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N. N. Klemparskaya, C. G. Alekseyeva, R. V. Petrov
and V. F. Sosova.

PROBLEMS OF INFECTION, IMMUNITY AND ALLERGY
IN ACUTE RADIATION SICKNESS

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Annotation.

Monograph of N. N. Klemparskaya, O. G. Alekseyeva, R. V. Petrov and V. F. Sosova reviews the literature and their experimental investigations over several years conducted by them and numerous co-workers.

The significance of the normal flora in an irradiated body, the state of natural and induced immunity, peculiarities of allergic reactivity and the course of infections in an irradiated organism are clarified in this book.

The book is of interest to physicians, biologists and scientists.

N. N. Klemparskaya, O. G. Alekseyeva,
R. V. Petrov and V. F. Sosova.

PROBLEMS OF INFECTION, IMMUNITY AND ALLERGY
IN ACUTE RADIATION SICKNESS

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I n t r o d u c t i o n .

Extensive use of radioactive substances and radiation in different fields of the national economy and scientific investigation leads one to pay a special attention to the studies of their influence on humans, animals, and plants.

The effect of ionizing radiation at certain intensity induces severe sickness in humans and animals with a typical complex of symptoms, in which phenomena connected with the change in the immunobiological reactivity of the irradiated organism play a leading role.

It is known, that the vital activity of all living creatures proceeds in continuous interaction with the world of microorganisms inhabiting its integument and cavities, which are in communication with the environment. A healthy organism possesses a certain stage of stability with regard to the surrounding microbes, but after a change in reactivity of the organism due to impairment of its vital activity, induced by various agents such as radiation, this symbiosis is disrupted and the phenomena of autoinfection appear.

Numerous experimental data and clinical observations of humans after radiation indicate the appearance of inflammatory processes induced by the penetration of microorganisms. Often they are also detected in blood (bacteriemia). A detailed investigation of microbes isolated from blood and internal organs of irradiated bodies showed that they are species ordinary inhabiting living organisms and thus they compose the normal microflora of the host. The microflora of the intestines are the most frequently found.

Besides the presence of autoinfection, it has been established that after irradiation the susceptibility to pathogenic microorganisms increases. Infectious diseases in exposed animals proceed uniquely, and much more severely than in nonirradiated ones: death rate and duration of the sickness increase, latent infections take a clinically pronounced course.

It is known, that disturbances in the resistance of the organism to the germs increase with increasing radiation dose. Some information has been obtained as to the causes for the increase in sensitivity of the irradiated bodies to various microbes. A substantial change in the reactivity of the organism following irradiation, manifested as a decrease in intensity of nonspecific natural immunity and a lowered level of acquired immunity, is one of the principal reasons. The << defenselessness >> toward germs which appears after irradiation has a practical significance for the sick body itself (because the infectious complications increase the severity of the course of the basic process) as well as for the surrounding bodies, because the irradiated organism may become a hazardous infection source continuously infecting the environment and persons who come in contact with it.

It is obvious, that investigation of peculiarities in the inter-relation between the irradiated organism and microbes, in order to detect means for struggling with the development of infection, is the goal of the microbiologists working in the field of radiobiology.

In the complex process of interaction between micro- and macroorganisms which are under the effect of definite environmental conditions, one must take into account changes in any of them as well as the complex phenomena of their interaction.

In order to study the biological peculiarities of germs, which change under a direct effect of radiation as well as under the effect of excretions and substances of the tissues of an irradiated organism, the use of various bacteriological, biochemical, morphological and serological methods is necessary. Investigations on immunobiological reactivity of the exposed organism have to be varied. Use of immunity reactions in vivo and in vitro, use of biochemical analysis and marked atoms and experimentation on animals infected with germ cultures enable one to obtain the necessary information on changes in reactivity appearing after irradiation.

In this interesting and extremely fascinating field of studies the accumulation of data does not restrict further investigations, but, on the contrary allows one to detect new problems which require solution.

