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THE ROLE OF THE HYPOPHYSEAL-ADRENAL SYSTEM IN IMMUNOLOGICAL AND INFECTION PROCESSES

V.I. Ioffe and L.P. Kopytovskaya

In the study of the role of individual physiological systems in the regulation of immunological and infection processes, interest has justifiably been attracted to the hypophyseal-adrenal system; interest in this system has increased substantially with the concept of the adaptation syndrome, which is developed in such detail in the works of Sel'ye. The immediate occasion for our work in this field was certain problems in the pathogenesis and immunology of whooping cough, but our investigation very soon acquired a more general character. We have attempted to summarize the results of our work which are applicable to the general problem of infection pathology and immunology. We will consequently dwell on only three problems.

1. THE ROLE OF THE HYPOPHYSEAL-ADRENAL SYSTEM IN THE DEVELOPMENT OF AN ALLERGIC REACTION

The data in the literature and our experiments have shown that, after removal of the suprarenal glands, lethal anaphylactic shock develops on administration of small doses of an appropriate antigen to white mice, in which it is extremely difficult to induce an anaphylactic reaction under ordinary conditions. This was clearly manifested under conditions of active, passive, and inverse anaphylaxis, both indirect and direct.

In all of the experiments, the anaphylactic reaction clearly developed immunological specificity in adrenalectomized mice; the animals

well withstood the administration of control antigens. No anaphylactic reaction developed if specific desensitization was performed before the reacting dose was administered.

The fact that, no matter form the anaphylactic reaction took in the adrenalectomized mice, it could not be suppressed by adrenalin, but was inhibited by cortisone is of substantial importance.

We will not stop to enumerate the mechanisms which characterize an anaphylactic reaction in adrenalectomized animals, but will dwell only on the problem of which portion of the anaphylactic reaction is affected by removal of the suprarenal glands. Our investigations have shown that there is no basis for associating the development of an anaphylactic reaction in adrenalectomized mice with any quantitative or qualitative differences between the interaction of antigen and antibody in this case and that which is observed in control animals under identical experimental conditions. It must be concluded that removal of the suprarenal glands has no direct effect on the immunological (actuating) portion of the anaphylactic reaction and we must turn to the pharmacological (more precisely, the toxicological) portion. We are apparently dealing with an increase in the sensitivity of adrenalectomized animals to the product of the immunological reaction. This assumption is quite well confirmed by the established fact that there is a substantial (by a factor of several hundred) increase in the sensitivity to histamine of mice whose suprarenal glands have been removed.

In this connection, attention has been attracted by the problem of the sensitivity of adrenalectomized mice to an antigen-antibody complex; this problem was investigated in our laboratory by A.G. Artemova. The experiments were conducted not only with complexes formed when the appropriate ingredients were mixed in vitro in different ratios, but also with so-called natural immune complexes, which are formed in the organ-

TABLE 1

Toxicity of Immune Preparations Produced in Vitro for Adrenalectomized Mice (data compiled by A.G. Artemova)

A Иммунный препарат	B Лошадиная сыворотка + кроличья иммунная сыворотка	C Стрептококковый полисахарид + кроличья иммунная сыворотка
D Разведения иммунных сывороток	1:1 1:4 1:8 1:16	1:1 1:2 1:4 1:8 1:16 1:32
E Доза антигена	1:50—1:200	1:1000
F Результаты	12/12 7/12 5/12 4/12	4/4 4/4 4/4 2/4 3/4 1/4

Notes: 1) In tables 1 and 2, the number of animals is shown in the denominator and the number of animals dying is shown in the numerator.
2) Administration of the same preparations to normal mice did not cause them to die.

A) Immune preparation; B) horse serum and rabbit immune serum; C) streptococcal polysaccharide and rabbit immune serum; D) dilutions of immune serums; E) antigen dose; F) results.

ism during the immunization or infection process. The following results were obtained.

Immune preparations produced artificially by mixing various anti-

TABLE 2

Toxicity of a Natural Immune Complex (Sera from Rabbits Immunized with Horse Serum) for Adrenalectomized Mice (data compiled by A.G. Artemova)

A Срок взятия пробы	B Серология: D антиген	B Серология: D антитело	E Гибель адреналэктомированных мышей	
F До иммунизации . . .	—	—	0/12	
24 часа G	+	—	4/20	
4 дня H	+	+—	9/17	
7—8 дней H. . . .	+	+	11/14	
11—12 »	+	+	12/16	36/45
14—16 »	+и±	+	13/15	
20—35 »	—	+и— I	2/33	

A) Time at which sample was taken; B) serology; C) antigen; D) antibody; E) death of adrenalectomized mice; F) before immunization; G) hours; H) days; I) and.

gens with the appropriate immune sera and suspended in a buffer solution caused a substantial portion of the adrenalectomized mice to die, but they were not toxic for the control animals.

The toxicity of the immune complexes decreased as the dosage of immune serum used to form the complex was reduced and, to a lesser degree, depended on the amount of antigen added (when the immune serum dosage remained constant).

Sera from animals immunized with various antigens proved toxic for adrenalectomized mice when their aggregate state was changed. The toxicity of a serum differed during various periods of the immunization process and was correlated with the serological data; the most toxic samples were those in which the presence of the immune complex could be established by the serological method. These data are illustrated in Tables 1 and 2.

2. THE INFLUENCE OF THE HYPOPHYSEAL-ADRENAL SYSTEM ON SENSITIVITY TO MICROBIAL POISONS

Examination of this problem was a logical extension of the investigation described in the section above. Above all, this was an obligatory and necessary stage in the study of the role of the physiological system which we are considering in the infection process.

The data obtained reduced to the following.

After removal of the suprarenal glands, the sensitivity of the mice to endotoxins of gram-negative microbes increased sharply (by a factor of several hundred). This has been established in experiments with preparations prepared by Buaven's method, as well as in experiments with filtrates of broth cultures of salmonella, dysenteric microbes, and Bacterium coli. Conversely, sensitivity to exotoxins (diphtheritic, erythrogenic, streptococcal, staphylococcal, and Shiga's dysenteric toxins, as well as the toxins of pathogenic anaerobes were investigated)

increased by a factor of only 2-10 or did not increase at all.

It must be assumed that the principal differences between these two types of microbial poisons result from large differences in their properties, their points of application, and the mechanisms by which they act. A discussion of this problem would be far beyond the scope of this report. It should only be noted that, in the case of exotoxins, we are dealing with poisons which are specialized both with respect to point of application and with respect to the functional disruptions which they cause. For certain exotoxins, this may also be considered to be true of disturbances in the general biochemical processes which occur in the organism. Conversely, the endotoxins of microbes of the intestinal group seem to us to be nonspecialized poisons with a wide field of action. It is logical to assume that the regulating role of the hypophyseal-adrenal system should manifest itself to a greater degree for endotoxins than for exotoxins.

It should be recalled that certain manifestations of the action of endotoxins on the organism result from their influence on the suprarenal glands. The latter are also affected in immunological processes. Thus, according to our data, mice sensitized with horse serum to which a small dose of dysenteric endotoxin was added were observed to go into lethal anaphylactic shock three times as often as control animals which were sensitized only with horse serum. It is also known that small doses of endotoxin have a stimulating influence on antibody formation. It must be assumed that, under ordinary conditions, the administration of a sensitizing dose of endotoxin to an animal causes a corresponding compensatory reaction, intended to eliminate the disturbances, in which the suprarenal glands participate. This reaction is not realized in animals whose suprarenal glands have been removed.

In addition, the sharp increase in sensitivity to endotoxins

which occurs in andrenalectomized animals is of interest for infection pathology and in connection with the fact that it furnishes us with an approach to the study of the nebulous problem of antiendotoxic immunity. It is known that the very presence of a specific antiendotoxic immunity raises doubts. The cause of this uncertainty is the absence of large differences between the resistance of normal and immunized animals to endotoxins. This difference is usually no greater than four- to six-fold. This relationship is substantially altered if the experiments are conducted on andrenalectomized mice. In this case, as a result of the sharp decrease in the lethal dose, the difference between the resistance characteristics of immune and normal animals may be expressed as a factor of 35-40; this generates a substantially wider range for the appearance of specific immunity.

In addition, the possibility of using small doses of endotoxin (smaller than ordinary doses by a factor of several hundred) affords us an opportunity of making a volumetric analysis of the antiendotoxic properties of sera.

We must point out the characteristics of titration; when the mixture contains certain ratios, an immune complex whose administration can cause andrenalectomized mice to die despite neutralization of the poison is formed. The antiendotoxic titre of the serum must consequently be determined before the end-point.

3. THE ROLE OF THE HYPOPHYSEAL-ANDRENAL SYSTEM IN THE DEVELOPMENT AND COURSE OF INFECTION PROCESSES

In this section, we must dwell primarily on infections whose causative agents have a marked influence on the hypophyseal-adrenal system. According to the data in the literature and our data, Bacillus pertussis has such a marked inhibitory effect. The administration of a large dose of this bacillus to mice causes them to enter a state

similar to that which is observed after bilateral adrenalectomy; their sensitivity to histamine and to endotoxins of microbes of the intestinal group increases sharply. If the animals were sensitized with a foreign protein, a quite marked anaphylactic reaction can develop in them, this not being true of the control animals. The agent has not as yet been identified with any of the known antigens of Bacillus pertussis; it is impossible to be completely certain that we are not dealing with a substance of nonantigenic nature. The action of whooping cough vaccine is temporary; it is inhibited not only by cortisone, but also by ACTH.

We will not dwell just now on the problem of the role of these properties of Bacillus pertussis in the pathogenesis of the corresponding disease or on its significance for the important problem, as yet unstudied, of the high reactogenicity of whooping cough-diphtheria vaccine.

The question of the influence of disruptions of the function of the hypophyseal-adrenal system on the course of infection processes plays a substantial part in the problem which we are considering. In order to study this influence, we selected three experimental infections which differed in the localization and character of the infection process, the nature of its course, and the properties of the causative agent; these infections were of the paratyphoid, pertussal, and streptococcal types.

In the first case, we are dealing with an acute generalized process (which results in lethal sepsis when the causative agent is of sufficient virulence and dosage) caused by an agent which produces an endotoxin the sensitivity to which increases sharply when the activity of the hypophyseal-adrenal system is inhibited. It was shown that removal of the suprarenal glands caused a higher percentage mortality and

an earlier occurrence of death among these animals than among the control animals, which were innoculated with the same dose. This was not a result of more vigorous development of the microbial foci and an intensification of the infection process in the experimental animals. The more rapid occurrence of death and higher percentage mortality may be explained by a high sensitivity to the small doses of endotoxin acting within the organism, which the control animals withstood.

A similar phenomenon was recently observed by P.V. Osipova in experiments on mice innoculated intracerebrally with Bacillus pertussis. Using this innoculation method, an infection process could be induced in the control mice only if selected strains of Bacillus pertussis well adapted to brain tissue were employed. On innoculation with several tens of cells from such a culture, the microbial focus in the brain contains hundreds of millions of bacteria after 7-10 days. The process takes the course of a suppurative meningo-encephalitis and the animals die at the onset of aggravated symptoms of injury to the nervous system (paralysis). Innoculation of the brain with a virulent pertussal culture which is not adapted to brain tissue produces no effect even when large doses (hundreds of thousand of microbes) are used. Experiments on andrenalectomized mice yielded different results: firstly, the mice were observed to die both when innoculated with a neurotropic culture and when innoculated with an ordinary culture; secondly, the animals died earlier, 2-3 days after innoculation; thirdly, the microbial foci in the brains of the dead animals were quite small, rarely reaching 1 million cells. In these cases, we are thus dealing with death from toxicosis, which sets in even when the microbial foci are only slightly developed.

The experimental pertussal infection has also been studied under different experimental conditions, i.e., with aerosol innoculation. In

this case, a localized process developed in the lungs (acinic pneumonia); this process was characterized by a severe, prolonged cyclic course involving a gradual growth of microbial foci over a period of 3-4 weeks, a subsequent freeing of the organism from microbes, and a safe recovery. In this case, the animals respond by developing specific antibodies. The nature of the process made it possible to trace the influence of disruptions of the functioning of the hypophyseal-adrenal system after specific sensitization. This influence was evaluated from the epizootic among innoculated animals after adrenalectomy; in contrast to what occurred among the control animals, removal of the suprarenal glands caused a substantial percentage mortality, ranging from 13 to 45%, among the innoculated mice. In this case, the number of mice dying increased as the process developed, reached its maximum (45%) during the 3rd-4th week, and then decreased.

