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TRANSLATION

ANTIBACTERIAL IMMUNITY AND RADIORESISTANCE

By

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FOREWORD

During the years of Soviet rule a great deal of data has been amassed in studying the effect of various types of vaccines on the body. Scientists attempted to develop vaccines which would not have a detrimental influence on the organism and would ensure a maximum level of immunity. It was found that parenteral or enteral administration of foreign microbial products causes substantial changes in reactivity. This forced the development of special contraindications for a number of vaccines.

It was established that administration of antigens not only promotes the development of specific immunity to the microbe in question, but also considerably alters the activity of many physiological systems, causes proliferation of special cellular elements, and changes the reaction of the organism to various external agents. It was found that a vaccinated organism reacts not only to inoculation with the causitive agent of the disease in question, but also to infection with other microorganisms, to the influence of a number of physicochemical agents, and, especially interesting, to exposure to ionizing radiation.

Reports appeared ever more frequently in the literature that if vaccinated animals were irradiated a portion of them survived without any treatment, despite the fact that all unvaccinated animals died; however, no explanation of this phenomenon was given.

As part of the joint work of our laboratory and that headed by

Professor P.D. Gorizontov a special study was made of the increase in radioresistance which occurs on inoculation with bacterial vaccines. The role of the dosage and type of vaccine, the interval between inoculation and irradiation, and the other factors which cause a very large increase in the radioresistance of vaccinated organisms was established.

Research in this direction has now moved out of our laboratory. That an increase in radioresistance occurs in vaccinated animals has been confirmed by the work of the laboratories of the Kursk and Vovonezh Medical Institutes, as well as by the Institute imeni Acad. N.F. Gamaleya. The expansion of research in this field has enabled investigators to discover many interesting facts which further our understanding of this phenomenon and has consequently made it possible to direct this research toward the desired end.

However, there is already a rather large amount of data, systematization of which would make it possible for our scientific colleagues to avoid repeating experiments which have been performed, so that they can move further toward solving this problem, which is of theoretical and practical interest.

Our opinion on the nature of this phenomenon will undoubtedly give rise to controversy, which will result in the appearance of new views; this will be useful for an ultimate understanding of the essence of the facts presented. In addition, general practitioners, who make wide use of prophylactic immunization, are frequently unfamiliar with radiobiology and many of them are scarcely acquainted at all with the fact that radioresistance increases in vaccinated organisms (this was confirmed by our dealings with physicians at the Central Institute for Advanced Training). As a result of the ever-increasing use of ionizing radiation both for therapeutic purposes

and in various branches of the national economy the number of persons coming into contact with it is increasing. They may include both vaccinated and unvaccinated individuals, the extent of irradiation varying within each category and frequently producing opposing effects.

All of this impelled us to publish this monograph, in which an attempt is made to draw theoretical implications from the available data on the action of antibacterial immunity on radioresistance and to present certain considerations about the nature of this phenomenon and the possibilities for its practical utilization. Unpublished data obligingly furnished by T.V. Kalyayeva, M.F. Sbitneva, I.I. Urucheva, G.A. Shal'nova, and O.V. Smirnova are used in this monograph and the authors extend their deep thanks to these individuals.

Chapter 1

REACTION OF THE ORGANISM TO ADMINISTRATION OF BACTERIAL ANTIGENS AND SIGNIFICANCE OF INDIVIDUAL MANIFESTATIONS OF THIS REACTION FOR THE ACCOMPANYING INCREASE IN RADIORESISTANCE

It was established in studying the influence of radiation on active artificial immunity that the effectiveness of the immunization depends on its temporal conjunction with the irradiation. If the vaccination was carried out first and then the irradiation (within 1-24 hours) development of immunity and antibody production were observed. Conversely, if the animal was irradiated first, vaccination was not sufficiently effective, even when performed within 15 minutes to 1 hour. These experiments revealed peculiarities in the course of radiation sickness in vaccinated animals. A number of works* contained descriptions of curious facts which indicated that animals vaccinated before irradiation withstood radiation sickness better than unvaccinated subjects and frequently survived without any treatment. We conducted special investigations which confirmed that this is so.

In order to understand correctly the reason for the favorable influence of preliminary immunization on radioresistance it is first necessary to analyze the characteristics of the phenomenon which develop in the body after administration of antigens.

In this case we must evaluate the significance of the changes observed not only from the participation of certain tissue or physio-

logical systems in them, but also from their duration, since the protective influence of vaccination with respect to irradiation is not limited to hours or days, but lasts for several weeks or months.

Immunologists were interested in studying the reactive changes which occur after administration of antigenic substances, primarily from the standpoint of the actual process of immunity formation. Detailed examination of the blood serum and organ extracts at various intervals after immunization showed that the accumulation of antibodies is associated with definite organs and tissues. It was natural that the cellular structure of these organs should be subjected to a similar study; it was found that there are characteristic changes which indicate a system-wide reaction of the endothelial tissue, in addition to signs of a nonspecific reaction (Grabar, 1959).

It is impossible to make a detailed study of the reaction of the body to administration of antigens without a histological investigation of the cellular changes in the antibody-producing organs and the tissues at the vaccination site.

In numerous works on the morphology of immunogenesis (Ya.L. Rapoport, 1957; G.A. Gurvich, 1960; etal.) the qualitative and quantitative changes observed in the cellular composition of various organs (the spleen and lymph nodes) were compared with data obtained by measuring the antibody concentrations in these tissues serologically, with data obtained in studies of the microchemical characteristics of tissue components, and with the results of the direct display of antibodies in cells by means of adsorption of tagged antigens (the tagging being done with a radioactive tracer or fluorescent stains) (Ruth et al., 1957; Coons et al., 1955, and others). It was established that endema developed at the site of microbial-antigen administration, with subsequent leucocytic infiltration and a slight proliferation of

local tissue elements. Signs of lymphadenitis (an increase in the number of pseudoeosinophils, lymphoblasts, and basophilic lymphocytes) are observed in the regional lymph nodes.

The majority of authors characterize the response of the organism to antigenic stimulation as a pluricellular reaction in which various types of cells (macrophages, plasma cells, reticular elements, and lymphocytes) participate (M.P. Pokrovskaya and L.S. Kaganova, 1947; M.P. Pokrovskaya and M.S. Makarov, 1942; MacNell, 1950; Wissler, etal., 1957; Makinodan, Ruth, and Wolf, 1954).

The development of a characteristic cellular reaction in the spleen and lymph nodes precedes the appearance of antibodies in these organs and in the peripheral blood. Witch and his colleagues (1956) observed that exposure to ionizing radiation (which is known to depress antibody production) on the 4th day after immunization does not affect the typical development of the cellular reaction and antibody formation. Cells which are already in a state of active antibody production are apparently more stable to unfavorable influences than resting elements.

It was thus established that immunization with various antigens causes a prolonged specific activation of certain cellular elements of the lymph nodes, spleen, bone marrow, and connective tissue (primarily at the site of administration). Types of cells were detected whose appearance was extremely closely related to the formation of immunity (plasma cells and lymphoid elements).

However, this narrow study of the morphology of immunogenesis at the site of antibody formation alone somewhat reduced the amount of attention paid to morphological investigation of the changes in important systems which participate in immunization, e.g., of the state of the hematogenic system. The matter consists not only in

studying the accumulation of plasma and lymphoid cells in this system, but also in evaluating its functional capacity for producing blood cells. The prolonged proliferation of the cells which actively produce antibodies cannot help but affect the continuous processes involved in the multiplication of cells associated with the reproduction of the cellular elements of the blood.

Another problem, on which little light has been shed in the literature, is that of the characteristics of the cellular reaction to various types of antigens. The state of hematogenesis in inoculated organisms, the proliferation of certain cells, and the qualitatively unique reactions to individual types of antigens are of great importance in analyzing the mechanism by which vaccination acts on radioresistance.

TABLE 1

Dynamics of Changes in the Cellular Composition of the Peripheral Blood, Spleen, and Bone Marrow in Rats

Days	After administration of bone-marrow cells	After inoculation with a vaccine prepared from Bacillus Breslaviensis
3rd	Slight increase in reticuloendothelial cells in the spleen alone (from 5 to 8%). Depression of myelopoiesis, decrease in total quantity of myeloid elements in the bone marrow to 35% from a normal level of 55% and marked reduction in content of regenerative myeloid forms. Sharp (from 0.5 to 1.8%) increase in number of cells with chromatinolyzed nuclei. Decrease in total number of leucocytes in the peripheral blood.	Increase in number of reticuloendothelial cells in the bone marrow (to 10%) and spleen (to 7%). Inhibition of myelopoiesis in the bone marrow and decrease in total number of myeloid elements (to 44% from 55%). Reduction in number of leucocytes in the peripheral blood.

7th	Maintenance of somewhat elevated quantity of reticuloendothelial cells in the spleen and sharp increase (from 5 to 13%) in the number of these cells in the bone marrow. Slight increase in number of regenerative myeloid forms and considerable chromatinolysis (1.6%). Further decrease in total number of leucocytes and sharp increase in number of monocytes in the peripheral blood.	Further increase in content of reticuloendothelial elements in the spleen (to 12%). Decrease in the number of these elements in the bone marrow (to 4%). Inhibition of myelopoiesis (total number of myeloid elements was 41%). Normalization of leucocyte count in the peripheral blood.
12th	Increased content of reticuloendothelial cells in the spleen and especially in the bone marrow (to 14%); increase in number of monocytes and reticuloendothelial cells in the peripheral blood. Tendency toward normalization of myelopoiesis. Decrease in number of cells with chromatinolyzed nuclei. Reversion to normal of total number of leucocytes in the peripheral blood.	Maintenance of reticuloendothelial elements at a high level in the spleen (14.5%) and bone marrow (10%). Considerable decrease (36%) in the total number of myeloid elements in the bone marrow, at the expense of mature forms. Considerable reduction in percentage content of lymphocytes in the spleen (to 68% from a normal level of 84-88%). Considerable increase in number of monocytes in the peripheral blood and slight increase in the total number of leucocytes.
12th	Maintenance of number of reticuloendothelial elements at an elevated level in the spleen and a very high level (15%) in the bone marrow. Con-	Considerable increase (by a factor of more than 3 in comparison with the normal level, 16.5%) in the number of reticulo-

	tinued reduced content of myeloid elements in the bone marrow (38.4%). Continued elevation of the total number of leucocytes, monocytes, and reticuloendothelial cells in the peripheral blood.	endothelial cells in the spleen and maintenance of a somewhat elevated level in the bone marrow (9%). Continued reduced total number of myeloid elements (38%). Decrease in number of monocytes and normalization of total number of leucocytes in the peripheral blood.
30th	Myelopoiesis not completely restored. Second increase in number of cells with chromatinolyzed nuclei (1.3%). Number of reticuloendothelial elements in the spleen within normal limits, but somewhat increased (10%) from the normal level of 5.2% in the bone marrow. Total quantity of leucocytes and monocytes in the peripheral blood somewhat elevated.	Below-normal number of reticuloendothelial cells in the bone marrow, but maintenance of a considerably increased content of these cells in the spleen (14%). Myelopoiesis remained at a reduced level (45.0%). Normalization of number of monocytes in the peripheral blood.
45th	Number of reticuloendothelial cells in bone marrow and spleen reverted to normal. Myelopoiesis remained at a reduced level (41.5%). Total number of leucocytes and percentage of monocytes in the peripheral blood were somewhat elevated.	Quantity of reticuloendothelial cells in the spleen reverted to normal. The content of these cells in the bone marrow remained somewhat elevated (9 %). A slight inhibition of myelopoiesis (49.2%) persisted; the composition of the peripheral blood was normalized.

In order to study the functional properties of the hematogenic organs on immunization and the reactions to different types of antigens

N.N. Klemparskaya, M.F. Sbitneva, T.V. Kalyaycva, and T.A. Fedorova conducted a series of experiments involving immunological, hematological, and biochemical research methods.

They compared the reactions to a microbial antigen and to homologous cellular antigens.

The experiments were conducted on 100 male rats weighing 160-225 g. 35 of these animals were immunized by intramuscular injection of 1 billion microbial bodies in the form of heated vaccine prepared from Bacillus breslaviensis. Another group of animals (52 rats) was given a single intravenous injection of a suspension of bone marrow cells from the femora and tibiae of healthy rats from the same batch in Tyrode's solution ($1.0 \cdot 10^8$ - $1.7 \cdot 10^8$ nucleate elements). In order to study the reactions of the hematogenic organs and the state of the peripheral blood rats were killed with ether at intervals of 1, 3, 7, 12, 21, 30, and 45 days after administration of the antigens. Table 1 and Figs. 1 and 2 show the changes in the cellular composition of the peripheral blood, spleen, and bone marrow.

It was found that both types of antigen treatment caused the typical response to immunizational stimulation, a proliferation of reticular elements in the bone marrow and spleen accompanied by an increase in the number of monocytes in the peripheral blood. However, the intensity of this proliferation in individual organs differed after administration of the microbial and tissue antigens.

Treatment with the microbial antigen caused an extremely marked reaction on the part of the reticuloendothelial elements of the splenic tissue, while administration of the homologous bone-marrow cells caused a similar reaction on the part of another organ, the bone-marrow tissue (the splenic content of reticular cells being considerably elevated only during the first few days).

During the period of maximal elevation of the number of reticuloendothelial elements in the spleen the rats inoculated with the microbial vaccine exhibited a considerable (by 20%) decrease in the lymphocyte count of the splenic tissue. A similar phenomenon has been described in the literature. It is very important to note that both types of antigen caused a marked and prolonged inhibition of myelopoiesis. Special attention should be paid to this fact in conducting special investigations on immunization with various types of bacterial antigens in order to work out more precisely the indications and contraindications for vaccination. This is important for developing a rational system of periatric vaccination prophylaxis.

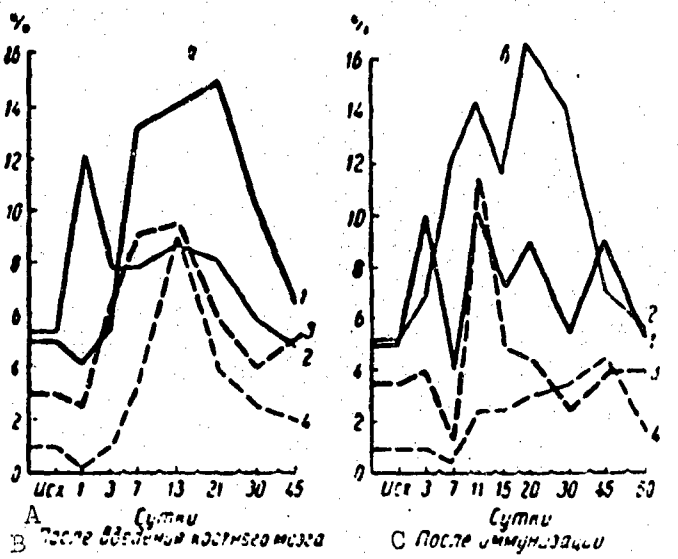

Fig. 1. Change in the percentage content of reticuloendothelial elements in impressions made from the bone marrow and spleen and in the peripheral blood (average data). a) After a single injection of bone marrow; b) after immunization with vaccine prepared from Bacillus breslaviensis; 1) Percentage content of reticuloendothelial cells in the bone marrow; 2) the same, in the spleen; 3) percentage content of monocytes in the peripheral blood; 4) percentage content of reticuloendothelial cells in the peripheral blood (from the data of M.F. Sbitneva and T.V. Kalyayeva). A) Start; B) days after administration of bone marrow; C) days after immunization.

The depression of myelopoiesis was more marked after injection of the homologous cells than after vaccination with the microbial antigen. There was a disturbance of both the formation and maturation

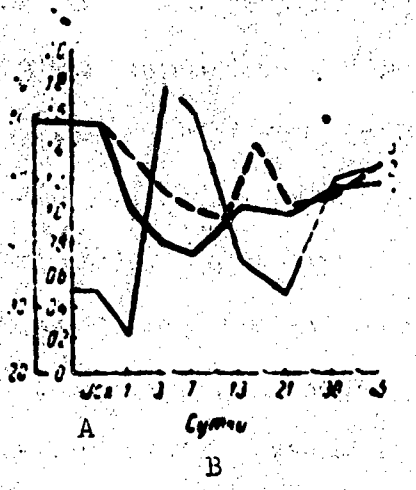

Fig. 2. Change in the percentage content of myeloid elements and the number of cells with chromatinolyzed nuclei in impressions of rat bone marrow after administration of bone marrow and immunization (average data). 1) Percentage constituted by total number of myeloid elements after administration of bone marrow; 2) the same, after immunization; 3) percentage of cells with chromatinolyzed nuclei after administration of bone marrow (from the data of M.F. Sbitneva and T.V. Kalyayeva). A) Start; B) days.

of myeloid cellular elements, primarily the latter being disrupted under the action of the paratyphoid vaccine. Another peculiarity noted after treatment with the homologous cellular antigens was a sharp increase in the number of cells with chromatinolyzed nuclei (on the 3rd, 7th, and 30th days after administration of the antigens).

From the data cited it follows that the rapidly-developing proliferation of reticuloendothelial elements has a substantial inhibiting influence on the functioning of hematogenic tissue over a long period. At the same time, myelopoiesis is at a reduced level, this probably playing a definite role in the development of radioresistance, since it is known that myeloid tissue is highly radioresistant and that depressing its functioning before irradiation may contribute to its sustaining somewhat less damage.

The proliferation of plasma cells and other elements of the reticuloendothelial system may be important in retaining the capacity of irradiated animals for immunogenesis with respect to heterogeneous antigens (M.I. Ravich-Shcherbo and L.G. Prokopenko, 1960). In addition, it is impossible to exclude the nonspecific intensification of the phagocytic reaction observed after vaccination. Activation of this function should also be of positive value during radiation sickness, although it lasts only a short time, disappearing within 2-3

weeks. It is consequently impossible to explain the increase in radioresistance observed over a period of 1-2 months after vaccination as being due to this factor alone.

In analyzing the possible mechanisms for the rise in radioresistance which occurs in vaccinated organisms we cannot overlook the considerable change which takes place in the general condition of the organism and the activities of a number of its vitally-important physiological systems.

Immunologists and epidemiologists working in the field of public health knew during the first few years when prophylactic vaccines (against smallpox, typhoid, cholera, etc.) were being given that vaccination causes certain painful symptoms; however, these disappear within several days. A reaction to vaccination was considered inevitable during the 19th century (Pfeiffer, 1897) and certain authors even asserted that active immunity developed better as this reaction became more intense (Friedberger, 1920). However, subsequent epidemiological practice and the scientific investigations which were conducted showed that an intense post-vaccination reaction is not at all necessary to the development of immunity (N.F. Gamaleya, 1939) and may even be detrimental (F.N. Berngof, 1928; Meyer, 1943).

Thus, for example, V.A. Snopkova (1959) established that active immunity to infection with live paratyphoid bacteria developed in rabbits only if they exhibited no marked reaction (hypothermia, loss of weight) to vaccination. Small doses of vaccine (50-800 million microbial bodies) yielded better results than immunization with 500-1500 million microbial bodies, which led to an intense post-vaccination reaction.

The entire history of the development of vaccine production is a story of the struggle for high effectiveness and low "reactivity."

There gradually accumulated a number of data on serious complications and even death occurring after vaccination in certain cases (N.S. Kheyfets, 1936; Robinson, 1937; Bannwarth, 1948; Love and Driscoll, 1943; Peacher and Robertson, 1945; Lindsag, 1905). This forced the development of a list of contraindications for vaccination (which is pasted onto the ampules containing the vaccines, together with the instructions for vaccination) and an attempt was made to reduce the post-vaccination reaction by means of drugs (N.V. Sergeyev, 1952). A medical examination before immunization and a check on the temperatures of vaccinated persons are obligatory. In order to prevent the distribution of vaccines which might cause an intensified reaction each batch is checked on a limited group of healthy persons after laboratory testing and, if reactions occur in no more than 5-7% of the vaccinated individuals, is released for distribution.

All of this indicates the marked general influence of vaccination on the state of human health; the change in radioresistance is apparently associated with this. How does this general effect of vaccination manifest itself?

It is first necessary to point out that this reaction of the body is nonspecific and displays the same general traits and symptoms when vaccines prepared from different microbes are administered.

The post-vaccination reaction manifests itself clinically* in complaints of chills, headaches, weakness, and nausea 2-3 hours after vaccination. A rise in body temperature, intestinal disturbances, and vomiting may be observed after 3-6 hours. The tissues at the site of the injection become edemic, hyperemic, and painful and the regional lymph nodes are occasionally enlarged (S.I. Zlatogorov, 1915, 1928; L. Falin, 1930; I. Itskovich, 1937; G.I. Besedin, 1940; G.I. Grennaus and T.K. Guseva, 1940; L.A. Shvartsman and A.R. Kroytman,

1942). The large number of investigations directed at reducing and completely eliminating these undesirable symptoms made it possible to establish the importance of a number of factors in the development of the post-vaccination reaction (a detailed analysis of these factors is given in the dissertation of N.N. Klemparskaya, 1948).

It was found that the toxicity of the bacterial strains from which the vaccines are prepared and the preparation method (for heat-killed, formalin-killed, AD, and delayed-action vaccines etc.) are significant. No less important is the method of administration (oral immunization is the least reactive), the season of the year, and the condition of the macroorganism (age, character of diet, existence of avitaminosis, state of nerves, endocrine, and cardiovascular systems, conditions encountered at work and during liesure time, previous illnesses, etc.). The presence of specific immunity is of special importance in the development of unpleasant post-vaccination symptoms. The first vaccination is usually the most reactogenic, although it usually involves a lower dose than subsequent vaccinations (I.M. Malyy, N.N. Chalyy, and T.A. Margolin, 1942). However, cases have been described of an unusual sensitization to subsequent administration of a given antigen, in the form of an intensification of the post-vaccination reaction (V.A. Yaroslavskiy and A.V. Fedorova, 1946, L.I. Aleksandrina, 1944).

In the production of vaccines certain tests have been developed for evaluating their toxicity. This evaluation is based on the mortality among animals undergoing experimental immunization with a given batch of vaccine, the temperature reaction and variation (decrease) in weight which occur, and the changes in the general condition of the animals. In addition, methods which make it possible to evaluate the intensity of the local tissue reaction to administration

of toxic vaccine products were tested. These include, for example, the intracutaneous injection of vaccines into rabbits. The size of the inflammatory infiltrations which develop and the presence of hemorrhages and necroses are used to judge the toxicity of the vaccine.

N.N. Klemparskaya (1948) established the following phases in the development of the local reaction by studying the cellular composition of preparations from the inflammatory focus produced at the site of intracutaneous injection of vaccines produced from gram-negative bacteria (typhoid, dysenteric, paratyphoid).

1. A stage of edema accompanied by the formation of a hemorrhagic exudate (30 minutes to 1-3 hours). The number of erythrocytes in the specimens increased continuously (to 100 in the microscopic field of view); petechia appeared, usually being of large size when toxic vaccines were administered. When the tissue was compressed the number of petechia increased, this indicating brittleness of the vessels.

2. A stage of local tissue leucocytosis (from 3 to 6 hours) with a prevalence of neutrophils. Leucocytosis appeared earlier in blood taken from the injection site than in other regions of the body. Leucopenia was observed in the peripheral blood during this period, being replaced by leucocytosis after 5-6 hours.

3. A stage of involution of the local symptoms (from 24 to 72 hours). At this time the edema disappeared, the petechia resorbed, the local leucocytosis disappeared, and there was almost no proliferation of tissue elements at the injection site.

The local symptoms which develop in response to injection of the killed vaccines generally used for human immunization disappear within 2-3 days. The tissue reaction to injection of live microbes is natural-

ly more severe and more prolonged than that to injection of killed vaccines. According to our observations (1956, 1961; the data from these investigations is given in Chapter 2), intracutaneous injection of 200-500 million-one billion cells from a live culture of Bacterium coli causes the development of an inflammatory focus, which undergoes involution over a period of 7-10 days. In this case culturing the blood of healthy animals does not show the development of a prolonged bacteriemia, while the bacteria introduced into the cutaneous tissue die rather than multiply. However, dissemination of bacteria from the focus of inflammation apparently occurs all the same, since N.N. Klemparskaya performed experiments involving intramuscular and subcutaneous injection of 100 million cells from a live culture of Bacterium coli on 48 mice and detected these bacteria in the liver, spleen, and kidneys during the first 3-5 days. The considerable quantity of Bacterium coli found in the urine of these animals when their bladders were opened indicates that the kidneys play an important role in purifying the internal environment of the organism of these bacteria.

What significance does the local inflammatory reaction have in the development of subsequent radioresistance? In our opinion it is of very great importance.

It is known that the development of a local inflammatory focus can activate the process of immunogenesis. The action of the so-called delayed-action vaccines (mixtures of antigens with slowly-resorbed substances) is based on this principle. Besides oils and mineral antigen adsorbants (calcium and aluminum phosphates and alum), whose action on tissue has been studied in detail in a number of works (Holt, 1950; Yu.B. Volgin, 1955; etal.), the stimulator developed by Friend (1954-1955) has now come into wide use; this preparation includes

mycobacterial cell components in addition to oils and an emulsifier. As histological investigations have shown (Weigle, Dixon, and Deichmiller, 1960; Mckinney and Davenport, 1961; Laufer, Tal Chloe, and Behar, 1959), Friend's stimulator causes a prolonged inflammation not only in the tissue at the injection site, but also in the liver, spleen, and lungs. It ensures a continuous flow of antigen into the organism, the concentration of antigen in the tissues rather than its total quantity being important. The presence of an inflammatory focus has a considerable influence on reflex activity, metabolism, alimentary excitability (P.N. Aleksandrov, 1956), the functioning of the reticuloendothelial system, and phagocytosis, causing nonspecific activation of the latter two (P.A. Vershilova and M.I. Chernysheva, 1960; A.I. Chuchukalo, 1957). It may be assumed that an organism having such an inflammatory focus will react differently to exposure to ionizing radiation.

