed by Marchuk used computer experiments to check dozens of situations involving different values that comprise these four basic equations. The infecting doses and the rates of virus accumulation, the original antibody level, the dynamics of accumulating plasma cells, the weight of the affected organ and other parameters were changed. The basic equations became more complex as new parameters were introduced, like T- and B-immunity systems, the temperature coefficients of production of the antibodies of IgM, IgG, IgA classes, and others. The author arrived at several important biological conclusions. Some of them are given below.

"Maximal virus concentration in the body depends on the state of the immune system and viral characteristics, rather than the infecting dose."

"It is disadvantageous for a system to respond to small doses of viruses with sluggish dynamics."

"To achieve normal functioning of the immune system, antigen-stimulated T-cells should provide the feedback signal to stem cells that trigger their differentiation towards immunocytes."

"The artificial reduction of body temperature results in lingering or chronic forms of a disease."

"Chronic forms of a disease are due to insufficient stimulation of the immune system."

When T- and B-systems of immunity "get cut off" from the immune system, a chronic form of a disease turns into the acute one."

"To turn the chronic form of a disease into

the acute one with subsequent convalescence, the virus concentration in the body should be increased. This can be achieved by treating the body with alien antigen substances (the author calls them biostimulants) like pyrogenal, prodigiozan, and others. Diverting the immune system to themselves, biostimulants create the conditions for the explosive proliferation of the "chronic" virus. Its antigen weight grows. The chronic process turns into the acute one with subsequent convalescence."

Marchuk not only advocates the use of antigen biostimulants, but insists that this kind of therapy controlled by modern methods of clinical evaluation of the immune system should be introduced into practice in accordance with WHO recommendations. He also suggests the mechanism of action for the second antigen: the competition of T-complexes for the receptors on macrophages, since it is macrophage that triggers B-cell proliferation by capturing these T-complexes.

Now, together with Marchuk, we are working on more complex versions of this model. We have distinguished between humoral and cell-mediated arms of the immune response and expanded the model to include the phenomenon of double recognition and T-helper activity. Other factors to be embraced by the model in the future are suppressors, idiotypes, and antiidiotypes.

In the nearest future, computer simulation is likely to map out the shortest and most reliable immunological roads for experimenters and clinicians.

Where Do Immunologists Come from?

It so happened that in September 1963, a letter was sent to Meditsina Publishers proposing a textbook on immunology. On November 10, the answer arrived: "The state publishers on medical literature inform you that your proposal on publishing a textbook for students cannot be accepted, since there is no such subject as "immunology" in the list for college students, approved by the USSR Ministry of Public Health."

In former years, when immunology was not yet "new" and dealt mainly with preventing contagious diseases by means of vaccines, it totally belonged to the realm of microbiology. Its problems were totally bound to microorganisms, the pathogens of infectious diseases. And though the immune system belongs to the body a microorganism invades, not to this microorganism itself, it was looked upon "in light of microorganisms" Very few people delt with non-infectious immunology, but there were some of these nevertheless. Most modern immunologists came from microbiology, and some of them sprang from biochemistry, physiology or surgery.

The most natural path is from microbiology. In this case the scientific fate of a researcher kind of duplicates, in a concentrated form, the history of immunology. Microbiological problems of a certain infectious disease give rise to the problems of immunization against it. The work in this direction poses more general questions on studying the immune system. If they become the crux of the matter for a scientist, then. "the horse has galloped off and taken away the brid-

le,": the researcher virtually ceases to be a microbiologist.

Whether or not he would become a good immunologist is not clear. To achieve this, he will not only have to work much in this unknown area, he will have to cognize it, to get to know it by himself, without any universities, to get acquainted with it with the help of scientific magazines, books, libraries, conferences and through communication with good immunologists.

The path from biochemistry passes through studying the structure of molecules involved in immune reactions, the regularities of their synthesis and interaction. It is a real challenge to find out what is the chemical language of the machine for recognizing alien substances. But the body's immune system is not only able to recognize an alien newcomer, but is also able to produce a weapon for its destruction and to fire shots. These shots must be of fantastic accuracy, so that in the cell hurly-burly, the "loyal citizens" are not hit.