The percentage mortality of the innoculated animals was thus a function of the level of specific sensitization and the size of the microbial foci formed, i.e., the amount of antigen in the organism. It should be noted that adrenalectomy alone has no marked influence on the size of the microbial foci and does not lead to generalization of the process. In addition, the administration of pertussal antigen (agglutinogen) to the innoculated animals after removal of their suprarenal glands induced lethal anaphylactic shock in a substantial portion of the animals. In this case, the highest percentage mortality was observed to occur at times corresponding to the maximum development of the process and the beginning of its waning.

The problem of the influence of disruptions of the functioning of the hypophyseal-adrenal system on the development of a bacterial allergy is of basic interest in connection with infections having a prolonged course (in a number of cases, having a recurrent course). Such

phenomena include chronic infection processes of streptococcal etiology. L.M. Khay succeeded in reproducing a form of streptococcal focal nephritis having a prolonged course (extending over a number of months) in mice. The process caused an obvious immunological reaction in the animals. Using this experimental model, A.G. Artemova was able to establish that sera taken from innoculated mice at certain stages of the process were toxic for adrenalectomized animals; this must be attributed to the formation of an immune complex as the process developed. It must also be noted that removal of the suprarenal glands caused the same percentage mortality among the innoculated mice as did the experimental pertussal infection. Finally, the innoculated adrenalectomized animals died when a streptococcal polysaccharide hapten which the control (innoculated but intact) animals withstood well was administered to them.

The investigations whose results we have attempted to present briefly here are, in the last analysis, far from exhaustive in many respects. Our interference was limited by including only the final link in the system, although this link is very important and possibly decisive in the processes which we studied. We have not touched on any correlations with other systems, even similar ones. The role of the hypophyseal-adrenal system in the processes which interest us was evaluated from a complex, multistage reaction of the organism as a whole, with no proper and complete differentiation of individual stages. Finally, we did not deal with the intimate mechanisms of the reactions. All the same, it is necessary to attempt to generalize the established facts.

The role of the hypophyseal-adrenal system in the development of infection processes must be evaluated in different ways, in accordance with the nature of the process, the type of course which it takes, and the properties of the causative agent. This system is of the greatest

importance and can apparently manifest itself most clearly in infections having a prolonged (chronic) course and involving marked sensitization of the organism (infection allergy), as well as in diseases which entail the participation of endotoxins, which have a wide field of action, in contrast to the specialized exotoxins.

The thesis advanced above also makes it possible for us to map out our next tasks, which include a broadening of the study of the role of the hypophyseal-adrenal system in chronic infections, the study of the effects of the immune complexes formed during infection processes, the importance of the hypophyseal-adrenal system in the development of autosensitization, and a reexamination of the problem of antiendotoxic immunity and the role of the hypophyseal-adrenal system in the pathogenesis of specific diseases.

Finally, if we are to speak of the place which the study of the role of individual physiological systems occupies in the general scheme of immunological research, we must keep in mind the following fact. Modern immunological research has developed on different levels. The biochemical and morphological characteristics of immunogenic processes and immunological changes form the subject matter of research at the cellular and subcellular levels. The study of the regulation of these processes by individual physiological systems comprises the next level. Both areas of research lie within the field of experimental immunology. Another phase of such research, the investigation of immunity at the level of the entire organism, belongs chiefly to clinical immunology. This branch of research deals not only with immunological and specific pathological processes, but also with the immunological characteristics of a disease as a whole. Finally, the next level comprises epidemiological immunology, which deals with the immunological characteristics of an epidemic process, research being conducted at the level

of the human race. It is understood that the areas enumerated above are interrelated.

DISRUPTIONS OF NATURAL IMMUNITY IN IRRADIATED ANIMALS AND METHODS OF STIMULATING IT

V.L. Troitskiy

Disruptions of immunity resulting from exposure to radiation have attracted the attention of investigators for the past 10-15 years. A great many investigations have been reported in both Soviet and foreign publications. At the VII International Conference on Microbiology one session was spent on radiation effects on immunity. An International Symposium on this topic was held in the USA in 1961. The 3rd Inter-institute Conference on Radiation Immunology recently met in the USSR.

There are two reasons for interest in these problems. Firstly, disruptions of immunity play a large role in the pathology and therapy of radiation sickness. Secondly, radiation-induced disruptions of immunity provide science with a remarkable instrument for studying the mechanism of immunity and immunological processes.

If we consider the first aspect of the problem, we are faced with the question of whether the wealth of data on the role of the infection factor in the course and outcome of radiation sickness which have been obtained in experiments on laboratory animals and monkeys can be extrapolated to man. Sufficient material has now been amassed that we can answer this question in the affirmative.

The role of the infection factor in the course and outcome of radiation sickness, convincingly substantiated by Soviet investigators in experiments on laboratory animals and monkeys, is completely confirmed by data now available on persons suffering from radiation sick-

ness incurred as a result of the atomic bombings in Japan or as a consequence of accidents.

At the beginning of the last decade, the work of Lorenz, Jakobson, Barnes, Loutit, et al. showed that the percentage mortality of animals irradiated with a lethal dose of ionizing radiation could be sharply reduced by internal administration of hematogenic tissue cells from nonirradiated animals.

It has been established in experiments on mice that not only isologous, but also homologous and even hetrologous bone marrow can be implanted in irradiated animals. In this case, it was proved that the cells transferred from the donor are accepted by the recepient organism, multiply therein, and continue to function normally, maintaining their physiological properties in the recepient organism.

A fact of great theoretical importance was thus established. It is not only natural immunity to infection which decreases under the influence of ionizing radiation. The possibility of transplantation, especially heterotransplantation, of bone marrow in an irradiated organism is a conclusive proof of severe depression of natural immunity in its broadest sense. Ionizing radiation disrupts the earliest and most general mechanism of natural immunity, the mechanism of differentiation between "own" and "foreign." It is precisely this fact which makes the transplantation of foreign bone marrow and other tissues to an irradiated organism possible. The possibility of successful transplantation becomes even greater when dealing with massive irradiation doses which exceed the absolutely lethal ionizing radiation doses.

Among the many factors of natural immunity disrupted by irradiation, depression of hematogenesis plays an especially important role, since it is directly linked to two basic mechanisms involved in the protection of the organism, phagocytosis and antibody formation, which

ensure that the internal environment of the organism remains constant with respect to the microorganisms which enter it from the day of its birth.

Stimulation of hematogenesis by transplantation of hematogenic tissue is consequently of special importance in reestablishing natural immunity in irradiated animals.

The majority of work on the treatment of radiation sickness with bone marrow has been conducted on mice. When working with rats, we are confronted with a situation where the difference between the irradiation dose necessary for acceptance of nonisologous bone marrow, which is a dose sufficient to cause depression of immunity to transplantation, and the dose sufficient to cause an intestinal syndrome is very small. When rats are irradiated in doses sufficient to produce the bone marrow syndrome, the immunological system of the recepient is thus frequently not depressed to a sufficient extent, this causing the foreign bone marrow to be accepted only for a very short time. Under these conditions, one of the methods of bone marrow therapy developed in our laboratory reduces to the repeated administration of bone marrow. We did not observe the treatment of rats with homologous bone marrow to have any favorable effect when the marrow was administered once, on the day after irradiation. Only repeated daily administration of homologous bone marrow to the rats over a 10-25 day period after irradiation proved effective. Under these conditions, treatment with bone marrow averted death in 30% of rats irradiated with x- or γ-rays in absolutely lethal doses; the animals lived for 3 months or more.

Another method of bone marrow transplantation in rats when immunity to transplantation has not been completely depressed consists in suppressing the remaining immunological capacity with cortisone, which selectively depresses lymphoid tissue. This method is based on

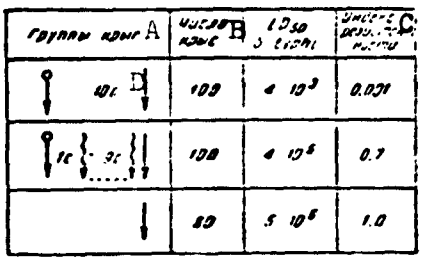

Fig. 1. Influence of bone marrow on the natural immunity of rats to S. typhi. A) Groups of rats; B) number of rats; C) index of resistance; D) S.

that proposed by Tulon, which entails homo- and heterotransplantation after the recepient has been treated with x-rays and cortisone. Our preliminary data showed that the therapeutic effect of a single administration of bone tissue to irradiated rats also treated with cortisone was more successful than the administration of marrow to animals which were only irradiated.

Experiments conducted in our laboratory on a large number of rats showed that bone marrow, which reestablishes hematogenesis, sharply increases natural immunity to infection in irradiated animals, raising it to the level of resistance in animals not exposed to radiation.

In order to investigate the influence of bone marrow on the natural immunity to infection of irradiated rats, an experiment was conducted on three groups of animals (Fig. 1) (M.A. Tumanyan and A.V. Izvekova). The rats in the first two groups were irradiated in a dose of 550 r. The animals in one group were treated with bone marrow on the day after irradiation. The bone marrow was administered daily for a 10-day period.

After 10 days, the rats in all three groups were innoculated with different doses of a culture of live Bacillus typhosus strain 4446. The LD_{50} and the index of resistance to innoculation were determined for the rats in all of the groups. The LD_{50} for nonirradiated, untreated rats was 5.48 million bacteria. The index of resistance to infection for the animals in this group was assumed to be equal to 1. The indices of resistance for the rats in the other groups is the ratio of the LD_{50} for the animals of the experimental group to the LD_{90} for

the animals in the control group. As may be seen from Fig. 1, the index of natural resistance for irradiated rats treated with bone marrow (0.7) was essentially greater than the index of natural resistance for the untreated irradiated rats (0.001), although still at less than 1.

The data obtained in this experiment thus show that transplantation of bone marrow to irradiated animals increases their natural resistance to infection.

We have available similar data on the stimulation of the natural resistance of irradiated animals to bacterial toxins (Fig. 2). D.R. Kaulen showed that the administration of homologous bone marrow cells to irradiated guinea pigs may substantially increase the resistance of the animals to diphtheritic toxin, which decreases under the influence of radiation. The index of resistance for the animals in the two groups treated with bone marrow thus equalled 0.69 and 0.87, as compared with 0.34 for the irradiated but untreated animals.

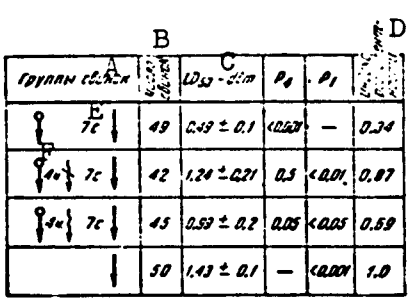

Fig. 2. Influence of bone marrow on the natural immunity of guinea pigs to diphtheritic toxin. If P is equal to or less than 0.05, the statistical difference between these values is reliable. A) Groups of guinea pigs; B) number of guinea pigs; C) LD_{50} – MLD; D) index of resistance; E) S; F) Ch.

A number of investigators, having ascertained that bone marrow cells are accepted when administered to irradiated animals, observed that animals cured of radiation sickness died later, 1-2 months after irradiation and treatment. These animals were subject to severe emaciation, dermatitis, shedding and depigmentation of the coat, and necrotic changes in the liver, intestinal tract, and skin. This syndrome and the death which follows it are called the "secondary reaction," "secondary illness," or "secondary syndrome." Curves representing the percentage survival of mice which

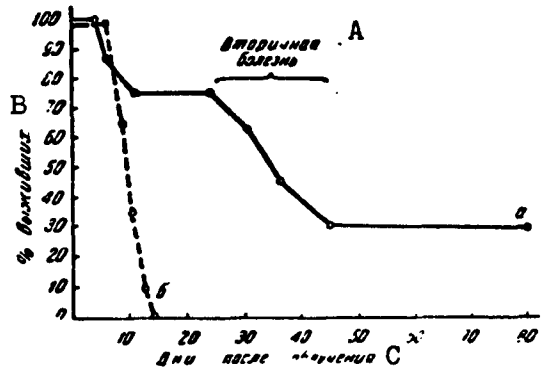

Fig. 3. Percentage survival of irradiated mice treated with bone marrow. a) Treated with bone marrow; b) untreated. A) Secondary illness; B) % surviving; C) days after irradiation.

developed this secondary illness are given in Figs. 3 and 4.

A great many experimental investigations have been devoted to the study of the mechanism of the secondary mortality of irradiated animals to which bone marrow cells had been administered. It is now acknowledged that the reason why these animals died later is an immunological conflict between the accepted cells from the donor and the regenerating tissues of the recepient.