In our first experiments on the influence of vaccination on radioresistance we noted that it was primarily those animals in which local inflammation developed in response to vaccination which survived. In order to intensify this reaction, in our later experiments we turned from injecting killed vaccines to immunization with a live microbial culture.

The research which has been done on tissue changes at vaccination sites and the data in the literature indicate that some cellular elements die in the inflammatory focus, this resulting in a change in the vital activity of the tissue and the formation of biologically active substances, the so-called endogenous agents (Kh.Kh. Planel'yes, 1955). In our opinion, the action of these agents may be of great importance in increasing (or decreasing, under certain conditions) the radioresistance of the organism. Thus, for example, tissue decomposi-

tion products denatured by the action of microbial toxins absorbed from the local focus of inflammation participate in the unusual process of autoimmunization, whose role in the development of radioresistance has not as yet been studied at all and is apparently very large. In addition, as T.A. Fedorova's observations have showed, during the period when antibodies are appearing (12-15 days after the first vaccination) depolymerization of deoxyribonucleic compounds is considerably intensified. This leads to an increase in the amount of their decomposition products (deoxycytidin) in the urine and indicates that the liberation of antibodies from the cells is accompanied to some extent by symptoms of cellular decomposition (Fig. 3).

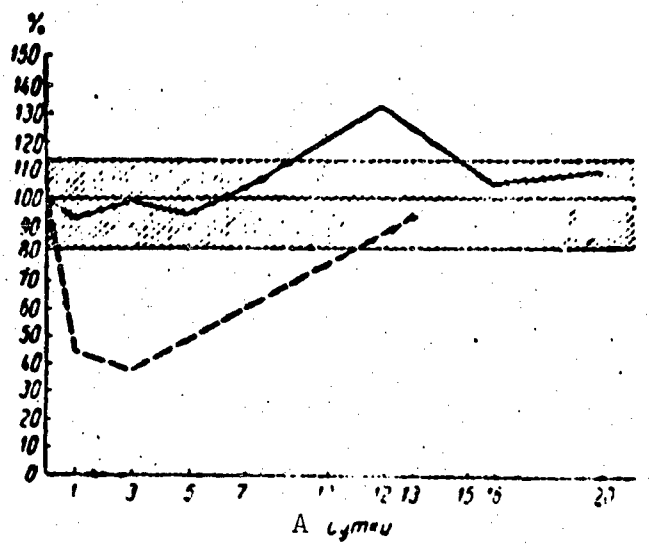

Fig. 3. Dynamics of changes in the quantity of deoxycytidin in the urine of rats inoculated with a vaccine prepared from Bacillus breslaviensis (solid line) and after injection of live bone marrow cells (dash line) (from data obtained by T.A. Fedorova). A) Days.

As a result of this autoimmunization by decomposition products the body begins to develop autoantibodies to the natural tissue products released during the compliment-bonding reaction. The investigations of P.N. Kiselev and his colleagues (1953) showed that after vaccination inoculation with staphylococcus or paratyphoid bacteria and autohemotherapy causes antibodies to denatured (with alcohol or

by heating) protein from the same organism to appear in the blood.

In 1960 Hackett and Beech published a work in which they reported that seven girls subjected to prophylactic immunization against poliomyelitis, tetanus, and typhoid-paratyphoid produced autoantibodies to their hepatic and renal tissues and the thyroglobulin of their lungs. The production of these antibodies reached a maximum during the 2nd-4th week and dropped to zero during the 5th week. Fresh tissue from a human cadaver rather than denatured tissue was used as the antigen.

In our investigations (N.N. Klemparskaya and N.V. Rayeva, 1961) on the formation of antibodies to tissue proteins in vaccinated dogs we also employed no additional denaturation of the tissue antigens. Physiological-solution extracts (30 minutes at room temperature) from healthy animal tissue were used as the antigens. In our experiments we and P.N. Kiselev compared the formation of antibodies to natural tissue decomposition products after inoculation with microbial vaccines with data on the serological reactions to these same antigens in irradiated animals. Irradiation causes cellular destruction in many organs and, increasing the permeability of the blood vessels, facilitates absorption of tissue substances into the blood stream.

I.I. Mishchenko and M.M. Fomenko in 1934 and P.N. Kiselev and his colleagues in 1955-56 proved that whole-body and local irradiation leads to the appearance in the blood of antibodies to denatured homologous protein and autoprotein. With the aid of the reaction by which complement is bonded to tissue antigens it was found possible to clarify the general characteristics of the action of vaccination and irradiation on the body.

In addition to establishing this similarity, we attempted in our investigations to reveal differences, which are of definite practical

importance.

The data which we have obtained in recent years have enabled us to recognize the important role of autosensitization in the development of radiation sickness. It is possible that the favorable effect which vaccination with bacterial antigens before irradiation has on the course of radiation sickness results from the autoimmunizing influence of vaccination with respect to tissue products.

In order to study the similarities and differences among the reactions of sera from vaccinated and irradiated dogs with tissue antigens we (N.N. Klemparskaya and N.V. Rayeva) used the complement-bonding reaction and our modification of Uan'ye's method (1955). After a number of preliminary experiments on denatured and fresh extracts of homologous organs and blood serum we selected fresh tissue from the mucous membrane of the small intestine of a healthy dog as the antigen for the complement-bonding reaction. This antigen had a minimal anticomplement activity and yielded the greatest number of positive results. The tissue was stored in a frozen state; an extract prepared from it with physiological solution and diluted to 1:100 served as the antigen. The complement-bonding reaction was carried out with dog sera taken before the beginning of the experiment, 15-20 days after vaccination, and 3, 10, and 15-20 days after irradiation.

The following modification of Uan'ye's method, which he used for establishing the presence of drug allergies, was employed to show autosensitization. Distilled-water lysates of washed erythrocytes* in dilutions of 1:30 to 1:15,360 was used as the antigen. At intervals of 2 minutes 0.1 ml portions of the erythrocyte lysate, beginning with the greatest dilution (1:15,360), were added to a plasma specimen (1.7 ml). After each addition the optical density of the mixture was measured with the aid of a Soviet-made FEKN-57 photoelectronephelo-

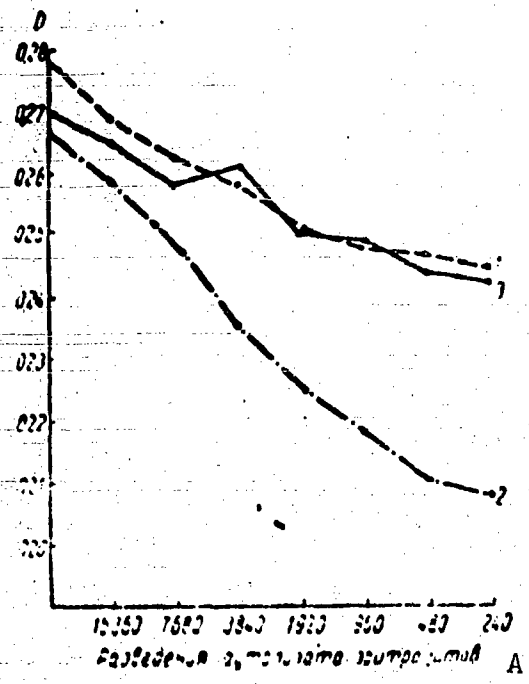

Fig. 4. Curves showing the change in the optical density (D) of plasma from dog No. 951 after addition of erythrocyte autolysate (the animal died on the 16th day after irradiation). 1) Before irradiation; 2) 3 days after irradiation; 3) 10 days after irradiation (positive reaction); A) Dilution of erythrocyte autolysate.

meter. The addition of the clear antigen solution to the plasma causes a progressive decrease in the optical density of the mixture. In the positive reaction, which occurs only at the optimal allergen dilution, the optical density remains at its prior level (plateau) or even somewhat increases rather than decreasing (Fig. 4). Positive reactions were obtained from 3 days after irradiation onward. The serum was investigated by this method at the same intervals used for the complement-bonding reaction. Serum from 43 dogs was studied, both methods being employed simultaneously for 21 of the subjects.

20 dogs were vaccinated by means of two intracutaneous injections of a suspension of living cells from a day-old agar culture of Bacterium coli, strain M_{17}, in physiological solution (dogs weighing 6-14 kg received 500 million cells and dogs weighing more than 14 kg 1 billion cells). The local inflammatory focus underwent involution after 7-10 days. The interval between vaccinations was 14 days, while 18 days elapsed between the last vaccination and the irradiation. The latter was carried out on the special EGO-2 apparatus and involved a lethal dose of γ-rays from Co^{60}. For dogs, this γ-ray dose from the source indicated has a biological effect equivalent to that of a dose of 600 r of x-rays. All of the control dogs (irradiated only) died

between the 15th and 20th days.

A considerable decrease in the severity of radiation sickness was observed among the vaccinated animals and a number of them survived. An analysis of these clinical peculiarities is given in Chapter 2. Of the 20 vaccinated dogs, 8 received antibiotics after irradiation. Since the basic observations were made before irradiation and, judging from their serological reactions, the sera of these dogs did not differ from those of the remaining 12 animals, we lumped them into one group in analyzing the results.

The data obtained with the aid of the complement-bonding reaction are shown in Table 2, from which it may be seen that the number and intensity of the positive reactions with the tissue antigen increased considerably (by a factor of approximately 3 according to the summed data) after vaccination and remained at this level after irradiation (increasing slightly). Whole body irradiation did not cause any increase in the number of positive reactions.

TABLE 2

Influence of Vaccination and Irradiation on the Results of the Complement-bonding Reaction (summed data is given for the reactions on the 3rd, 10th, 15th, and 20th days after irradiation)

Группа собак (A)	Число собак (B)	Число реакций (C)	Положительные реакции в разведении D: 1:5 число (E)	1:5 %	1:10 число	1:10 %	1:20 число	1:20 %	Общее число положительных реакций F: количество (G)	Общее число положительных реакций %
H 1. Непривитые необлученные	31	45	5	11	3	6,6	0	0	8	17,7
I 2. Только привитые[1] . . .	20	22	2	9	3	13,6	5	22,7	10	45,4
J 3. Только облученные . . .	11	33	2	6	3	9	0	0	5	15,1
K 4. Привитые и облученные	20	64	4	6,6	14	21,9	14	21,9	32	50

*These same dogs were included in group No. 4 for the investigation conducted after irradiation.

A) Group of dogs; B) number of dogs; C) number of reactions; D) positive reactions to following dilutions; E) number; F) total number of positive reactions; G) number; H) unvaccinated and unirradiated; I) vaccinated only; J) irradiated only; K) vaccinated and irradiated.

The difference between our data and those of P.N. Kiselev and I.I. Mishchenko may be explained by the different character of the antigen which we used, fresh-frozen homologous tissue rather than denatured autoserum. It is possible that our antigen showed changes in the blood serum which resulted from the active reaction in the surviving animals and were not present in the animals which underwent irradiation alone. When we used this method to examine the groups of dogs which survived single, twofold, and even threefold irradiation without treatment we found that three of the 7 animals exhibited positive reactions on repeated examination over a period of 5-8 months.

At the same time that the complement-fixation reaction was performed we determined the amount of agglutinins to the live culture Bacterium coli M_{17} used for vaccination. It was found that, possibly as a result of the rapidly-developing local inflammatory process, this immunization method did not produce any substantial increase in the titre of agglutinins over the level of normal antibodies to this microbe (1/40-1/160). There was only a temporary increase in the titre of agglutinins in 1 or 2 cultures, but this disappeared at the instant of irradiation. In addition, it must be noted that the majority of autostrains of Bacterium coli (multiplication of which leads to an indogenous infection in irradiated dogs) have no antigens in common with strain M_7 [sic], which was used in the vaccination. Their effectiveness consequently cannot be explained as resulting from the development of immunity to Bacterium coli.

Table 3 gives the results of an investigation of the serological

characteristics of the blood of the vaccinated and irradiated dogs by a modification of Uan'ye's method.

It may be seen that negative serological reactions predominated (90.6%) in the healthy dogs. The number of such reactions was considerably reduced (9.3%) after irradiation, i.e., the blood plasma acquired a new property, the ability to precipitate soluble substances produced from its own erythrocytes. A single vaccination caused a similar change, although the decrease was not as great in extent (to 27.7%). It is most interesting that there was no further decrease in the number of negative reactions in these vaccinated dogs after irradiation, just as many occurring as previously.

Using both methods in conjunction with the aforementioned data from the literature it was thus established that intracutaneous immunization with a live culture of Bacterium coli leads to a change in the body's reactivity both to the microbe in question and to tissue antigens. This change appears as an ability of the blood serum to react with antigen from the intestinal mucosa (the complement-fixation reaction) or with lysates of the erythrocytes themselves (by Uan'ye's method). The similarity between the reaction caused by vaccination and that produced by irradiation is demonstrated especially graphically by Uan'ye's method, since both factors cause positive reactions and one, as it were, "immunizes" against the other. A difference was discovered when the complement-fixation reaction was performed with a tissue antigen; vaccination with a live culture of Bacterium coli, accompanied by the formation of a local inflammatory focus, caused positive reactions, which somewhat increased in number under the influence of irradiation. A single irradiation fatal to the animals in the control group did not have this effect on the vaccinated subjects, but the survivors also exhibited positive reactions on

TABLE 3

Influence of Vaccination and Irradiation of Dogs and a Combination of These Two Factors on the Variation in the Number of Positive and Negative Reactions Obtained With an Autoerythrocyte Lysate by Uan'ye's Method

Группа собак A	Число собак B	Реакция до опыта C	Реакция после прививки D	Реакция после облучения по суткам E					Суммарно за период* 3 — 20-е сутки F			H % отрицательных реакций
				3-е	10-е	15—20-е	30-е	45-е	всего G	+	—	
I До опыта	32	29/32	—	—	—	—	—	—	32	3	29	90,6
J После двух прививок .	18	—	5/18	—	—	—	—	—	18	13	5	27,7
K После прививки и облучения	16	—	—	5/16	4/16	4/14	4/9	3/9	46	33	13	28,2
L Только после облучения	16	—	2/16	1/15	1/12	Собаки пали M			43	39	4	9,3

* Survival time for control dogs.

Note: The numerator gives the number of negative reactions and the denominator the total number of reactions at the time in question.

A) Group of dogs; B) number of dogs; C) reaction before experiment; D) reaction after vaccination; E) reaction after irradiation, by days; F) total for period* extending from 3rd to 20th days; G) total; H) % negative reactions; I) before experiment; J) after two vaccinations; K) after vaccination and irradiation; L) after irradiation alone; M) dogs died.

repeated irradiation.

Organisms inoculated with bacterial vaccines consequently develop antibodies to tissue products as well as to the microbes in question. It may be assumed that when such animals or persons are irradiated these antibodies fix the tissue-decomposition products which develop and thus keep them from acting on the organism; in our opinion, this latter is of great importance in the pathogenesis of radiation sickness.

The results of R.V. Petrov's experiments (1961) are a direct proof of the important role of tissue antigens and antitissue antibodies in the development of radiation affections; Petrov found that the survival rate among irradiated rats may increase after they are given antiserum to their intestinal tissue. The serum was adminstered in a dose of 0.1-0.5 ml 18 hours before irradiation and 6 hours afterward. Its effectiveness depended on the titre of the antibody to the intestinal tissue. Of the rats which received the serum 52% survived; 90% of the animals in the control group died. However, administration of large doses of serum aggravated the course of the radiation sickness.

All of the data cited above consequently enable us to conclude that autoimmunization increases the radioresistance of a vaccinated organism. One might suppose that the more intense the autoimmunizing influence, the higher will the radioresistance which develops be. However, this was not found to be so. There is apparently a definite optimum influence and exceeding it (hyperimmunization with tissue products) may even lead to the converse phenomenon, an increase in sensitivity to the action of radiation. In analyzing these very complex processes it is impossible to overlook the fact that in this case the antibodies are directed not at microbes, but at tissue substances and

formation of a large quantity of them may cause an undesirable cytotoxic influence on the tissues, which enhances the action of radiation.

In the experiments of N.N. Klemparskaya and N.V. Rayeva (1960) an attempt was made to intensify the formation of the local inflammatory focus by increasing the number of cutaneous injection sites for a live culture of Bacterium coli in rabbits and dogs (these data are given in detail in Chapter 2). In addition to increasing the radioresistance observed when a given dosage was injected at one point on the skin, there was a clear increase in sensitivity to radiation, this taking the form of a curtailment of survival time and an increase in the severity of radiation sickness. Ainsworth and Chase (1959) note that multiple vaccinations are less effective than single vaccinations with respect to the survival rate among irradiated mice. P.N. Kiselev and his colleagues (1956, 1959) obtained similar results in experiments on the effect of preliminary x-irradiation in small doses. If they subjected mice to single irradiations in doses of 119 r, their survival rate increased on subsequent lethal irradiation. If the same low-dosage irradiations were repeated three times (and the action of tissue-decomposition products consequently increased) the survival rate dropped rather than increasing after exposure to lethal doses. In experiments involving antigens prepared from denatured protein P.N. Kiselev was able to establish that the effect of single and triple preliminary irradiation in small doses varies as a function of the titres of the antibodies to the denatured serum protein.

Discovery of the antibodies to tissue substances, the quantitative laws governing their accumulation, and the dependence of the development of radioresistance on their formation is of theoretical and, doubly so, practical interest. This would make possible a direct

evaluation of the effectiveness of individual types of immunization in increasing radioresistance. This possibility began to appear in the experimental data cited above, when antigens prepared from fresh tissue from healthy animals was used, since the vaccinated dogs reacted to it differently than the irradiated animals. Further investigation is necessary for a final solution to this problem.

It must be noted that in analyzing the changes which vaccination produces in the body it is necessary to take into account their effect as an active stimulus which alters the functions of many important physiological systems. In 1952 A.D. Ado published his monograph "Antigens as Extreme Stimuli for the Body," in which he cited numerous data obtained in studying the effects of antigens on the activity of various organs and systems. It was established that administration of antigens alters the activity of the central nervous system, causes an increase in the pulse and respiration rates, and affects the metabolic rate (for example, it causes a substantial temporary increase in the blood sugar level, a decrease in the quantity of calcium in the blood, and a change in the K/Ca ratio). The excitability of the vascular chemoreceptors and the ratio among the protein fractions of the blood are altered (L. Olitskiy, etal., 1942; Loonely, 1945; I.A. Cheresh-iyev, V.G. Boreyko, and A.S. Nechayeva, 1959).

The reaction which develops during the first few days after immunization may be characterized as a stress reaction (H. Selye, 1960). However, all of these changes in the vital activity of the organism usually disappear within 1-2 weeks and it is thus interesting to study their effect on radioresistance only when irradiation is carried out during the first 2 weeks after vaccination, but never later (when the effectiveness of the action of vaccination on radioresistance is frequently even more marked). The importance of the tachyphylaxis

which develops after parenteral administration of a number of foreign substances must be judged in similar fashion. As is well known, the term tachyphylaxis is used to refer to the rapid increase in the resistance of the organism to protein and infectious agents which occurs after administration of foreign proteins and microbial vaccines.

In 1894 V.I. Isayev described the rapid development of resistance to administration of Vibrio comma in pigs after injections of milk, urine, bouillon, and tuberculin. B.V. Polushkina's book (1960) gives a detailed survey of the literature on tachyphylaxis.

It is interesting that the vaccine dosage is of great importance in producing this rapid increase in resistance after injection of microbial preparations. As T.A. Levina noted (1960), increasing the dosage of a streptococcal antigen leads to a decrease rather than an increase in the resistance of the organism. According to V.V. Skorodinskiy and N.E. Shchastniy (1960), the nonspecific increase which occurs in the resistance of the organism after administration of medicinal sera results not from the action of their protein fractions, but from the thermostable substances of the proteinless dialysate.

It is thus possible to alter the reactivity of the organism considerably by injecting microbial preparations and foreign protein and this cannot help but affect the characteristics of its reaction to ionizing radiation. However, the tachyphylaxis is very short, being limited to a few hours or, at most, 2-7 days. This phenomenon consequently plays no role in explaining the survival of irradiated animals vaccinated 2-8 weeks or more before irradiation.

Finally, in analyzing the changes which occur in the body after vaccination we believe it necessary to take into account still another phenomenon which usually accompanies administration of various vaccines. We are speaking of the variations in the number of leucocytes

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Finally, in analyzing the changes which occur in the body after vaccination we believe it necessary to take into account still another phenomenon which usually accompanies administration of various vaccines. We are speaking of the variations in the number of leucocytes

in the peripheral blood. A number of authors (Robertson, and You, 1938; Olitzki, Avinery, and Bendersky, 1941; V.L. Troitskiy and R.A. Veger, 1944; N.N. Klemparskaya, 1946) established that a marked leucopenia develops within 1-4 hours after administration of various vaccines, peptone solutions, and foreign proteins; this is replaced by leucocytosis and subsequent normalization of the leucocyte count (over a period of 1-2 days). It was also established that this phenomenon had a "threshold," since it is produced only when the vaccine dosage exceeds a certain level. In studying the leucopenic reaction to repeated vaccination it was found that leucopenia does not develop in this case. This was termed antileucopenic immunity (Olitzki, etal., 1941; N.N. Klemparskaya, 1952). In 1948 N.N. Klemparskaya conducted investigations directed at the use of this phenomenon as a test for evaluating the toxicity of vaccines (the minimum leucopenic dose decreases as tocicity increases) and for measuring the specific resistance of the inoculated organism (it was found possible to determine the intensity of antileucopenic immunity from the number of minimum leucopenic doses of vaccine which the organism can withstand without developing leucopenia).

This same author established that the time required for leucopenia to develop as a manifestation of a redistribution of leucocytes depends on the vaccine dosage and the manner in which it is administered. Leucopenia resulting from enteral administration of microbes was the first to be described; it was found that this mode of immunization creates an antileucopenic immunity to parenteral but not enteral administration of microbial products (the same being true of subcutaneous vaccination). The specific characteristics of the leucopenic reaction in rabbits, pigs, cats, and humans were studied. It was shown that man is the most sensitive to the leucopenic action of

vaccines prepared from typhoid, dysenteric, and paratyphoid bacteria. Finally, this work established the important role of vaccine-soluble bacteriolysis products and the rate at which they are absorbed in the development of leucopenia (the killed microbial bodies of a vaccine and precipitated vaccines do not cause leucopenia, but the liquid component of such a preparation is very active).

The relationship between the toxicity of a vaccine and the intensity of its leucopenic action enables us to consider this test to be suitable for evaluating the toxic influence of a vaccine on man and animals and, in additon to its interest for the vaccine industry (in choosing strains of low toxicity and selecting a method for preparing an areactive vaccine), may be of great importance in determining the effect of vaccination on radioresistance. The toxic action of a vaccine results from destruction of a number of cellular elements, possible denaturation of tissue antigens, conversion of the latter to autoantigens, etc.

The comparative action of vaccines of high and low toxicity on radioresistance must be investigated, on the basis of calculation of their leucopenic action on primary immunization.

As N.I. Krugla's investigations (1959) showed, the leucolytic properties of the blood serum and the phagocytic activity of the leucocytes increase as the leucopenic reaction develops. I.I. Mechnikov showed that leucocyte-decomposition products play an important role in stimulating the functions of these cells. However, despite the great importance of this problem, very little research has been done on the role and nature of the leucolytic substances in the blood. They have attracted a great deal of interest in recent years as a result of the discovery of leucolysins in certain autoimmune blood diseases (J. Dosse, 1959), in radiation sickness, and in homosensitiza-

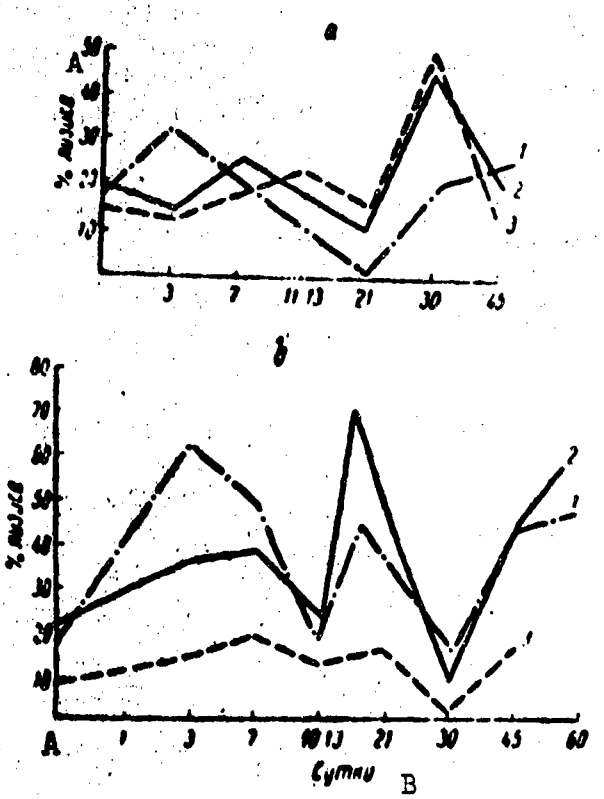

Fig. 5. Dynamics of the cytolytic activity of the blood and organs of a rat after administration of homologous bone-marrow cells (a) and a bacterial antigen (b). 1) Blood; 2) bone marrow; 3) spleen; A) Lysis; B) days.

tion (N.N. Klemparskaya, 1957).

It has been established that an increase in leucolytic activity is regularly observed after exposure of the organism to microbial and tissue antigens (heterologous antigens, homologous antigens, and autoantigens). N.N. Klemparskaya discovered that they appear (in definite amounts) not only in the blood plasma of healthy unvaccinated animals, but also in extracts of their organs. A special study of the dynamics of the change which occurs in cytolytic activity in rats after administration of tissue and microbial antigens, conducted by N.N. Klemparskaya (by the method developed by the author in 1957) in conjunction with the hematologists M.F. Sbitneva and T.V. Kalyayeva, and the biochemist T.A. Fedorova made it possible to determine

the characteristics of the appearance of leucolysins and their distribution in various organs.