Physiologists emerge onto immunological paths mostly through the jungle of the endocrine system, that is the system of organs producing hormones, the regulators of the body's activities. A small gland at the base of the cranium, called the hypophysis, produces growth hormones. The thyroid gland located at the neck in the forepart of the larynx secretes the hormone that regulates metabolism. Just above the kidneys there are small organs, the adrenal glands, that produce a range of hormones directly affecting the lymph tissue, that is the immune system.

A physiologist might get carried away with

the hormonal regulation of the immune system's activities and this would draw him deep into immunology. It is difficult to find one's ways in a new area. Friends look at him askance: he betrayed physiology, and, trying to keep up with a fad, got involved into some new subject. But immunology absorbs one, and absorbs one forever.

For dozens of years no colleges trained immunologists, while the specialists in this field became more and more numerous. No university, no medical, veterinary or agricultural institute had a chair of immunology, while the science on immunology developed.

This was the situation until 1965, not only in the USSR, but all over the world. The greatest world immunologists mentioned in this book came to immunology by different ways. Frank Burnet was microbiologist, Peter Medawar, a zoologist, and Roney Porter, a biochemist.

Beginning in 1965, the situation started to change. A new subject started to be taught in medical colleges of Great Britain, France, and the USA. Immunologists started to be trained.

In 1979 the Research Institute of Immunology was set up in Moscow. Beginning in 1980, a periodical *Immunologiya* (Immunology) was published. In 1981 another immunological institute in Novosibirsk, the Institute of Clinical Immunology of the Siberian Division of the USSR Academy of Medical Sciences was established.

In 1981 the programme for developing immunology, worked out and supervised by the State Committee on Science and Technology under the USSR Council of Ministers, was introduced. And,

finally, in 1982, a textbook on Immunology was issued by Meditsina Publishers.

Once, a journalist asked me: "What do you think is the starting point for a new profession, when does it acquire civic rights?" My answer was this: "If, in spite of certain accepted definitions, the word 'profession' is given a social meaning, then, from the moment when a profession assumes a humanitarian and social significance, the society takes the next step, starting to teach this profession. This, I think, is the starting point."

More than 100 students study annually at the chair of immunology at the N. I. Pirogov Moscow Medical Institute. Dozens of young researchers and doctors become post-graduates or come to work in attempts to master modern immunological methods. The students entering the Institute know that they are going to learn this subject. Some of them have some misgivings about it, and some are pleased. In fact, most are pleased, since entering the institute is a big joy.

The Union of Immunological Societies

There is a bronze memorial medal that I always have in front of me on my desk, and that is very dear to me. The blackened bronze shines through only in the most raised parts of the embossing, letters, and symbols. It is uncommon in its shape, rectangular, nearly a centimetre thick, a weighty plaque with the sides cut as the arches of some circles. It is about half a palm big. The point, though, is not in its irregular shape. This medal was handed to the participants of the 1st International Congress of Immunologists, that

had both scientific and organizational implications. At this congress, the International Union of Immunological Societies (IUIS) was set up.

A portrait is embossed on the face of the medal. A scientist is looking searchingly through old-fashioned glasses in fine frames. He has small moustache and beard. At the side of the medal there is an inscription: "Paul Ehrlich, 1854-1915". You should remember the story of Paul Ehrlich, the one who created the humoral theory of immunity at the time when Ilya Mechnikov developed the phagocytal theory of immunity. It was Ehrlich who shared with Mechnikov the honour of winning the first Nobel Prize in Immunology.

On the back side of the medal along the perimeter an inscription reads: "The First International Immunological Congress. Washington, 1971" The picture in the centre features two lymphocytes placed at an obtuse angle of the rhomb and connected with each other with two sling-shot-like molecules of antibodies. This is the symbol of two specifically acting "heroes" of the immune system.

Barnard Cinader, a prominent immunologist from Canada, head of the chair of immunology at Toronto University, was elected as President of the International Union of Immunological Societies. I have already mentioned his name in the beginning of this book.