When the immunologically competent cells of the host begin to function as a result of the incipient restoration of hematogenesis, an immunological reaction of the "host against the transplant" sets in. If the transferred bone marrow cells are accepted at a site where there is also a sufficient number of lymphoid cells, an immunological reaction of the "transplant against the host" sets in.

The majority of investigators believed that both reactions took the form of transplantation immunity reactions, i.e., reactions involving the cells themselves. Medovar showed that transplantation immunity is distinguished by the fact that it requires direct contact

between the transplant and the lymphoid cells of the host.

Trentin (1956-1957) and Uphoff (1957-1958) later advanced the hypothesis that the cells accepted from the donor develop an antibody against the cells or cellular antigens of the host in the reaction of the "transplant against the host."

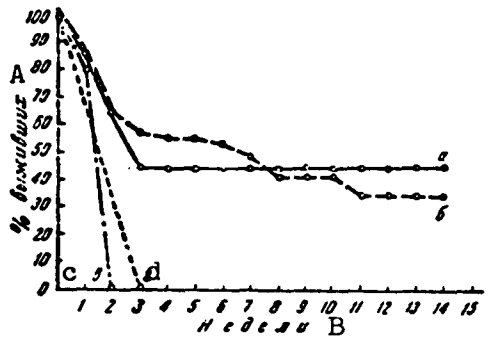

Fig. 4. Prevention of secondary illness by the administration of cortisone to irradiated mice treated with bone marrow. a) Treated with bone marrow and cortisone; b) treated with bone marrow; c) treated with cortisone; d) untreated. A) % surviving; B) weeks.

During the past 3 years, many investigators working on the processes which occur during the transplantation of homologous and hetrologous hematogenic tissue cells to an irradiated organism have shown an ever greater tendency to believe that, in this case, reactions occur which take the form of an ordinary immunological interaction between an antigen and an antibody. The reaction of the "host against the transplant" is also involved in the mechanism of the secondary illness. Makinadan, Gengozian, Shekarchi and Perkins, Carter and Peterson (1958, 1959, 1961), and Porter (1961) adhere to this viewpoint.

An unusual immunological situation arises when bone marrow is transplanted to irradiated animals. This situation is most marked when treating leukemia in mice by irradiation and the administration of bone marrow cells and, as was proved by Mate, when treating leukemia in man by the same method. Animals dying of severe secondary syndrome do not exhibit the symptoms of leukemia, since the accepted transplant acts against the leukemic cells. The transplanted cells are not accepted by animals which exhibit no symptoms of the secondary syndrome and the

animals die of leukemia.

Just as in treating leukemia by the combined use of radiation and bone marrow transfusions, the basic difficulty in treating radiation sickness with bone marrow is thus the secondary illness. This is the viewpoint of the most outstanding investigators in this field.

What is the nature of the immunological reaction which causes the secondary illness after transplantation of bone marrow to irradiated animals?

The following experiments were conducted in our laboratory (M.A. Tumanyan and A.V. Izvekova). White mice of the BALB strain were irradiated in doses of from 600 to 650 r; a suspension of bone marrow cells (15-20 million) from another strain of mice, C-57, was administered internally 1 1/2-3 hours after irradiation. The mice were bled at various intervals after the bone marrow was administered and 1.5-2 ml of a mixture of sera from 8-12 mice (pooled serum) was administered subcutaneously to guinea pigs. After 24 hours, suspension of bone marrow cells (30-40 million) from mice of the donor strain (C-57) was administered internally to these guinea pigs. An experiment in passive anaphylaxis in which the guinea pigs are prepared with serum from a sensitized mouse is thus set up; bone marrow cells from the donors are used as the reacting injection. An outline of the experiments and the principal results obtained are given in Fig. 5.

We will not dwell on a number of important technical details of the experiments, but will only note that lethal anaphylactic shock was very rarely observed. For the most part, we were dealing with anaphylactic reactions which may be designated by one, two, or less frequently three X's in the customarily used system. We could not attempt to assign any value to the difference in the intensity of the reactions, since it was clear that the differences in intensity were determined

to a large extent by the individual reactivity of the animals. The important fact was whether an anaphylactic reaction occurred or not.

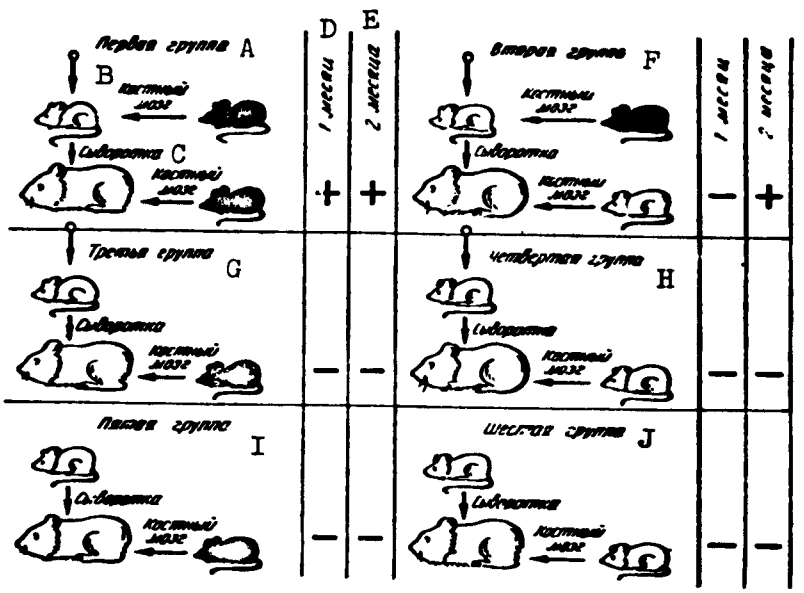

Fig. 5. Passive anaphylaxis in guinea pigs treated with serum from irradiated mice treated with bone marrow. A) First group; B) bone marrow; C) serum; D) month; E) months; F) second group; G) third group; H) fourth group; I) fifth group; J) sixth group.

As may be seen from Fig. 5, positive reactions were observed only if sera from the recepients which were injected with bone marrow were administered to the guinea pigs.

It should also be noted that positive reactions were exhibited by the guinea pigs of the first group even when the sera which they received were taken from the mice on the 8-9th day after administration of bone marrow. The intensity of the reactions increased and reached a maximum on the 40th day after administration of bone marrow. This intensity then decreased and weak reactions (+) were observed by the 60th day. In certain cases, positive reactions, albeit of low intensity, were observed in guinea pigs to which sera taken from the mice on the

3rd-4th day after administration of bone marrow had been administered. These isolated cases are still within the scope of our interpretation.

Positive anaphylactic reactions were also obtained when bone marrow from mice of the same strain as the recepients was administered to sensitized guinea pigs (the second group of guinea pigs). The fact that, in this case, positive reactions were observed only when the guinea pigs were sensitized with sera taken during the second month after they were treated with bone marrow is quite important. No anaphylactic reactions were observed in any of the guinea pigs treated with sera from irradiated and nonirradiated animals which did not receive bone marrow cells. Injection of these guinea pigs with bone marrow cells from either of the strains of mice as the reacting dose did not cause any reactions (the third, fourth, fifth, and sixth groups).

Our experiment on passive anaphylaxis in irradiated mice treated with bone marrow cells thus established that antibodies against these cells are formed. The irradiation apparently only reduced the level of the immunological response, but did not completely attenuate it; a reaction of the "host against the transplant" consequently developed. The antibody-forming fraction is restored as a result of the regeneration of hematogenic tissue, particularly of immunologically competent lymphoid tissue, and the quantity of antibodies formed by the host against the transplant reaches its maximum by the 40th day.

On the other hand, an antibody which interacts with the bone marrow cells of the host develops during the 2nd month after administration of bone marrow to the mice (the second group of guinea pigs). We are consequently dealing with a reaction of the "transplant against the host." Antibodies are thus developed against the host by the cells of the transplant and against the cells of the transplant by the cells of the host during the period in which the animals exhibit the greatest

mortality from secondary illness, the 2nd month after irradiation.

This experiment, conducted in our laboratory, shows that the fact that the animals die later is associated with immunological reactions which proceed in two directions, "host against transplant" and "transplant against host." We also wish to suggest that the possible role of anaphylactic reactions is underestimated when studying transplantation immunity and the secondary illness.

Of itself, the occurrence of passive anaphylaxis indicates only that the transferred serum contains antibodies against the antigen which was used for the reacting injection. However, the similarity between the morphological changes observed in mice during the secondary illness and the anaphylactic reactions which we have described, the increase in sensitivity to infection which occurs in both cases, and, finally, the fact that the secondary illness may be prevented in rats by repeated injections of antigen (bone marrow cells) permits us to advance the hypothesis that an anaphylactic reaction takes part in the secondary illness.

In this interpretation, the absence of later death on repeated administration of bone marrow cells is explained, from one standpoint, by the fact that the cells of the host are in a state of densensitization. The cells from the donor are not accepted (they only survive) and consequently do not become immunologically active.

The results of our experiments thus incline us to the viewpoint of those investigators who believe that the later death of animals to which homologous bone marrow has been administered results from immunological reactions which proceed in two directions. Our viewpoint approximates the position of Czech investigators (Lengerova and Zeleni), but we believe that a reaction of the "host against the transplant" occurs in addition to the immunological reaction of the "transplant against

the host" in the later stage of the secondary illness.

We also believe that there are certain grounds for assuming that an anaphylactic reaction takes part in the secondary disease of the homologous marrow chimeras. A knowledge of all these interrelationships is very important to the development of immunological methods for preventing a secondary illness.

Another method of preventing secondary reactions, which somehow are a symptom of tissue incompatibility, is the formation of new hematogenic tissue in irradiated animals from the tissues of the irradiated organism itself, this new tissue replacing the destroyed tissues.

A.A. Maksimov in 1907 and Huggins in 1931 showed that it is possible to induce bony tissue (occasionally accompanied by marrow) in the connective tissue of normal animals with the aid of transitional epithelium. The conditions for such induction were analyzed in work conducted by A.Ya. Fridenshteyn in our department. It was shown that it occurs in the presence of atypical submerged ingrowths of transitional epithelium into the surrounding connective tissue. As a result, an inductive factor is excreted from the epithelial cells and osteogenesis and hematogenesis develop in the connective tissue (Fig. 6).

Further experiments were directed toward determining the possibility of such induction in irradiated animals. It was shown that the capacity for induction is maintained in guinea pigs and rabbits irradiated with sublethal doses of x-rays which caused substantial disruptions of their own bone marrow.

We know know that different stages of the induction of bony and hematogenic tissue have a different sensitivity to the action of x-irradiation. If irradiation is conducted against a background of incipient induction (i.e., after transplantation of transitional epi-

Fig. 6. Homotransplant of transitional epithelium of a guinea pig. Time – 15 days. Fixation – Geli. Dye – azan. Induction of bony tissue. Objective – 20X.

thelium or after ligation of the blood vessels of the kidney), irradiation conducted during the period when immature osteogenic tissue is being formed around the epithelium (8-11 days in guinea pigs and 20-30 days in rabbits) depresses the induction of myeloid tissue. Conversely, irradiation conducted a longer or shorter interval after the beginning of induction does not prevent the development of hematogenic tissue, which can be obtained 5-8 days after irradiation, when regeneration is still weak in the bone marrow.

We now also know that the hematogenesis which develops in the vicinity of the transitional epithelium in irradiated animals lasts at least somewhat more than 2 months and, as may be seen from Figs. 7 and 8, proceeds in very intensive fashion.

Induction with the aid of live mucous tissue from the urinary tract, which is lined with transitional epithelium, is naturally only an experimental model which made it possible to demonstrate that the induction of hematogenic tissue in irradiated animals is possible. This

obviously is based on the fact that, as a result of relatively low sensivity, connective tissue cells are damaged to a lesser degree by irradiation than are the hematogenic cells and they thus retain a capacity for transformation. We still cannot exclude the possibility that transitional epithelium sets up especially favorable conditions about itself for the acceptance and myeloid transformation of the surviving cells after irradiation of lymphoid cells. A great deal of attention is now being attracted by the active inductive factor, free from living cells, which is execreted from transitional epithelium. General investigations conducted in this direction have shown that homogenates of transitional epithelium have an inductive activity equal to that of the secretion produced by this epithelium under certain conditions.