Despite the large number of published experimental and review papers in the field in which the studies of changes in the properties of micro- and macroorganisms subjected to irradiation are carried out, there are still many problems of theoretical and direct practical significance which have to be investigated.

Thus, for instance, the dynamics of the development of autoinfection in relation to the severity and stage of radiation sickness requires detailed study; disturbances in various factors of natural immunity have not been investigated sufficiently; investigation of the course of compensation and restoration of immunobiological reactivity after irradiation is urgent, too.

The peculiarities in the course of allergic reactions in an exposed organism are almost entirely unknown. As is known, many painful reactions, such as serum sickness, anaphylactic shock, food and drug idiosyncrasy, and also allergic reactions to allergens of pathogenic bacteria used in diagnostics (Pirko test and others) can be attributed to these reactions.

All these kinds of scientific investigations can be separated into two groups, one of which is preparatory for the other.

Papers of a descriptive character, in which the effect of radiation on these or other factors is clarified, pertain to the first group. The majority of the published papers regarding the effect of radiation on inter-relations of micro- and macroorganisms are of such a character. The data of these investigations provide the basic characteristics of the state of the irradiated organism, but they fail to disclose the reasons for the apparent changes. On the basis of the data obtained, the papers of the second group appear; they contain experimental investigations of the reasons and mechanisms of the phenomena discovered. These are mainly research, experimental papers; their execution is more difficult. They require tireless searching and comparing of facts as interpreted by a definite working hypothesis, without which it is impossible to carry out these experiments.

The present monograph is a collective work, which reviews a series of data from the literature of the recent years (without reference to widely known published reviews and papers), it contains data of our own investigations in the region of the first descriptive group of works as well as the results of experiments dedicated to studies of the reasons for the changes in reactivity of the irradiated organism.

These investigations were carried out over a period of many years by a group of co-workers, which included, besides the authors of the book, G. A. Chekatilo, O. R. Nemirovich-Danchenko, G. M. L'vitsina, and A. F. Kosov, whose nonpublished data are included in the data presented by us. Acute radiation sickness induced by total-body external irradiation or by the effect of radioactive substances is the investigational object.

White mice, guinea pigs, rabbits and dogs served as experimental animals.

Problems pertaining to all basic sections which characterize the interrelations between the microbes and irradiated organism were investigated. The phenomena of autoinfection and infection caused by pathogenic microbodies, changes in the natural and induced immunity and allergic reactivity were studied. Special attention was paid not only to the theoretical significance, but also to the possible practical use of the data obtained.

Chapter 1

Change in Automicroflora and Microinvasion in the Irradiated Organism.

There are no data in the literature delineating the specific role (relative to other reasons) of infectious complications as cause of death in radiation injuries. However the clinical and experimental data show the important role of the germ factor during the course and on the end result of radiation sickness. Radiation dose proved to be essential. In the cases of massive radiation which result in death, the germ factor is not the cause of death (219, 274); in exposing humans (253, 324) and animals (265, 317, 325) to sublethal radiation doses the effect of infection emerges when the pathologic process persists for weeks or months. It aggravates radiation sickness and can lead to a lethal outcome. Actually the development of infectious complications is the consequence of weakening of the natural stability of the organism. The autoinfections appear as a result of definite changes in the environment, which brought the organism and its microflora out of the state of symbiosis.

The weakening of natural barriers--induced by various factors--which preserve the organism from its commensals--leads to spreading beyond the limits of the ordinary reservoirs; the microflora invades the tissues and organs. The later course of microbes as well as of the infected organism, depends on the efficiency of internal natural defense mechanisms, and on pathogenicity and number of penetrating viruses.

By changing the temperature conditions, I. E. Trop (160, 162) has detected intestinal bacteria in liver and spleen of overheated animals; he found microbes in lungs of cooled animals. Arnold (185) has disclosed the dependence of the microinvasion and change in the intestinal microflora on feeding and weather conditions.