The congress is convened once every three years. After being held in the USA, it was held in Great Britain (1974), then in Australia (1977), and in Paris (1980). Next, the 5th Congress was held in August 1983, in Kioto, the an-

cient capital of Japan. Thousands of immunologists get together at every congress to listen to interesting reports, exchange opinions, map out new ways and elect a new President. The Nobel Prize winner Baruch Benacerraf was replaced by the Director of the Institute of Clinical Immunology in Bern, Professor Alen De Veck.

The Paris congress brought together about 4000 people. This interest in immunology struck even Paris organizers of the congress who had witnessed many events of this kind. A geological congress held in Paris at the same time drew one third this number of delegates.

The International Union unites more than 30 national immunological societies of countries around the world. The total number of IUIS members is nearing 20 thousand people. There are several standing commissions working under the auspices of IUIS. Two of them are of special interest.

The first one is the committee on the standardization of immunological methods and preparations. The member countries can compare their reagents, treatment and diagnostical immune preparations, tests and techniques, with the unified international immunological references. They can exchange samples, make up joint collections and banks of sera, hybridomas, etc. The second is the committee on immunological education. It launches immunological teaching projects in various countries, holds workshops and seminars on the most urgent problems. For a number of years this committee has been headed by the British immunologist Ivan Roitt. It was he who introduced the denotations of T- and B-lymphocytes. At

a committee session in 1974 my proposal to start a special work on training teachers was approved. This is really important for a new and rapidly developing science, since in many countries where immunology has not yet "stood on its own feet" there is nobody to teach it.

The greatest number of IUIS members is in the USA Immunological society, where there are over 2000 members; then comes Great Britain (1600), Japan (1300), and West Germany (over 500). There are large Immunological Societies in GDR, Yugoslavia, Czechoslovakia, and Poland.

Why is immunology so popular? Why has the number of IUIS members grown more than threefold during the twelve years of its existence? Does it mean that immunology is so important not only as an interesting theoretical subject, but also from the standpoint of the national economy? Yes, this is precisely so. In the introduction to my textbook on immunology published in 1982 that I mentioned earlier, I formulated ten economic tasks that depend on the achievements in immunology.

1. To prevent the infections that are not yet overcome in humans and agricultural animals, including influenza, parasitic diseases, gonorrhea, syphilis, African pig fever, and others (i.e. to find new principles for vaccines and synthetic vaccine preparations).

2. To find ways for stimulating immunity against "artificial" microorganisms designed by gene engineering methods, as well as against artificial and natural toxins and allergens. This is connected with the rapid development and big prospects of gene engineering, as well as the in-

creasing level of new chemicals and allergens in the environment.

3. To develop methods for correcting secondary immunodeficiencies causing acute and chronic infectious complications in surgical, obstetric, pediatric, and other clinics ("staphylococcus plague"), as well as chronic pneumonia, mastitis, artritis, and the like due to conditionally pathogenic microorganisms. This is based on finding stimulants for specific links of the immune system or the means for their compensation.

4. To prevent and treat rheumatic and other autoimmune diseases. Even now, the use of immunodepressors yields certain results. Still, the present-day immunodepressive therapy has only the means for total depression of all immunocompetent cell populations. The discovery of functionally alternative lymphocyte subpopulations, in particular, T-helpers and T-suppressors, maps up new ways for developing immunotherapy. Now researchers are looking for ways to affect selectively various lymph subpopulations for selective suppression of effector or helper cells and selective stimulation of suppressor cells, whose defectiveness results in autoimmune diseases.

5. Immunoprevention, immunodiagnostics and immunotherapy of tumours, and, primarily, of lymphoproliferative processes, lymphomas, and the like. Progress in tumour immunotherapy also depends on the ways and means for selective immunosuppression-immunostimulation. This task is opposite to the previous one: here we need to stimulate effectors and helpers while inhibiting and blocking suppressor cells.

6. To prevent and treat allergies. A promising

path in this direction lies in finding ways for switching the synthesis of allergic antibodies (IgE) over to the synthesis of normal antibodies (IgG).