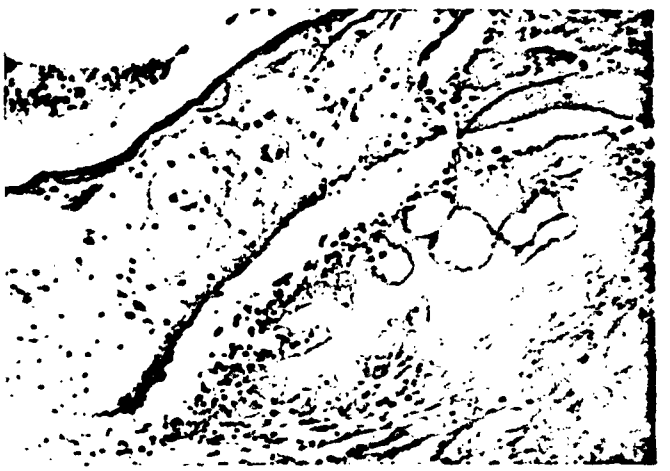

Fig. 7. Homotransplant of transitional epithelium in guinea pigs irradiated 20 days after transplantation. Time - 28 days. Bony and myeloid tissue. Fixation - Shabadash. Dye - ShIK-hematoxylin. Objective - 10X.

Interest in the induction of hematogenic tissue with the aid of transitional epithelium in irradiated animals is not only a result of theoretical problems. In order to evaluate this phenomenon, it is necessary to keep two facts in mind. Firstly, cases of the formation

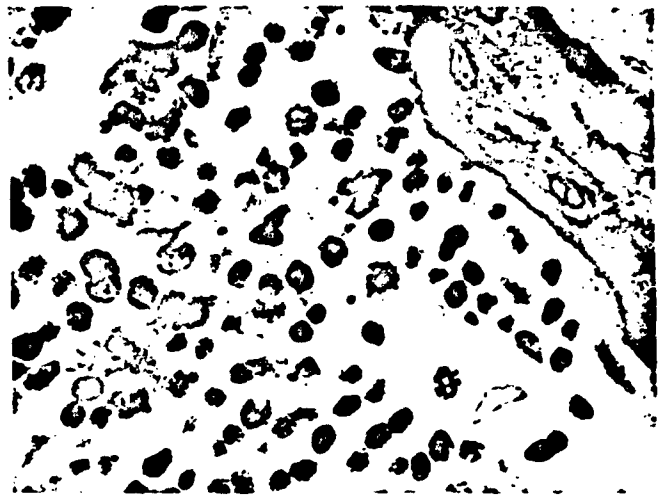

Fig. 8. The same subject as in Fig. 7. Objective - 40X.

of bone in the vicinity of growths of transitional epithelium were first observed in man and were described by pathologists and anatomists at the end of the 19th century. Secondly, hematogenic tissue induced from the tissues of an irradiated organism is obviously not immunologically foreign to it and consequently should cause no secondary illness, which is, as has been said, a fundamental obstacle to the use of bone marrow transplantation for treating radiation sickness and reestablishing the natural immunity of irradiated animals.

However, a great deal of work is still necessary in order that the inductive properties of transitional epithelium may be used in practice.

Summing up, we may make the following statements.

1. The reestablishment of hematogenesis is an important factor in the stimulation of natural immunity disrupted under the action of radiation on the organism.

2. Homologous transplants of bone marrow are a powerful stimulus

to hematogenesis and the reestablishment of the disrupted natural immunity of irradiated animals.

3. For preventing secondary reactions in such animals, it is wise to use an immunological method based on recognition that the immunological reaction in irradiated bone marrow chimeras proceeds in two directions, "host against transplant" and "transplant against host," and a method of inducing ectopic myelogenesis which eliminates the problem of tissue incompatability.

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[List of Transliterated Symbols]

16 с = s = sutki = days

17 ч = ch = chasy = hours

THE INFLUENCE OF CHEMOTHERAPEUTIC AGENTS ON THE PROTECTIVE FUNCTIONS OF THE ORGANISM

Kh.Kh. Planel'yes

At the very beginning of the development of chemotherapy, Ehrlich and Koch raised the problem of the importance of the protective reactions of the organism for the successful use of chemotherapeutic drugs in infectious diseases. However, at that point, the question was merely one of the relative importance of immunological reactions for ending the infection process against a background of suppression of the development of the causative agent or partial destruction of this agent and lysis. It was consequently assumed that the destructive action of etiotropic drugs on the microorganisms sensitive to them would favor an intensification of the answering immune reactions of the organism, this being defined as "ictus immunisatorius."

Further study of this problem has attracted a great deal of attention in recent years. The influence of new chemotherapeutic drugs (sulfonamides, antibiotics) on the extent to which immune bodies accumulate during treatment and convalescence has been studied in particular detail. Experimental investigation of the influence of chemotherapeutic drugs, particularly antibiotics, on the dynamics of antibody accumulation has established that a suppression of immunogenesis occurs. A detailed analysis of the results obtained showed that this suppression of immunogenesis may depend on the influence of the antibiotics on the antigenic structure of the causative agent, on the decrease in the quantity of antigen, and on the direct depression of the immunological

reactivity of the organism. However, it should be noted that, under clinical conditions, chemotherapeutic drugs are observed to have no such negative influence or only a slight influence on the development of the answering immunological reactions of the organism. This results from the fact that the use of chemotherapeutic drugs for treating patients usually is begun when the disease is in that stage of development in which a quantity of antigens sufficient to cause development of the corresponding immune reactions has already accumulated in the patient. In addition, the rapid destruction (lysis of microbes under the influence of antibiotics, many of which have bactericidal properties) which occurs can facilitate a rapid increase in antigenic stimulation, this causing an intensification of the immune reactions. Under clinical conditions, antibiotic therapy is observed to have no negative influence on the immune reactions of the organism, this resulting from the fact that the marked etiotropic effect of chemotherapy not only compensates for, but completely eliminates the complications which may arise as a result of depression of the immune reactions of the organism.

However, while the study of problems associated with the influence of chemotherapeutic drugs on immune reactions has received a great deal of attention among both domestic and foreign investigators, the study of the action of these drugs on other systems and functions of the organism which play a large role in protecting it from infection have not attracted sufficient attention. Among the neglected aspects of this subject is the fact that, while depression of the answering immune reactions is of no substantial importance for the course of the infection process, a decrease in the resistance of the organism or a change in its reactivity to infections can facilitate the emergence of complications caused by the development of other microorganisms which are present among the so-called normal flora of the organism or

which chance to be inhabiting the mucous membranes of the patient's body.

Over a number of years, it has been shown that the nature of the action of antibiotics (in particular, their property of only depressing the vital activity of specific types of bacteria and their inability to exhibit a marked antibacterial action in the lymph nodes of the diseased organism) generates conditions which may facilitate the development of severe forms of illness or superinfections.

Among the complications of antibiotic therapy, so-called superinfections are worthy of special attention. It is true that superinfections are not observed very frequently, usually present no great danger, and often pass unnoticed by the physician, but they may occassionally become of serious import, a fact which may be inferred from the increasing number of papers on this subject which have appeared in the world literature.

The study of the genesis of these complications and the mechanism by which they develop is currently occupying the workers of the department which I head, since discovery of the pathogenesis of the the superinfections which occur during antibiotic therapy may be of great theoretical and practical importance for infection pathology.

Ten years ago, in 1952, in a study of the action of antibiotics on healthy laboratory animals, we established that it was possible to develop generalized autoinfections in the animals, these infections being caused by temporarily pathogenic bacteria which usually inhabit the intestinal content and are insensitive to the administration of a preparation to the organism. There was every reason to suppose that superinfections arise in the same fashion, independently of the nature of the disease, in patients undergoing antibiotic therapy.

It is necessary to mention that the majority of authors who dealt

with this problem believed that the principal cause of superinfections was the changes in the "normal" microflora of the organism which developed under the action of antibiotics and, moreover, that the causative agents of superinfections could only be strains or types of microorganisms stable to the preparations used. It was established that a selection of less sensitive or totally insensitive species, strains, and types occurs under the action of active antibacterial substances and that this selection depends on the preparation used, on the size of the single and daily doses, and on the duration of the treatment. However, in studying the various factors which determine the development of superinfections, it becomes quite clear that a generalized infection caused by permanent or accidental inhabitants of the mucous membranes of the organism cannot arise merely as a result of qualitative or quantative changes in the bacterial flora of the oral cavity or the content of the gastrointestional tract. It has long been known that all experiments on the artificial stocking of the intestine with new types of bacteria end in failure and, if it is possible to develop certain strains of bacterial which have a high "antagonistic index" (after Nissl), these bacteria can cause temporary changes in the "landscape" of intestional microflora, but never cause infections.

A change in the "normal" microflora, so-called disbacteriosis, essentially determines only the specific type or strain of microorganism which can serve as the causative agent for the developing superinfection. However, the superinfection itself or, more precisely, the conditions favorable to the development of a superinfection arise as a result of the action of antibiotics on the various systems and functions of the macroorganism which determine its susceptibility to infections.

According to our data, the following factors which may facilitate

the development of superinfections during antibiotic therapy should be noted: 1) disruption of the barrier function of the epithelium, expecially the epithelium of the nasopharynx and the intestinal tract; 2) intensification of hormone secretion from the adrenal cortex, these hormones having"proinfection properties"; 3) depression of the phagocytic and antitoxic activity of the endothelial-microphagic cells of the lymph system and the capillaries of the blood channel; 4) "biotic release" of types of temporarily pathogenic microorganisms insensitive to the antibiotic; 5) infection within the hospital itself with strains of microorganisms which are naturally insensitive or resistant to the drug used.

When analyzing these factors, it is necessary to take into account the fact that neither qualitative nor quantative changes in the composition of the microorganisms which are permanent or accidental inhabitants of the mucous membranes have any influence whatsoever on their virulence. The ability of these microorganisms to cause superinfection results from an increase in the susceptibility of the macroorganism. This increased susceptibility results primarily from disruption of the integrity of natural barriers, especially from damage to the protective epithelium under the action of antibiotics, and possibly from the combined influence of the antibiotics and the toxic products liberated when sensitive bacteria are decomposed and the vital activity of the "new" microflora. Sole had already shown in 1950 that a marked decrease in the viability of the cells of the mucous membrane of the oral cavity is observed under the influence of penicillin (i.e., an antibiotic which is generally considered to be a virtually nontoxic drug). The works of A.M. Kharitonova (1957, 1958) proved that, no matter what the method by which they are administered (per os, parenterally), antibiotics of the tetracycline group cause various affections of the mucous membranes of

the gastrointestinal tract (total exfoliation of the epithelium, necrosis of the mucous layer, and edema of the muscular and serous layers in certain cases and tumefaction and partial desquamation of the epithelium in other cases), the extent of these affections depending on the sensitivity of the type of animal to these antibiotics and even on the individual sensitivity of specific species. Similar phenomenon are observed when patients dying of diseases not associated with the gastrointestinal tract (pneumonia) are treated with tetracyclines, as well as in patients to whom drugs of the neomycin group have been administered per os (Jacobson, Prior, Faloon, 1960).

Severe affection of the mucous membranes of the gastrointestinal tract is observed only when penicillin or erythromycin is administered to highly sensitive animals (guinea pigs, hamsters) (Tonutti, 1953; Rolle and Mayer, 1953; Tigertt and Gochenour, 1957). Disruption of the barrier function of the mucous membrane of the intestine is also observed when large doses of actinomycin and aurantine are administered parenterally (intravenously, in a dose of 100 - 150 γ per kg of body weight). N.P. Gracheva and A.M. Kharitonova conducted experiments to prove that the nonpathogenic bacteria detected in the organs of the experimental animals came from the intestinal content. They succeeded in establishing that a culture of B. prodigiosus administered per os to rabbits disappeared from the intestinal content one and one-half hours after administration, while in animals which received aurantine, B. prodigiosus could be detected after 30 minutes not only in various regions of the intestinal tract, but also in the mesenteric glands, the kidneys, and the spleen. After administering chloromycetin to mice, K.A. Akhundova extracted Bacillus pyocyaneus and Proteus pyocyaneus, which predominate in the intestinal microflora of the animals, from their tissues and organs.

Our attention is also drawn to the fact that the implantation of specific foreign strains of bacteria in the intestine is especially easy to accomplish if bacteria stable to tetracyclines are used and antibiotics are administered simultaneously (Lischka and Prohaszka, 1958). This may also explain the well-known fact that, under hospital conditions, so-called intrahospital infections spread more readily and occur more frequently among patients treated with various antibiotics, naturally provided that the corresponding causative agents are insensitive to the antibiotic used (Moebius, Moebius and Waumgaertner, 1958; Berntsen and McDermott, 1960). It is impossible to explain these superinfections as being the result of a disruption of the "normal landscape" of intestinal microflora by the antibiotic, since superinfections caused by Pseudomonas aeruginosa have frequently developed in patients treated parenterally with penicillin (Stanley, 1947). The same may be said of the cases of bronchopneumonia, meningitis, and pyelonephritis, accompanied by bacteremia, which occur after administration of streptomycin (Weinstein, 1947).