It was found that there are three periods during which the leucolytic activity of the blood and organs increases after administration of antigens:

1. The early reaction, the first 1-3 days after immunization;

2. The intensification of leucolytic activity during the second week (during the period when antimicrobial immune bodies are appearing);

3. The late reaction, encompassing the 45th-60th days (the period of autoimmune complications following administration of tissue antigens).

Administration of a vaccine prepared from Bacillus breslaviensis to rats caused an increase in the cytolytic activity of their blood plasma and bone marrow during all three of these periods. It is interesting that the leucolytic activity of splenic extracts remained within the limits of normal variation over a period of 60 days (Fig. 5).

Intravenous injection of homologous bone-marrow cells ($1-1.7 \cdot 10^8$ nucleate elements) led to another type of reaction; after a slight rise in leucolytic activity in the blood plasma no increase in the quantity of leucolysins in the blood or organ extracts was noted for an extended period. However, a considerable increase in the quantity of leucolysins in the spleen and bone marrow was detected on the 30th day; this index reverted to normal toward the 45th day. This late reaction coincides with the development of autoimmune complications produced by the therapy employed; these probably resulted from the production of leucolysins by the transfused bone-marrow cells, which are known to be capable of forming antibodies to the proteins of the

recipient. Observations of the dynamics of leucolytic activity consequently make it possible to detect certain peculiarities in the reaction of the organism to antigenic substances of different natures.

According to our data, there is a considerable increase in the cytolytic activity of the plasma and organs during the first 3-7 days in animals irradiated with lethal doses of x-rays or γ-rays from Co^{60}. This increase in the quantity of leucolysins present precedes the development of the leucopenia which is so characteristic of radiation affections.

What value can these data have in studying the effect of vaccination on radioresistance? It seems to us that studying the leucolytic activity of the blood and organs of vaccinated and irradiated animals shows the common character of these two agents, which are antigenic stimuli. Tissue autoantigens, whose existence was discovered by a number of authors (R.V. Petrov and L.I. Il'ina, 1956; L.A. Zil'ber, V.A. Artamonova, G.M. Frank, and A.D. Snezhko, 1956), become the antigens on irradiation; this role is played by the microbial substances on immunization and by the transplanted tissues on transplantation.

In addition, observation of leucolysin dynamics makes it possible to evaluate the duration of the reaction of the organism to the antigens used and the predominance of the participation of certain organs in this reaction. The nature of leucolytic substances requires further study, but it is now clear that they play an important role in autoimmune diseases and radiation affections. The presence of a definite quantity of these substances in the blood plasma before the development of leucopenia indicates their relationship to leucocyte destruction, not only in vitro, but also in vivo.

The dynamics of the cytolytic reactions which occur in vaccinated and irradiated animals consequently show that there is a definite similarity between the action of vaccination and that of irradiation. This is also indicated by the active participation of certain physiological systems (reticuloendothelial, nervous, endocrine). Taking into account the mutual attenuating influence of vaccination and irradiation, we can assume the presence of known concurrent relationships as a function of their temporal sequence, since it is well known that if the body is actively reacting to a given antigen administration of another antigen (in the case of irradiation after vaccination these later antigens are the tissue antigens) may be ineffective and no reaction may develop to it. It is possible that this is also the essence of the favorable influence of vaccination on radiation sickness, a phenomenon which we (N.N. Klemparskaya, O.G. Alekseyeva, R.V. Petrov, and V.F. Sosova, 1958) feel to be based on the development of an autoimmune reaction to the tissue antigens.

There are now a great many works devoted to studies of antigen concurrency. Inhibition of the immunological reaction after sensitization to another protein antigen has been described by P.F. Zdrodovskiy (1947), A.A. Kiimentova, (1949), and I.Ye. Alatyrtseva and S.A. Usmanova (1955). F. Adler (1957) has pointed out the special importance of the dosage and quality of the antigens, as well as of the existence of an anamnestic reaction to one of them. V.I. Ioffe, and A.P. Kopytovskaya (1957, 1958) made a detailed study of the characteristics of the intensity of antigenic stimulation in cross-sensitization and desensitization. The works of M.A. Frolova, etal. (1958, 1960) show the possibility of completely suppressing the development of sensitization to a "second" allergen by altering cholinergic processes. Concurrent suppression of allergic processes by immunization

with bacterial vaccines has been used to prevent and treat bronchial asthma and hemolytic anemia in newborn infants (Kemmerer, 1936; O. Gunter, 1955). The article by P. Abramov (1960) gives an exhaustive survey of a number of works on antigen concurrency.

A number of data which are analyzed in detail in this article show that not all antigens enter into concurrent relationships (strong and weak antigens); it was discovered that the vaccination dosage and the intervals between injections play an important role, a number of other factors also having their effect. Tissue autoantigens and homologous antigens for a given organism are weak antigenic stimuli and their action on the body, which leads to autointoxication and autosensitization and plays an important role in the development of radiation sickness, may apparently be suppressed by preliminary administration of "strong" microbial antigens.

The existence of concurrent relationships between tissue and microbial antigens is also confirmed by the fact that concurrency is observed when the situation is reversed. It is well known that if irradiation precedes immunization a reaction (radiation sickness) develops to the former; while the reaction to the microbial antigen is suppressed and immunogenesis is disrupted.

Many of the processes which occur in the organism after administration of antigenic substances are brief in character and revert to a normal level after 5-15 days. Others such as the cellular reaction and the suppression of antibodies to tissue antigens last for a long time. The nonspecific activation of immunological reactivity which occurs under the influence of vaccination may be very prolonged and, as the observations of a number of authors (G.G. Karapetyan, 1957; Palesi and Vannucchi, 1958) have shown, manifests itself in the fact that vaccinated (with BCG vaccine) children grow and develop

better, are less sickly, and have lower mortality.

Administration of microbial antigens consequently may have a substantial and frequently a long-lasting influence on the vital activity of the organism, altering various of its functions. The specific importance of these changes in the development of resistance to irradiation is still unclear in many respects, but there are now data which make it possible to deduce from all of the facts which we have noted that the local reaction is of great significance, because of its autoimmunizing influence and its ability to cause the formation of antibodies against tissue antigens, and that antigen concurrency, the mechanism of which requires further study, also participates. Familiarity with the materials given above indicates the importance of further study of various types of post-vaccination reactions in solving a number of immunological and radiobiological problems of both theoretical and practical significance.

[Footnotes]

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4	Literature data on this problem are given in Chapter 2.
14	Here we are concerned with vaccines prepared from gram-negative bacteria and antitoxins.
21	Distilled water is added to the erythrocyte sediment to restore the original volume of the blood.

Chapter 2

REACTION OF THE IMMUNIZED ORGANISM TO IRRADIATION

All data given in this monograph on radiation affections relate to only one type of reaction to radiation, acute radiation sickness with its typical symptomatology, which is characterized by stages of development and a definite prolonged course. We will not dwell here on the well-known characteristics of the four periods of acute radiation sickness (the period of initial reactions, the latent period, the height of the illness, and convalescence or death), but will only remark that the specific radioresistance of animals is far from uniform; guinea pigs are the most sensitive in this respect, dying after whole-body irradiation in doses of 200-300 r. Mice, dogs, and monkeys die after exposure to 400-600 r. Rabbits are the most resistant, dying after receiving doses of 800-1000 r. The age and weight of the animal are of great importance. As a rule, the larger the animal the greater is its resistance. The conditions under which the animal is kept (diet, vitamins, isolation) and the presence or absence of diseases and traumatic agents play an important role. All of this makes it necessary to use different species of animals in studying the action of any agent on radioresistance, carefully selecting the subjects by weight, sex, and age and carefully watching control animals, i.e., irradiated animals from the same batch which have not been subjected to the agent in question, over an equal period of time.

It has now been established that the action of the active ions which develop in tissue on irradiation causes complex physicochemical processes (in particular, depolymerization of high-molecular-weight compounds), alters tissue permeability and metabolic reactions, and disrupts the activity of the nervous, endocrine, and cardiovascular systems. The destruction of a large number of cells (for example, lymphoid tissue, the epithelium of the intestine and gonads, etc.) and change in permeability which occur on irradiation are, in our opinion, the most important factors in the pathogenesis of radiation sickness. Absorption of autotissue-decomposition products into the blood stream leads to the development of primary toxemia, which can also be induced without irradiation, by intravenous injection of tissue homogenates or cellular organelle fractions (N.N. Klemparskaya, R.V. Petrov, and L.I. Il'ina, 1958). The action of these products on the general chemoreceptor fields later (after the latent period) leads to the development of an autoallergy, i.e., an increase in sensitivity to autotissue products and formation of cytotoxic antibodies, which disrupt the mal functioning of the organism.

The increased sensitivity of irradiated animals to autotissue products may appear during the latent period. In order to induce this it is sufficient to inject 0.1-0.5 ml of sterile distilled water into certain areas of the skin; this causes local decomposition of the tissue cells. When an autoallergy is present such a "reacting" stimulus causes a local hyperergic reaction of a hemorrhagic character, which develops 6-24 hours after injection rather than immediately. A hyperergic reaction may be induced in the tissue of the upper lip by injection of a suspension of homologous tissue, as well as by injection of distilled water (N.N. Klemparskaya, N.A. Krayevskiy, and V.V. Shikhodyrov, 1958). Autoimmunological processes, i.e., reactions to tissue products

which generally do not enter the lymph and blood streams, consequently play an important role in the development of radiation sickness. It is interesting that the antigenic properties of these products may vary, thus facilitating the development of autosensitization (R.V. Petrov and L.I. Il'ina, 1956; L.A. Zil'ber, etal., 1956). Autosensitization is especially marked during the height of radiation sickness. During this period the blood has intense cytotoxic properties and autoantibodies to tissue antigens may appear in it; the dermal reactions to injection of distilled water are sharply positive. If the animal recovers the intensity of these phenomena gradually decreases, but the aforementioned reactions persist considerably longer than the clinical symptoms of the disease. In our opinion, investigation of immunological processes is extremely important in searching for a way to alter the course of acute radiation sickness, since many of its symptoms – the hemorrhagic syndrome, leucopenia, and affection of the gastrointestinal tract – remind one of an allergy. It is not accidental that desensitizing substances (dimedrol, calcium chloride, hormones, etc.) are effective in radiation sickness. However, we believe that, in addition to medicinal desensitization, employment of measures which reduce the reaction of the organism to the perception of tissue antigens may also affect the development of autosensitization. These methods include preliminary vaccination, an analysis of whose action on the course of radiation sickness is the subject of this discussion.

A study of the characteristics of the course of radiation sickness in animals vaccinated before irradiation was undertaken by immunologists engaged in investigating the development of specific immunity under the action of ionizing radiation. Numerous investigations showed that if an antigen is administered before irradiation (even several hours before exposure) an active immunity develops, its intensity decreasing durin-

the appearance of the clinical symptoms of radiation sickness. If vaccination is carried out after irradiation (even 1-2 hours later) the formation of active immunity is considerably disrupted (M. Hazek and A. Lengerova, 1960; N.N. Klemparskaya, O.G. Alekseyeva, R.V. Petrov, and V.F. Sosova, 1958; V.L. Troitskiy and M.A. Tumanyan, 1958; N.N. Klemparskaya, 1956; V.L. Troitskiy, O.V. Chakhava, and N.L. Koslova, 1956; etal.). It was noted that some of the animals vaccinated before irradiation survived without any additional treatment (L. Hectoen, 1915).

Another approach to observation of the reactions of the vaccinated organism to irradiation was the attempt made by certain authors (F. Smith, U. Smith, L. Gonshery, and M. Grenan, 1954) to protect their subjects from the bacteremia which regularly develops during radiation sickness. For this purpose they preliminarily immunized mice and rats with heat-killed vaccines prepared from colon and paracolon bacilli and protei. Single and sixfold (over a period of 21 days) vaccinations were carried out. The presence of immunity was checked by inoculating the animals with a live culture of the microbe in question, but no correlation was found between the titre of antibodies and the survival rate of the animals. No increase in survival rate was noted after $LD_{60-90/30}$ x-irradiation, but the number of cases in which bacteria cultured from the irradiated animals was smaller among the subjects which were vaccinated first.

F. Smith, U. Smith, H. Andrews, and M. Grenan (1955) established that parenteral administration of inorganic substances pulverized to 44-177 μ (pyrex glass, quartz, limestone, calcite) to mice during the first hour or two after irradiation in a dose of 600 r has a favorable effect. The development of a local inflammatory process promoted an increase in the survival rate, a decrease in the intensity of bacteremia,

and an increase in the effectiveness with respect to inoculation with live protei of preliminary immunization with vaccines prepared from colon commensals.

Aseptic inflammatory processes induced by injection of turpentine were observed to have a positive effect in animals poisoned with polonium in the laboratory headed by Prof. I.A. Pigalev. According to the data of A.I. Chuchukalo (1957), survival time increased, there was less damage to the blood system, and reticuloendothelial functioning was activated in animals with inflammatory foci. V.V. Vasilyevskaya observed a more favorable course of radiation sickness on subcutaneous injection of 0.1-0.03 μcuries of polonium in rabbits vaccinated with typhoid vaccine or diphtheria toxoid than in unvaccinated animals. For example, the mean survival time among the vaccinated rabbits was 25-30 days, while that among the unimmunized animals was 19 days; the loss in weight for the animals in the first group was 3-13%, while that for the animals in the second group was 21%. The leucopenic reaction was also less marked among the vaccinated rabbits.

In their investigations Rowan, Moos, and Samter, (1955) showed that the resistance of various species of animals to radiation differs and is correlated with their sensitivity to histamine. In their experiments, injecting mice with pertussis vaccine 5 days before irradiation in a dose of 300 r increased not only the subjects' sensitivity to histamine, but also the mortality among them; 75-85% of the vaccinated mice died, as against 12% of the unvaccinated animals. Vaccination with diptheria toxoid and typhoid vaccine at the same interval did not have a favorable influence either; 25-30% of the mice died. Conversely, D.R. Kaulen noted an increase in survival rate among pigs vaccinated with diptheria toxoid one month before irradiation in a dose of 300 r.

The experiments which N.N. Klemparskaya (1957) conducted to study the effectiveness of antityphoid immunization at various times before and after irradiation showed that it is possible to increase specific immunity in irradiated animals by conjoining preliminary immunization (first vaccination) before irradiation with revaccination after exposure. These experiments were the first in which attention was called to the fact that the external appearance of mice vaccinated once with typhoid-paratyphoid tetravaccine 15-20 days before irradiation in a dose of 300 r differs considerably from that of irradiated unvaccinated animals. The vaccinated mice continued to grow and gain weight, maintained a good appetite, exhibited no disturbances of intestinal functioning, and did not die on exposure to ionizing radiation. These data force us to assume that preliminary vaccination not only promotes an increase in the effectiveness of specific immunization, but also affects the radioresistance of the organism.

In order to verify this hypothesis a group of authors (N.N. Klemparskaya, V.F. Sosova, O.R. Nemirovich-Danchenko, and G.M. L'vitsyn, 1957) conducted special experiments on mice and rabbits, which were given various bacterial vaccines (prepared from typhoid, paratyphoid, colon, and dysenteric bacteria and BCG) 2-4 weeks before irradiation. The data obtained in these experiments showed that preliminary vaccination increases the resistance of mice and rabbits to x-rays and polonium-induced affections.

The protective effect of immunization was confirmed by the research of E. Ainsworth and H. Chase (1959). Thus, these authors traced the influence of preliminary immunization with heat-killed vaccines prepared from various types of microbes on the survival rate among mice irradiated in a dose of 700-600 r. When 100% of the control animals died (at a dose of 700 r) 5.5% of the animals vaccinated with

vaccines prepared from typhoid and paratyphoid types A and B microbes died. Vaccines prepared from colon bacilli, protei, and capsular bacteria protected 46-56% of the mice to which they were administered, while injection of heat-killed vaccines prepared from streptococci, staphylococci, and a zymose preparation proved to be ineffective. Both corpuscular vaccines (prepared from whole microbial cells) and endotoxin preparations extracted from these bacteria were found to have a protective action. The authors note that it is possible for 16-27% of the surviving mice to die later (after 180-200 days). It is interesting that intensifying the immunizing stimulus by increasing the vaccine dosage for the number of injections did not increase the survival rate. Preliminary immunization at various times (from 24 hours to 7 days inclusive) was also studied in this work, vaccination 24 hours before irradiation in a dose of 600 r being found to be most effective (all of the immunized mice survived; 63% of the control mice died).

An intensification of immunogenesis under the influence of vaccination with homologous and heterologous antigens before irradiation was observed in the experiments on rabbits conducted by M.I. Ravich-Shcherbo and L.G. Prokopenko (1960) and A.P. Duplishcheva and O.V. Chakhava (1960). They also noted a considerable decrease in the severity of radiation sickness in vaccinated animals.

The works of different authors consequently confirm the fact that administration of bacterial vaccines has a protective influence with respect to subsequent irradiation. There is also an intensification of immunogenesis in response to administration of antigens (of the same or a different type) to an irradiated animal, this indicating the nonspecific character of preliminary immunization.

An increase in radioresistance may also be achieved by employing

protective-prophylactic measures, of which there are many (sulphur-containing amino acids and amines, sulphides, cyanide compounds, polysaccharides, methemoglobin producers, hormones, vitamins, carbon monoxide, and narcotics). The most effective chemical protective agents are derivatives of l-cysteine and cystamin and bacterial polysaccharides. Many of these drugs are quite toxic and cause symptoms of anoxemia soon after they are administered; certain authors (Ye.F. Romantev and A.Ye. Savich, 1958) believe this anoxemia to be basic to the mechanism by which they act.

How can the favorable influence on the irradiated organism of such chemically different substances be explained from the immunological standpoint?

It was found that, despite differences in their natures, all of these substances have one common property, which we feel to govern their radiation-protection effect. This common property is their ability to alter and occasionally even completely halt the functioning of the chemoreceptors, primarily in the vascular network (L.N. Bogatskaya, and Yu.S. Kogan, 1953; G.I. Smorodintseva, 1955; M.A. Frolova, 1951; [sic]). It may be remembered that the protective effect of these substances is usually manifested only when they are administered 15 minutes to 4 hours before irradiation. An organism protected by these agents is consequently exposed to the action of radiation with its chemoreceptor apparatus considerably depressed. This decrease in the sensitivity of the chemoreceptors reduces the effect of the toxic influence of the tissue-decomposition products formed in the irradiated tissues and absorbed into the blood stream, alleviating the symptoms of the primary reaction. The blocking of the chemoreceptors makes it impossible for the tissue products to have an autosensitizing influence and the subsequent radiation sickness is consequently less severe.

Many investigators have occupied themselves in studying effective protective agents. However, chemical protection against the action of radiation has serious drawbacks. These include the necessity for parenteral administration and the toxic effect of such chemicals on the body, which limits the usable dosage, as well as the brevity of their action, since they are either excreted from the organism comparatively rapidly or are soon destroyed in it. The brief duration of their protective effect requires that such substances be administered as close as possible to the time of irradiation (from 15-30 minutes to several hours beforehand) and this is very difficult in certain cases.

Considering these substantial drawbacks on the part of chemical protection, we must note the advantages of increasing radioresistance by administering vaccines, the principal gain being the duration of their protective influence, which is measured in months rather than hours. Modern vaccines have a very slight toxic influence and their protective effect may be intensified by repeated immunization (revaccination). It is understood that there are cases in which the action of chemical protective agents is more intense than that of vaccination. In this connection vaccination can be recommended for protection against moderate doses of radiation (for example, for workers handling radioactive substances or in the therapeutic use of ionizing radiation).

In addition to its practical significance, studying the action of vaccination on radioresistance is important in investigating the pathogenesis of radiation sickness. The fact that there are concurrent relationships between the antigenic influence of microbial substances and the effect produced by irradiation indicates that immunological processes participate in the development of radiation sickness and shows the paths which we should take in searching for improved therapeutic-prophylactic measures.

Different vaccines, animals (mice, pigs, and rabbits), and irradiation doses have been used in experiments conducted to study the action of vaccination on radiation sickness, since these involve different goals and different problems. The greatest number of experiments have been performed by administering vaccines several days before irradiation, i.e., during the period when the specific reaction to the antigen administered is still far from developed and there are only nonspecific changes in a number of physiological systems. There is now a need for one author to conduct a detailed investigation of the protective action of immunization before irradiation, studying the influence of various immunization conditions, e. g., vaccine dosage, number of injections, interval between vaccination and irradiation, type of preparation, etc.

Our work began with experiments on small laboratory animals, which made it possible to elucidate a large number of basic mechanisms (before using vaccines in experiments on larger animals).

The vaccine dosages ordinarily employed to obtain a high-level antibacterial immunity were employed in our investigations. We paid less attention to studying the action of small vaccine doses (experiments of this type were conducted only with EV antiplague vaccine), since use of such doses in epidemiological practice is prohibited by the fact that they have a sensitizing rather than an immunizing effect. In order to investigate the action of bacterial vaccines on radioresistance we consequently gave our subjects the immunizing doses normally employed for animals of their species.

In N.N. Klemparskaya's experiments the influence of immunization before irradiation with various bacterial vaccines made from live and killed microbes and endotoxins on radioresistance was studied in 1186 male white mice weighing 16-25 g.

The mice were irradiated in a RUM-3 x-ray apparatus under standard conditions: 180 kv, 15 ma, skin-focus distance-50 cm, dose rate 22-23 r/min, filter - 1 mm Al + 0.5 mm Cu. With the exception of the first experiments, which involved immunization with a tetravaccine (irradiation in a dose of 400 r), a dose of 500 r was used in all of the other experiments, this being the LD_{50-80} after 30 days, depending on the subject's age. The vaccines were administered subcutaneously, intracutaneously, intraperitoneally, and enterally at intervals of from 15 minutes to 2 months before irradiation. Sterilized cream was used as a control to determine the specificity of the action of the bacterial vaccines.

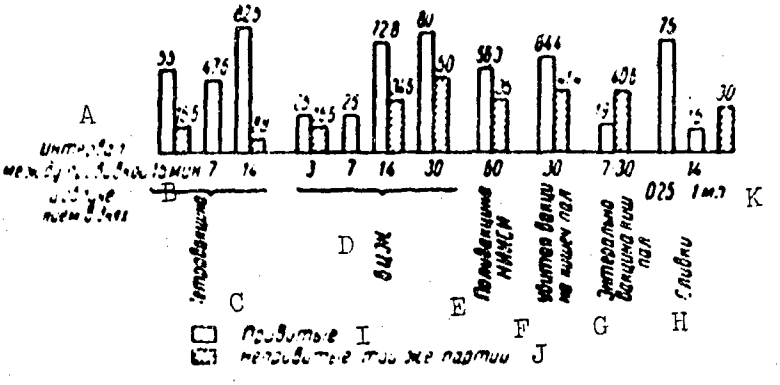

Fig. 6. Percentage survival among mice vaccinated with various vaccines before irradiation in a dose of 500 r. A) Interval between vaccination and irradiation, in days; B) min; C) tetravaccine; D) BCG; E) NIISI polyvaccine; F) killed vaccine prepared from colon bacilli; G) enterally-administered vaccine from colon bacilli; H) cream; I) vaccinated animals; J) unvaccinated animals from the same batch; K) ml.

Since the experiments were not conducted on animals of pure lineage and because the role of the interval between vaccination and irradiation was studied in mice of different weights (from 16 to 25 g), each experiment was necessarily accompanied by controlled irradiation of unvaccinated mice from the batch in question. The total number of control animals (477) was less than the number of subjects (709), since

mice from one batch inoculated with different vaccines or with different doses of the same vaccine were irradiated simultaneously, which made it possible to use a single control group.

As the killed vaccines we used a tetravaccine (prepared from typhoid and paratyphoid A and B bacteria and Flexner bacilli), NIISI polyvaccine prepared from endotoxins of pathogenic colon bacteria, a vaccine prepared from Bacterium coli M_{17} killed by 600,000-r irradiation or by heat, and a live culture of the same strain. Live vaccines (BCG and EV) were also tested. Sterilized cream was used as the nonmicrobial antigen.

The animals withstood vaccination well, continuing to gain weight and having a good appearance and appetite. After 2 weeks to 1 month the experimental animals had gained 0.8-2g more than the unvaccinated control animals.

Before conducting experiments involving immunization with a live culture of Bacterium coli we made a special study of the propagation of this microbe throughout a mouse's body from an intracutaneous focus into which 100-200 million cells from a day-old agar culture of strain M_{17} was injected. Cultures made of the blood, organs (site of injection, spleen, liver, and kidneys), and urine from the bladder showed that the body cleanses itself of the injected bacteria within 6-7 days; the number of bacteria decreases continuously rather than increasing (these experiments were performed on 62 mice). V.F. Sosova came to the same conclusions in studying the dissemination of a live culture of Bacterium coli in the tissues of a dermal focus in rabbits injected with it and the quantity of live bacteria therin.

The immunity created by vaccination was evaluated by inoculating the vaccinated animals with typhoid and paratyphoid bacteria, using the generally employed methods. In order to determine the intensity of

tive immunity produced by vaccination with Bacterium coli the subjects were inoculated intramuscularly with 1-2 billion cells of strain M_{17} (1 DCI = 1 billion cells). No special check was made on the intensity of the active immunity created by immunization with BCG vaccine. An entirely sufficient degree of specific immunity (complete resistance to LD_{50} and a survival rate of 80-90% at LD_{100}) was noted within 7-10 days after a single immunization.

In addition, the dynamics of antibody (agglutinin) formation were determined, (except in the experiments on BCG vaccine). An increase in the agglutinin titres was noted after 7-10 days, with the maximum increase occurring at 15 days and a decrease beginning during the 3rd or 4th week.