7. To reduce obstetric immunopathology and infantile mortality due to disturbances in the immunological mother-foetus relationship and various forms of congenital immune deficiencies, including primary immunodeficiencies. To solve the problem of primary immunodeficiencies would mean to find keys to many other forms of immunodeficiencies. No doubt, the one who will learn to treat primary immunodeficiencies will learn to treat cancer.

8. To work out clinically acceptable methods for cancelling immune protection and developing specific tolerance to achieve transplantation of the bone marrow, as well as other tissue and organs.

9. To learn to compensate for immunity-disturbing external impacts such as cytotoxic poisons, ionizing radiation, magnetic fields, and other types of energy, as well as a number of physical and chemical exposures due to occupational hazards.

10. To develop new supersensitive and highly specific methods and to improve the ones already available for detecting microquantities of organic substances. This task, important for a wide range of medical, biological and chemical fields, was called immune biotechnology.

The Constitution of the International Union of Immunological Societies reads: "Immunology has always been the field in which outstanding fundamental works and revolutionary practical ad-

vances have been closely connected, both in time and in the minds of leading research workers. It may safely be predicted that equally great gains may soon be achieved in the alleviation of allergic and rheumatic diseases, and even, perhaps, the treatment of cancer."

In the end of February 1983 a group of Soviet immunologists was invited to take part in the conference of the Immunological Society of the German Democratic Republic. The conference, held in the ancient German city of Erfurt, brought together over 300 participants. We were met by the President of the GDR Immunological Society, Dr. Ambrosius. He told us that the main subject of the conference was clinical immunology and the problems of allergy treatment with an emphasis on treating and preventing allergic diseases in agricultural workers, especially those working at big livestock and poultry farms.

Immunologists from the GDR develop hygienic norms and indices of the workers' health, in order to prevent occupational hazards and timely discern the very initial signs of possible allergies to chicken fuzz, swine bristle, hay dust, fodder additives, and others. They conduct systematical medical examinations of rural workers, making use of diagnostical and allergological tests.

Of course, the conference was not of a purely applied character. It is quite impossible to develop practically valuable means for prevention, diagnostics, and treatment without deep fundamental research. Ambrosius himself is a well-known specialist in molecular and cell immunology. In his talk at the conference, he gave a profound analysis of the state of the art in mod-

ern theoretical immunology and pointed out the achievements with the most important practical implications.

As the tradition would have it, a new President of the GDR Immunological Society was elected at this conference. It was the famous clinical immunologist Professor Eger. The manual on clinical immunology written by him is known well beyond the borders of the GDR.

In the same way, immunologists of the Soviet Union spare no efforts to solve most urgent theoretical and practical problems. One of the All-Union Conferences on immunology was held in Alma-Ata in summer 1981. This was a very big conference that marked the basic guidelines for the development of immunology in our country, demonstrated the achievements of research teams from various Union republics, and stressed the significance of the two national leading immunological centres, Moscow Institute of Immunology and Novosibirsk Institute of Clinical Immunology.

In conclusion, let me say a few words on the activities of Moscow immunologists.

Two years ago, on the initiative of the State Committee on Science and Technology, a standing workshop under the title "Modern Problems of Immunology" was set up under the Interdepartmental Scientific and Technological Council on the Problems of Molecular Biology and Molecular Genetics. The objective of this workshop is the permanent exchange of information on the latest problems of theoretical and applied immunology in order to achieve more effective work and introduce the achievements of fundamental

immunological research into everyday medical practice and other fields of the national economy. In organizing the work of the seminar, the Interdepartmental Council was guided by the fact that immunology is now a "hot area" of biology, and the achievements in this field underlie a number of important areas of our life, primarily health protection.

The chairman of this standing workshop is the chairman of the State Committee on Science and Technology, Academician Guri Marchuk. On the last Thursday of every month the conference hall of the State Committee brings together over a hundred prominent immunologists, well-known scientists from allied fields (microbiology, virology, biochemistry, genetics, and clinical medicine) and many young researchers.