It was long ago established that bacteria capable of passing through the lymphoid tissues of the tonsils or intestine under normal conditions or even through the epithelial coat of the digestive tract when it is damaged are, as a rule, immobilized and destroyed in the regional lymph nodes (Linzenmeier, 1961). In cases where superinfections develop when antibiotics are used, it may be established that not only is the barrier function of the epithelial coats of the nasopharynx and the gastrointestional tract disrupted, but there is also a marked depression of the barrier function of the regional lymph nodes and of the endothelial macrophagic system as a whole. This may explain the fact that intramuscular administration of streptomycin in small (nontherapeutic) doses to mice innoculated intraperitoneally with a

culture of S. typhosa hastens the animals' death (Welch, Price, and Randall, 1946) and that the death of mice innoculated intraperitoneally with a culture of P. aeruginosa is also hastened when the animals are treated by subcutaneous administration of chlortetracycline (Jackson and Axelrod, 1954). Similar cases in which patients died from superinfections caused by P. aeruginos during treatment of postnatal sepsis with large doses of penicillin have been described in the literature (Hodges and de Alvarez, 1960).

We now have available a sufficient number of data which demonstrate that, under certain conditions, the microflora of the mucous membranes can become of pathogenic importance when the epithelial barriers of an organism insensitive to the antibiotic administered are first disrupted, this resulting from a depression of the phagocytic ability of the macrophagic-endothelial system. N.A. Kalinina (1959) observed such depression under the action of chloromycetin administered per os to mice and rabbits.

In this connection, it is especially important to note that a definite intensification of the functions of the adrenal cortex is observed when antibiotics are used. Thus, Trams, Kashiwa, Cornman, and Klopp (1955) established that, after intraveneous administration of 50-100 mg/kg of chlortetracycline to rats, the content of ascorbic acid and cholesterol in the adrenal glands and the number of eosinophils in the blood channel decreased, this indicating considerable stimulation of the functions of these organs under the influence of the antibiotic. A decrease in the number of eosinophils in the circulating blood under the influence of the administration of penicillin or chlortetracycline to rats and guinea pigs was also observed by Brvegemann. Karg, and Scholl (1956), who concluded that these drugs have a stimulating influence on the adrenal cortex. In his experiments, Mosonyi

(1959) showed that the functional activity of the adrenal cortex of a rat can increase substantially under the influence of penicillin or streptomycin. Our colleagues N.A. Ozeretskovskiy and O.Sh. Dzheksenbayev also established that there is a substantial increase in the concentration of 17-oxycorticosteroids in the plasma of the peripheral blood of guinea pigs to which actinomycin has been administered.

Hence it follows that a definite increase in the quantity of circulating adrenal hormones may be expected during antibiotic therapy, this, as is well-known, depressing the activity of the cells of the macrophagic-endothelial system and exerting a negative influence on the protective function which comprises the absorption of bacteria and the neutralization of toxins. Under these conditions, the bacteremia can develop into a lethal septicemia and the circulating bacterial toxins may continue to have a necrotizing effect on the blood vessels. It has long been known that the depression of phagocytosis, immunogenesis, and the activity of the reticuloendothelial system which results from activation of the functions of the adrenal cortex (Thomas, 1953; Kass, Geiman, and Finland, 1953) at the onset of an infection whose causative agent is insensitive to the antibiotic used inevitably leads to disruption of the protective barrier mechanisms, an intensification of the dissemination of pathogenic agents, an increase in the severity of the disease, and the death of the patient.

In this connection, we should note the fact that the similar negative influence of stimulation of the functions of the adrenal cortex during antibiotic therapy which occurs when superinfections develop is not counteracted by the favorable effect of the combined administration of active adrenal preparations (cortisone, hydrocortisone), ACTH, and antibiotics in the treatment of tuberculosis or other infections, provided that the causative agent of the disease is sensitive to the pre-

paration used. In such cases, despite the "proinfection" properties of the glucocorticoids of the adrenal cortex, the depression of the inflammatory reaction and the formation of granulation can facilitate diffusion of the antibiotics in the damaged organs and increase their antibacterial effectiveness (Lambros, 1958; Fusco, Alexanian, and Tozzi, 1956). The combined use of adrenocorticosteroids and antibiotics (when it has been indisputably established that the causative agent is sensitive to the antibiotic) is undoubtedly one of the most effective methods for treating acute and persistent bacterial infections in which marked intoxication, hypotonia, and an inflammatory reaction are observed (Ribble and Braude, 1958; Des Prez and Organick, 1958). On the other hand, we have come to understand why a bacteremia, which frequently complicates the clinical picture of the disease substantially, may frequently be observed in many cases involving a reaction to stress and a state of shock (Frank, MacDonald, Palmerio, Schweinburg, and Fine, 1961).

A whole series of factors thus participate in the genesis of the superinfections which develop during the antibiotic therapy of bacterial infections, these factors leading to an increase in the susceptibility of the organism to infections whose causative agents may be only slightly pathogenic and may even be the saprophytes which are always present on the surfaces of the mucous linings of man or animals, provided that they be insensitive to the preparations used. A change in the microflora of the intestinal content or the nasopharyngeal mucus is important insofar as it establishes the strain or species of the next infection-inducing microbe. Maintaining the same virulence, it can become pathogenic as a result of: 1) disruption of the barrier function of the epithelial coats, 2) depression of the barrier function of the regional lymph nodes, and 3) activation of the functions

of the adrenal cortex, which secretes a hormone having a "proinfection" effect. All this proceeds against a background of the administration of antibiotics which are inactive with respect to the causative agents in question. Hence we may understand how necessary it is that the sensitivity of the causative agent to the drug used be carefully determined not just at the beginning of the treatment of a given infection; when superinfections develop, we must keep in mind that the initially active drugs have probably already lost their effectiveness and only treatment based on identification and study of the new causative agent and determination of its sensitivity to other drugs can ensure success.

However, the development of superinfections during the treatment of bacterial infections with antibiotics is encountered relatively rarely. It is very probable that the three factors on whose presence the development of superinfections depends do not arise when antibiotics are used for short periods or when specific conditions of patient reactivity exist. Timely cessation of the use of antibiotics also facilitates the rapid reestablishment of disrupted functions (the barrier function of the epithelium, the functions of the adrenal cortex, and the activity of the reticuloendothelial system) and the elimination of conditions favorable to an increase in susceptibility to slightly pathogenic causative agents.

STIMULATION OF THE PROTECTIVE FUNCTIONS OF THE ORGANISM WITH PHYSICAL AGENTS

A. N. Obrosov, F.D. Vasilenko and Ye.B. Markovnikova

The human organism possesses various systems and functions which ensure that it adapts to the conditions of its surrounding environment. Equilibration of the manifold influences exerted by this environment is carried out by these systems, with the direct participation of the nervous system.

The physical factors of the external environment are very powerful sources of various influences, adaptation to which is one of the most important facets of the vital activity of both healthy and ill individuals. The organism responds to the action of these factors with a number of reactions, which are intended to maintain the most important physiological functions such as blood circulation, respiration, temperature regulation processes, metabolism, etc. and sustain them at the level necessary for normal functioning. On this basis, it has long been customary to consider the physical factors used in physiotherapy as a means of exerting a nonspecific influence. However, each of the physical factors has a number of properties characteristic only of itself; it is natural that the organism should respond to the action of such a factor with a reaction general to a number of factors, responding not only as it would to the action of a nonspecific stimulus, but also as it would to the action of a specific stimulus. It is precisely this dependence of certain reactions of the organism on the specific characteristics of various physical factors used in modern physiotherapy

for therapeutic and prophylactic purposes which is the most important single feature of the nature and value of physiotherapeutic treatment. It is this characteristic property which makes it possible to use a certain factor or method of treatment in order to act purposefully and specifically on a certain physiological function and facilitate its normalization when it has been disrupted as a result of pathological processes.

It is very important for the theory and practice of public health that the general mechanisms by which the reactions of the organism to the action of physical stimuli are effected be established and that the actual physicochemical nature of these stimuli be studied. Determination of the specific nature of the action of physical stimuli on the organism makes it possible for the physician to establish the method which enables him to treat the pathological process and which itself may be used for prophylactic purposes. Consequently, the choice of an agent and of the method by which it will act on the appropriate link in the pathological process, constantly keeping in mind the functional state of the organism as a whole, is the basis of pathogenetic therapy with physical agents. Treatment with these factors in order to reinforce and increase the resistance of the organism to the unfavorable influences of the external physical environment is the basis of physical prophylactics. In order to maintain the functional mobility of the physiological systems, the effect produced by the use of a physical agent must manifest itself in an intensification and stimulation of the mechanisms of adaptation or protection against disease which the organism has at its disposal and which may be weakened by a pathological process.

The form and extent of the organism's responses to the action of physical agents are very diverse, but their direction and differentia-

tion obey definite rules. Specific characteristics of the protective capabilities of the organism manifest themselves in the first stage of the action of physical stimuli on the neuroreceptor apparatus of the skin. Thus, carbon dioxide and hydrogen sulfide, which are powerful stimuli, act at the level of excitability of the dermal receptors. An increase in the excitability of the dermal receptors and phase changes in the excitability which are characteristic of functional reorganization are observed under the action of these stimuli (K.D. Gruzdev). The reaction of the dermal receptor apparatus to the action of stimuli specific for them (for example, carbon dioxide and hydrogen sulfide) is a partial manifestation of the reactivity of the organism as a whole. The relationship between excitation and inhibition processes in the cerebral cortex, as determined from a conditioned-reflex reaction, is substantially altered under the action of these two stimuli. When carbonic acid, used in the form of a bath in the experiment, acts on the dermal analyzer, it causes an intensification of the excitation process, while hydrogen sulfide preferentially affects inhibition processes. The use of a series of baths is accompanied by the formation of a qualitatively new and maximally intensified functional state in the central nervous system; this state is stably maintained, a fact which may be evaluated from the conditioned-reflex activity of the cerebral cortices of dogs and electroencephalographic data (T.I. Afonina). It is obvious that the specific action of these mineral waters on the basic processes in the central nervous system (V.N. Peshchikov) make it possible to use them for therapeutic purposes in disorders of higher nervous activity - neuroses (B.V. Likhterman).

The systematic use of carbon dioxide baths for dogs which have been subjected to physical stress facilitates a more rapid development of the adaptational reactions of the organism. Carbon dioxide baths

administered after brief but intensive physical stress reduce the recovery period, which is determined from the state of the neuromuscular and respiratory systems and the body temperature, this being a manifestation of one of the forms of stimulation of the adaptational functions of the organism (A.I. Zol'nikova).

Stimulation of the protective capabilities of an organism by physical stimuli may be observed especially clearly in an organism weakened by pathological processes. It is quite distinctly displayed when physical stimuli are used on experimental models of certain diseases.

One of the central problems of radiobiology is the search for effective methods for the prophylaxis and treatment of radiation damage. Data on the use of aeroionization (positive and negative) in acute radiation sickness in an experiment on rats are consequently of definite interest. According to the experimental data, therapeutic use of this agent does not lead to any positive results, but prophylactic aeroionization involving both positive and negative charges substantially increases the animals' resistance to γ-rays. In this case, the threshold of sensitivity for positive ions was less than that for negative ions; the effective courses corresponding to these two cases were of different durations. In the experimental groups, 50-70% of the animals lived to the 20-30th day after irradiation, while all of the control animals died during this period (L.V. Serova). The indices of peripheral blood and gaseous interchange at specific intervals after irradiation coincided with the clinical picture of the disease (L.V. Serova, M.I. Fedotova).

During hyperergic inflammation, Arthus' phenomenon developed substantially more slowly under the influence of small doses of ultrasound than it did in the control group, resorption of infiltrates and tissue regeneration were accomplished more rapidly, and the percentage

mortality from anaphylactic shock was halved (N.F. Svadkovskaya). It is obvious that small doses of ultrasound had a desensitizing effect in this case.

A manifestation of the same desensitizing action of physical agents may be seen in an experimental model of rheumatism under the action of ultraviolet rays applied in erythema doses; the pathological condition of the joints disappears 2-3 times as rapidly as in the control group, the phagocytic activity of the leucocytes increases rapidly, and the intensity of the proliferative-infiltrative changes in the endomyocardium decreases (T.V. Karachevtseva). The desensitizing action of ultraviolet rays and the stimulation of immunobiological activity by these rays is also clinical observed in active rheumatism; against a background of a clinical course of the disease which is more favorable than that which occurs in the control group, an earlier and more complete reestablishment of such indices of immunity as the phagocytic activity of the leucocytes, an increase in the titre of complement, a rise in the antihyaluronidase level, an increase in the diphenylamine index, etc. quite clearly manifested itself. These positive results lasted for a number of years in children subjected to a course of irradiations with ultraviolet rays; relapses into progressive rheumatic damage to the heart were observed only half as often as the frequent relapses which occurred in children who were only treated by drug therapy (T.V. Karachevtseva, K.S. Kustareva).