Irradiation was thus carried out during the period when the development of specific immunity was ending and it was beginning to fade away (judging from the titre of antibodies). In this book we will not give any special analysis of the results obtained in calculating the intensity of specific immunity for each series of vaccines, since this does not enter into the tasks which we set outselves. According to the mean data shown in Table 4. the percentage survival among the mice vaccinated before irradiation was 55.5%, while that among the unvaccinated mice was 33.9%. However, it must be taken into account that these are mean data obtained for different preparations administered at various times to different groups, which included mice from different strains and of varying age and weight.

A comparison of the data from each experiment with that from the corresponding control group is far more precise. As may be seen from Table 4 and Fig. 6, all of the bacterial vaccines tested and the sterile cream increased the survival rate among mice contracting radiation sickness when they were administered before irradiation, i.e.,

TABLE 4

Influence of Preliminary Vaccination on Survival Rate Among Mice After X-irradiation

A Вид, доза вакцины и путь введения	B Привитые						Контрольные (непривитые) C				
	интервал до облучения D	доза облучения в *р* F	всего мышей F	из них G: выжило H	из них G: пало I	% выживаемости J	доза облучения в *р* E	всего мышей F	из них: выжило H	из них: пало I	% выживаемости J
K Тетравакцина ТАБФ, однократно 0,2 *мл*, подкожно и внутрибрюшинно	15 минут V	400	54	30	24	55	400	24	4	20	16,6
	1 неделя W	400	42	20	22	47,6		—	—	—	—
L То же	2 недели	400	40	33	7	82,5	400	34	3	31	8,8
»											
M БИЖ, однократно 1 *мл*, внутрикожно	3 суток X	500	24	6	18	25	500	24	4	20	16,6
	7 суток	500	24	6	18	25	—	—	—	—	—
То же	14 суток	500	96	70	26	72,8	500	91	32	59	34,6
»	1 месяц Y	500	26	21	5	80	500	18	9	9	50
N Поливакцина НИИСИ, трехкратно, по 0,1 *мл* с интервалом 14 дней, подкожно	2 месяца	500	48	27	21	56,3	500	40	14	26	35
O Третья вакцина из кишечной палочки, однократно, 200 млн. подкожно	1 месяц	500	78	38	40	48,8	500	41	13	31	29,5
P Вакцина из кишечной палочки, убитая облучением 600 000 *р* однократно 250 млн. подкожно	2 месяца	500	76	49	27	64,4	500	41	17	24	41,4

CONTINUATION OF TABLE 4

Вид, доза вакцины и путь введения	Привитые						Контрольные (непривитые)				
	интервал до облучения	доза облучения в *р*	всего мышей	из них		% выживаемости	доза облучения в *р*	всего мышей	из них		% выживаемости
				выжило	пало				выжило	пало	
Живая культура кишечной палочки, двукратно по 100—200 млн. подкожно	1—2 месяца	500	122	59	63	48,3	500	87	41	46	47,1
Вакцина из кишечной палочки, убитая облучением 600 000 *р*, троекратно с интервалом в 7 дней по 10 млрд.	7—30 дней	500	42	8	34	19	500	32	13	19	40,6
Сливки двукратно по 0,25 *мл* с интервалом 2 недели подкожно	14 суток	500	32	24	8	75	500	42	12	30	30
Сливки двукратно по 1 *мл* с интервалом 2 недели подкожно	14 „	500	20	3	17	15	—	—	—	—	—
Суммарно по всем группам			709	394	315	55,5		477	162	315	33,9

A) Type of vaccine, dosage, and mode of administration; B) vaccinated animals; C) control (unvaccinated) animals; D) interval before irradiation; E) irradiation dose, in r; F) total number of mice; G) of which; H) survived; I) died; J) % survival; K) TABF tetravaccine administered subcutaneously and intraperetonially in single injections of 0.2 ml; L) the same; M) BCG administered intracutaneously in a single injection of 1 mg; N) NIISI polyvaccine administered subcutaneously in three injections of 0.1 ml at intervals of 14 days; O) heat-killed vaccine prepared from Bacterium coli and administered subcutaneously in a single injection of 200 million cells; P) vaccine prepared from Bacterium coli killed by irradiation in a dose of 600,000 r and administered subcutaneously in one injection of 250 million cells; Q) live culture of Bacterium coli administered subcutaneously in two injections of 100-200 million cells; R) vaccine prepared from Bacterium coli killed by irradiation in a dose of 600,000 r and administered in three injections of 10 billion cells at intervals of 7 days; S) cream administered subcutaneously in two injections of 0.25 ml at an interval of 2 weeks; T) cream administered subcutaneously in two injections of 1 ml at an interval of 2 weeks; U) total for all groups; V) minutes; W) weeks; X) days; Y) months; Z) days.

they had a nonspecific action. X-irradiation in a dose of 500 r caused 50-80% of the mice to die, primarily during the period extending from 10 to 16 days after irradiation. The animals became dirty and lay passively in one spot with their eyes closed, huddled against one another; their coats were disheveled and they did not eat. Diarrhea and loss of weight were frequently observed. The remaining animals died later, between the 20th and 30th days.

When inoculation was effective the vaccinated animals had an entirely different external appearance. They retained their cleanliness, good appetites, and active behavior. If the vaccinated mice died it was somewhat later than was the case for the control animals. In analyzing the results given in Table 4 it may be seen that the effectiveness of the protective action of certain preparations (BCG vaccines) depended directly on the time of vaccination, i.e., on the interval between inoculation and irradiation. Thus, vaccination 3-7 days before irradiation did not materially increase the survival rate among the irradiated mice, while vaccination 14-30 days before irradiation produced a considerable difference in radioresistance between the vaccinated and unvaccinated

animals.

There are also vaccines which increase radioresistance when administered shortly before irradiation and have no effect when administered 2-4 weeks beforehand. Thus, our experiments and the data in the literature showed that preparations of gram-negative bacteria had a protective influence when administered as little as 15 minutes before irradiation. The live EV antiplague vaccine is an example of such a preparation. O.V. Smirnova, a graduate student in our laboratory, obtained an increase in survival rate among pigs vaccinated subcutaneously in a dose of 1.5 million microbial bodies 3 days before γ-irradiation with Co^{60} (in a dose of 250 r). The survival rate among the vaccinated animals was 41.6%, while that among the unvaccinated animals was only 16.6%. Administration of the same or larger doses of EV vaccine 7 and 14 days before irradiation did not have a favorable effect, even aggravating the course of the radiation affection in certain cases (when a lysate of the vaccine strain or killed cells were administered).

An increase in survival rate was also obtained in experiments on white mice vaccinated 3 days before irradiation in a dose of 500 r. The survival rate among the vaccinated mice was 68% and that among the unvaccinated mice 34%. Large (10 million cells) and small (2.5 million, 500 thousand, 50 thousand, and 50 cells) doses of vaccine did not a favorable influence when administered 3-7-30 days before irradiation. Immunization with a toxic lysate of the vaccine strain aggravated the course of radiation sickness and reduced the survival rate among the irradiated animals.

O.V. Smirnova observed an aggravating effect in experiments on rabbits inoculated with increasing doses of vaccine (from 1.5 to 20 billion cells) and irradiated in a dose of 900 r with γ-rays from Co^{60}.

Enteral vaccination with a killed vaccine prepared from colon ba-

still prove to be completely ineffective, even leading to an increase in mortality among the irradiated mice, possibly because of a sensitizing effect with respect to the intestinal flora, which is the basic source of indogenous infection in radiation sickness.

In order to investigate the mechanism by which preliminary immunization affects the course of radiation sickness N.N. Klemparskaya used experiments on mice to study the intensity of autoallergic reactions, employing a high modifiable test involving distilled water. If 0.1 ml of sterile distilled water is injected into the skin of the upper lip of a mouse 3 days after x-irradiation in a dose of 500-600 r, cellular decomposition products are formed under the influence of the osmotic trauma at the injection site; these cause a local hyperergic reaction to develop in the autosensitized organism (within 24-48 hours). This reaction takes the form of edema of the lip and hemorrhagic necrosis. This test was performed on 37 mice vaccinated before irradiation (with vaccine prepared from heat-killed colon bacilli) and 42 unvaccinated animals 3 days after irradiation in a dose of 500 r. Sharply positive reactions were noted in only 10% of the vaccinated mice and in 40% of the unvaccinated mice.

Preliminary immunization consequently had an inhibiting influence on the development of autoallergies after irradiation.

The experiments on mice confirmed the wisdom of studying this problem in different species of animals. For this purpose the change in radioresistance under the influence of preliminary vaccination was traced in 46 guinea pigs. The results of the experiment are set forth in Table 5. They are of double value as a guide line, since they were preliminary experiments on the use of two types of antigen: a live culture of Bacterium coli and heat-killed vaccine prepared from Bacillus breslaviensis.

TABLE 5

Influence of Immunization 30 Days Before Irradiation in a Dose of 250 r (with γ-rays from Co^{60}) on the Postirradiation Survival Rate Among Pigs

№ групп	Вид вакцины и способ прививки	Число свинок	Из них выжило	Пало до 10-го дня	Всего	Средняя продолжительность жизни павших в днях	% выживших
A	B	C	D	E	G	H	I
J Первая	Живая культура кишечной палочки (200 млн. внутрикожно, однократно) . . .	13	1	4 (30,3%)	12	11,8	7
K Вторая	Живая культура кишечной палочки (в 6 мест кожи по 200 млн. в каждую точку)	9	2	2 (25,5%)	7	10,6	22
L Третья	Убитая вакцина Бреслау 200 млн. + живая культура кишечной палочки (200 млн. в одно место кожи)	8	5	2 (25%)	3	10,6	62
	Суммарно по всем привитым	30	8	8 (26,6%)	22	11	26,6
M Четвертая	Контрольные — непривитые	16	4	8 (50%)	12	9,1	25
	R						

Note: The pigs weighed 250-300 g.

A) Group number; B) type of vaccine and method of vaccination; C) number of pigs; D) number surviving; E) number dying; F) before the 10th day; G) total; H) mean survival time of animals which died, in days; I) % survival; J) first; K) second; L) third; M) fourth; N) live culture of Bacterium coli (200 million cells administered in one intracutaneous injection); O) live culture of Bacterium coli (administered at 6 places on the skin in doses of 200 million cells); P) killed Bacillus breslaviensis vaccine in a dose of 200 million cells and a live culture of Bacterium coli (administered in a dose of 200 million cells at one place on the skin); Q) total for all vaccinated animals; R) control animals, unvaccinated.

The total data for the vaccinated animals showed that there was a very slight increase in survival rate and mean survival time and a marked decrease in mortality during the first 10 days after irradiation (26.6% in comparison with 50% among the unirradiated guinea pigs). However, the increase in radioresistance was quite marked in individual groups (the third group) and this indicates that it is possible to work out modes of immunization for a given species of animal which will insure a considerable percentage survival after irradiation.

Rabbits were a third species of animal in which the influence of

TABLE 6

Influence of Preliminary Immunization on Survival Rate Among Rabbits x-Irradiated in a Dose of 800 r

A	B	C	D	E			Среднее число лейкоцитов	
№ группы	Вид вакцины и способ прививки	Интервал между прививкой и облучением	Всего кроликов	выжило	пало	% выживаемости	J 9–11-е сутки	K 15-е сутки
L Первая	O Живая культура кишечной палочки (200 млн. в одно							
M Вторая	P место кожи)	30	22	19	3	86	3,4	5,05
	Живая культура кишечной палочки (в 6 точек кожи по 200 млн. на каждую							
N Третья	Q точку)	30	8	0	8	0	1,2	1,3
	Контрольные—непривитые		20	8	12	40	1,8	3,2

Note: The rabbits weighed 2.7-3 kg.

A) Group number; B) type of vaccine and method of vaccination; C) interval between vaccination and irradiation; D) total number of rabbits; E) of which; F) survived; G) died; H) % survival; I) mean leucocyte count; J) 9th-11th days; K) 15th day; L) first; M) second; N) third; O) live culture of Bacterium coli (200 million cells administered at one place on the skin); P) live culture of Bacterium coli (administered in doses of 200 million cells at 6 points on the skin); Q) control animals, unvaccinated.

preliminary immunization on radioresistance was investigated.

In the experiments of N.N. Klemparskaya (Table 6) rabbits were vaccinated before irradiation with a live day-old agar culture of Bacterium coli, strain M_{17} administered intracutaneously on the lateral surface of the body in a dose of 200 million cells suspended in 0.2 ml of physiological solution. A mild hyperemic infiltration approximately 2-3 cm in diameter formed at the injection site; it resorbed over a period of 6-8 days. This vaccination had no effect on the animals' general condition; the young rabbits continued to gain weight and had a good external appearance and appetite. A slight leucocytosis was noted during the first two days after vaccination.

In order to study the effect produced by increasing the antigen dosage we employed (in addition to single vaccinations) injections of 200 million microbes at 6 points on the skin, 3 on each side 3-4 cm

TABLE 7

Influence of Preliminary (fourfold) Immunization With Killed Vaccines on the Survival Rate Among Rabbits Irradiated in a dose of 800 r (Terapiks or RUM-3 apparatus, at a dose rate of 10-15 r/min)

Группа животных A	Вакцина B	Число животных в группе C	Из них пало D		% выживаемости G
			в первые 10 дней E	всего за 30 дней F	
H 1 Непривитые	K	35	7	12	65,7
I 2 Вакцинированные	Кишечная палочка . . .	17	2	3	82,3
J 3 То же	Дизентерийная палочка L	5	1	4	
4 » »	Паратифозная Б M	11	3	3	72,7
5 » »	Палочка перфрингенс N	4	1	1	

Note: Vaccination was carried out intravenously in doses of 0.2-0.4-0.8-1 million microbial bodies, at intervals of 4 days. The animals were irradiated on the 9th day after the last injection.

A) Group of animals; B) vaccine; C) number of animals in group; D) number which died; E) during first 10 days; F) total after 30 days; G) % survival; H) unvaccinated; I) vaccinated; J) the same; K) Bacterium coli; L) Shigella dysenteriae; M) paratyphoid B; N) Clostridium perfringens.

apart. Such "multiple" vaccination caused a slight (30-50 g) loss of weight during the first 24-48 hours and leucocytosis (12,000-16,000 leucocytes per mm^3), which lasted 2-3 days longer than that which occurred on injection of a single dose.

Unvaccinated animals were used as the control. All of the animals were irradiated on the RUM-3 apparatus in a dose of 800 r 20-30 days after vaccination. As may be seen from Table 6, the survival rate among the rabbits vaccinated at one point was twice that among the unvaccinated animals. Another sign which indicated that radiation sickness took a milder course in these animals was the lesser severity of the leucopenia and the more rapid reestablishment of the leucocyte count. Increasing the antigen dosage and the number of injections not only failed to produce an increase in effectiveness, but even caused radio-

resistance to drop below the level observed in the control animals. We observed a similar negative influence when we increased the dosage of the antigens given to guinea pigs (see Table 4); it has also been described in a work by E. Ainsworth and H. Chase (1959). Detailed study of this interesting phenomenon requires further experiments on various species of animals.

The action of heat-killed vaccines on the radioresistance of rabbits receiving the same irradiation dose has been studied by V.F. Sosova. As may be seen from Table 7, an increase in survival rate was noted after vaccination with vaccines prepared from Bacterium coli, paratyphoid bacteria B, and Clostridium perfringens. A dysenteria vaccine did not have the same influence, but the experiments must be repeated on a larger number of animals before a final judgement is rendered.

The influence of preliminary vaccination on the rate of early death (during the first few hours after irradiation) among rabbits was clearly revealed at the higher irradiation dose (1100 r) used in Sosova's experiments. Of the 6 control rabbits in this batch 3 died during the first day after irradiation and one during the second day; none of the preliminarily immunized rabbits died during the first 3 days.

However, experiments on small animals can be used only for obtaining guide line data. It is possible to study the clinical characteristics of the reaction of the organism to vaccination and the course of radiation sickness in vaccinated animals quite thoroughly in experiments on large animals such as dogs.

Experiments have been conducted on 89 dogs, male and female, 2 to 6 years old and weighing 8-25 kg. The reactions to administration of various antigens and subsequent γ-irradiation with Co^{60} were traced.

The majority of the experiments involved immunization of the dogs with a live culture of Bacterium coli. Washings from a day-old agar culture of this bacterium were administered to 29 dogs intracutaneously on the lateral surface of the body. This antigen was selected in accordance with the results of preliminary experiments on rabbits and dogs in which we observed that radioresistance increased when a local inflammatory reaction developed at the injection site. Since such a reaction rarely occurs in dogs in response to subcutaneous injection of killed vaccines we were obliged to change the mode of administration (injecting the vaccine intracutaneously) and to replace the killed vaccine with a live culture of Bacterium coli M_{17}. Experiments on mice and rabbits convinced us of the safety of administering the aforementioned doses of this microbe. When injected in certain doses into the skin of a healthy animal (the inoculations were always given before irradiation) colon bacilli begin to die and never cause a generalized infection. Involution of the local inflammatory focus, which is a soft or dense hyperemic infiltration 2-3 cm in diameter, occurs between the 5th and 7th days in dogs. In some cases, administration of a large dose of this culture to light-weight dogs caused necrosis of the center of the infiltration and hemorrhaging.

In addition to intracutaneous injection of live Bacterium coli, we tested subcutaneous injection of a polyvaccine and diphtheria toxoid for preliminary immunization in 6 dogs, intracutaneous immunization with a BCG vaccine in 3 dogs, and intramuscular injection of sterile milk in 8 dogs. There were 41 animals in the control groups. All of the animals received a careful clinical examination (daily observation of behavior, appetite, body temperature, character of stool, weight, and state of visible mucosae). The leucocyte, erythrocyte, hemoglobin, thrombocyte, and reticulocyte counts of the blood were investigated.

The sedimentation rate and leucocyte ratio were determined. The bone marrow was examined in individual cases. Agglutination reactions with a live culture of the same strain M_{17} used for immunization and with a number of autostrains of Bacterium coli cultured from the feces of the vaccinated dogs were used to determine the extent of antibody production. In order to clarify the autosensitizing influence of the local inflammatory focus blood serum from the vaccinated dogs was reacted with tissue antigens before and after immunization. One reaction, the ordinary complement-fixation reaction, was performed with an extract from fresh organs from a healthy dog, while another (performed by a modification of Uan'ye's method) involved measurement of the antigen-antibody reaction in a photoelectronephelometer (see p. 21).

The extent of the local reaction was determined by palpitating the injection site and measuring the infiltration along two mutually-perpendicular diameters. Since the animals differed in weight we were forced to employ different dosages of the live Bacterium coli culture. This dosage amounted to 500 million cells for dogs weighing from 6 to 14 kg and 1 billion cells for animals weighing from 14 to 21 kg. The BCG vaccine was tested both in a large dosage, 2.5 mg, and in a dose of 0.05 mg. It is well known that the effectiveness of antimicrobial immunization depends to a considerable extent on the number of vaccinations and the intervals between them. In order to investigate the possibility of extending this rule to the development of radioresistance we conducted experiments to study immunization with Bacterium coli and by repeated injection of this antigen with revaccination after 1-3 months.

In addition, in order to intensify the immunizing effect intracutaneous injections were given both at one point on the skin and at 6 points (three points on each side) 10-15 cm apart, depending on the

size of the animal. In this case 100 or 200 million microbes were injected at each of the six points. The dogs thus received approximately the same total number of microbes as in single vaccination.

In this case the local reaction manifested itself in the formation of infiltrations 0.5-1 cm in diameter at all six injection sites. N.V. Rayeva observed the development of the general and local reaction of dogs to vaccination over the entire immunization period, until subsequent irradiation. There was not a single case in which any marked deviation from normal could be observed. The animals had a good appetite, a healthy external appearance, and a normal stool. The variations in temperature and body weight remained within normal limits. Changes in the peripheral blood indicated the presence of a general reaction. As a rule, after intracutaneous injection of colon bacilli the dogs developed leucocytosis involving an increase in the absolute leucocyte count to a level 1.5-2 times the initial level. This reaction developed within 3 hours after vaccination and increased in extent for 24-48 hours; the leucocyte count then dropped to normal (it was only when BCG vaccine was administered that there were no marked changes in the leucocyte count). The sedimentation rate was frequently accelerated during the first two days after vaccination.

The dogs thus well withstood intracutaneous injection of the live culture of Bacterium coli, developing no illnesses. After 7-14 days the only sign that a vaccination had been performed was a slight thickening of the blood at the injection site. At the time of irradiation the animals' condition exhibited no deviations from normal and all traces of the effect of the local inflammatory process on the organism had disappeared.

Formation of antibodies to Bacterium coli was noted in all of the vaccinated animals within 5-7 days after inoculation, but the titre of

agglutinins was not particularly high (above normal in only one or two cultures). This is explained by the fact that absorption of the antigen from the rapidly-formed enclosed inflammatory focus in the skin is difficult. The antibody level reverted to normal within 2-3 weeks. In addition, the strain of Bacterium coli M_{17} which we used for inoculation differed considerably in antigen structure from the colon bacilli which inhabit the intestines of mice, rabbits, guinea pigs, and dogs. This was shown in experiments involving immunization of rabbits and mice, serological cross-reactions, and inoculation with various strains of colon bacilli.

We consequently feel that the increase in survival rate which occurs among animals irradiated and vaccinated with this strain of bacteria cannot be attributed to the development of immunity to colon bacilli. Firstly, with the vaccination method in question this immunity is not of great extent and, secondly, the nonuniformity of the antigenic composition of colon bacilli makes such attempts unsuccessful. However, in addition to antibodies to the microbe administered, vaccination with a live culture of colon bacilli causes the organism to develop another type of antibody. These are antibodies to the tissue antigens formed as a result of autoimmunization with the tissue-decomposition products absorbed from the inflammatory focus (see Chapter 1). This fact makes it possible for us to explain the mechanism by which vaccination before irradiation affects radioresistance in the following manner: the anti-tissue immune bodies which develop after vaccination may combine with and neutralize the tissue antigens and decomposition products which enter the blood stream during irradiation and the first few days afterward.

In addition to their experiments on dogs, N.N. Klemparskaya and N.V. Rayeva studied the reaction of 12 male monkeys (rhesus) weighing

2.2-3.5 kg to injection of live colon bacilli and BCG vaccines. In addition to careful daily clinical observation and measurements of body temperature and gain in weight, the animals' blood was investigated (general analysis, determination of intensity of leucocyte luminescence, and microchemical glycogen tests) and the titre of agglutinins to Bacterium coli was followed.

A suspension of live colon bacilli was injected into the skin of the lateral surface of the body in the same doses used for the dogs (500 million or 1 billion microbial bodies from a day-old agar culture of Bacterium coli M_{17}). From 1 to three injections were given. In a number of cases vaccination was carried out in the same fashion as for the dogs, injections being given at 6 points on the skin of the lateral surface of the trunk (three on each side, 5-7 cm apart).

It must be noted that the reactivity of a monkey's skin to this microbe is far less than that of a dog's skin. As a rule, no reaction whatsoever, not even an infiltration, was observed in the monkeys at doses of 100-500 million cells. Injection of large quantities of microbial bodies caused a soft, nonhyperemic infiltration which disappeared within 2-3 days. There was not a single case of elevated body temperature. The change in body weight took the form of a slight (1-3%) decrease during the first two days, especially after the first vaccination. Another sign of the general reaction were the changes in the peripheral blood. Each injection of the live culture of Bacterium coli caused the leucocyte count to increase by 30-94% in comparison with its initial level.

The leucocytosis persisted for 2-3 days and was succeeded by a drop in leucocyte count to a normal or subnormal level.

The increase in leucocyte count occurred at the expense of neutrophils, the lymphocyte count decreasing. No marked changes were noted in

TABLE 8

Survival Rate Among Vaccinated and Unvaccinated Dogs After Irradiation (First Group)

Группа животных A	B Доза в млн. микробных тел	C Число прививок	D Число собак	E Средний вес в кг	F Интервалы между прививками в днях			G Интервал между прививкой и облучением в днях	H Доза облучения в *р*	I Средняя продолжительность жизни в днях	J Число выживших собак
					1—2-я	2—3-я	3—4-я				
Привитые K	600	Однократно N	3	10,6	—	—	—	18	300	17	0
	500	Двукратно O	4	8,8	14	—	—	18	300	19	0
	1 000	»	4	21,2	14	—	—	20	350	19	1
	1 500	Троекратно P	3	11,7	12	35	90	30	300	14	0
	500	Четырехкратно Q	4	10,9	14	30	90	22	300	18	1
Непривитые L			8	10,4	—	—	—	—	300	14,8	0
Контроль M	—	—	10	21,0	—	—	—	—	350	13,8	0

A) Group of animals; B) dose, in million microbial bodies; C) number of vaccinations; D) number of dogs; E) mean weight, in kg; F) intervals between vaccinations, in days; G) vaccination-to-irradiation interval, in days; H) irradiation dose, in r; I) mean survival time, in days; J) number of surviving dogs; K) vaccinated; L) unvaccinated; M) control; N) one; O) two; P) three; Q) four.

any of the other formed elements.

Injection of 5 mg of BCG vaccine caused a local reaction in the monkeys, but this was less marked than in the dogs. Thus, the former developed a nodular dense infiltration approximately the size of a small pea, but there was no necrotization or hyperemia and the inflammation persisted for 1-2 months. The axillary and inguinal lymph nodes were not enlarged; in the dogs a dense hyperemic infiltration with a necrotic center developed at a far lower dose (2.5mg) and lasted for 1-1.5 months.

N.V. Rayeva made her basic observations on the characteristics of the symptomatology of radiation sickness in vaccinated and unvaccinated animals in experiments on dogs. Except for special diet, the animals were given no therapy whatsoever. Just as in treating allergic illnesses, meat and fish were excluded from their diet and 20-40 g of butter, 1 or 2 eggs, and milk were substituted for them. In the control experiments use of this diet did not cause any increase in survival among irradiated dogs.