The environment affects the macro- and microorganism, and consequently, also their symbiosis directly and indirectly (by means of their symbionts). Penetrating radiation, one of the physical environmental factors, affects the indicated symbiosis in an identical manner.

During irradiation, naturally, radiation affects the organism and microflora dwelling in it directly. Thereafter, reciprocal effects of the components of symbiosis, which undergo constant change, join the developing radiation sickness. Radiation with incorporated radioactive substances, where the radiation source is active continuously or throughout life, complicates the situation even more.

In studying the role of the germ factor in radiation pathology, on one hand one had to clarify the state of automicroflora of the irradiated organism, on the other hand it was important to find out which changes in the mechanism of the natural immunity induced by radiation stimulate the development of autoinfection.

The intestine is the most important reservoir of microflora in the animal organism. Bacteriological investigations of the feces of humans who were subjected to local X-irradiation (120), and those of animals after total-body irradiation, exhibited quantitative and qualitative changes in the content of the microflora of the intestines. Furth, Coulter and Howland (219) have observed an increased number of gram-negative forms, staphylococci and streptococci in the feces of irradiated dogs (450 r). R. V. Petrov (123) has noted an initial decrease in the total number of bacteria (among them the coliform bacterium) in the large intestine followed by an increase (especially in B. coli) in 30 irradiated (600 r) rats to the end of their lifetimes. The mean number of B. coli in per cent of the total number of bacteria in feces before irradiation amounted to 45, on the first postradiation day -25, 4th-49, 6th-47, 9th-54 and on the 11th-75. On the day the rats died, mainly B. coli was cultured from their feces. The number of hemolytic, proteolytic indole- and hydrogen-sulfide-producing stocks increased which indicates an increase in the pathogenic properties of bacteria inhabiting the intestines.

A significant increase in the number of bacteria in intestines of mice after a total-body irradiation (300 r) was observed also by B. G. Avetikian and A. G. Artemova (1). Besides, these authors have studied the effect of radiation on the formation of a focus of autoinfection under special experimental conditions: a ligature was placed on the caecum of the mouse, but the blood supply of the isolated section was maintained. Proof was obtained that the number of microbes in the content of this section of the intestine as well as in its wall, where the focus of autoinfection appeared, was manifold larger, than in nonirradiated animals operated in the same way.

The change in the intestinal microflora induced by irradiation of the body with radioactive substance (polonium, an α -emitter) was observed by O. R. Nemirovich-Danchenko (116). The experiment was carried out on 32 dogs, that received subcutaneously (in the hip) a single injection of 0.05 mC of polonium per 1 kg of weight. The animals died within 18-35 days. Part of the dogs received a complex of therapeutic measures inclusive of antibiotics, vitamins, blood transfusion, unithiol (a preparation which accelerates the excretion of polonium) and others. Two hundred sixty bacteriological investigations of feces were carried out. In contrast to the data reported above, which were obtained after an external total-body irradiation, O. R. Nemirovich-Danchenko has observed a decrease in the number of typical B. coli in fecal masses of animals poisoned with polonium (treated and untreated), which progresses with the development of radiation sickness; in treated dogs this phenomenon was less pronounced. A decrease in the number of coliform bacteria in the cultures of feces corresponded to a decrease in the number of gram-negative forms in the smears of feces. At the same time the number of stocks of coliform bacteria with altered biochemical properties and capable of giving hemolysis-increased. An increase in the number of anaerobic microbes (B. perfringens), whose number grew very large up to the time of death of the animal, was observed.

By comparing the content of intestinal microflora of individual animals that received identical doses of polonium, but whose clinical syndromes of radiation sickness were of a different degree of severity, O. R. Nemirovich-Danchenko notes a dependence of the content of microflora on the general condition of the animal—a decrease in the number of intestinal coliform bacteria was

greater in dogs with a more severe form of radiation sickness. The same author discloses also the fact, found in her experiments, that the microorganisms of intestines acquire an increased resistance to antibiotics (levomycetin, streptomycin) not only in irradiated dogs that received these preparations continuously, but also in exposed dogs that failed to receive antibiotics (116).