Favorable changes in the content of phagocytes in the blood as compared with the phagocyte content in persons in the control groups were observed in patients who were irradiated with x-rays in small doses both in the region of the heart and in the vicinity of the damaged joints and simultaneously received salicyl preparations (N.K. Pozdenyeva).

According to the data of L.V. Iyevleva, the titre of complement during the active phase of the cardioarticular form of rheumatism decreased in 78% of his patients, the extent of the decrease corresponding to the severity and acuteness of the disease. The use of a complex treatment involving x-rays (in a dose of 100 r) appliedto the region of the damaged joints and the simultaneous administration of salicyl preparations had a marked positive influence on the course of the rheumatic process and was accompanied by favorable shifts in the titre of complement in the majority of patients. Thus, when they entered the clinic, 22% of the patients exhibited a normal titre of complement, while 50% had reached this titre when they left the clinic. The decrease in the number of patients with a sharply reduced titre of complement (20% as compared with 49%) is especially characteristic and indicates an intensification of the protective reactions of the organism in these patients. No similar favorable effect could be observed in the patients of the control group, who were treated only with salicylates.

The severe disruptions of carbohydrate and protein metabolism (pathological diabetoid-type glycemic curves after intravenous administration of glucose, a sharp decrease in the albumin-globulin index) observed during the rheumatic process disappeared in the patients who were irradiated with x-rays in the region of the damaged joints and the heart and simultaneously received salicyl preparations. During the period of clinical improvement, the majority of patients who were irradiated with x-rays in the region of the heart and simultaneously received salicylate therapy (Z.S. Kuleshova) exhibited a normalization of the pathological glycemic curves and the indices of serum protein composition, this normalization manifesting itself as an increase in the albumin content and a decrease in the α_2- and γ-globulin fractions (Ye.B. Markovnikova). Favorable dynamics were also observed for the

indices of serum protein composition of the blood in rheumatism patients irradiated with ultraviolet rays in the region of the damaged joints and also receiving salicylate therapy (T.M. Kamenetskaya); no such favorable dynamics could be observed in patients who received only salicylate therapy. Dynamic observations made during the use of various physical therapeutic methods on patients suffering from lobar pneumonia also showed that a more rapid normalization of the phagocytic activity of the neutrophils and disappearance (as determined from the glycemic curve and the protein formula of the blood) of metabolic disruptions occurred in patients treated with sulfanilamides in conjunction with inductothermy (Ye.B. Markovnikova, N.K. Pozdneyeva); this was also reflected in the clinical status of the patients. The process of inverse development of the inflammatory infiltrate in the lungs set in earlier under the influence of the combined therapy than under sulfanilamide therapy alone. In addition, combined therapy of lobar pneumonia leads to a decrease in the work time which the patients lose as compared with that lost by patients who received only sulfanilamide therapy (N.A. Glagoleva).

Physical factors affect the deep-seated, intimate processes which occur in the organism, including the activity of its fermentative systems. Stimulation of fermentative activity in the organism under the action of physical agents may be observed during single therapeutic baths or courses of such baths. At a temperature of 46-44°, the activity of the respiratory ferments - succinodehydrogenase and cytochromoxidase - increases in comparison with the control, cholinesterase activity increases, and alkaline phosphatase activity decreases (I.A. Ul'm). The same results were obtained in experiments involving the use of a pulsed ultrahigh-frequency electric field in extrathermal doses (V.L. Kardashev).

It has been noted that oxidation-reduction processes in the tissues are intensified and the activity of cellular ferments such as the carbonic anhydrase and succinoxidase of the adrenal glands is stimulated under the action of negative aeroions in therapeutic doses (Worden, Nielsen, Harper).

Under the action of hydrogen sulfide baths, the fermentative activity of anaerobic dehydrogenases in animals with experimental arteriosclerosis is more intense than that of the aerobic dehydrogenases, a fact confirmed by data gathered in studying the state of the oxidation-reduction potential of tissue. This important biological fact indicates that there is a stimulation of the adaptational functions of the organism, this stimulation being effective by changes in the direction of the oxidation-reduction processes (V.A. Shalimov). Under the action of small doses of ultrasound, stimulation of tissue respiration and glycolysis in various sections of the brain is observed both immediately after exposure and two months later (N.F. Svadkovskaya). Many authors (I.K. Talanova, Yu.D. Zhilov, V.S. Svarichevskiy, et al.) have observed a normalization of alkaline phosphatase activity under the action of whole-body irradiations with ultraviolet rays conducted for prophylactic purposes. In passing, we should mention that these authors also indicated that there is an increase in the vital capacity of the lungs, an increase in hemoglobin content, a rise in the number of erythrocytes, normalization of the number and composition of leucocytes, a decrease in morbidity and a gain in weight for infants, and a substantial decrease in the period of temporary incapacitation for adults.

A tendency toward stimulation or normalization of the depressed functioning of the endocrine metabolism mechanisms was observed to occur under the action of physical agents. For example, in active rheumatism, the functions of the adrenal cortex were activated under

the action of irradiation with ultraviolet rays both experimentally and in the clinic. The same effect has also been observed to result from exposure to inductothermy in the vicinity of the adrenal glands during nonspecific infectious polyarthritis; the increased content of potassium in the blood simultaneously decreases and the amount of sodium returns to a normal level (Ya.Ye. Shapiro, Ye.M. Miloslavskiy, et al.). The use of a course of hydrogen sulfide and carbon dioxide baths in experimental arteriosclerosis in animals and during the arteriosclerotic process in man reduces the quantity of cholesterol in the blood, changes the ratio of the lipoprotein fractions of the blood, which was disrupted by the pathological process, and improves the hemodynamic indices, i.e., the new protective capabilities of the organism inhibit further development of the disease (F.D. Vasilenko, S.Kh. Kubli, Z.A. Sokolova, A.F. Tkachenko, et al.). Similar results have been obtained in various stages of the development of arteriosclerosis under the action of novocain-electrophoresis used in a special manner (N.A. Kaplun, N.K. Pozdneyeva, Ye.B. Markovnikova, et al.). The physiological mechanism of this favorable effect is complex. This process involves primarily the nervous and endocrine systems. In particular, it is known that these stimuli produce their effect by mobilizing the sympathetico-adrenal resources of the organism, a fact which has been shown in special pharmacological tests and on animals subjected to experimental physical stresses (N.R. Chepikova, V.L. Kardashev).

These isolated examples far from exhaust the many data on the marked influence of various physical agents on the physiological mechanisms which protect the organism from disease or increase the resistance of the organism to unfavorable influences exerted by its external physical environment. They only serve to give some idea of the diverse

results obtained in experimentation and in the clinic which indicate that the use of physical agents makes it possible to change the dynamics of immunobiological, fermentative, and oxidation-reduction processes, etc. In addition, the data cited confirm the statement made above that only the differential use of physical agents in accordance with the characteristics of the pathological process can produce a favorable effect.

The experimental material demonstrates the intensification of adaptational and trophic processes which occurs under the action of physical stimuli. Its biological significance is increased by the fact that the importance of the psychological factor which is generally encountered in man when conducting an investigation is substantially reduced in experiments on animals. The experimental data enable us to understand the mechanism by which physical stimuli exert their stimulating action under various conditions and permit us to devise methods for the correct use of these stimuli for therapeutic and prophalactic purposes in the clinic. On the other hand, the clinical data, which are in agreement with the results of experimental investigations, permit us to assume that the differential use of a certain physical agent in strict accordance with the characteristics of the pathological process in question can have a favorable effect and consequently can and should be used in the complex treatment of various illnesses.

THE ROLE OF ANAEROBIC AND OXIDATIVE PHOSPHORYLATION PROCESSES IN THE PROTECTIVE FUNCTIONS OF THE ORGANISM

S.Ye. Severin

The object of this paper is to show that ATP plays a multifaceted role in the various functions of the organism and that this compound in formed in two ways, anaerobic and oxidative, in cells and to emphasize the high efficiency and regulability of this latter mode. In conclusion, we will dwell in detail on the significance and utilization of this regulability in the protective functions of the organism*.

The energy produced in metabolic processes is partially dissipated in the form of heat and partially stored within the organism in the form of energy-rich phosphorus compounds. A characteristic property of these compounds is the fact that the free energy liberated during their cleavage can be used for any of the organism's needs - muscular work, neural impulses, absorption or secretion processes, biosynthesis of protein, or oxidation of fatty acids. Hence we may understand the enormous significance of the energy-rich phosphorus compounds and particularly of ATP (Fig. 1), which is the most important and most universal of these compounds, in vital processes.

This substance contains three phosphoric acid groups, two of which are unstably bonded. The breaking of these bonds is accompanied by the liberation of energy, 8-10 kilocalories per gram-molecule of phosphate. The energy produced by the cleavage of ATP is used for the various needs of the organism. This is schematically represented in Fig. 2

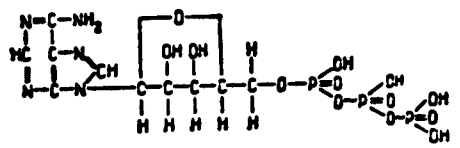
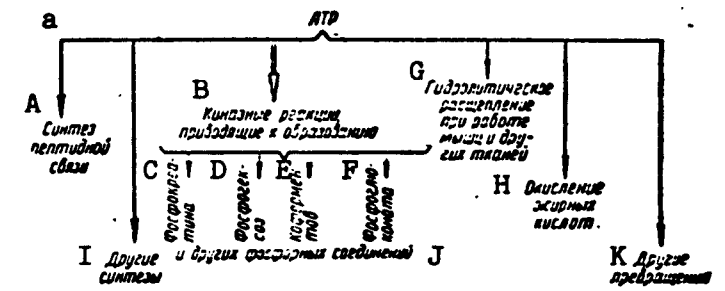


Fig. 1. The structure of ATP. The OP bond in the last two phosphoric acid groups is characterized by a large energy reserve.

(a and b) where the use of the energy liberated by ATP for muscular work, biosynthetic processes, etc. is shown.

What is the mechanism by which ATP is formed, i.e., what is the mechanism by which a molecule of inorganic phosphoric acid is converted into an energy-rich phosphate group of this organic substance? This process is called phosphorylation and is effected in two ways.



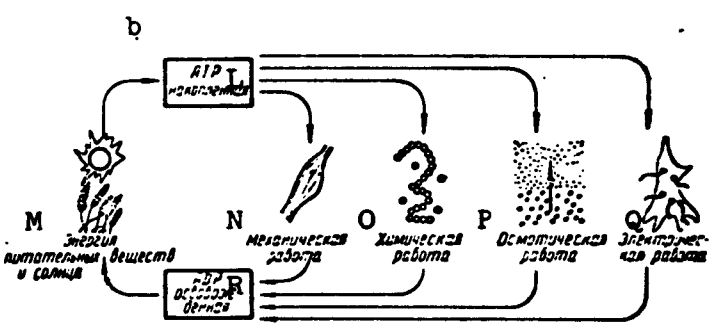


Fig. 2. Ways in which ATP is used (a and b). (After Leninger.) A) Synthesis of peptide bonds; B) kinase reactions leading to the formation of; C) phosphocreatine; D) phosphohexoses; E) coferments; F) phosphogluconate; G) hydrolytic cleavage during work performed by the muscles and other tissues; H) oxidation of fatty acids; I) other syntheses; J) other phosphorus compounds; K) other conversions; L) stored ADP; M) energy from nutritive substances and the sun; N) mechanical work; O) chemical work; P) osmotic work; Q) electrical work; R) liberated ATP.

The first mode of ATP formation is the glycolysis or anaerobic decomposition of carbohydrates; this process begins with glycogen or glucose and ends with the formation of lactic acid (Fig. 3).