The irradiation was carried out on an experimental EGO-2 apparatus, with γ-rays from Co^{60}; the irradiation dose was 300 r for dogs weighing up to 14 kg (this being equivalent to 600 r of x-rays). The dose was increased 50 r for animals weighing more than 14 kg. In the majority of experiments irradiation was performed 3-4 weeks after the last vaccination.

All of the animals were examined daily and their temperatures were taken. Every 5 days after irradiation they were weighed, their blood was investigated, and serological analysis were made. Autopsies were performed on the animals which died, in order to show the typical changes characteristic of radiation sickness.

The first group was composed of dogs which were vaccinated with

TABLE 9

Survival Rate Among Vaccinated and Unvaccinated Dogs After Irradiation (second group)

Группа животных A	B Доза на одно введение в млн. микробных тел	C Суммарная доза в млн. микробных тел	D Число прививок	E Число собак	F Средний вес в кг	G Интервал между первой и второй прививкой в днях	H Интервал между прививкой и облучением в днях	I Доза облучения в *р*	J Средняя продолжительность жизни в днях	K Число выживших собак
Привитые L	100	600	Двукратно N	2	10,5	30	30	300	19	1
	200	1200		7	10,3	30	30	300	16	—
	200	1200	Однократно O	2	10,4	—	3	300	16	1
Непривитые M	—	—	—	8	9,3	—	—	300	16,8	0

A) Group of animals; B) dose per injection, in millions of microbial bodies; C) total dose, in millions of microbial bodies; D) number of vaccinations; E) number of dogs; F) mean weight, in kg; G) interval between first and second vaccinations, in days; H) interval between vaccination and irradiation, in days; I) irradiation dose, in r; J) mean survival time, in days; K) number of surviving dogs; L) vaccinated; M) unvaccinated; N) two; O) one.

TABLE 10

Survival Rate Among Irradiated Dogs Inoculated With Different Vaccines and Unimmunized Control Dogs

Группа животных A	Вид вакцины и доза B	Способ введения и число прививок C	Число собак D	Средний вес в кг E	Интервал между первой и второй прививкой в днях F	Интервал между прививкой и облучением в днях G	Доза облучения в р H	Средняя продолжительность жизни I в днях	Число выживших собак J
Привитые K	M Поливакцина (3 мл в сочетании с 5 мл дифтерийного анатоксина)	Q Подкожно, двукратно	6	21 / 3 / 11,2 / 3	21	3	350	13,8	0
Контрольные L	—	—	5	13,8	—	—	350	14,2	0
Привитые K	N Стерильное молоко (5 мл)	S Внутримышечно, однократно	8	6,6 / 5 / 21,8 / 3	—	21	300 / 5 / 350 — 400	17,0 / 14,5	0 / 1
Контрольные L	—	—	5	6,8 / 3 / 24,6 / 2	—	—	300 / 2 / 350 — 400 / 2	15,6 / 11,5	0 / 0
Привитые K	O Вакцина БЦЖ (2,5 мг)	S Внутрикожно, однократно	3	6,3	—	3	300	14	0
Контрольные L	—	—	3	6,8	—	—	300	15,6	0
Привитые K	P Вакцина БЦЖ (0,05 мг)	S Внутрикожно, однократно	2	20,6	—	30	350	17	0
Контрольные L	—	—	2	21,2	—	—	350	18	0

Note: The numerator shows the number of dogs of a given weight for the number of dogs receiving the indicated irradiation dose.

A) Group of animals; B) type of vaccine and dosage; C) mode of administration and number of vaccinations; D) number of dogs; E) mean weight, in kg; F) interval between first and second vaccinations, in days; G) interval, vaccination to irradiation, in days; H) irradiation dose, in r; I) mean survival time, in days; J) number of surviving dogs; K) vaccinated; L) control; M) polyvaccine (3 ml in conjunction with 5 ml of diphtheria toxoid); N) sterile milk (5 ml); O) BCG vaccine (2.5 mg); P) BCG vaccine (0.05 mg); Q) two subcutaneous injections; R) one intramuscular injection; S) one intracutaneous injection.

a live culture of Bacterium coli M_{17} at one point on the skin 18-32 days before γ-irradiation with Co^{60} in a lethal dose (Table 8). 2 of the 18 animals in this group (i.e., 11%) survived, all of the unvaccinated animals dying. The mean survival time for the vaccinated animals which died, with the exception of those which received three injections, was 3-5 days longer than for the dogs in the control group. As may be seen from the data cited, increasing the vaccine dosage and the number of injections did not lead to any rise in the effectiveness of the influence of preliminary immunization on radioresistance. We have already noted a similar regularity in discussing the results obtained in experiments on mice.

The second group was composed of dogs vaccinated at six points on the skin with a live culture of Bacterium coli (Table 9). The survival rate in this group (18.1% or 2 of 11 dogs) was slightly higher than in the preceding group. It is interesting that one of the dogs which was vaccinated only 3 days before irradiation survived.

In the other variants of preliminary immunization (Table 10) the only survival occurred among the dogs which received injections of sterile milk; one of these animals survived.

It was thus shown in the experiments on dogs that, in certain cases, animals which have been preliminarily immunized can withstand an irradiation dose absolutely lethal for the control (unvaccinated) dogs without any additional treatment.

However, the survival rate is not the only criterion for evaluating the influence of immunization on radioresistance. We believe that analysis of the clinical symptoms of radiation sickness is very important. The vaccinated and control dogs developed a typical radiation sickness, which began after a latent period of 7-15 days; the animals became sluggish and dirty, their appetites decreased, there were bloody dis-

charges from the mouth, their stools were liquid and frequently blood-tinged, there was blood in the urine, and hemorrhaging occurred in the skin and mucosae. Their body temperatures rose to 40-41° and dropped to 37° just before death.

These symptoms may also appear at the end of the first week after irradiation in severe cases. Since the time at which the typical symptoms develop plays a great role in characterizing the severity of radiation sickness, an analysis of the clinical course of the disease in vaccinated and unvaccinated dogs was carried out from this standpoint (Table 11). It must first be noted that the radiation sickness not only was no more severe in the vaccinated animals, but their symptoms developed later and less frequently than in the unvaccinated dogs and were less marked.

This was especially clearly shown in the dogs which were inoculated with a live culture of colon bacilli in small doses at 6 points on the skin (100-200 million cells at each point). Thus, during the period from the 6th to the 9th days, when the unvaccinated dogs were already exhibiting a deterioration of their general condition, loss of appetite, fever, and hemorrhaging, the vaccinated subjects displayed no signs whatsoever of radiation sickness. Their symptoms developed from the second half of the second week onward, or 2-3 times more slowly than in the unvaccinated animals.

Symptoms of radiation sickness appeared on the 6th-9th day in the dogs immunized intracutaneously with a live culture of Bacterium coli at one point, although they appeared somewhat less frequently than in the unvaccinated subjects. The difference in the times at which the symptoms appeared in the vaccinated and unvaccinated animals was least for the development of hemorrhagic diathesis. Hemorrhages occurred in almost the same percentage of both groups, but were more diverse and wide-

TABLE 11

Time of Appearance of Clinical Symptoms of Radiation Sickness in Vaccinated and Unvaccinated Irradiated Dogs (the percentage of subjects with a given symptom is shown)

Группа животных A	Характер признака B	Принятые (29 собак) C		Непринятые (26 собак) E	
		период появления признака в сутках D		период появления признака в сутках D	
		6–9	10–14	6–9	10–14
F 18 собак, привитых живой культурой кишечной палочки в одну точку кожи	G Ухудшение общего состояния	5,5	44,5	16,7	72
	Потеря аппетита H	11,1	33,4	16,7	61
	Повышение температуры I	11,1	33,4	11,1	55,5
	Понос J	11,1	16,7	5,5	33,4
	Геморрагические явления K	0	55,5	0	55,5
L 11 собак, привитых в шесть точек кожи живой культурой кишечной палочки	G Ухудшение общего состояния	0	18,2	12,5	50
	Потеря аппетита H	0	18,2	12,5	37,5
	Повышение температуры I	0	18,2	37,5	25
	Понос J	0	9,1	0	25
	Геморрагические явления K	0	18,2	12,5	25

A) Group of animals; B) character of symptoms; C) vaccinated (29 dogs); D) period required for symptoms to appear, in days; E) unvaccinated (26 dogs); F) 18 dogs vaccinated with a live culture of Bacterium coli at one point on the skin; G) deterioration of general condition; H) loss of appetite; I) rise in temperature; J) diarrhea; K) hemorrhaging; L) 11 dogs vaccinated at 6 points on the skin with a live culture of Bacterium coli.

spread in the unvaccinated dogs. In addition to hemorrhages in the skin and mucosae, there was frequently blood in the urine and feces and bloody discharges from the mouth.

Table 11 is based on an analysis of the clinical course of radiation sickness in all of the vaccinated dogs which died and survived and the unvaccinated animals which died. However, it must be pointed out that 5 of the immunized animals survived and the course of the radiation sickness which they incurred differed considerably from that observed for the other animals in this group. Throughout the entire period (60 days) after irradiation, the surviving animals had a good external appearance, exhibited no symptoms of hemorrhagic diathesis,

remained alert and clean, ate well, and had normal stools. Their body temperature remained within the limits of normal fluctuation. Dog No. 797 (which was vaccinated twice with a live culture of Bacterium coli at one point on the skin) was the only exception, exhibiting a soft stool and an elevated body temperature over a period of one week, hemorrhaging in the skin of the inguinal region, and, on the 25th day, bloody discharges from the nose and edema of one paw. It is interesting that in all of the dogs which survived irradiation as a result of immunization with a live culture of Bacterium coli the local reaction to vaccination was moderate and manifested itself primarily in the formation of a soft hyperemic infiltration with no necroses or hemorrhages.

All of the dogs which reacted to vaccination with a severe inflammatory process died. The number of observations is naturally still too small to enable us to assume that a moderate reaction to intracutaneous injection of colon bacilli is a prognostic sign which makes it possible to evaluate the subsequent course of radiation sickness. However, these facts do indicate the important role which the characteristics of the immunobiological reactivity of the organism play in the development of its reaction to ionizing radiation.

In analyzing the data which N.V. Rayeva obtained in investigating the peripheral blood it must be noted that leucopenia developed among both the inoculated and the control animals, but that it was somewhat less severe in the vaccinated animals. The changes in the sedimentation rate, thrombocyte count, and erothrocyte count had the same dynamics in both groups.

All of the animals which died were subjected to pathologoanatomical examination. No substantial differences were noted between the dead vaccinated and dead unvaccinated dogs when the macroscopic changes were studied. The symptoms of hemorrhagic diathesis were most

marked; copious hemorrhaging was observed in the lungs, stomach, large and small intestines, and subcutaneous cellular tissue.

The observations cited above, although few, thus indicate that preliminary vaccination with bacterial antigens increased the radioresistance of the dogs even when they were exposed to doses of radiation sufficient to cause all of the control and unvaccinated dogs to die. Our attention is struck by the unusual course of the radiation sickness incurred by the vaccinated dogs which survived; they displayed no clinical symptoms, exhibiting only the characteristic changes in their blood cells.

In our opinion, vaccination may serve as a method of creating a very favorable "background" for the action of other drugs and complexes of drugs. We became convinced of the effectiveness of conjoining immunization before irradiation with subsequent administration of medicinal drugs in experiments on dogs and monkeys.

The six dogs in the first group were vaccinated with 10 mg of a BCG vaccine 3-7 weeks before γ-irradiation with Co^{60} in a lethal dose. During the first few days after irradiation the animals received a complex of antibiotics orally twice daily, in accordance with the scheme worked out by N.V. Rayeva (200,000 units of streptomycin, oxytetracycline, phenoxymethylpenicillin, and vitamins C, B_1, and B_2, over a period of 25 days). The six irradiated dogs in the second group received only antibiotics and 7 animals were used as the control group. The control dogs all died of radiation sickness (their mean survival time was 14.7 days). Half of the dogs in the first and second groups survived. However, the course of the radiation sickness which they incurred was not uniform. While the animals treated only with the complex of antibiotics exhibited marked symptoms of an acute radiation syndrome (adynamia, diarrhea, loss of appetite, and hemorrhaging),

in the animals which were both vaccinated and treated these symptoms were either entirely lacking or quite weak and brief (lasting only one-half to one-fourth as long as in the unvaccinated subjects). The vaccinated dogs which died had a survival time 4 days longer than the dogs treated with antibiotics alone.

The advantage of conjoining vaccination with oral administration of antibiotics after irradiation appeared especially clearly in experiments on monkeys. In this case we obtained a greater effect from single vaccinations than in the experiments on dogs. A total of 16 rhesus monkeys weighing 2.5-3 kg were used in the experiment; six of them were vaccinated intracutaneously with live BCG vaccine 3 weeks before irradiation, three receiving a dose of 0.05 mg and no further treatment, while the other three received a dose of 5 mg and were given antibiotics and vitamins orally after irradiation according to the scheme described above, the only difference being that the antibiotic dosages were halved. Five animals were treated only with the antibiotics and five constituted the control group.

The animals were subjected to whole-body γ-irradiation with Co^{60} on an apparatus with a uniform irradiation field (EGO-2), receiving a minimum lethal dose of 300 r, which is equivalent in biological effect to 600 r of x-rays.

Of the five control monkeys four died between the 7th and 16th days after irradiation. They exhibited typical clinical symptoms and characteristic changes in the blood. One monkey survived. All of the animals treated with antibiotics alone contracted a severe form of radiation sickness, displaying marked clinical symptoms. Two animals were in serious condition on the 52nd day, since they developed infectious complications.

Of the 3 monkeys which received 0.05 mg of vaccine and no addi-

tional treatment one survived and two died on the 24th and 34th days, having considerably outlived the control animals. The clinical symptoms of radiation sickness which they displayed were less marked. They died as a result of diplococcal sepsis, while the control monkeys developed an endogenous infection caused by colon and paracolon bacilli.

It is interesting that the character of the body flora also changed in the animals which survived, a phenomenon which was discovered by culturing mucus from the oral cavity (data compiled by G.A. Shal'nova). Thus, in the control monkeys the flora of the mouth on the 17th day consisted of 99.3% Bacterium coli; in the animals inoculated with BCG vaccine these bacteria constituted 6.3% of the oral flora, in which diplococci predominated, while in the subjects treated with antibiotics colon bacilli comprised 96% of the bacteria in the oral cavity.

Preliminary immunization thus somewhat increased the monkeys' resistance to radiation and caused a change in the character of the endogenous infection which ordinarily develops. It may be assumed that supplemental prescription of antibiotics to halt the development of the endogenous diplococcal infection can prevent death. This is confirmed by experiments conducted on 3 male monkeys vaccinated with 5 mg of BCG vaccine and treated after irradiation by oral administration of antibiotics. These monkeys not only all survived, but generally were not ill and exhibited no clinical symptoms of radiation sickness, if we do not count the development of leucopenia and thrombopenia between the 15th and 25th days after irradiation. However, these changes were less severe than in the control animals and restoration occurred earlier and more rapidly than in the single control monkey which survived. Even during the height of radiation sickness all three experimental monkeys had a good external appearance and appetite, were

lively, and did not lose weight.

It may consequently be seen, even from these few data, that the changes in reactivity caused by preliminary immunization promote an increase in the effectiveness of therapeutic measures during radiation sickness. We are able to attribute the effect which we obtained to the vaccination, since the monkeys treated with antibiotics alone developed marked clinical symptoms of radiation sickness, in contrast to the vaccinated animals.

The majority of investigations conducted to study the effects of vaccination involved external irradiation with x-rays or γ-rays from Co^{60}.

At present there are still very few data available on the action of vaccination on the course of radiation sickness produced by entry of radioactive substances into the body. However, observations made in our laboratory indicate that in this type of affection preliminary vaccination increases radioresistance. In experiments on mice which received 0.1μ curie of polonium per kg of body weight it was established that animals preliminarily immunized with tetravaccine had a mean survival time of 24 days, while the control animals lived only 14 days. Triple vaccination was less effective in this case than single or double inoculation. There is no doubt that further research is necessary to study the influence of vaccination in the presence of affections caused by radioactive substances.

Analysis of the data in the literature and the experimental data cited in this chapter enable us to conclude that it is possible to increase radioresistance by preliminary vaccination with bacterial antigens. Research on this aspect of the action of vaccines involved the use of lethal irradiation doses and no additional therapy whatsoever. Since the animals were observed to survive and incur a less severe ra-

diation sickness in this case, it may be assumed that the effect of preliminary immunization will be far greater when smaller irradiation doses are encountered (as occurs in working with ionizing radiation and using it for therapeutic purposes). Timely immunization (before contact with ionizing radiation) of the personnel of therapeutic institutions and laboratories may prevent the development of pathological conditions resulting from irradiation. Since the change (increase) in radioresistance occurs soon after vaccination, it is quite possible that the latter can be used to alleviate the reaction of a subject undergoing radiation therapy. We and other investigators have naturally studied only a small number of vaccines. A great deal of further work must be done in studying the change in radioresistance which occurs under the influence of various bacterial preparations and we believe that vaccines will be found which will ensure the survival of a majority of irradiated organisms, even though they receive lethal doses.

Chapter 3

REACTION OF IRRADIATED ORGANISMS TO ADMINISTRATION OF BACTERIAL VACCINES

There are several aspects to be considered in studying the immunological reactivity to vaccination of an irradiated organism. We are able to make certain basic observations about one of these aspects, clarification of the role which disruption of immunogenesis plays in the development of bacteremia on massive irradiation.

Many works on the biological effect of ionizing radiation on the living organism contain references to a depression of immunogenesis as one of the causes of bacteremia. Unfortunately, we encounter this widely-held notion in immunological works of recent years. Thus, M.I. Ravish-Shcherbo and G.L. Prokopenko (1960) write: "death caused by x-irradiation in sublethal and moderate lethal doses most frequently results from bacteremia, whose development is promoted by a depression of immunogenesis."

Here, just as in other works, no account is taken of the sequence of phenomena, i.e., that the potential capacity of the organism to form antibodies (its capacity for immunogenesis) is realized in the presence of a general infectious process only as a consequence of bacteremia, which results from the action of microbial antigens entering the blood stream on the organism.

Let us take typhoid fever as an example. The causitive agent can be isolated from the blood during the first few days of illness. It

is only from the second week of illness onward, when antibodies appear in the patient's blood, that the Widal serological reaction can be used for diagnostic purposes.

It is quite obvious that, even in unirradiated animals, antibody formation lags considerably behind the development of bacteremia and, as a result, regardless of the state of the immunogenetic mechanisms they cannot be included among the causes of the bacteremia.

Even if an irradiated organism does develop specific antibodies as a result of bacteremia, their role in the struggle with this condition is slight, since they cannot act on the microbes. Antigens such as opsonins and bacteriotropins act only in collaboration with phagocytes, whose number is sharply reduced during radiation sickness, while bacteriolysins cannot destroy the microbes because of a lack of complement in the blood of the irradiated organism.

Experiments performed on vaccinated irradiated animals confirm the correctness of this evaluation of the antibodies. A test of immunity in mice vaccinated against typhoid fever before irradiation and subjects inoculated with the same culture after irradiation showed that a considerably larger number of irradiated mice died (experiments conducted by N.N. Klemparskaya and R.V. Petrov, 1958).

M.A. Tumanyan and A.V. Izvekova (1956) were able to obtain a rather high titre of antibodies in mice vaccinated after irradiation in a dose of 400 r. However, when the intensity of immunity as evaluated from the antibody level was the same the irradiated immunized animals were less resistant to infection than the unirradiated immunized subjects.

Analyzing the data obtained by his colleagues, Prof. V.L. Troitskiy (1958) concluded that when natural protective mechanisms are depressed or damaged under the influence of ionizing radiation anti-

bodies do not determine the state of immunity to infection. This explains the low effectiveness of passive immunization, i.e., administration of prepared antibodies, in the irradiated organism.

Study of the role of antibodies in producing immunity in the presence of a radiation affection thus confirmed an important hypothesis of general immunology, that such natural mechanisms of immunity as the undisrupted permeability of the cell membranes of the barrier structures, the bactericidal properties of body fluids and tissues, and full-valued phagocytosis play the principal role in protecting the organism from infection. It is damage to these protective mechanisms rather than disruption of immunogenesis which promotes the development of bacteremia.

In our opinion, an essential link in this process is the early disruption of the ability of an irradiated organism to limit the multiplication of commensal microbes in the mucosae of the gastrointestinal tract and respiratory passages and in the dermal tegmina.

In her experiments, N.N. Klemparskaya (1955) traced the increase in the number of microbes in the intestinal mucosa of white mice during the period extending from 3 to 144 hours after irradiation in a dose of 750 r ($LD_{100/6}$). She found that the normal microbial level was maintained for only the first 24 hours after irradiation. Some of the animals exhibited a marked increase in the number of bacteria in the intestinal flora at the end of the first day, this phenomenon being observed in all of the subjects between the 48th and 144th hours.

This increase in the number of bacteria in the intestine causes them to enter the lymph and blood streams continuously, the majority of them being retained in the lymph nodes, liver, and spleen at first; it is only when the barrier capacity of these organs is exhausted that bacteremia develops (R.V. Petrov, 1957).

N.N. Klemparskaya (1959) also showed that the absorption of microbes from the intestine is limited only to a certain extent in healthy animals. If a healthy mouse is given a large dose of microbes (3-5 billion colon bacilli) orally, they may be detected in its internal organs within an hour. Indeed, the body is eventually cleansed of these microbes, a considerable number of them being excreted through the kidneys. The threshold of impermeability for bacteria is consequently easily exceeded, even in healthy animals, if the number of microorganisms is increased. In irradiated animals this phenomenon is promoted by a considerable rise in the permeability of the mucosae and a spontaneous increase in the number of microorganisms.

The process which leads to the endogenous infection is thus based on an early disruption of the ability of the organism to limit the accumulation of commensal bacteria and an increase in the permeability of the mucosae and vessels. This makes it possible for a considerable number of microbes to enter the blood and lymph continuously. The microbes are at first absorbed by organs having barrier functions, but continued stress exhausts this type of protection and the bacteria begin to circulate in the blood. The destruction of bacteria in the blood and organs is disrupted at the same time, as a result of depression of phagocytic functioning and a decrease in the bactericidal properties of the blood plasma.

These are, in our opinion, the bases of the mechanism by which the endogenous infection develops in an irradiated organism.

The principles of the prevention of infectious complications in the presence of radiation affections have now been determined. They consist in using specific measures, in the form of a complex of antibiotics with a rather wide spectrum of action, and nonspecific measures of the type employed in replacement and stimulation therapy,

which accelerate the regeneration of various systems, including natural protective mechanisms.

In coming to this conclusion we are by no means disparaging the role of microbiologists and immunologists in the further study of radiation affections. Quite the contrary, we believe that their participation must be expanded. This belief is based on materials cited in Chapter 2, which conclusively show that immunological reorientation of the organism by vaccination before irradiation favorably affects the course of the radiation-induced process. The essential role of immunological processes in the pathogenesis of radiation sickness is thus proved.

There is another branch of radiation immunology which we consider to be no less important and interesting, i.e., study of the phenomena observed when vaccination after irradiation is employed.

Surveys of works on this subject are given in the articles by I.X. Taliaferro and L.G. Taliaferro (1951), I.A. Pigalev (1955), and V.L. Troitskiy (1958) and in the books by N.N. Klemparskaya etal. (1958) and V.L. Troitskiy and M.A. Tumanyan (1958).

What problems of radiation immunology can be solved by vaccinating irradiated animals?

In the course of research it was found that these problems are rather wide in scope and pass far beyond the study of radiation affections.

Vaccination after irradiation was perhaps employed at first only to determine the characteristics of the reactivity of the irradiated organism. Changes in the capacity for antibody formation after whole-body irradiation always took the form of a decrease, whose extent depended on the radiation dose and the stage of radiation sickness. In essence, the vaccine test was used as one method of studying radiation

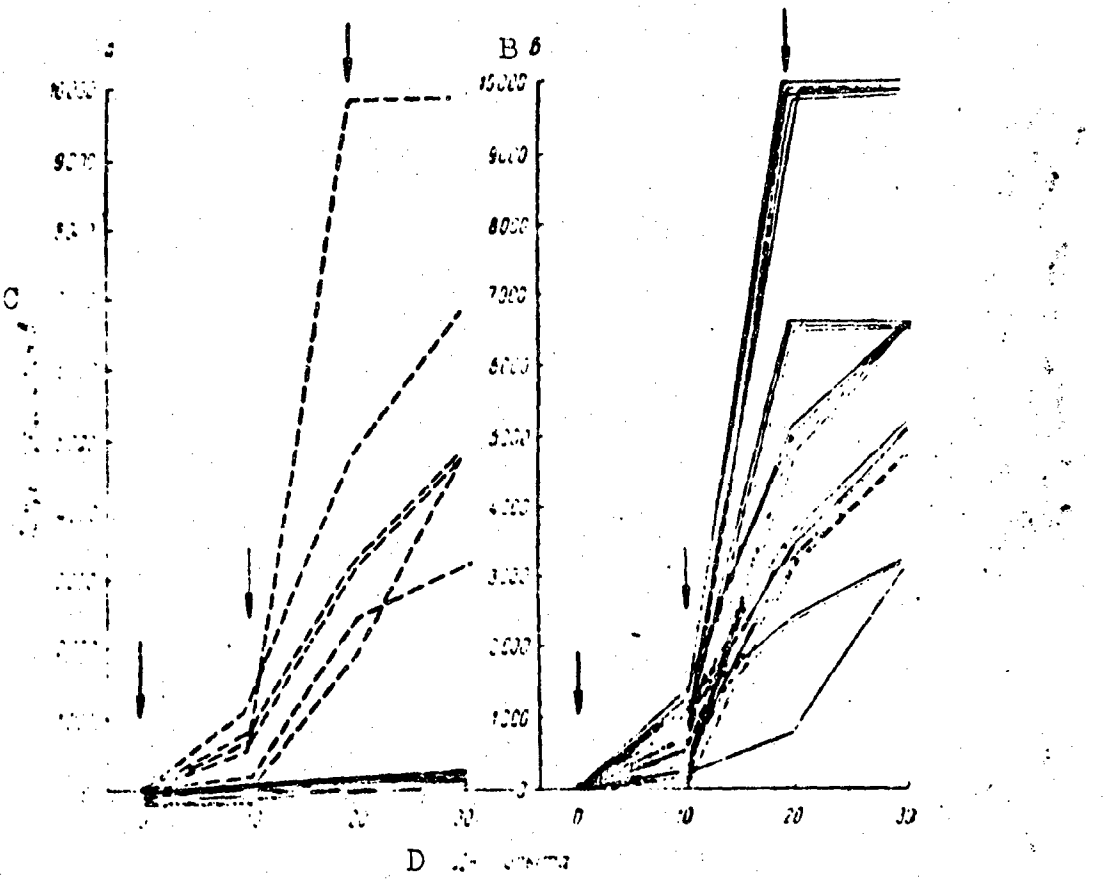

Fig. 7. Dyanamics of the change in precipitin titres in irradiated and control rabbits (according to data compiled by M.I. Ravish-Shcherbo and L.G. Prokopenko). A) Single immunization with two antigens 24 hours after irradiation; B) preliminary immunization with vaccine. The solid lines represent irradiated rabbits and the dash lines control rabbits (the arrows designate times of immunization). C) precipitin titres; D) days of experiment.

in experimental animals. However, this trend did not receive the requisite development, although tests to evaluate the effectiveness of various prophylactic drugs and measures used in the experimental therapy of radiation affections could successfully augment indices of immunogenetic capacity, which reflects the state of reactivity.