Experts in immunology from various ministries and departments, researchers from any different cities take part in the workshop. The presence of immunologists, physicians, biochemists, molecular biologists, and other representatives of medical and biological fields is quite natural. But the seminar is also attended by mathematicians, who not only listen to the others but also give talks themselves.

The workshop is so popular because the participants manage to have maximally concise, 2-3 hour-long, acquaintances with the latest theoretical, experimental, and clinical findings, the problems and the ways to their solution. The discussions generate new discussions, help pave new and more effective ways and, what is even more important, strengthen creative ties between researchers and scientific teams. Interlaboratory

and interinstitute complex research groups established at these seminars are very fruitful. The proceedings of the workshop are published timely in separate volumes in the series "Itogi Nauki" ("The Results of Science"), and very soon become available for all those interested in immunology: scientists, physicians, and students, all over the country.

I'll speak about the very first meeting of this workshop. The honour to give the first talk was given to Professor Rahim Khaitov. The title of his talk was brief but weighty for fundamental immunology: "B-suppressors" Why weighty? Because prior to Khaitov and his co-authors, T-lymphocytes were considered to be the only cells regulating immune response. I have already told you that T-lymphocytes include both helper and suppressor cells, and it is T-cells that "conduct" immune response.

So, in his first talk at the workshop, Khaitov gave strong evidence that there are regulatory suppressor cells that belong to the family of B-lymphocytes. In other words, the major conductor has some assistants. It appeared that they exist in the bone marrow and prevent the antibody-producing cells from accumulating there. They "preclude" the development of immune response on the territory of the bone marrow, since the bone marrow is meant for other purposes. It is the place where blood cells, such as red blood cells, blood platelets, leucocytes and B-lymphocytes themselves, are generated, a kind of cell factory from which they come into being. B-Lymphocytes generated there enter the blood and settle down in the spleen. There they have a

right to receive immunological signals and start the production of protective antibodies, since the spleen is the place where the immune response is realized. For a number of years it was unknown why the bone marrow, where there is a lot of B-lymphocytes, where T-lymphocytes arrive, where there are macrophages, why the organ that has all the components needed for immune response, nevertheless does not develop it. By 1980 all the participants to the workshop already knew the answer to this question. It is because the bone marrow contains a special sort of B-lymphocytes that were called B-suppressors. These cells perform the crucial function of preventing the bone marrow from doing the work it is not meant for. The principle of specialization is safeguarded: each factory works on its specific sort of production, each skillful craftsman deals with his own job.

The participants to the seminar knew about this scientific news a year before all the rest of the world. This is very important in science. It takes some time for information to come out, reach your desk and for you to get around to reading it. And who knows whether or not this magazine finds its way to you? And will you be able to comprehend it all by reading a small article? Will you be able to feel all the importance of it? And here you can do it all at once, to clarify all the minor details, including the subtleties of methods and techniques. This in itself dictates the need for the specialists to get together at the meetings of seminars, conferences, and scientific societies.

Learn to Stick to a Goal

There is a good tradition in the 2nd Moscow Medical Institute. Late in August, on the 30th or 31st, the ceremony when students are accepted is held. Professors, lecturers, people from the administration and the students themselves speak at this ceremony. Professors make serious speaches, and students stage merry performance.

On August 25, 1981 the academic pro-rector of the institute Ivan Novikov gave me a call. He invited me to speak at the ceremony that was to take place in the Column Hall of the House of Unions. I agreed and hung up the receiver, and then the law of paired contingencies started to work: the second call came, this time from the youth newspaper "Komsomol'skaya Pravda". I got a similar offer; to write an article for the newspaper for the first of September, the beginning of the school year. I was to "speak" on the same subject, not only in front of the students of the 2nd Medical Institute, but to the youth of the entire country who were starting their studies on this day. Every year "Komsomol'skaya Pravda" marks the Day of Learning with such contributions. On August 31, I spoke in the Column Hall, and on September 1 "Komsomol'skaya Pravda" published my article "Learn to Stick to a Goal". It is the best thing to finish the book with this topic.