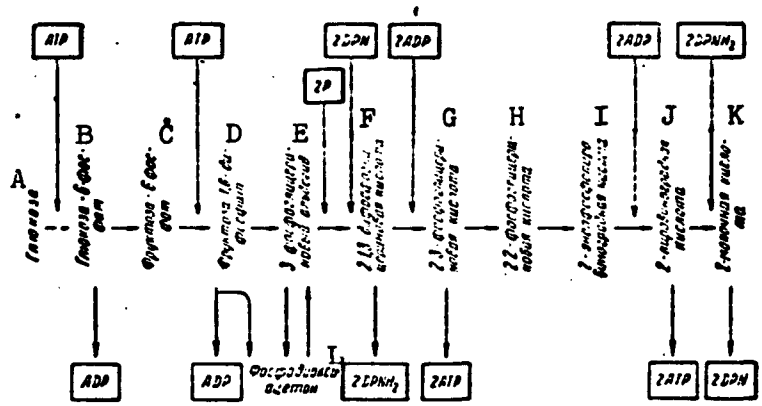


Fig. 3. Glycolytic formation of ATP. A) Glucose; B) glucose 6-phosphate; C) fructose 6-phosphate; D) fructose 1,6-diphosphate; E) 3-phosphoglyceraldehyde; F) 2 1,3-diphosphoglyceric acid; G) 2 3-phosphoglyceric acid; H) 2 2-phosphoglyceric acid; I) 2-enolphosphopyruvic acid; J) 2-pyruvic acid; K) 2-lactic acid; L) phosphodihydroxyacetone.

Before cleavage, the glucose must be converted into a phosphoric ester of glucose and the addition of phosphoric acid must later be repeated during the formation of fructose diphosphate.

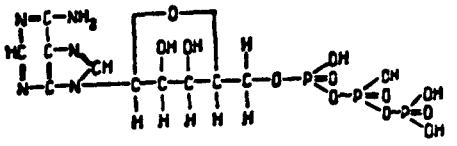
In these reactions, two phosphate groups separate from two ATP molecules and two ADP molecules are formed.

1. Hexose + 2ATP $\rightleftarrows$ hexose$\overset{P}{\underset{P}{<}}$ + 4 ADP.

With the formation of a hexose or, more precisely, fructose diphosphate, glycolysis itself sets in, passing through a number of stages and leading to the formation of two molecules of lactic acid.

2. Fructose$\overset{P}{\underset{P}{<}}$ + $2P_{mineral}$ + 4 ADP $\rightarrow$ 2 lactic acid + 4ATP.

When Reaction 1 and Reaction 2 are compared, it may be seen that two molecules of ATP split during the initial stages of the fermentation of glucose, while four ATP molecules are formed during the final stages of this process; consequently, the overall equation for the



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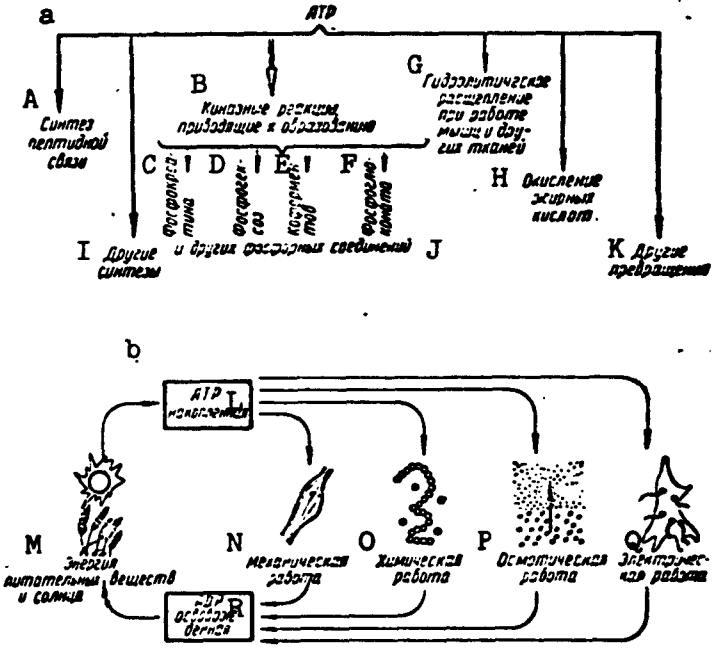

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reaction has the form

$$\text{glucose} + 2P_{\text{mineral}} + 2ADP \rightarrow 2 \text{ lactic acid} + 2ATP,$$

i.e., the overall result will be the conversion of two molecules of mineral phosphate into two energy-rich groups in the ATP molecule. This phylogenetically ancient process has a number of special properties. Among these we should note, firstly, the relatively low efficiency with respect to energy (two new energy-rich bonds are formed by the cleavage of one glucose molecule) and, secondly, the high interconditionality between the cleavage of glucose and the formation of energy-rich bonds. The formation of each molecule of lactic acid is rigidly correlated with the formation of one energy-rich bond in the ATP structure. Glycolytic phosphorylation may consequently be characterized as highly conjugate, since the formation of lactic acid is impossible without the simultaneous formation of ATP.

The process of anaerobic phosphorylation can only be altered quantitatively, i.e., it can only be regulated by increasing or decreasing the activity of the anaerobic fermentative decomposition of the carbohydrates.

The second mode of energy-rich phosphorus bond formation, oxidative phosphorylation, which is further along on the evolutionary scale, is characterized by entirely different properties. The discovery of this process, one of the greatest achievements in the field of cellular energetics, was made by V.A. Engel'gardt in 1930. In 1939-1940, V.A. Belitser elucidated the most important and most fundamental mechanisms characteristic of oxidative phosphorylation. After these works appeared, study of the process of oxidative phosphorylation was centered in many foreign laboratories. Gradually, parallel with the development of the study of biological oxidation (cellular respiration), it became possible to establish a number of the details of

oxidative phosphorylation processes (Fig. 4), of which the following may be considered to be the most important.

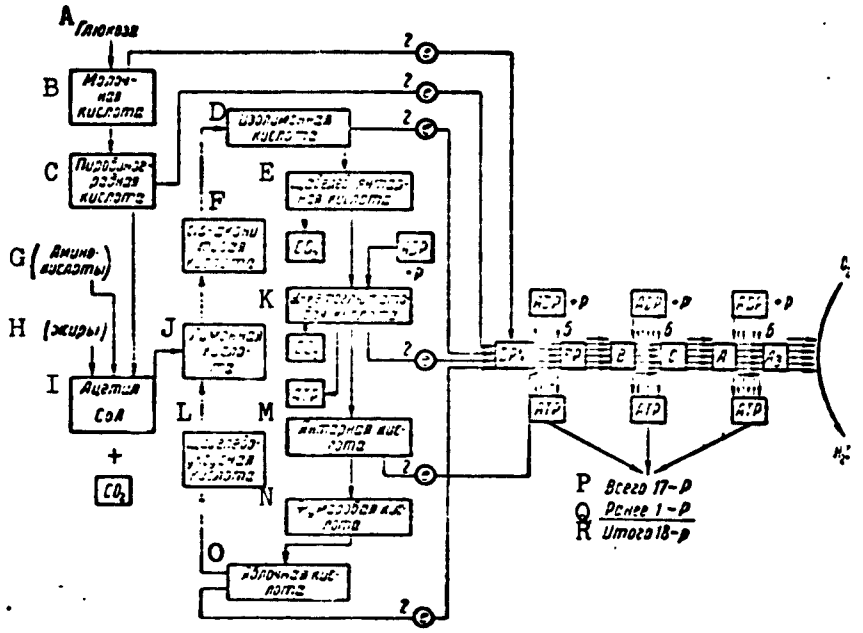

Fig. 4. Oxidative formation of ATP. (After Leninger.) A) Glucose; B) lactic acid; C) pyruvic acid; D) isocitric acid; E) oxalosuccinic acid; F) cis-aconitic acid; G) amino acids; H) fats; I) acetyl CoA; J) citric acid; K) α-ketoglutaric acid; L) oxalacetic acid; M) succinic acid; N) fumaric acid; O) malic acid; P) total; Q) previously; R) altogether.

1. All acts of oxidative phosphorylation center in the mitochondria.

2. One energy-rich phosphate bond is formed by the cleavage of succinyl CoA, a product of the oxidative conversion of ketoglutaric acid. Since ketoglutaric acid is the oxidation substrate, this type of phosphorylation is called substrate phosphorylation. It is characterized by strong conjugation and is of little interest to us, since it is similar to glycolytic phosphorylation.

3. The remaining acts of phosphorylation are associated with the transfer of electrons from DPNH to O_2 in the respiratory chain. We may consider the discovery of a number of phosphorylations and their rela-

tionships with definite stages in electron transfer to be one of the greatest recent advances in biochemistry. It is believed that the transfer of electrons from DPNH to flavoproteins causes one phosphorylation, that from flavoproteins to the cytochrome system causes a second, and that from the cytochromes to oxygen causes a third.

4. The cleavage and complete oxidation of the products of the glycolytic conversion of glucose can cause the formation of 38 energy-rich bonds, i.e., 19 times as many as in the anaerobic decomposition of carbohydrates.

This latter fact is naturally very important. However, in this paper we do not so much wish to emphasize the high efficiency of oxidative phosphorylation as to stress its regulability. This term implies the possibility of transferring electrons into the respiratory chain over a two-fold path (Fig. 5): 1) by the formation of energy-rich phosphorus bonds, this being respiration coupled with phosphorylation, or phosphorylation oxidation; 2) respiration not coupled with phosphorylation or (as we term it) free oxidation. However, we must note first that oxidation can actually be both phosphorylation and free, secondly that it can be regulated both in vitro and in vivo, and thirdly that this regulation can be used in the protective functions of the organism.

In analyzing the process of oxidative phosphorylation in isolated mitochondria, determination of the quantity of absorbed oxygen (Δ O in micro[gram]-atoms) and bonded P (Δ P in micro[gram]-atoms) and of the ratio P/O is employed (it is occasionally more convenient to use the inverse ratio O/P).

In experiments conducted in vitro, it was possible to repeatedly change free oxidation into phosphorylation oxidation and vice versa. The change in the value of P/O as a result of repeated switching

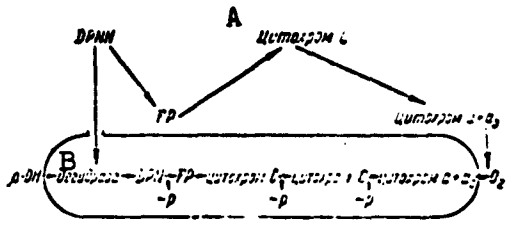

Fig. 5. Two modes of DPNH oxidation: nonphosphorylation at the surface of the mitochondria and phosphorylation within the mitochondria. A) Cytochrome; B) dehydrogenase.

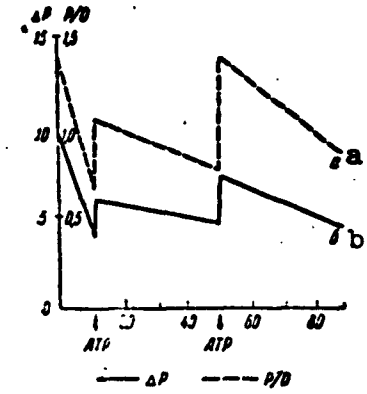

Fig. 6. Repeated changing of free oxidation to phosphorylation oxidation and vice versa. a) Δ P; b) P/O.

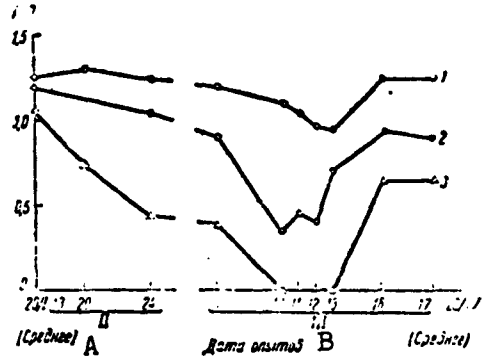

Fig. 7. Dynamics of the seasonal fluctuations in the P/O ratio for mitochondria from the liver of a pigeon. A) Mean; B) date of experiments.

from one oxidation mode to the other is shown in Fig. 6. This switching was achieved by adding ATP to a suspension of mitochondria; ATP severely affects the state of the mitochondria, prevents intumescence, and shifts oxidation to the phosphorylation mode.

The possibility of a change in the relationship between phosphorylation oxidation and free oxidation can be demonstrated on mitochondria taken from the organs or tissues of a living organism under various physiological conditions. This can be illustrated by two examples.

As our first example, we cite the dynamics of the seasonal fluctuations in the P/O ratio for mitochondria from the liver of a pigeon during the period from January to April (Fig. 7).

The gradual decrease in the P/O ratio from February to the middle

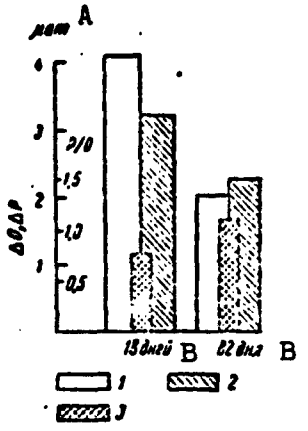

Fig. 8. Oxygen absorption and phosphorus fixation by the mitochondria of the muscles at various stages of growth. 1) Oxygen absorption per gram of muscle; 2) phosphate fixation per gram of muscle; 3) P/O; A) Micro[gram]-atoms; B) days.