One investigation conducted on irradiated animals in which indices of immunogenesis on vaccination were used to augment clinical, hematological, and histological data is the work of M.I. Ravich-Shcherbo and L.G. Prokopenko (1960).

The results of this work confirm our assertion that bacterial vaccination may prevent the serious after effects of irradiation. It

was found that not only did the hematological picture improve and survival increase among animals vaccinated and then irradiated, phenomena which we also observed, but also that the subjects' capacity for immunogenesis was retained to a greater extent.

We will now describe the method used in and the data obtained from (Figs. 7 and 8) this noteworthy work. Rabbits were vaccinated with three subcutaneous injections of paratyphoid B vaccine (500 million, 1 billion, and 1 billion cells) or human blood serum (0.5, 1, and 1 ml) at intervals of 10 days and were irradiated in a dose of 600 r 10 days later. 24 hours after irradiation the same antigens were administered in accordance with a cross-version of the same scheme, i.e., the rabbits which had preliminarily been vaccinated with paratyphoid bacillus received serum and the subjects which had been inoculated with serum received paratyphoid vaccine. The antibody (agglutinins and precipitins) titres were compared with data obtained from nonirradiated and irradiated animals which had been given the two antigens simultaneously 24 hours after irradiation. Using this experimental setup it was conclusively shown with the aid of the vaccine test that the method which we have recommended, vaccination before irradiation, ensures maintenance of a level of reactivity so high that the depression of immunogenesis characteristic of radiation affections does not occur after irradiation.

Vaccination after irradiation may consequently be used as a diagnostic test in conducting experimental work on radiation sickness.

Numerous bacteriological investigations have confirmed the important role of the microbial factor in the etiology of radiation affections, which was established by C. Miller, C. Hammond, and M. Tompkins (1951). The susceptibility of irradiated organisms to infection was demonstrated by inoculation with various microorganisms.

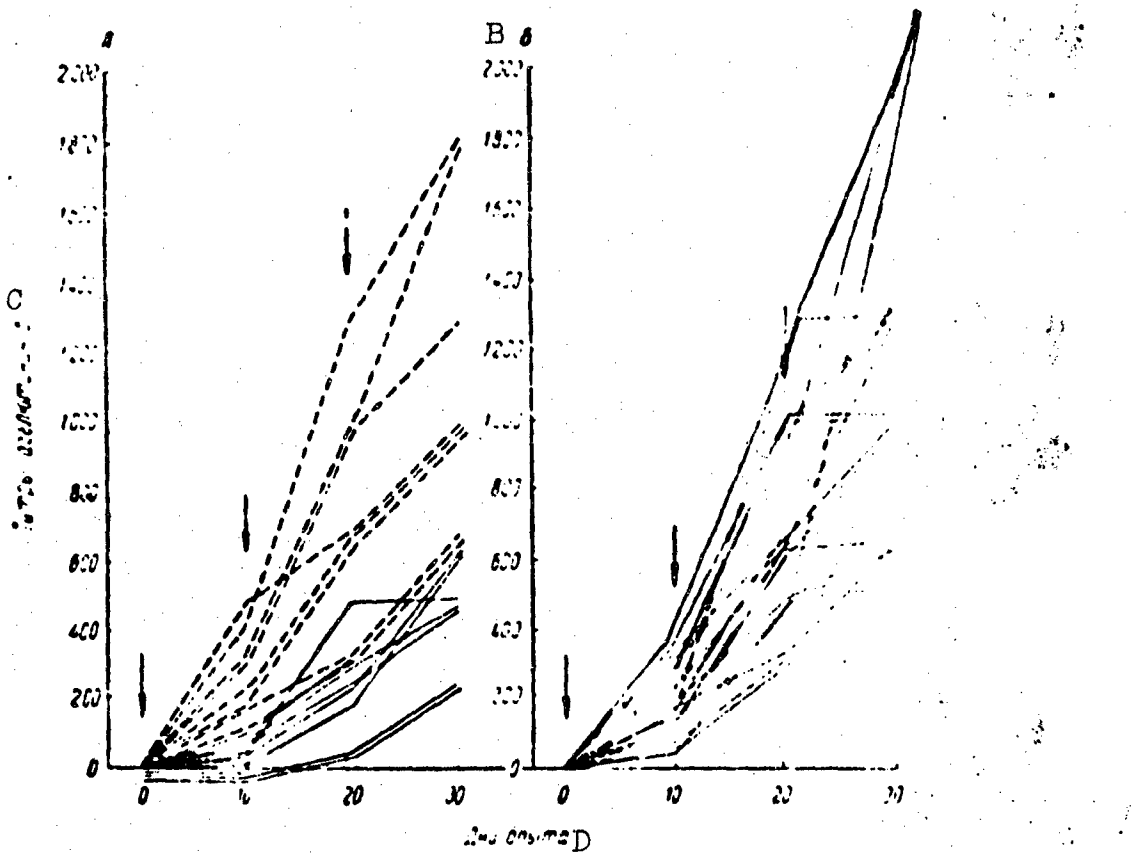

Fig. 8. Dynamics of the change in agglutenin titres in irradiated and control rabbits (according to data compiled by M.I. Ravich-Shcherbo and L.G. Prokopenko). A) Preliminary immunization with blood serum; the other symbols are the same as in Fig. 7. C) agglutenin titres; D) days of experiment.

As a result, it naturally became necessary to elucidate the capacity of irradiated animals for generating artificial immunity. Study of this problem was in accord with epidemiological goals.

There is no necessity to describe here the large number of works whose general results reduced to the fact that there is a period in any acute radiation affection, lasting from approximately the 3rd to the 20th day, when vaccination does not produce immunity or this immunity is considerably attenuated. It is consequently clear that under unfavorable epidemiological conditions there is no sense in resorting to vaccination during the acute period of radiation sickness.

Still another important fact was discovered during the course of research. It is widely known that there exists among immunologists the

concept of "vaccination reactivity." This term is used to define the reaction of the organism to vaccination, as manifested in clinical and hematological changes. This reaction depends both on the properties of the vaccine itself and on the characteristics of the organism. Vaccination is contraindicated under certain conditions. In addition, a postvaccination reaction is not necessary for active immunity to develop. It might be expected that vaccination during acute radiation sickness would be accompanied by unfavorable symptoms. Such was found to be the case; vaccination reactivity in a number of irradiated animals was very high, reaching a level sufficient to cause death.

According to the data of G.A. Shal'nova, a colleague in our laboratory, almost half of mice x-irradiated in a dose of 300 r ($LD_{10/30}$) and vaccinated intracutaneously one day after irradiation died within three days after vaccination (Table 12).

Mortality also increased as a result of triple vaccination, which was begun on the first day with simultaneous administration of paratyphoid formalin vaccine and human serum; 5 of 12 rabbits died within 25 days after irradiation in a dose of 600 r (M.I. Ravich-Shcherbo and L.G. Prokopenko, 1960).

In Z.V. Shevtsova's experiments (1960) guinea pigs were vaccinated subcutaneously with live brucellosis vaccine before and after irradiation in a dose of 200 r (at a dose rate of 37 r/min). When the vaccine was administered 3 days after irradiation mortality was 3 times as great as in the animals subjected to irradiation alone, while when the vaccine was administered after 10 days it was twice as great.

A.I. Maslov and G.N. Krivenkov (1961) carried out aerogenic immunization and reimmunization with live brucellosis vaccine against a background of exposure to ionizing radiation. Guinea pigs were irradiated in a dose of 250 r (21.3 r/min) 5 hours before administration

TABLE 12

Mortality Resulting from Vaccination Among Irradiated Male Mice, During First Three Days After Vaccination (data compiled by G.A. Shal'nova)

	A	B	C
D Моновакцины	F B·Breslau 200 млн.	0/20	10/20
	G БЦЖ, 1 мг	4/35	18/40
	H B·coli живая 100 млн.	3/20	8/20
E Дивакцины	I B·Breslau 200 млн. + БЦЖ 1 мг	4/35	20/40
	J B·Breslau 200 млн. + B·coli живая 100 млн.	1/20	11/20

Note: The mice were immunized during the first day after irradiation in a dose of 300 r. The numerator shows the number of mice which died and the denominator the total number of mice.

A) Type and dosage of vaccine; B) unirradiated mice; C) irradiated mice; D) monovaccines; E) divaccines; F) Bacillus breslaviensis, 200 million cells; G) BCG, 1 mg; H) Bacterium coli, live, 100 million cells; I) Bacillus breslaviensis, 200 million cells + BCG, 1 mg; J) Bacillus breslaviensis, 200 million cells + Bacterium coli, live, 100 million cells.

of the vaccine aerosol. A group of animals irradiated and vaccinated subcutaneously was used for purposes of comparison. The authors observed a substantial increase in mortality among the guinea pigs vaccinated by the aerogenic method; 60% of these animals died, as against 10% of those vaccinated subcutaneously or subjected to irradiation alone. Revaccination did not cause death. However, this aggravating effect produced by administration of live vaccines is easily explained from another standpoint. It is well known that both pathogenic and commensal microbes multiply intensively in the tissues of the irradiated organism (V.F. Sosova, 1956; B.G. Avetikyan and A.G. Artemova, 1956). As a result, brucellosis microbes of the vaccine strain may cause a rather severe process to develop (Z.V. Shevtsova, 1959).

It was shown in Chapter 2 (see Table 7) that quadruple vaccination

with Bacterium coli (administered intravenously) before irradiation had a favorable effect on the course of radiation sickness. However, when V.F. Sosova carried out quadruple vaccination (with 1 ml of a one-million-cell suspension) with rabbits during the first few hours after irradiation in a dose of 800 r he observed precisely the opposite effect; the animals soon died, exhibiting symptoms of shock, while in those which survived this serious state the number of leucocytes in the blood dropped to a leucopenic level (3000 per 1 mm^3), during the first day, which confirms that shock developed.

A severe reaction in which the rabbits rapidly died of shock was also noted on intravenous injection of 2 ml of a filtrate from a six-day bouillon culture of Bacterium coli 25 hours after irradiation in a dose of 1100 r, the Shwartzman phenomenon being induced (V.F. Sosova, 1956). The irradiated organism thus becomes extremely sensitive to bacterial products during the initial period of radiation sickness. In Sosova's opinion, this sensitivity gradually decreases during the following days, until the animal is completely areactive during the height of the illness.

We may also conclude that this areactive state is present from the fact that rabbits do not die after being injected intravenously with a filtrate from a bouillon culture of Bacterium coli 3-4-5 days after irradiation in a dose of 1100 r (V.F. Sosova, 1956), from the attenuation of the Shwartzman (Becker, 1948; V.F. Sosova, 1956) and Sanarelli-Zdrodovskiy (Ye.P. Sidorik, 1957) phenomena, and from the inflammatory reaction to intracutaneous injection of a large quantity (3.5 billion cells) of killed colon bacilli. Finally, the areactivity of the organism is also shown by the necrotic-hemorrhagic character of the reaction to dermal administration of live microbes; the destructive processes in the focus are considerably stronger than the

protective inflammatory processes.

It is well known that stimuli become extreme or subthreshold in accordance with the state of certain links in the nervous system. In 1896 Acad. I.R. Tarkhanov was the first to show the role of the nervous system in the pathogenesis of radiation affections. He noted that the time required for the acid reflex of an x-irradiated frog was prolonged, amounting to 30-100 metronome beats, as against 5-7 beats before irradiation. Irradiation carried out after administration of strychnine kept frogs from having convulsions. A decrease in reflex excitability was observed by D. Lapitskiy (1935); the greater the dose of x-rays the frog received before administration of the strychnine, the later convulsions set in. Ye.I. Bakin (1951), expressed the same opinion, assigning special importance to the afferent portion of the peripheral nervous system, on the basis of the fact that transection of the sensory nerve trunks of an irradiated segment of the spinal cord prevents the convulsive seizures usually observed on radium irradiation of this organ. In studying the state of neural reception in irradiated areas of the skin M.N. Livanov and N.S. Delitsyna used records of the action currents which develop in a sensory nerve trunk on tactile stimulation through the skin to establish that irradiation causes a weakening of the receptor reaction. (depending on the irradiation dose and the time at which the investigation is conducted). N.N. Garvey (1956) observed morphological changes in the exteroreceptors of the skin on whole-body irradiation of rats.

B.B. Moroz (1952) showed that sensitivity to tetanus toxin decreases under the special conditions created by the action of the incorporated radioactive substance in polonium poisoning; while the mean survival time for rats which received a lethal dose of this toxin was 6.5 days, rats simultaneously poisoned with polonium lived twice

as long.

The investigations of M.I. Nemenov and V.V. Yakovleva (1940), Z.A. Yanson (1956), A.A. Grafov (1958), and others have shown that the conditioned-reflex activity of the cerebral cortex is attenuated by irradiation.

In the opinion of the Bulgarian researchers G.Tencheva, S. Baluyeva, and A. Sakhatchiyeva (1955), the decrease in tissue-colloid dispersion, increase in the permeability of cell membranes, acidosis, depression of the aerobic phase of cellular respiration, and prolongation of chronaxia (especially that of the afferent links of reflex arcs) observed on whole-body irradiation indicate that inhibition of the parabiotic type develops. H. Yamashita and T. Miyasaka (1952) used physiological methods to show that exposure to penetrating radiation leads to a state of parabiosis. Recording the biocurrents of the cerebral cortex made it possible to discover that the processes which occur in the central nervous system have a phase-like activity during radiation sickness; at the beginning of a radiation affection the bioelectric effect is increased, but during the period of acute symptoms it decreases, this indicating the development of severe inhibition (M.N. Livanov, 1962).

It follows from these observations that one might expect different reactions on vaccination of an irradiated animal, depending on the character of the perception of this stimulus during a given phase of radiation sickness.

The problem of why vaccination aggravates the course of radiation sickness is still far from solution. Even in our laboratory contradictory observations have been made when vaccines were administered at the height of radiation sickness, i.e., during the period when the areactivity of the organism is at its greatest. We do not exclude the

possibility that the results were affected by use of microbial suspensions which differed in their content of toxic substances and by the fact that different species of animals were employed.

Thus, N.N. Klemparskaya (1958) noted an increased mortality among mice irradiated in a dose of 300 r and then vaccinated with heat- or formalin-killed typhoid bacillus. It was found that while a total of 7% of the mice died after irradiation, triple subcutaneous vaccination between the 1st and 15th days after irradiation increased mortality to 81.8%. Single intraperitoneal vaccination during the first day after irradiation caused 10.6% of the mice to die, while vaccination during the second week produced 83.4% mortality and inoculation during the 3rd-4th weeks 57.3% mortality.

V.F. Sosova obtained different data in experiments on rabbits to which he administered a suspension of Bacillus breslaviensis killed with formalin vapors and carefully purified of toxic substances. The rabbits were injected intravenously with the vaccine one hour and 3 days after irradiation in a dose of 800 r; nonirradiated animals were also immunized in the same manner. A very large vaccine dosage (25 billion microbes in 5 ml of physiological solution) was employed. Vaccination of 4 rabbits one hour after irradiation caused a typical state of shock characterized by dyspnea, adynamia, convulsions, screaming, and severe leucopenia (2000-3000 leucocytes per mm^3 of blood); all of the animals died within several hours.

The reaction of the rabbits vaccinated 3 days after irradiation was entirely different. Some of them exhibited only a slight depression. Not one of the 16 animals died, even though this vaccine dosage occasionally caused death among the nonirradiated rabbits (3 of 12 rabbits died over a period of one day). However, there is apparently a tolerance limit in this case. In one series of experiments three

daily injections of this vaccine dosage 3-4-5 days after irradiation did not cause acute shock in 4 rabbits irradiated in a dose of 800 r, but their general condition deteriorated and all of them died within 8-11 days after irradiation. One of the three control (unirradiated) rabbits used in this experiment died soon after the third vaccination.

Whole-body irradiation of rabbits in rather high doses (1100 r) frequently caused lethal shock to develop soon after exposure. However, "spontaneous" shock is very rarely encountered after irradiation in a dose of 800 r. As our experiments showed, the provoking factor at this irradiation dose may be the additional stimulus provided by the foreign proteins of the intravenously-administered vaccine. The reliability of this observation was confirmed later, during another season of the year, when 4 rabbits were vaccinated one hour after irradiation. The same effect was again obtained: all of the animals died of shock.

Wishing to check how another species of animal would react to the same vaccine, we conducted experiments on mice irradiated in doses of 500, 600, 700, 800, 900, and 1000 r. Twenty to 24 mice were used for each irradiation dose. Half of them were given washed Bacillus breslaviensis vaccine (2.5 billion cells in 0.5 ml of physiological solution) by injection into the caudal vein 1-2 hours after irradiation. The mice withstood this procedure quite satisfactorily; shock did not develop in a single case, even after irradiation in a dose of 1000 r. Moreover, early vaccination of irradiated mice with the washed vaccine did not have any detrimental influence on their survival time. Specific differences in the reactivity of organisms to the action of these two factors, irradiation and antigen stress, clearly appeared in these experiments.

In making a more thorough investigation of the reaction of the

organism to vaccination in the presence of an acute radiation affection it is very important to study the effectiveness and safety of vaccination during chronic forms of radiation sickness. The literature contains no material on experimental work in this direction, probably because of the difficulties created by keeping experimental animals for extended periods. Our laboratory has available certain data relating to this important problem.

V.F. Sosova, I.K. Petrovich, and B.A. Markelov (1961) conducted a complex investigation of the clinical, serological, and hematological data which characterized the reactions of animals with chronic radiation sickness to vaccination and revaccination.

The experimental animals were 12 dogs aged 5-6$\frac{1}{2}$ years which were suffering from an acute radiation affection resulting from a single intravenous (8 dogs) or inhalation (4 dogs) administration of radioactive strontium. Four dogs of the same age were used as the control. The animals were injected with 0.1-0.25 mcuries/kg of Sr^{90} or a mixture of Sr_{89+90} intravenously. In inhalation the dogs breathed air containing a mist made up of a mixture of isotopes in a concentration of 0.17-0.08 mcuries/l for 15 minutes.

The dogs were satisfactorily sensitive 3-4$\frac{1}{2}$ years after administration of the strontium, i.e., at the beginning of vaccination. Their blood was characterized by a normal erythrocyte count, a leucocyte count reduced by 50%, and a lowered thrombocyte count.

Immunization was carried out with Bacillus breslaviensis formalin vaccine administered subcutaneously. The suspension contained 1 billion microbes per ml. A dose of 1 ml of vaccine for each 10 kg of body weight was used for the first injection (vaccination), while a dose of 1.5 ml per 10 kg was used for the second injection (revaccination). The interval between vaccinations was 4 months. The total observation time

was 8 months.

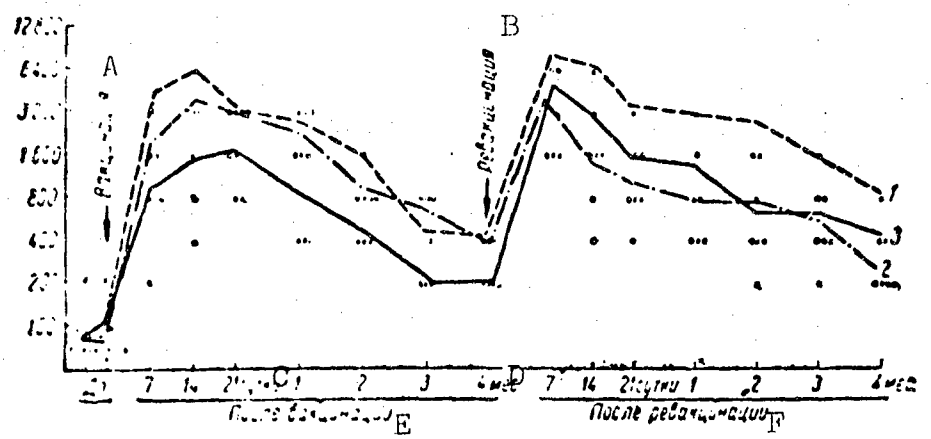

Fig. 9. Antibody formation in dogs as a function of dosage of Sr^{90} administered intravenously 3-4 years before vaccination. The time before and after vaccination and revaccination with Bacillus breslaviensis is shown along the abscissa; the extent of blood-serum dilutions is shown along the ordinate. 1) Mean agglutinin titres in immunized control dogs; 2) the same, in dogs which received 0.1-0.15 mcuries/kg of Sr^{90} (individual data are indicated by circles); 3) the same, in dogs which received 0.2-0.25 mcuries/kg of Sr^{90} (individual data are indicated by crosses). A) Vaccination; B) revaccination; C) days; D) months; E) after vaccination; F) after revaccination.

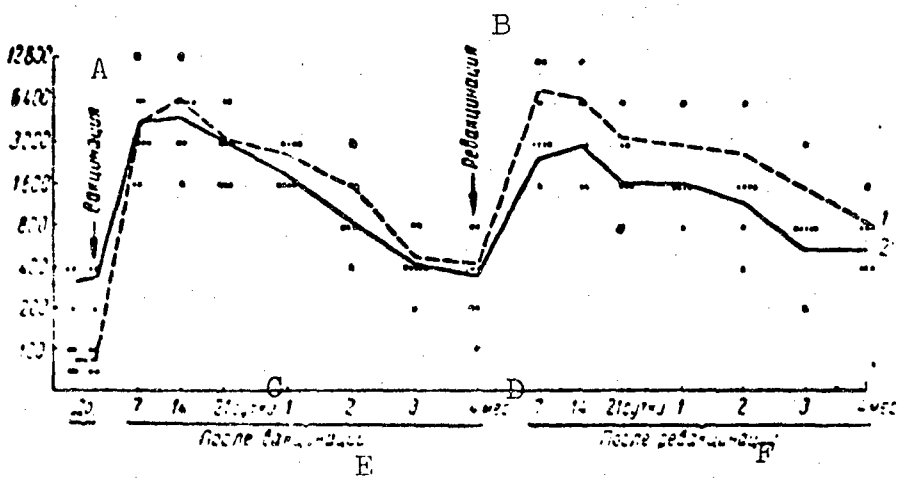

Fig. 10. Dynamics of agglutinins to Bacillus breslaviensis in dogs immunized 3-4 years after administration of radioactive strontium by inhalation. 1) Mean antibody titres in immunized control dogs (individual data are represented by crosses); 2) the same, in immunized irradiated dogs (individual data are represented by circles). A) Vaccination; B) revaccination; C) days; D) months; E) after vaccination; F) after revaccination.

The extent of antibody formation was determined from the titre of agglutinins in the blood serum 7, 14, and 21 days and 1, 2, 3, and 4

months after vaccination and revaccination. The leucocyte ratio, leucocyte count, thrombocyte count and erythrocyte count were determined at the same intervals and the sedimentation rate was measured. In addition, the leucocyte count was determined and temperature and body weight measured daily during the first week after vaccination and revaccination.

The observations showed that both the control and radiostrontium-irradiated dogs withstood the initial and repeated vaccinations well. Their body temperature increased by 0.5-1° and reverted to its initial level within 2-4 days; there were no substantial changes in weight or behavior.

In processing the data obtained from the agglutination reaction it was found that at the height of the immunizing reaction (during the first three weeks after vaccination) some of the animals with chronic radiation sickness exhibited agglutinin titres markedly below those of the control animals (Fig. 9). The majority of low agglutinin titres were obtained from the 4 dogs which were injected intravenously with radioactive strontium in large doses (0.25-0.2 mcuries/kg). The minimum antibody titre in these animals was equal to a serum dilution of 1:200 and the maximum titre was equivalent to a dilution of 1:3200. At the same time, the corresponding titres in the immunized control animals were equivalent to serum dilutions of 1:3200 and 1:12,800, i.e., the immunizing reaction in the irradiated animals was less intense by a factor of 4-8. This difference persisted until the 3rd or 4th month. Antibody production was also slightly reduced after revaccination, being almost identical in all of the animals, regardless of the radiator dose administered. The animals which were poisoned intravenously with small doses of the β-radiator (0.1-0.15 mcuries/kg) or were given the preparation by inhalation (Fig. 10) exhibited no great dif-

ferences in the extent of the rises in agglutinin titres after vaccination; however, they reacted to repeated administration of the antigen with a reduced antibody production.