Today, on the Day of Learning, I was invited to the traditional speaking place of the department. This is not a simple task. Of course it is nice to utter a word of praise for learning, but there have been so many words said, weighty and

meaningful words. Is it at all possible to add something new to them?

Still, I was happy to accept the offer. Happy because I am convinced: each of us has always had and will surely retain his own special and deeply personal attitude to this day. I mean those who come back to classes after the holidays, those who prepare a new course of lectures, and those who, for the first time in their life, will see their offspring to the school doors. Each of us has something to say on this day, be it to ourselves, to our children, students or colleagues. In the hope that these words won't be wasted, in the hope that somebody will hear them and heed them, I will dare, in this day's lecture, to formulate some of my personal observations. Let's say: the observations of a studying man.

So, the first observation: a professional one.

In contrast to all the other forms of living matter, people have two types of heredity. Biological heredity is written down by the genetic code. This is characteristic of all living beings. While the inheritance of knowledge and other cultural values gained by previous generations, the achievements of fine arts, music, poetry, and literature is typical only of people. A newborn baby knows nothing of it. But he is endowed by the abilities of his brain and soul to absorb the cultural inheritance. It is not written down in his genetic code. It is written down by means of letters, figures, drawings, paints, notes, light on photo- and cine-films, electromagnetic field on recorder tapes, and in the electronic memory of computers.

Knowledge is passed from generation to generation. Each generation not only perceives previously acquired knowledge to pass it further, but increases and multiplies it. The Palace of Knowledge becomes larger and more beautiful with every year. The duty of us all is to contribute our might to creating this palace. This means that each of us is to do something new, something unknown to all previous generations.

What is done, then, what is already known, what is discovered, what humankind is capable of doing, and what is not yet done, what is yet unknown, not yet discovered, what is still to be learned? We must know this to offer our might to the progress of human knowledge and culture.

And here is high time to exclaim: long live school, college, institute, post-graduate courses! Long live the teachers, tutors, professors, no matter where they teach: at school, at a factory, in the field or in a university. Long live students, the basic molecules of cultural heredity, the heredity of knowledge!

The second observation: a principal one.

The first of September is the day when a teacher and a student first meet. One passes knowledge on, the other receives it. He receives knowledge not only to get information and use it in his work, but also to develop it and create new knowledge. To create new knowledge—this is the supreme goal of any education. To do something better than it used to be done, to make it faster, more convenient, to make it wiser, more beautiful or more surprising. To make a name for oneself, a violin player or a singer should stand out from all the others. A potter or a carpenter

should somehow surpass his fellow craftsmen. A farmer or an engine-driver, a miner, agronomist, tailor, engineer, doctor or scientist—each of them should strive to excel his precursors and contemporaries. This is what one should learn to do. This is why teachers pass over their knowledge, and the students receive this knowledge.

Of course, teacher and student are relative concepts. The teacher himself is learning all the time. The student also teaches somebody, sometimes even his teacher. Still, there is the teacher and there are students, for instance, a professor and his students, post graduates, his fellow researchers. What is the crucial point in their relations? What purpose does a teacher serve, if everything can be learned from books and other forms of storing knowledge? What should one learn? What should one teach? You cannot possibly teach one to make discoveries (Can you even do it yourself?). Nevertheless, you should make your student an educated person capable of creatively using and developing the knowledge obtained. This means to make him a Man, since only Man inherits culture. It is education that makes man a Man.

The third observation: a practical one.

No one can create a person except for the person himself. It is not a teacher that creates him. Spoken out, this opinion always rouses ardent objections: "How come! We know, for instance, of the school of Academician Yoffe that generated a number of very prominent Soviet physicists, we know of Russian ballet school, and the like. One is extremely lucky if he chances to have a remarkable supervisor for one's studies, such as

a gifted teacher or great scientist. What can one do without a tutor? Not only will he learn nothing, he will not be able to distinguish between true and false, light and dark, good and evil. To be a student of an Expert, in the truest sense of this word, means to become an Expert oneself."