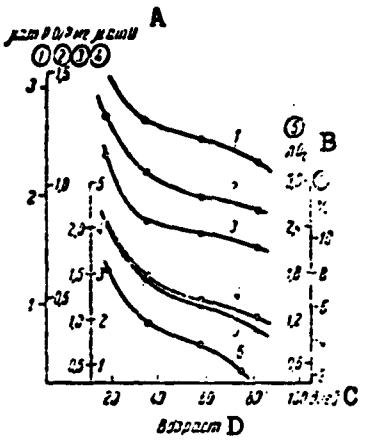

Fig. 9. Oxidation, phosphorylation, degree of decoupling, quantity of mitochondrial protein, and growth rate as a function of chicks' age. 1) Phosphorylation per 30 minutes per gram of muscle; 2) O/P; 3) quantity of mitochondrial protein per gram of muscle; 4) oxidation in vitro per 30 minutes per gram of muscle; 5) oxidation in vivo per hour per kilogram of body weight; 6) growth rate (gain in weight per day, in percent). A) µg-atoms, P/O, mg, µg-atoms; B) liters; C) days; D) age.

of March and the renewed increase in this ratio during the second half of March and April (curve 1) draws our attention. These fluctuations, which only slightly exceed the limit of error, may be more clearly seen if the mitochondria are subjected to brief aging as a result of preliminary incubation for 10-11 minutes (curve 2). Preliminary aging of mitochondria for 30 minutes is sufficient to cause very marked results which indicate that mitochondria possess varying degrees of resistance to preliminary incubation (curve 3). This manifests itself in their varying ability to maintain phosphorylation oxidation during various periods of the year.

Our second example pertains to the evaluation of oxidative phosphorylation in mitochondria from the muscles of chicks during periods of development which are marked by different growth rates (Fig. 8).

As may be seen, it is not only the rate of oxygen absorption and of energy-rich phorphorus compound formation which vary. The P/O ratio increases substantially as the growth rate slows down. The curves shown in Fig. 9 reflect the identical nature of the changes per gram of muscle in the: 1) phosphorylation rate, 2) ratio of oxygen consumed to phosphorus fixed, O/P, 3) quantity of mitochondrial protein, 4) oxygen absorption rate in vitro, 5) oxidation in vivo (per kg of body weight), and 6) growth rate.

The fact that all the curves have the same shape makes it possible to conclude that there is a correlation between the processes described and that the ratio between free and phosphorylation oxidation is regulable.

Both of the examples cited are concerned with the variability of the P/O ratio under various physiological conditions. We might also cite other data on the variability of the relationship between respiration and phosphorylation in the mitochondria of the liver in hyperthyroidism and in cardiac muscle homogenates during marked thyrotoxicosis. However, what has been said is sufficient to permit us to state that the ratio between free and phosphorylation oxidation varies as the physiological state of the organism varies.

Let us move on to the question of what role oxidative phosphorylation plays in the protective functions of the organism. It may be recalled that, for a substance such as pyruvic acid, approximately 50% of the energy of oxidation is dissipated in the form of heat and approximately 50% is stored in the form of energy-rich phorphorus compounds, chiefly ATP.

TABLE 1

Oxidative Metabolism in Cardiac Muscle Homogenate

	A	B	C			
			D	E	F	G
			в процентах H			
I Норма	11.4	6	100	100	100	100
J Спустя 2 дня после операции	9.4	11.2	45	120	71	6
K Спустя 14 дней после операции	4.4	2.8	60	260	240	25

A) Absorption of O_2 by endogenous substrates (in μA [microgram-atoms]); B) formation of phosphocreatine on oxidation of endogenous substrates (in μA P); C) composition of cardiac muscle; D) glycogen; E) lactic acid; F) β-OH; G) phosphocreatine; H) in percent; I) normal; J) 2 days after operation; K) 14 days after operation.

It is clear that, all other conditions being equal, the P/O ratio decreases as the portion of the energy of the oxidative processes which is converted into heat increases and as the portion stored in the form of ATP decreases. In this case, relatively unfavorable conditions are created for the use of ATP in directions such as those shown in Fig. 2, since only a small quantity of ATP is formed. Conversely, an increase in the P/O ratio in an organ indicates that it is possible for the specific functions of this organ to be efficiently realized, e.g., as muscular activity in muscle tissue.

M.F. Vyalykh has determined the respiration and phosphorylation rates after experimental constriction (4 times) of the lumen of the aorta. It is obvious how great a load this places on the cardiac muscle. Data obtained at brief intervals after the operation (2 and 14 days) are given in Table 1.

Our attention is drawn to the data on oxygen absorption and phosphocreatine formation on endogenous substrates, i.e., without the addition of any substance which would be subjected to oxidation, in the cardiac muscle homogenates. After 2 days, the potential capacity of the

tissue for oxygen absorption is slightly reduced; at the same time, phosphocreatine formation is sharply increased (by a factor of approximately 2). It must be added that the activity of the ferment which converts phosphate to creatine does not increase. The formation of a large quantity of phosphocreatine must consequently be considered as an intensification of oxidative phosphorylation, which ensures maximum systhesis of energy-rich phorphorus compounds. These compounds are necessary both for carrying out the increased work which must be performed by the heart and for intensified synthesis of muscular structures. Only a sharp intensification of phosphorylation processes 2 days after the operation can satisfy the demands for ATP necessitated by the increased work and intensified biosynthesis. It is interesting that the phosphocreatine content of the cardiac muscle decreases at this stage. This again indicates a very high consumption of ATP, since the efficiency of respiration with respect to its coupling with phosphorylation was very high at this time. The changes in cardiac metabolism as compared with its normal level which occurred 2 days after the operation are difficult to evaluate either as a mobilization of its protective resources or as a result of regulation intended to ensure maximally effective ATP formation.

Energy metabolism processes in the heart proceed differently 14 days after the operation. At this point, oxygen consumption is more than halved. Oxidative phosphocreatine formation is also sharply reduced. However, very large quantities of lactic acid are detected when the composition of the cardiac muscle is studied. It may consequently be assumed that the demand for energy-rich phosphorus compounds is satisfied by glycolytic phosphorylation. In this case, regulation can consist only in an intensification of carbohydrate decomposition, which culminates in an accumulation of lactic acid. Actually, the gly-

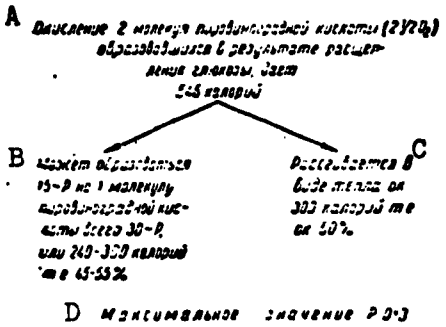

Fig. 10. Caloric effect of the oxidation of pyruvic acid. A) Oxidation of two molecules of pyruvic acid (2 1/2 O_2) formed as a result of the cleavage of glucose yields 546 calories; B) 15 P bonds can be formed per molecule of pyruvic acid, a total of 30 P bonds or 240-300 calories, i.e., 45-55%; C) approximately 300 calories, i.e., approximately 50% is dissipated in the form of heat; D) maximum value of P:O = 3.

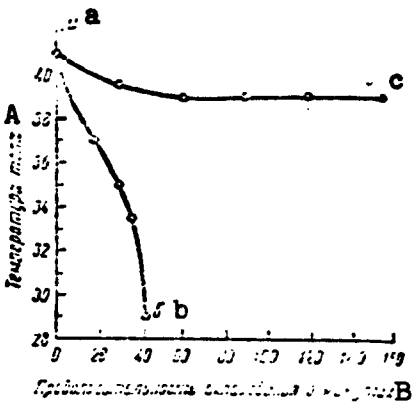

Fig. 11. Curves showing body temperature of pigeon during first cooling and repeated cooling. a) Before cooling (P/O = 1.82); b) during first cooling (P/O = = 1.06); c) during repeated cooling (P/O = 0.33); A) Body temperature; B) duration of cooling, in minutes.

cogen content is sharply reduced during this period.

Let us consider still another example of clear regulation of the oxidative phosphorylation process. This example is concerned with the adaptation of animal organisms to low temperature.

If a pigeon is placed in a ventilated room at −15°, after only 40 minutes it will be near death and its body temperature will have dropped to 28-29°. However, on repeated cooling, the temperature curve acquired an entirely different character (Fig. 11).

As may be seen from Fig. 11, even when the pigeon is subjected to prolonged cooling (3 hours or more), its body temperature does not drop below 39°. The experiments of V.P. Skulachev, S.P. Maslov et al. have shown that mice also adapt to cold (although with somewhat greater

difficulty than pigeons) and, after repeated cooling, withstand it far more easily.

How is this ability to maintain body temperature achieved? It has been shown that the ratio between free and phosphorylation oxidation varies sharply in mitochondria removed from the muscles of cold-adapted pigeons and mice, with the former mode predominating. In other words, if the energy of oxidation in muscle tissue (see Fig. 10) is normally divided approximately half and half between phosphorylation oxidation and free oxidation, a shift occurs toward free, nonphosphorylation oxidation on adaptation to cold. As may be seen from Fig. 10, this means that the proportion of direct heat production increases, this being that form of heat which is called "primary heat" by Neyfakh and is not associated with the preliminary formation of ATP.

TABLE 2

Oxidation and Phosphorylation in Mitochondria of the Muscles of Warm-Blooded Animals during Adaptation to Cold

A	B	До охлаждения C			После адаптации к холоду D		
Животные	Регулятор, добавленный in vitro	O	ΔP	P/O	O	ΔP	P/O
E Голубь	—	7,9	8,2	1,04	6,4	1,4	0,22
	АТР + ЭДТА	8,1	11	1,35	6,5	5,1	0,79
F Мышь	Ca^{++}	6	3,2	0,39	5,6	0	0
	—	7,5	7,5	1	7,9	5,1	0,65
G	Сывороточный альбумин	7,5	8,7	1,17	9	10,4	1,16

A) Animals; B) regulator, added in vitro; C) before cooling; D) after adaptation to cold; E) pigeon; F) mouse; G) serum albumen.

All of these relationships are shown in Table 2.

The P/O in the mitochondria of the muscles of the pigeon dropped from 1.04 to 0.22 after adaptation; the P/O ratio in the mitochondria of muscle tissue from the mouse dropped from 1 to 0.65 after adaptation.

When one of the decoupling agents - calcium ions - is added, this decrease becomes even sharper, the ratio falling from 0.39 to 0. In these experiments, phosphate fixation was also almost nil.

We are now faced with the problem of to what extent the disruption of phosphorylation can be considered to be the result of regulation, i.e., a negative physiological influence, and not of complete malfunctioning of the system which couples respiration and phosphorylation. This question may be very easily answered. A mixture of EDTA and ATP was used as a substance to reduce the "decoupling" between respiration and phosphorylation in experiments on the mitochondria of a pigeon.

It proved to be possible to increase Δ P from 1.4 to 5.1 and P/O from 0.22 to 0.79. The use of serum albumen was found to be more effective. The addition of albumen increased phosphorylation by a factor of 2 (from 5.1 to 10.4) and returned the P/O ratio to its initial level. The disturbance of the P/O ratio during adaptation to cold was thus wholly reversible and may be considered to be a protective function of the organism.

There is no doubt that many pathological states arise as a result of disruption of the regulation between respiration and phosphorylation. In such cases, an improvement can be obtained by using drugs which are capable of increasing the coupling between respiration and phosphorylation (for example, cardiac glycosides at certain stages of heart disease) or decreasing it (for example, dicumarol and its derivatives, Pelentan, salicylates, et al. as necessary). From this viewpoint, the broad treatment of problems of oxidative phosphorylation in the clinic must be regarded as more than justified. Moreover, the extremely diverse functions of ATP which were mentioned at the outset a priori give us the right to acknowledge the importance and expedience of detailed and many-faceted investigation of methods of regulating the

formation and use of ATP in the organism under normal and pathological conditions.

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[Footnote]

50 The following abbreviations will be used in this article: ATP -- adenosine triphosphate; ADP -- adenosine diphosphoric acid; DPNH -- diphosphopyridine nucleotide, reduced form; CoA -- succinyl coenzyme A; EDTA -- ethylenediaminetetraacetate; FP -- flavoprotein; β-OH -- hydroxybutyric acid.

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