Evaluating the general state of immunogenesis at long intervals after entry of radioactive strontium into the body in doses of 0.1-0.25 mcuries/kg, it may be concluded that, under the conditions of chronic radiation sickness, animals react to vaccination and revaccination (with a bacterial antigen) by active production of antibodies. However, at doses of 0.2-0.25 mcuries/kg antibody formation proceeds more slowly.

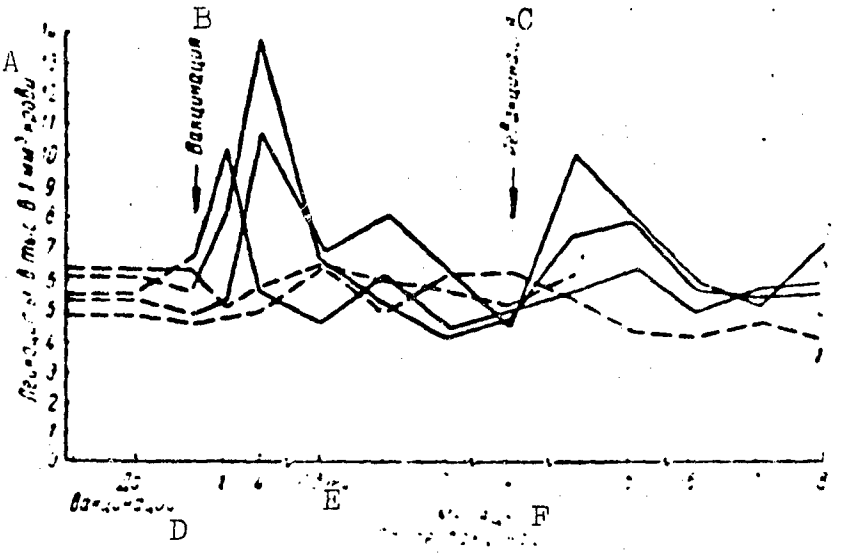

Fig. 11. Leucocyte count in vaccinated and unvaccinated dogs poisoned intravenously with Sr^{90} in doses of 0.1-0.2 mcuries/kg. 1) Two unvaccinated irradiated dogs; 2) three vaccinated irradiated dogs. A) Leucocyte count, in thousands per mm^3 of blood; B) vaccination; C) revaccination; D) before vaccination; E) days; F) months after vaccination.

As for the data obtained in hematological investigations, it is first important to note the absence of any unfavorable effect on hematogenesis resulting from vaccination and revaccination during chronic radiation sickness. No pathological forms of white or red blood cells were detected; nuclear pyknosis, chromatinolysis, and hypersegmentation were encountered no more frequently than in unvaccinated animals

which received the same dosage of radiostrontium and were examined at the same time. In all of the animals the leucocyte count increased sharply 1-5 days after vaccination (Fig. 11), a phenomenon which normally occurs in humans after vaccination. The increase in the white cell count was accounted for primarily by neutrophils and amounted to 100-200% or more. Revaccination yielded approximately the same type of leucocytosis; in a number of animals the leucocyte count remained at a high level, approximating the initial level, for as long as 4 months after revaccination. The thrombocyte and erythrocyte counts did not change materially during immunization. The sedimentation rate was somewhat accelerated for 2-3 days after revaccination, rising to 15-30 mm/hr in 2 dogs.

Development of an intense neutrophilic leucocytosis was also observed in x-irradiated rabbits suffering from acute radiation sickness. The rabbits were given 0.2 ml of a one-billion-cell suspension of paratyphoid B vaccine intravenously 40 days after irradiation in a dose of 800 r. On the following day their leucocyte counts reached 48,000-50,000, 6-8 times as great as the counts before vaccination. A second injection given after 15 days again caused a similar leucocytosis.

Experiments involving vaccination have shown that the hematogenic tissues of animals which survive acute radiation sickness are in a labile state. As a result, periods of stable leucopenia apparently frequently alternate with periods of temporary leucocytosis during chronic radiation sickness. This may be caused by infection, trauma, or changes in diet.

The Japanese authors Yaci, Nagata, etal. (1957) found that the disruption of the hematogenic functioning of the bone marrow produced in mice by injection of the isotope P^{32} (4 microcuries) into the ab-

dominal cavity can be eliminated to a considerable extent by subcutaneous injection of smallpox vaccine. According to our data, vaccination and revaccination long after irradiation with radioactive strontium also promote a slight increase in leucocyte count in the blood of dogs.

Antibody production is thus maintained at a rather high level during chronic radiation sickness caused by incorporated radiostrontium; vaccination and revaccination do not make the hematological picture or the clinical course of the affection worse.

A similar investigation, using the same vaccine and vaccination scheme, was carried out on dogs which had survived a normally-fatal affection resulting from subcutaneous injection of the α-radiator polonium in a dose of 0.0025 mcurie/kg (data compiled by V.F. Sosova, A.G. Izergina, and I.K. Petrovich). As a result of the special treatment method employed the animals did not die and were vaccinated after one year (2 dogs) and one year 9 months (6 dogs). They withstood vaccination well, responding with a completely satisfactory agglutinin production (Fig. 12) and a marked leucocytosis (Fig. 13). Vaccination did not have any unfavorable influence on hematogenesis and the clinical condition of the animals.

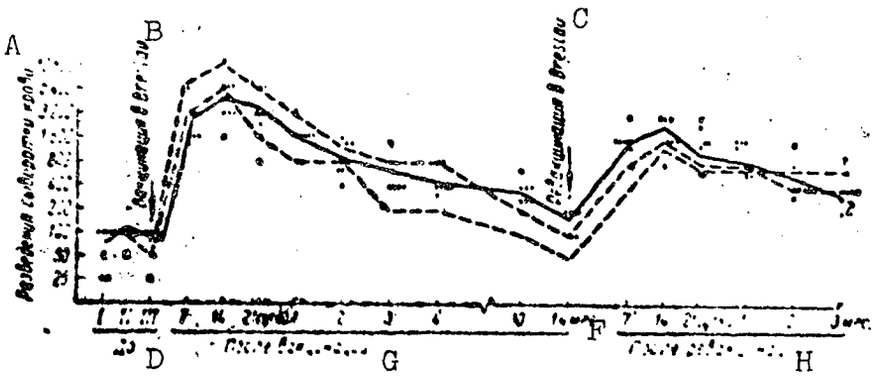

Fig. 12. Antibody formation in dogs poisoned with polonium. 1) Agglutinin titres in two unirradiated vaccinated control dogs; 2) mean titres in eight vaccinated irradiated dogs. A) Serum dilutions; B) vaccination with Bacillus breslaviensis; C) revaccination with Bacillus breslaviensis; D) before; E) days; F) months; G) after vaccination; H) after revaccination.

Data on Dogs Subjected to Multiple Irradiation

A Кличка или номер собаки	B Пол	C Возраст в годах	D Вес в кг	E Источники облучения: F 1-й раз	G 2-й раз	H 3-й раз	I Дата облучения: 1-й раз	2-й раз	3-й раз	J Интервал между облучением и вакцинацией: K первым	L последним	M дата вакцинации	N Условия облучения
BB Лань	Самка FF	4–5	21,8	Рентгеновский аппарат. O 3-трубочный 600 *p* P	12-трубочный 300 *p* Q	ЭГО-2 Co^{60} 300 *p* R	27 VII 1957	14 IX 1957	28 VIII 1959	3 года 2 месяца V W	1 год 1 месяц	20 IX 1960	X Рентгеновский аппарат 3-трубочный, 180 *кв*, 20 *мА*, фильтр 0,5 *мм* Cu + 1 *мм* Al, расстояние от анода до середины тела собаки 90 *см*, мощность дозы 16,1 – 16,5 *р/мин*
CC Галун	Самец GG	4–5	20,7	Рентгеновский аппарат. 3-трубочный 600 *p*	12-трубочный 300 *p*	ЭГО-2 300 *p*	27 VII 1957	14 IX 1957	29 VIII 1959	3 года 2 месяца	1 год 1 месяц	20 IX 1960	
DD Дармоед	»	4–5	20,0	РУМ-3 600 *p* S	ЭГО-2 300 *p*	–	5 VI 1957	27 VIII 1959		3 года 3 месяца	1 год 1 месяц	20 IX 1960	Y Рентгеновский аппарат 12-трубочный, 190 *кв*, 56 *мА*, фильтр 0,5 *мм* Cu + 1 *мм* Al, мощность дозы 121 *р/мин*, облучение со всех сторон
EE Бобик	»	3–4	16,0	ЭГО-2 350 *p*	ЭГО-2 300 *p*	–	29 VI 1959	27 VIII 1959		1 год 3 месяца	1 год 1 месяц	20 IX 1960	
662	Самка FF	3–4	28,3	ЭГО-2 350 *p*	ЭГО-2 300 *p*	–	1 VI 1959	29 VIII 1959		1 год 4 месяца	1 год 1 месяц	20 IX 1960	Z Гамма-облучатель ЭГО-2 (Co^{60}), мощность дозы 417 – 418 *р/мин*, облучение со всех сторон.
634	»	1,5	7,7		Контроль вакцинации (без облучения) T							20 IX 1960	AA Доза 300 – 350 *р* гамма-лучей по биологическому эффекту была равна 600 *р* лучей Рентгена $LD_{93/45}$ дней
293	»	4	18,0		То же U							21 III 1960	
300	Самец GG	5	21,2		» »							21 III 1960	

A) Name or number of dog; B) sex; C) age in years; D) weight in kg; E) irradiation source; F) first time; G) second time; H) third time; I) irradiation date; J) interval between irradiation and vaccinations; K) first; L) subsequent; M) vaccination date; N) irradiation conditions; O) x-ray apparatus; P) 3-tube, 600 r; Q) 12-tube, 300 r; R) EGO-2, Co^{60}, 300 r; S) RUM-3, 600 r; T) vaccination control (no irradiation); U) the same; V) years; W) months; X) 3-tube x-ray apparatus, 180 kv, 20 m amp, 0.5 mm Cu + 1 mm Al filter, distance from anode to center of dog's body – 90 cm, dose rate – 16.1-16.5 r/min; Y) 12-tube x-ray apparatus, 180 kv, 56 m amp, 0.5 mm Cu + 1 mm Al filter, dose rate – 121 r/min, irradiation from all sides; Z) EGO-2 γ-irradiator (Co^{60}), dose rate – 417-418 r/min, irradiation from all sides; AA) γ-ray dose of 300-350 r was equivalent in biological effect to 600 r of x-rays, $LD_{93/45}$; BB) Dan'; CC) Galun; DD) Darmoyed; EE) Bobik; FF) female; GG) male.

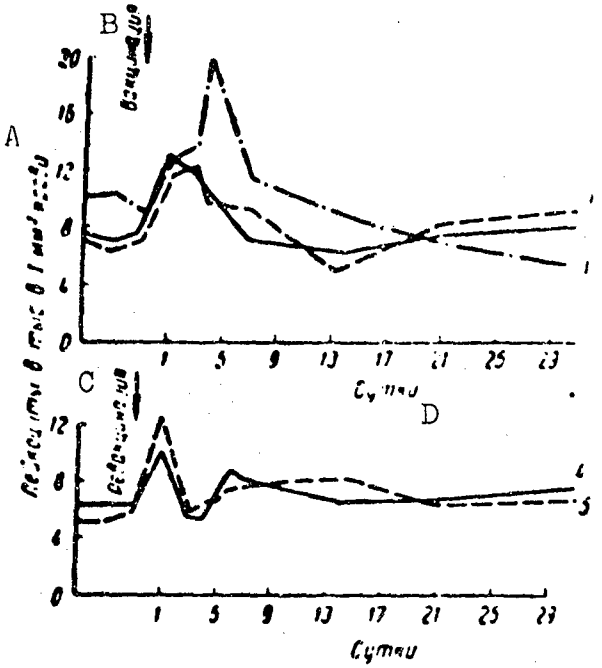

Fig. 13. Leucocytic reaction to vaccination and revaccination in dogs given polonium (averaged data). The arrows indicate vaccination. 1) Vaccinated dogs not irradiated with polonium; 2) dogs vaccinated 365 days after administration of polonium; 3) dogs vaccinated 650 days after administration of polonium; 4) dogs revaccinated 775 days after receiving polonium; 5) dogs revaccinated 1062 days after administration of polonium. A) Leucocyte count, in thousands of cells per mm^3 of blood; B) vaccination; C) revaccination; D) days.

We also had at our disposal 5 dogs which had survived double or triple x- or γ-irradiation (data compiled by V.F. Sosova and N.V.. Rayeva, Table 13). These animals were vaccinated with Bacillus bre-slaviensis (by the same method) 1 year 3 months to 3 years 2 months after their first irradiation or 1 year 1 month after subsequent irradiation. Their condition was completely satisfactory at this time; they behaved actively, ate well, and exhibited no material deviations in their hematological indices. However, these dogs differed greatly in appearance from the others used, large areas of their coats having turned gray (Fig. 14). Vaccinating them also provoked a temporary increase in the number of leucocytes in the peripheral blood (Fig. 15), but did not lead to any unfavorable clinical or hematological symptoms.

Antibody formation proceeded more slowly (Table 14) in the dogs

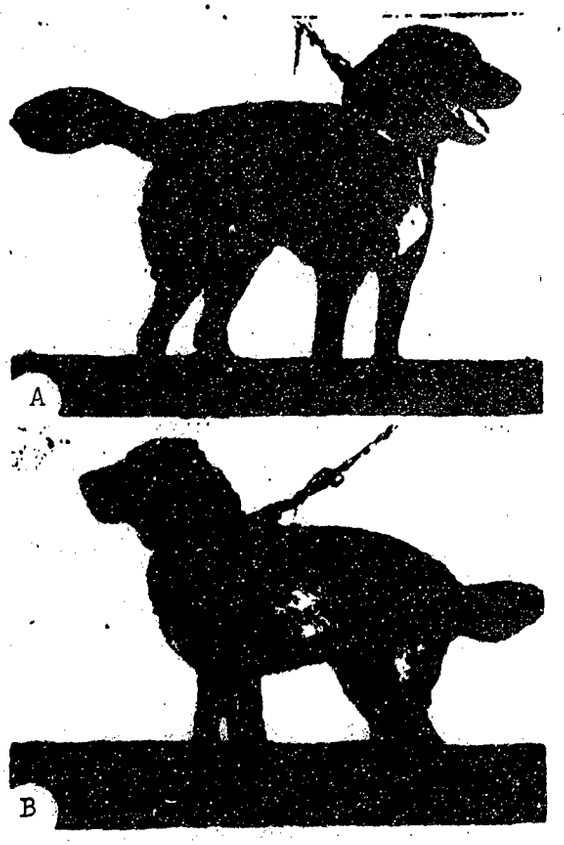

Fig. 14. Large areas of coat turning gray in a dog after triple external irradiation. A) Before irradiation; B) after irradiation.

of this group than in the animals of the control group or the unirradiated and irradiated dogs used in other experiments. In addition, no material differences were observed to result from the multiple irradiation, this indicating that the individual reactivity of the animals themselves plays a definite role. In essence, this group of dogs was made up of animals with an unusual resistance to irradiation, since the doses which they received were lethal. It is consequently still difficult to answer the question of what caused the depression of immunogenesis in these animals. It is possible that the multiple irradiation prevented the immunogenetic mechanisms from having their full

effect. However, we should not exclude the possibility that these animals, which were resistant to irradiation, also had a high threshold to the action of antigens.

There is still another important area in which vaccines can be used after irradiation, as hematogenic stimulators.

T.V. Kalyayeva (1958) made a detailed study of hemopoiesis in x-irradiated rabbits which were subsequently injected intravenously with killed microbial suspensions. The rabbits were irradiated in a dose of 450 r, which did not cause death although it substantially altered the hematological picture.

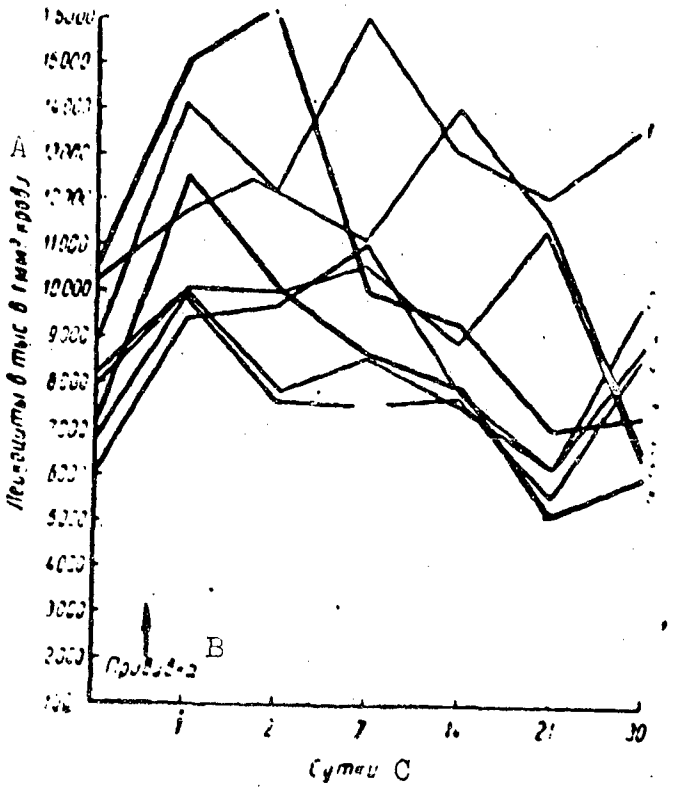

Fig. 15. Leucocytic reaction to vaccination in dogs which survived multiple external irradiation. The arrow indicates vaccination with Bacillus breslaviensis; 1, 2, 3, 4, 5, and 6) Vaccinated irradiated dogs; 7 and 8) vaccinated unirradiated dogs. A) Leucocyte count, in thousands of cells per mm^3 of blood; B) vaccination; C) days.

Injection of the experimental and control animals with B. mesentericus caused a brief toxic reaction, which manifested itself clinically in the development of adynamia and hematologically in the

TABLE 14

Titres of Agglutenins to Bacillus breslaviensis in Dogs Which Survived Acute Radiation Sickness 2 or 3 Times (external irradiation)

	A Кличка или номер собаки	B До вакцинации I	B До вакцинации II	C Доза вакцины в млрд. (1 млрд. на 10 кг веса)	D После вакцинации 7 суток E	14 суток	21 сутки	1 месяц F	2 месяца	3 месяца	4 месяца
G Облученные	Дань I	1:50	1:50	2,2	1:1 600	1:1 600	1:800	1:400	1:400	1:200	1:200
	Галун J	1:25	1:25	2,1	1:800	1:1 600	1:1 600	1:800	1:400±	1:400	1:400±
	Дармоед K	1:25	1:50	2,0	1:800	1:1 600	1:1 600±	1:400	1:200	1:100	1:200±
	Бобик L	1:100	1:100	1,6	1:400	1:3 200	1:3 200	1:1 600	1:400	1:200	1:400
	662	1:50	1:50	2,8	1:400	1:1 600±	1:800	1:400	1:200±	1:100	1:100
H Необлученные	634	1:100	1:100	0,8	1:3 200	1:3 200	1:3 200	1:1 600	1:200	1:200	1:200
	293	1:100	1:100	1,8	1:6 400	1:12 800	1:6 400±	1:3 200	1:1 600±	1:400	1:800±
	300	—	1:100	2,1	1:3 200	1:6 400	1:1 600	1:800	1:800	1:200	1:200

A) Name or number of dog; B) before vaccination; C) vaccine dose, in billions of cells (1 billion cells per 10 kg of body weight); D) after vaccination; E) days; F) months; G) irradiated; H) unirradiated; I) Dan; J) Galun; K) Darmoyed; L) Bobik.

appearance of degenerative forms of granulopoiesis and a slight depression of erythropoiesis and thrombopoiesis. At the same time, the hematological indices showed that it is possible for hematogenesis to be activated by killed microbes even during the period of its most severe depression after irradiation.

This stimulating influence on hemopoiesis took the form of an increase in the relative and absolute neutrophil counts, the total number of leucocytes in the peripheral blood, the total number of cellular elements, and the number of hemohistoblasts, cells in various stages of mitosis, and regenerative erythroblast elements (especially of the myeloid series) in the bone marrow and the spleen.

Signs of hematogenic stimulation were observed most clearly in irradiated rabbits inoculated with a killed culture of B. mesentericus in a small dose (0.5 ml) 7 times over a period of 12 days after irradiation, as well as in rabbits injected with 3 ml of a killed pneumococcus culture over a period of 3 days after irradiation.

Comparing the hematological shifts observed in the animals which received the killed microbial suspensions (vaccines) with those which occurred in animals infected after irradiation, T.V. Kalyayeva (1958) found a number of indices to be similar. In cases of spontaneous infection (plague in dogs) or artificial infection with diluted cultures of Bacterium coli and Clostridium perfringens hematogenic functioning was less disrupted by irradiation.

The author concludes that certain microbial products may be used as hemopoietic stimulators in the irradiated organism (provided that their toxicity is reduced).

These data indicate that, in addition to the use of bone-marrow cells and other hemostimulators, attempts to utilize microbial stimulants for this purpose also present good prospects. It might follow

to combine injection of killed microbes with administration of protein stimulators such as blood serum.

In this connection we might point out the experiments conducted by V.F. Sosova. Both unirradiated rabbits and rabbits irradiated in a dose of 800 r were given Bacillus breslaviensis vaccine intravenously 8 days after the beginning of the experiment and then injected with rabbit anti-Bacillus breslaviensis serum by the same method one hour or 24 hours later (methodological details are given on page 110). Both the experimental and control animals developed a very severe leucocytosis as a result of this treatment; the number of leucocytes per mm^3 of peripheral blood reached 13,700-38,700 in the irradiated rabbits and 53,700-178,000 in the unirradiated rabbits.

Fractional administration of the vaccine and the serum caused a comparatively slight rise in leucocyte count in all of the animals.

This was the first time in all of our many years of work with irradiated animals that we encountered the astounding phenomenon of an animal's passing out of a state of severe leucopenia at the height of radiation sickness without blood transfusions, essentially independently, albeit temporarily (for 2-3 days). If the figures given are expressed as percentages of the initial (before vaccination) leucocyte count, it turns out that the number of white cells increased after injection of the vaccine and serum by a factor of 7-15 in the irradiated animals and a factor of 4-12 in the unirradiated animals, i.e., to approximately the same extent (Fig. 16).

This experiment enabled us to discover that leucocytosis may develop in an irradiated organism. It is not clear whether this leucocytosis results from redistribution or from a stimulation of hematogenesis.

Comparing our observations with the data cited in the work of

M.I. Ravich-Shcherbo and L.G. Prokopenko (1960), who essentially combined vaccination with administration of serum and obtained very favorable leucocyte counts (no lower than 4200 leucocytes per mm^3 of blood among the experimental animals and an average of 2000 leucocytes per mm^3 among the irradiated control animals), we are inclined to believe that they have a common basis.

Our attention is struck by V.M. Alekseyeva's observation (1960) that injection of bacterial lipopolysaccharides causes leucocytosis in irradiated rabbits.

Thus, while vaccination of an irradiated animal is unwise because it is impossible to ensure complete resistance to infection by this method during acute radiation sickness, we feel that the use of vaccines as hematogenic stimulators merits further research. This problem is closely connected with the study of radioresistance.

Finally, we will dwell on research in which irradiated animals were used as models of damaged immunogenetic mechanisms in order to study a number of unsolved immunological problems.

Varying the time at which the antigen was administered (before or after irradiation), Dixon and Talmage (1952) concluded that the mechanisms which participate in antibody formation do not remain the same over the entire period for which antibodies are produced. They based this conclusion on the fact that the initial phase of immunogenesis proved to be very radiosensitive. If the immune reaction had already begun subsequent irradiation did not prevent antibody formation. It follows from this that antibody production itself is effected by different mechanisms, which are resistant to the action of radiation. The initial phase was found to be brief in these experiments, being limited to several hours, while the radioresistant phase occupied the entire remaining period of antibody formation.

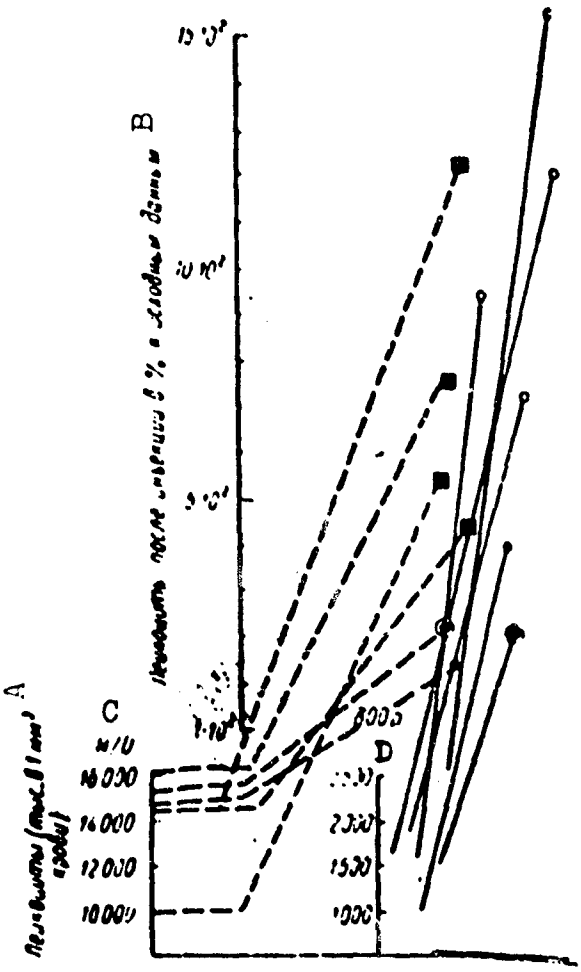

Fig. 16. Leucocytosis in rabbits in response to intravenous injection of vaccine and immune serum 3 days after irradiation in a dose of 800 r. The lower scales show the initial absolute leucocyte counts in the unirradiated (n/o) and irradiated (800 r) rabbits before vaccination. The circles indicate the leucocyte counts in the irradiated rabbits which preliminarily received vaccine and serum, the squares show the same for the nonirradiated rabbits, the dots the same for administration of serum alone, and the circle-and-dot combination for administration of the vaccine alone. A) Leucocyte count (thousands of cells per mm^3 of blood); B) leucocyte count after injections, as a percentage of the initial level; C) n/o; D) r.