When I hear monologues like this, I recall the quarrel between two biochemists who claimed each other's experiments were staged incorrectly. One of them, to make his arguments more weighty, exclaimed: "I'm a student of Academician Severin!" "There were a lot of fools among Severin's students!", the other retorted.

A good teacher inspires the craving for knowledge, the striving to know more, he teaches his students to choose untrodden paths. But will he manage to teach all of this, if a student has no such interest, no such craving? If he only repeats what others say or show him? If he does not go straight to the library to clear up uncertain points using books, and even perhaps to verify (yes, to check and verify!) a teacher's opinion and tell him that there is some other viewpoint other than his?

I know of some researchers (I wouldn't call them scientists) who work in the following way. They read the latest issue of the international journal "Nature" which publishes scientific papers sooner than all other journals, two or three months after receiving them. The work disclosed in a paper should be of a fundamentally new, pioneering character, to contain some ideas and information previously unknown. So, having received this journal, say, in January or February, these so-called researchers repeat the works, get

the same results, and manage to publish them in the same year, but in another "fast" journal.

Is this creative work? No, it is the work of an apprentice, not of a master. Is it at all useful? In general, yes, since they rapidly confirm the truth, and this confirmation is indispensable in science. Theirs is the honour of the first confirmation, provided, of course, they referred to the author of the original paper in their publication.

And if they did not? Then they seem to appear among the pioneers, the discoverers, since they have published their works in the same year. So the question arises: is this ethical? The answer is clear: this is not only far from creative work, this is dishonest. Fortunately, scientific journals thought of how to bar the way for such "researchers". Each paper to be published is accompanied by the date of its reception by the journal. For instance, a journal comes out in January 1981, and the line in the end of the article reads "received June 16, 1980". A teacher teaches scientific honour, but whether or not a student will learn it, is another question.

The fourth observation, perhaps, is a disputable one.

The main thing for a teacher is not to teach something, but to prevent mistakes. I foresee objections: how come a student didn't gain knowledge, didn't learn something? Yes, that's true, but we have already agreed that a student's mission is not only to assimilate the experience, but also to multiply it.

One can't help making mistakes, in the broad sense of the word, but one should be able to find

them. Fallacies are necessary in a scientific quest, and the truth cannot be found without them. But one should be able to overcome them. There is a very special kind of courage, the courage of objectiveness, the resolve to say to oneself: "I was wrong." We all need this courage, no matter what field we are working in. But this comes along only with education. An ignorant person, unaware of anything except that which is done by him, is not capable of saying: "I did that wrongly, I was mistaken."

But there is a very subtle borderline, the borderline between doubt and confidence. Excessive confidence leads to the loss of objectiveness. Excessive doubts can also block scientific endeavor. All his life one can keep telling oneself "I was wrong, I'm too far from perfection," thus depriving the world from a masterpiece which may have been created by him.

This is why the major task for a teacher is not just to beat knowledge into his students' heads, not just to teach them how to work, but also to teach them to intercept mistakes, fallacies, excessive confidence, as well as excessive doubts. A teacher must also make an objective and unbiased assessment of a student's work. A student should learn to see the crux of the matter. Norbert Wiener gave a good example illustrating the thought that real creative activities cut all that is unnecessary. A herd of monkeys can be taught to type. They will fill a heap of paper with all the letters of the alphabet, and, among the abracadabra they produce there will also be the text of Shakespeare's "Hamlet" But, Shakespeare is needed to cross out all the unnecessary letters,

and only then the text of the immortal tragedy will remain.

Now I'll give you two rules; one for the teacher and one for the student.

First: all you know on a certain question today, all you are capable of today, give it to your students. Take out everything from your stock, and only then you will have new knowledge and new skills. Both you and your students, or you together with your students will have it. A teacher who keeps something to himself, who never lays his trump card on the table, who wants to be unattainable fearing that his student might surpass him—this teacher rouses no respect. This teacher bars progress. To be sure, he will not cancel the growth of knowledge. Someone else will hit upon his trump. But is it reasonable to cling to this trump, isn't it better to give it away and create a new one? Look for a tutor, instructor or supervisor who gives his knowledge over to the last little bit. And learn to be like this yourself, creative work is impossible without it.