The change in sensitivity of the microflora of feces to antibiotics has been noted not only in using 0.05 mC/kg of polonium, but also in cases in which smaller doses of the agent (0.02 mC/kg) were used. Thus, for instance, of 72 stocks of intestinal germs of dog No 37, before the injection of polonium 92% were stable and slightly sensitive to penicillin, 32% to streptomycin, 6% to levomycetin, and 80% to biomycin. One hundred and five stocks of coliform germs were studied during the course of acute radiation sickness (the dog died on the 43rd day), and the number of stable and slightly sensitive forms increased: by 5% to penicillin, by 19% to streptomycin, by 41% to levomycetin and by 17% to biomycin. The 177 investigated representatives of the intestinal microflora were mostly varieties of the coliform bacteria (159 stocks), and, consequently, these data characterize the change in sensitivity to antibiotics of the basic dweller of intestines--the coliform bacterium.

In another variation of the experiments, namely, at a daily injection of 0.02 μ C/kg of Sr⁹⁰, O. G. Alekseyeva in a series of cases has observed in dogs an increase in the number of intestinal germs with a decreased sensitivity to antibiotics.

In order to show the effect of season on the content of microflora of the macroorganism and its sensitivity to antibiotics, the study of properties of intestinal germs at identical seasons of the year is presented as an example. Thus, before the treatment with Sr⁹⁰ in January 19% of 33% stocks of coliform bacteria, separated from the feces of the dogs Dick and Mike, were resistant (or only slightly sensitive) to streptomycin, 76% to biomycin and 0 to levomycetin. After 10-12 months from the beginning of the Sr exposure (also at a cold season) the sensitivity to antibiotics changed essentially to the side of an increase in the number of resistant and slightly sensitive germs: 42% of 65 stocks of coliform bacteria were resistant and only slightly sensitive to streptomycin, 99% to biomycin and 39% to levomycetin. The change in the response of coccal forms to the indicated antibiotics went in the same direction. The decreased sensitivity to antibiotics in germs isolated from an exposed organism can be explained by the fact that, due to the development of radiation sickness, essential changes appear in metabolism, in the quality and quantity of the secretion absorbed by the intestinal cavity, in the production of bactericidal substances and so on. In consequence new life conditions for the microorganisms are created. Dwelling in this new environment, evidently, induces these corresponding changes in their vital activity, in their metabolism. The presence of these changes, evidently, determines the different response of the autoflora of the organism, which continuously was exposed to radioactive substances, to antibiotic preparations. The correctness of this assumption is confirmed by the fact that a similar alteration in bacteria is described (104) for different states which also damage the vital activity of the organism, for instance, avitaminosis, application of painful stimulations, injections of heterologous protein and so on. L. G. Peretz (119) has observed an increased stability to drying out in microbes resistant to sulfonamids.

Thus, change in the environment may lead to the increased stability of microbes simultaneously to several unfavorable agents, among them to antibiotics. These facts confirm the correctness of the basic thesis of the Michurin biological science on a close connection between living organisms and their environment.

The difference in the dynamics of the change in the intestinal flora during external and internal irradiation at first sight may be easily explained by species variation in the responses of experimental animals to radiation. However, O. R. Nemirovich-Danchenko indicated that, at an identical dose of the radioactive substance, the changes in the microflora of feces are not identical in all dogs: they were more significant in animals, in which the radiation sickness was more pronounced. Consequently, to solve this problem definitely, further investigations must involve consideration doubly, of the clinical condition of the animal. Especially one has to take into account the presence or absence of enterocolitis, because it is well known that even with no radiation, the content of the microflora of fecal masses changes in diarrheas of different etiology.

As for the change in the bacterial flora of the upper respiratory tracts and skin after a massive total-body irradiation, we failed to find papers in the literature on these problems.