As will be shown later, their relationships to irradiation are not the only characteristics of these phases. In particular, the presence of passive immunity during the initial phase materially affects antibody production.

It seemed possible to discover a more definite role for the spleen in immunogenesis in the irradiated organism. Jacobson, Robson, and Marks (1950) screened the spleen during irradiation and concluded that

it is the principal site of antibody formation in response to intravenous injection of cellular antigens. However, a thorough analysis made with the aid of subsequent experiments (Jacobson and Robson, 1952) in which sheep corpuscles were administered 24 hours after removal of the screened spleen (the splenectomy was performed one day after irradiation) showed that this operation does not prevent antibody formation and that just as many antibodies are produced outside the spleen. The specific characteristics of the subjects and the mode of antigen administration are important in this case (Wissler etal., 1953).

Many investigators came to a hasty conclusion about the possibility of complete suppression of immunogenesis by ionizing radiation.

This conclusion was supported by scientists who were dealing with tissue transplants and were consequently interested in methods of suppressing immunogenesis. However, disappointment soon occurred, especially when it was found that transplantation of bone-marrow cells, strikingly successful during the acute period of radiation sickness, itself causes a severe immunological reaction which results in death after long intervals (Makinodan, 1957).

Recent works (N. Gengozian and T. Makinodan, 1958; O.P. Peterson and I.A. Kozlova, 1958) and our observations (V.F. Sosova, 1961) have shown that it is impossible to suppress antibody formation completely by sublethal irradiation. The error which was made can be explained by the brief time for which the irradiated and vaccinated experimental animals were observed and the use of small antigen doses (administered subcutaneously or intraperitoneally) and several vaccinations.

We may speak of attenuation of antibody formation after sublethal irradiation only for a certain period of time.

We believe that in order to solve the problem of immunogenesis it is necessary to select the most favorable conditions for obtaining

a direct antigenic stimulus, which would facilitate subsequent processing of the data.

These conditions must include:

1) intravenous injection of the antigen so that a measured dose comes into contact with the cellular immunogenetic mechanisms immediately after it is diluted in the blood;

2) a single injection of the antigen, since repeated injections may have an inhibiting effect even on healthy animals, especially when given at short intervals.

Observing these conditions experimentally confirmed that irradiation in doses which make it possible to prolong survival time considerably does not suppress antibody formation completely.

There are fields of medicine which are concerned not with methods of stimulating immunogenesis, but with methods of depressing it. This is primarily true of tissue-transplantation problems. It is well known that depression of the capacity for active antibody formation may be produced in a healthy animal if it is vaccinated soon after an injection of the corresponding immune serum (Ya.R. Kovalenko, 1958-1959; A.I. Kazran etal.,(1959); Popovici and Gogoasa, 1956). Assuming that irradiation and administration of an immune serum may have a summed depressive effect, V.F. Sosova attempted to trace the action of a combination of passive immunity and irradiation on immunogenesis, using a corpuscular microbial antigen.

The experiments were conducted on rabbits weighing 2800-3000 g, which were subjected to whole-body irradiation in a dose of 800 r with a three-tube field of a 12-tube x-ray apparatus (180 kv, 14 ma, 0.5 mm Cu + 1 mm Al filter, dose rate - 30-34 r/min). This dose is the $LD_{30/30}$.

The animals were vaccinated during the acute period of their

radiation sickness, i.e., 3 days after irradiation. Unirradiated rabbits of the same weight were used as the controls.

Passive immunity was created in the rabbits by single intravenous injections of 10 ml of rabbit anti-Bacillus breslaviensis serum, after which (1-24 hours later) agglutenins to Bacillus breslaviensis in titres of 1:400-1:1600 were detected in their blood. The immune sera were kept under refrigeration, no preservatives being added. Their titres were from 1:12,800 to 1:51,200. Serum (or a mixture of sera) of the same titre (from the same flask) was naturally taken for each series of experiments.

The vaccine was an agar culture of Bacillus breslaviensis killed with formalin vapor and suspended in physiological solution. On the day of the experiments the initial dense microbial suspension was washed five times in order to purify it of dissolved toxic substances and centrifuged (3000 revolutions over a period of 30 minutes). 5 ml of a five-billion-cell washed suspension was used for the intravenous injections. Such a large antigen dose was used because the antibody-formation reaction to a single injection of a small dosage could not be detected in the presence of passive immunity.

Preliminary examination of the rabbits showed that the majority of them exhibited normal antibodies to Bacillus breslaviensis at a serum dilution of 1:25, although a dilution of 1:50-1:100 was required for some.

The extent of passive immunity and antibody formation was evaluated from the change in the titres of agglutenins to Bacillus breslaviensis in serum taken from the rabbits' pinnal veins. Blood was taken daily for 10-15 days after immunization and at intervals of 3-5 days for a further 50-60 days.

In the first version of the experiments a group of rabbits re-

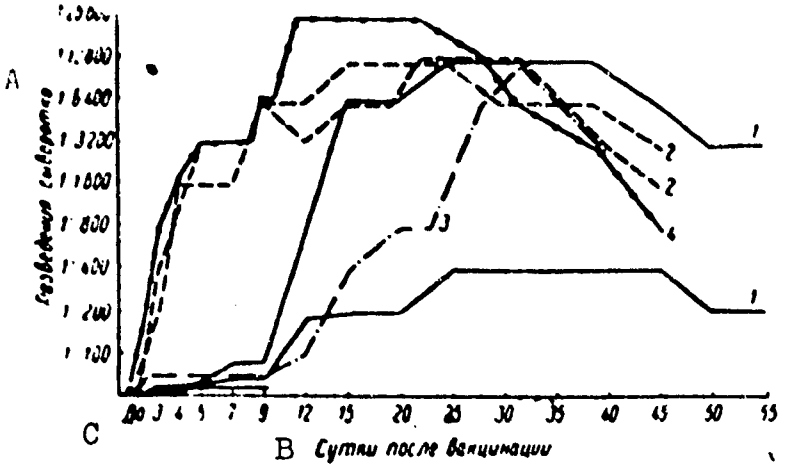

Fig. 17. Change in agglutinin titres in rabbits irradiated in a dose of 800 r and unirradiated rabbits receiving Bacillus breslaviensis vaccine only, as well as control data obtained on injection of 10 ml of normal serum one hour before vaccination. 1) Antibody titres in rabbits vaccinated 3 days after irradiation; 2) the same, in unirradiated vaccinated rabbits; 3) the same, in rabbits which received normal rabbit serum before vaccination; 4) the same, in unirradiated rabbits. A) Serum dilution; B) days after vaccination; C) before.

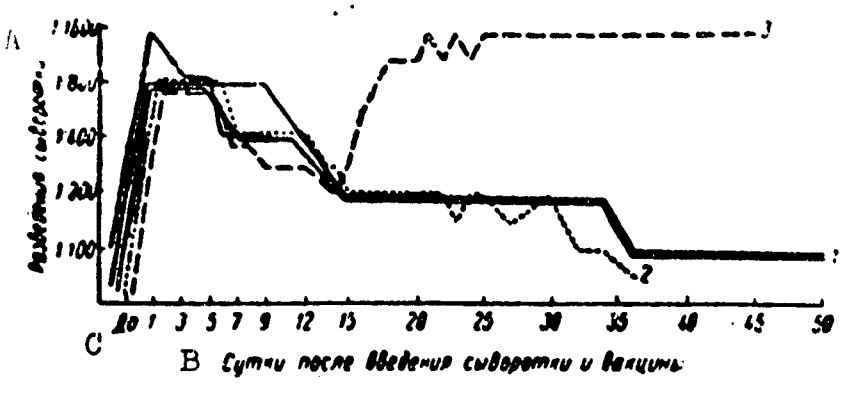

Fig. 18. Influence of administration of immune serum one hour before vaccination on active antibody formation in rabbits irradiated in a dose of 800 r. 1) Agglutinin titres in rabbits which received immune anti-Bacillus breslaviensis serum 3 days after irradiation and were then vaccinated with Bacillus breslaviensis; 2) the same, in an irradiated rabbit which received only immune serum; 3) the same, in an unirradiated rabbit vaccinated after administration of immune serum. A) Serum dilution; B) days after administration of serum and vaccine; C) before.

ceived immune serum one hour before vaccination, i.e., the rate of antibody formation was investigated against a background of passive immunity. In the second version the animals were vaccinated and then received immune serum one or 24 hours later. This clarified the role

which passive antibodies play after antibody formation has already begun.

The data obtained in these two basic series of experiments are given below.

Vaccination in the absence of passive immunity yielded the following results (Fig. 17).

A considerable accumulation of antibodies was noted 3-4 days after vaccination in the unirradiated rabbits. The maximum titres were reached on the 15th–20th day.

Antibody formation was only inhibited, not completely suppressed in the animals irradiated in a dose of 800 r and vaccinated with Bacillus breslaviensis 3 days later. A marked increase in the agglutinin titres was detected only at the end of the second week after vaccination; as is shown by the trend of the curves, the maximum titres were no lower than in the unirradiated vaccinated rabbits.

Administration of 10 ml of normal rabbit serum one hour before vaccination did not materially affect the course of the agglutinin-content curves for either the irradiated or unirradiated animals.

A completely different picture was observed when immune serum was injected an hour before vaccination (Fig. 18). During the first week all of the rabbits (irradiated and nonirradiated) maintained an almost identical quantity of passively-produced antibodies, the titres of which gradually decreased to a dilution of 1:200 on the 12th-18th day. However, the antibody curves diverged sharply at this time. While the quantity of antibodies remained at a low level in the irradiated animals until the end of the 2nd month (as in the control after administration of immune serum alone), in the unirradiated rabbits the antibody content increased 8 fold. This considerable rise in the quantity of agglutinins indicated the presence of active antibody produc-

tion. However, comparison with the control data (see Fig. 17) showed that the antibody titres in this case did not reach the levels obtained on administration of the vaccine alone.

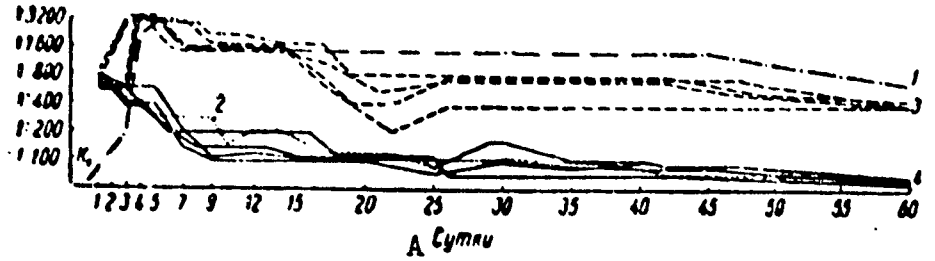

Fig. 19. Influence of administration of immune serum after vaccination on active antibody formation in irradiated and unirradiated rabbits. 1) Agglutinin titres in an unirradiated rabbit which received the vaccine alone; 2) the same, in an unirradiated rabbit which received the immune serum alone; 3) the same, in a group of unirradiated rabbits which received both the vaccine and the immune serum; 4) the same, in rabbits irradiated in a dose of 800 r. A) Days.

The presence of finished antibodies in the blood at the instant of exposure to the antigen thus had an important effect on antibody formation. Administration of immune serum before vaccination completely prevented active antibody formation in the irradiated organism. According to data in the literature this treatment slows down antibody formation and reduces the quantity of antibodies produced when used on a healthy unirradiated organism.

In the other experimental setup, i.e., when the immune serum was injected after vaccination, the passive antibodies also prevented active antibody production in the irradiated animals.

Figure 19 shows curves representing the quantity of agglutenins in 10 rabbits, which were experimented on simultaneously. Each pair of irradiated and unirradiated rabbits was given immune serum one or 24 hours after vaccination; the two unirradiated control rabbits received vaccine (1) or immune serum (2) in fractionated doses. It may be seen from the graph that the passive immunity which develop in all of the nine rabbits injected with serum was characterized by titres of

1:600-1:800. This antibody level was maintained for 3-4 days. The curves then diverged sharply, regardless of when the serum was administered. The curves for the unirradiated animals are grouped in the upper portion of the graph; their antibody titres increased fourfold, this indicating active production of antibodies to the vaccine administered. The curves for the irradiated rabbits at first dropped in the same fashion as that for the second control, thus pointing to a gradual depletion of passive immunity. The antibody levels in the irradiated animals remained within the limits of normal variation thenceforth, until the 60th day of observation.

The goals of this investigation did not include a detailed study of the reaction of the unirradiated animals, which were used only to obtain comparative data. Nevertheless, some account should be taken of the fact that the rise in antibody level reached a second peak in these animals on the 25th day. From the character of the curves we may hypothesize that the superimposition of specific passive immunity on vaccination may cause an unusual antibody-production reaction having two stages: the first involves discharge of antibodies into the blood stream on the 3rd-4th day and the second a similar discharge one month after vaccination.

It may not be fortuitous that two of the 4 irradiated rabbits exhibited a slight second peak in the antibody curves after 30 days. It is possible that we were dealing in these cases with the secondary antibody response observed in the experiments of Uhr and Baumann (1961), who studied antibody formation induced by administration of a precipitate of diphtheria toxoid and antiserum.

The essence of our experiments lies in the fact that we were able to find a method of augmenting the blocking action of irradiation on immunogenesis. The results of these experiments reduced to the fact

that administration of immune serum to irradiated animals before or shortly after vaccination may completely prevent active antibody production. Combining two factors, irradiation and passive immunity, which depress active antibody formation may completely suppress immunogenesis.

The effectiveness of conjoining irradiation and passive immunity to depress antibody formation indicates one possible direction in which research on methods of overcoming the immunological obstacles to tissue transplantation should proceed. Actually, great difficulties may be encountered here, if we take into account the cytotoxic properties of antitissue sera.

The data cited agree with those of S. Hollingsworth (1950) and D.R. Kaulen (1957) on the fact that passive antibodies persist for a long time in an irradiated organism, gradually decreasing in number in the same fashion as in an unirradiated animal. However, as our experiments showed, passive antibodies may serve as an agent which alters the conditions under which the antigen and the antibody-forming cells normally interact.

The mechanisms underlying the depressive effect of irradiation and that of immune serum on the immunological process are completely different. As the observations of I.Ya. Uchitel', E.L. Khasman, and A.S. Konikova (1961) showed, the healthy organism responds to vaccination with an intensification of protein synthesis in the liver and adrenal glands during the inductive phase, i.e., when it is still impossible to detect antibodies in the blood. These authors suggest that there is a stage of the inductive phase during which a serologically inactive protein, a predecessor of the antibodies, is formed. Since protein synthesis is disrupted in the presence of a radiation injury, we cannot discount the possibility that the basic cause of the tempo-

rary depression of antibody formation may be disruption of the synthetic capacity of the antibody-forming cells.

As a result of the excess of antibodies passive immunity creates unfavorable conditions for immunogenesis. This is apparently explained by the fact that the antibodies (i.e., the increased quantities of γ-globulin) prevent contact between the antigen and the cells, prevent antibody formation in the cells, or keep the antibodies from being discharged from the cells into the bloodstream. Passive immunity induced in a healthy organism prior to vaccination consequently often depresses active antibody production, prolonging the time required for it to develop and reducing the rate at which it proceeds. The death of a large number of cells and the disruption of cellular functioning which occur in the irradiated organism contribute to the complete suppression by the passive antibodies of the production of new antibodies.

On the basis of these data, we must include among the fundamental conditions for immunogenesis (in addition to healthy reticuloendothelial cells and a definite antigen concentration) the presence of favorable immunological conditions in the medium in which the antigen comes into contact with the cells. The presence of passive antibodies has a negative effect on immunogenesis.

The data of Leskowitz (1960) testify to the inhibiting effect of the excess of antibodies created by vaccination. This author studied the immune response of rabbits to vaccination with a specific precipitate prepared from bovine serum albumin and the corresponding rabbit antiserum. On administration of the precipitate antibodies developed to the bovine albumin only when it and the antiserum were given in equivalent quantities; if there was an excess of antiserum no antibodies were formed. Terres and Wolings (1961) also pointed out the material importance of the antigen dosage in the precipitate for active

antibody production.

Prepared antibodies consequently have a depressive effect on antibody formation when various antigens are used, be they cellular or simply protein.

In contrast to our experiments, in the work described above the antigen-antibody complex was prepared in vitro, for intravenous injection. We apparently produced a similar complex consisting of Bacillus breslaviensis and the corresponding antibodies in vivo in our rabbits, the prolonged passive immunity noted in these animals indicating an excess of antibodies. In addition, control experiments on the adsorption in vitro of anti-Bacillus breslaviensis serum by a suspension of Bacillus breslaviensis (in the doses used in our experiments) did not show any material change in the immune titre of the serum, i.e., an excess of passive antibodies was set up in the rabbit's body.

By injecting immune serum after vaccination we were able to limit the period of free contact between the antigen and the cells to a definite time interval. This method makes it possible to clarify certain characteristics of the initial period of immunogenesis.

Judging from the results of the experiments involving injection of immune serum one or 24 hours after vaccination, we may conclude that one hour (or less) of free contact between the antigen and the cells is sufficient to cause immunogenesis to begin in the healthy organism. However, a time this short (and even 24 hours) is clearly insufficient for the antigen to be effective in the irradiated organism; administration of immune serum after vaccination consequently had the same depressive effect which we observed for vaccination against a background of passive immunity.

We are correct in speaking of a disruption of the antigenic effect, since if the antigen were fixed in the tissues of the irradiated

rabbits for 24 hours we would observe a delayed immune response somewhat similar to that detected in the irradiated rabbits which received the vaccine alone (see Fig. 17). One gets the impression that an intravenously-administered corpuscular antigen continues to circulate in the blood of the irradiated organism for at least 24 hours or more. The presence of an excess of the prepared antibodies administered with the immune serum prevents it from coming into further contact with the cells and it is gradually eliminated, before cellular functioning is restored.

Studying the state of immunogenesis in irradiated rabbits by investigating the elimination of an isotope-tagged antigen from the blood, Talmage, Dixon, etal., (1951) concluded that the antigen circulates for an extended period in the blood, because of the absence of antibodies. Negative serological tests served as the basis for this conclusion.

The brief period for which the experimental animals were observed did not make it possible to detect the later appearance of antibodies. In our opinion, the value of these experiments lies in the fact that they showed that an administered antigen circulates for a long time in the blood of an irradiated animal, as well as that the fixation of the antigen in the animal's tissues is disrupted.

However, it must be noted that M.V. Tikhomirova (1958) injected guinea pigs irradiated in a dose of 500 r with normal horse serum and established that the fixation of this antigen by the tissues of the liver and intestines is intensified.

We attempted to produce specific agglutinins in mice, injecting them intraperitoneally with the blood of rabbits vaccinated intravenously with a bacterial vaccine. We intended to determine at intervals the time at which the antigen circulated in the blood of irradiated and unirradiated animals. However, no data which clearly indicate any

difference have as yet been obtained.

Summing up the material presented in this chapter, it may be concluded that vaccination after irradiation may be used to solve a number of practical and theoretical problems in radiation and general immunology.

CONCLUSION

The discovery and wide introduction of prophylactic immunization in addition to the other measures employed in the Soviet Union made it possible to completely eliminate or considerably reduce the incidence of many infectious diseases. However, as is shown by the data given in this book, the importance of vaccination is not limited to this alone. Experimental investigations carried out by biologists have shown that vaccines have still another noteworthy property; under certain conditions, they are able to increase radioresistance.

Ionizing radiation destroys cellular elements and increases the permeability of vessels and tissues. Favorable conditions are set up for cellular decomposition products to be absorbed and act on the body, this leading to autointoxication during the first few hours after irradiation and later to autosensitization of the irradiated organism.

Immunological mechanisms, the reaction to autoantigenic substances, thus play an important role in the development of radiation sickness.

It is possible to prevent the development of this reaction by immunological methods, one of which is vaccination.

Administration of foreign antigens causes a complex reaction, in which the nervous and endocrine systems participate. After the initial symptoms of stress, Selye's "alarm reaction," a prolonged characteristic reaction develops on the part of certain cellular elements, primarily the cells of the reticuloendothelial system and the plasmatic and lymphoid elements. The phagocytic functioning of the microphages and

macrophages is activated. As a result of the specific change in globulin synthesis immune bodies begin to develop not only to the antigen in question, the microbe, but also to tissue decomposition products, as recent investigations have shown.

Since the increase in radioresistance is observed months after vaccination it must be assumed that the most important factors in this process are the prolonged changes which affect the functioning of the reticuloendothelial system.

Study of the nature of the action of vaccination on the reaction of the organism to radiation has only just begun and is now attracting the attention of an ever wider circle of scientists. There is no doubt that the data obtained in various laboratories will aid in gaining a deeper understanding of this phenomenon and deciphering the details of the mechanisms on which it is based.

In contrast to foreign scientists, research workers in the Soviet Union have not only gathered data on the action of certain vaccines on radioresistance, but have also tried to explain this phenomenon and used it in practice, to improve the health of persons who come into contact with ionizing radiation.

At the level of our present knowledge, we may note two possible ways in which prophylactic vaccination may influence radioresistance.

The first is based on the phenomenon of antigen concurrency, which is well known in immunology. In this case "strong" microbial antigens (those having an active immunizing influence) administered before irradiation suppress the stimulating effect of "weak" tissue antigens.

The second is associated with the destruction of cells in the focus of local inflammation which develops as a result of microbial vaccination and with the resultant formation of antibodies to the tissue antigens. These antibodies are retained in the body for a long time

and may combine with and neutralize the tissue products formed as a result of irradiation. Many aspects of the action of these antibodies are still unknown. There is some definite optimum in their formation, since excessive hyperimmunization with autotissue products decreases rather than increases radioresistance. Since different antigens have different capacities for acting on tissue cellular elements (the local inflammatory reaction is of varying intensity) and activities in concurrent relationships a wide field of activity has been opened up for various investigators in searching for vaccines having the most active influence in increasing radioresistance. In our opinion, vaccines prepared from living microbes present the best prospects in this respect.

In studying the action of any vaccine special attention must be paid to the dosage, the mode of administration, the number of injections, and the duration of the interval between vaccination and irradiation. Very little research has been done on the influence of vaccination on animals poisoned with radioactive substances.

As the data cited in this work show, the effect of vaccination on the irradiated organism varies in accordance with the nature of the agent to which the animal was first exposed. It is in this fact that the concurrent character of immunization with microbial antigens and autoimmunization with tissue products appears most clearly.

If vaccination is carried out before irradiation immunity to the agent in question is not only maintained after exposure to ionizing radiation, but the course of the radiation sickness contracted is considerably altered. The survival rate is increased, fewer of the subjects show signs of damage to the gastrointestinal tract, symptoms of hemorrhagic diathesis are almost entirely lacking, the animals lose less weight, and active behavior and good appetite are maintained.

Vaccination conducted after irradiation does not have this effect on radioresistance.

Immunogenesis with respect to the microbial antigen administered is almost completely suppressed in this case. The toxic effect of the vaccine is especially strong; the vaccination makes the radiation-induced leucopenia more severe, increases the loss of weight, and intensifies the symptoms of hemorrhagic diathesis. A hemorrhagic inflammatory focus is formed at the site of injection of a vaccine prepared from live microbes and the number of microbes in this focus considerably exceeds the number of bacteria in the tissues of corresponding foci in unirradiated animals. Irradiated animals are regularly observed to die during the first two days after vaccination, this being especially marked when vaccination is carried out during the second week after irradiation. All of these facts indicate that irradiated animals must be vaccinated with special care and that contraindications must be worked out. Vaccination is apparently completely impermissible during certain periods after irradiation.

It is well known that after irradiation there is a considerable increase in the sensitivity of the organism to bacterial endotoxins and exotoxins, a depression of the phagocytic reaction and antibody production, destruction of the cellular elements of a number of organs, and a change in metabolism and the activity of the nervous system.

Against the background of such a severe disruption of vital activity any additional functional stress (and vaccination is a very severe stress) seriously affects the condition of the organism.

However, if the animal survives the radiation-induced affection a period of convalescence begins; general condition and external appearance improve, there is a gain in weight and appetite, the leucocyte count reverts to normal, the symptoms of hemorrhagic diathesis disap-

pear, body temperature is normalized, and the capacity for immunogenesis is restored. Active immunization is possible during this period, although its effect is considerably less at first than in healthy animals.

Vaccinations conducted long after γ-irradiation with Co^{60} or poisoning with radioactive substances showed a sufficient immunological reactivity to be present, although complete restoration of this reactivity does not occur for 3-4 years after exposure to large doses of radiation.

A further amassing of data on the effectiveness of prophylactic vaccination at various intervals before and after irradiation will make it possible to select the most suitable immunization conditions and types of preparations which will ensure a maximum increase in radioresistance; it will also enable us to develop indications and contraindications for vaccination of irradiated animals.

It is impossible in this book to mention the large number of epidemiologists and immunologists who have studied various methods of carrying out active immunization of the populace. The effect of vaccination on radioresistance is of interest to general practitioners and research workers as a proof of the immunological nature of the processes which occur during irradiation and as a possible method of safe and effective prevention of the development of radiation sickness in persons and animals who come into contact with ionizing radiation.

The authors of this work will be very satisfied if it contributes to an expansion of research in this field and if the results of this research are of use in public health work, in the preservation of human life and health.

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