A rule for the student, and since we all are always learning something, a rule for all of us: learn to stick to a goal.

All kinds of human activities, be it studying or work, are unthinkable without will. It seems so simple to finish something once started. But in actual fact how many things have we started only to drop them "in five minutes" The books not read to the end, the promises not fulfilled, the houses not completed, the dresses not sown, the ideas not thought through to the end, the efforts not reaching the goal. We find ourselves all

sorts of justifications: "What sort of goal is it? It is too small to care so much about it. When I have a goal big enough, I'll try and pool my energies, I'll achieve everything." No, you will achieve nothing at all if you don't learn to implement small and medium goals, if you don't learn "to stick to a goal". To stick to it all the way through, from conception to attainment.

Great scientists are commonly believed to be absent-minded. He would put his necktie on backwards, people say, and never notice it. Is it absent-mindedness or great concentration on some other things? It was not the falling apple which inspired Newton to discover the law of universal gravity. He focussed on a goal which had not yet taken shape, held it in his consciousness day and night, so that, at that very moment it could be realized having turned into a law.

One would be a little bit of a hound to be able to chase the target. A good hound that never lets a haunted beast alone, even if its legs are worn out and bleeding. One should be a little bit of a race horse that would rather fall dead than fall behind. But one should also be a person who knows a great deal so as to be able to formulate and firmly stick to the goals facing him.

Let this be my most important and sincere wish to you: learn to stick to a goal.

Other Books for Your Library

A. Lapo, Cand. Sc. (Min.)

Traces of Bygone Biospheres

This book serves two main purposes: (1) it is aimed at demonstrating that one of the leading factors which geologically transforms the face of the earth is life; (2) it is a tribute to the Russian biogeochemist Vladimir Ivanovich Vernadsky (1863-1945).

Topics covered in the book include the following: a general introduction to the biosphere; discussions of the distribution, chemical and mineral composition, enzymatic and catalytic importance, and abundance of living matter; and a review of the production and accumulation of biogenic material on the earth's crust. The final chapter deals with the "traces of bygone biospheres". Lapo briefly discusses the origin and occurrence of certain sedimentary rocks that are either generated by, or formed under the influence of, living matter. Examples of such rocks are limestones, dolomites, diatomites, radiolarites, coals, petroleum, phosphorites and certain ferruginous and aluminiferous rocks.

This volume is a good, non-technical introduction to biogeochemistry and the geological importance of life. It is fairly light, entertaining reading, well-referenced and profusely illustrated. Lapo includes brief biographical notes on important researchers who have contributed to the field.

This book could serve as supplementary reading for an introductory biogeochemistry course. Students will gain from it an appreciation of the importance of life to many geological processes on the surface of the Earth.

V. Pekelis

Realize Your Potential!

This book discusses the intellectual, psychological, and physical reserves which are possessed by every person. Also discussed are a number of scientifically founded examples and means for developing keenness of observation, memory, attention span, and creative capabilities as a whole. Touched upon are issues concerning the organization of creative work under conditions of informational overloading, the interrelationship between an individual and the collective, the control exerted by the collective and its improvement.

D. N. Trifonov, Cand. Sc. (Chem.) and
L. G. Vlasov, Cand. Sc. (Chem.)

Sillhouettes of Chemistry

The authors of this book have endeavoured to touch upon the most important and interesting problems of chemistry. Their book describes and explains the structure of the Periodic System of Elements; it guides you through a kind of chemical museum full of curious exhibits; it tells of the intricate substances chemists have produced and how they have learned to work even with single atoms of the elements; it acquaints you with the various chemical professions and shows how chemistry has penetrated all spheres of human activity. Reading these short but instructive stories about the exciting discoveries of chemistry will urge you to find out more about this fascinating science and to take up chemistry seriously.

To the Reader

Mir Publishers would be grateful for your comments on the content, translation and design of this book, we would also be pleased to receive any other suggestions you may wish to make.

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