O. G. Alekseyeva is the only one who has these data. She has investigated the natural microflora of skin of 13 dogs exposed to X-irradiation at a dose of 600 r. All these dogs died of radiation sickness. The investigational technique was as follows: before irradiation, over the period of an acute radiation sickness, and during the terminal period, glass plates with meat-infusion agar, containing mannite and alcoholic solution of bromothymolblue, were applied to a shaved section of skin of the lateral surface of the dog's body. After one-day's incubation at 37°, the total number of colonies and the number of yellow colonies, which breakdown mannite, on the agar plates were counted. The preliminary experiments showed that on plates with ordinary agar (with no stain), applied to the skin of dogs, the microbes give uniform growth.

Thus, O. G. Alekseyeva virtually studied the dynamics of microbes dwelling on the skin and resistant to the stain in the concentration used. According to her data (Table 1), under the conditions of lethal radiation sickness, the total number of bacteria on the skin increases. At the same time the number of microbes breaking down mannite grows larger, which verifies the activation of their biochemical properties.

However, in addition to a clear tendency to an increased microflora of the skin after a total-body irradiation, in some cases an opposite effect took place (judging by the average data)--a decrease in the number of bacteria. O. G. Alekseyeva noticed that this was observed only in those dogs whose initial number (background) of germs was large.

The relatively small number of experimental animals does not permit evaluation of the significance of the initial values for microflora, for comparison with the further change due to radiation. At the same time, taking into account the peculiarities of the technique one may justifiably draw conclusions on qualitative deviations in the content of microbes, in particular on the change in their sensitivity to the stain in the concentration used.

That is, taking into account the peculiarities of the technique, the increase in the number of microbes on the skin of the exposed dogs can be explained by their greater stability to the effect of the bactericidal stain, acquired by microbes over the process of radiation sickness of the animal.

The appearance of new properties--the decreased sensitivity to the effect of antibiotics (as noted above) and to bactericidal stain, obviously, may be explained by the adjustment of the microorganisms to unusual conditions appearing in the exposed organism.

Table 1

Dynamics of change in the microflora of skin of 13 irradiated dogs (600 r)

Characteristics of microflora	Before irradiation (the initial background of the microflora)	Over the period of acute radiation sickness (on the 3rd through the 15th day).	Ante-mortem period (the 10th-20th day) ¹⁾
Mean number of germs on 12 cm ² of skin	97	153	156
Comparative evaluation of the data in % of the initial	100	157	160
% of microbes, breaking down mannite	23.2	32.8	80.5
Comparative evaluation of the data in % of the initial	100	141.3	346.9

Note: Death of the dogs began during the course of an acute radiation sickness from the 10th day and ceased by the 20th day.

In analyzing the reasons for the quantitative and qualitative change in the automicroflora of the exposed animal, the fact of the presence of different radiosensitivities of higher organisms and microbes must be considered. If doses in the range of decimal fractions of roentgens (in the case of chronic irradiation), or hundreds of roentgens (in a single irradiation), cause pathologic effect in higher organisms, then identical radiation doses do not change microbes noticeably. A radiation flux of several ten thousand roentgen is required to damage the structure and metabolism of the bacterial cell (105), (O. G. Alekseyeva and M. P. Domshlak, (7)).

It follows from this that the quantitative and qualitative shifts in the content of automicroflora of animals exposed to lethal and sublethal radiation doses, are not consequences of the direct effect of radiation on microbes. These shifts occur as a result of a change in the microbes' environment, i.e. because of a change in the macroorganism due to the irradiation.

The data from experimental infection of animals after irradiation, when the direct effect of radiation on microbes is excluded, also confirm that the main factor affecting the composition and properties of automicroflora is the altered environment of the animal. Under these conditions the microorganisms acquire other biochemical properties and changes in virulence and reproductive power.

There is only one paper--Liu and co-workers (256)--in the foreign literature, which indicates, that the inoculation of typhus rickettsia in irradiated mice (450 r) increased the virulence of the rickettsia in young mice.

G. A. Chekatilo (172, 173) has vast experimental data concerning the variability of microbes in an irradiated host. The data show a possible increase in the virulence of microbes under these conditions.

G. A. Chekatilo has studied the dynamics of the virulent and biochemical properties of microbes isolated from induced focal infections in the skin of irradiated guinea pigs and rabbits. X-irradiation was carried out before infection, which excluded, then, a direct effect of radiation on microbes. The virulence of microbe stocks was checked using the method of intraperitoneal infection of white mice.

The white, saprophytic staphylococcus, examined in the experiments of G. A. Chekatilo after dwelling in the skin of exposed guinea pigs (484 stocks separated from irradiated animals, and 248 from controls, were studied) in a series of cases acquired pathogenic properties--broke down mannite, induced hemolysis and necrosis, produced hyaluronidase.

The stocks of typhoid bacillus isolated from induced inflammatory foci of irradiated animals led to a higher death rate of healthy mice in comparison with the cultures of typhoid microbes separated from the foci of nonirradiated control animals, i.e. an increase in the virulent properties was observed (Table 2).

Table 2

Change in the virulence of typhoid microbes dwelling in cutaneous inflammatory foci of guinea pigs dependent on the radiation dose of the latter (according to the data of G. A. Chekatilo).

Typhoid cultures, isolated from the foci of guinea pigs	Number of stocks	Number of mice infected with 15 mil. microbes	Died		Number of mice infected with 30 mil. microbes	Died	
			absolute number	%		absolute number	%
Non-irradiated	64	150	39	26.0	150	75	50.0
Irradiated with							
100 r	43	116	48	41.3	113	73	65.4
200 r	28	68	28	41.1	67	46	68.6
300 r	22	49	19	38.7	49	38	77.5

As can be seen from Table 2, the difference between the death rates of mice infected intraperitoneously with cultures of typhoid bacilli at a dose of 15 mil. microbes, from cutaneous inflammatory foci of irradiated guinea pigs (100-300 r), and animals not subjected to irradiation, amounted to 12-15%. A greater difference, which increases with increase in radiation dose, has been obtained in experiments in which a dose of 30 mil. germs was used for infection. While the death rate in the group of mice infected with cultures from the foci of control animals (nonirradiated guinea pigs) was 50%, in the other group of mice that received cultures from irradiated guinea pigs, the death rate was higher. The higher the radiation dose of the guinea pigs, the more mice died.

The virulence of typhoid bacilli increased especially in the cases in which the germs remained in the body for a prolonged time. However, if such cultures were in the inflammatory foci of nonirradiated animals over the course of five days, then they caused death to 28% of the control animals, but after 10 days--46.9%, while the germs separated within five days after the infection

of irradiated animals induced death in 38-62% of mice; isolated at more prolonged times (10 days after infection) they caused death to 60-70% of the mice (Table 3). Thus, the swelling of germs in an exposed organism, with no radiation effect on the microflora, leads to an increased virulence.

Table 3

Dependence of the virulence of typhoid bacilli on the duration of their dwelling in cutaneous inflammatory foci of irradiated and nonirradiated guinea pigs. (according to the data of G. A. Chpaktilo).

Typhoid cultures isolated from the foci of guinea pigs	Death rate of mice infected ¹⁾ with cultures preserved in the focus.							
	within 5 days (45 stocks)		6-10 days (86 stocks)		more than 10 days (31 stocks)			
	number of infected mice	died abs. no. %	number of infected mice	died abs. no. %	number of infect. mice	died abs. no. %		
Nonirradiated	82	23 28.0	152	60 39.4	66	31 46.9		
Irradiated with a dose: 100 r	16	10 62.5	134	58 43.2	79	53 67.0		
200 r	14	17 88.6	68	40 58.8	23	17 73.9		
300 r	--	--	78	45 57.6	20	12 60.0		

- 1.) The mice were infected intraperitoneally with a suspension of 1 day old agar culture at a dose of $15-30 \times 10^6$ germs in a volume of 0.2 ml.

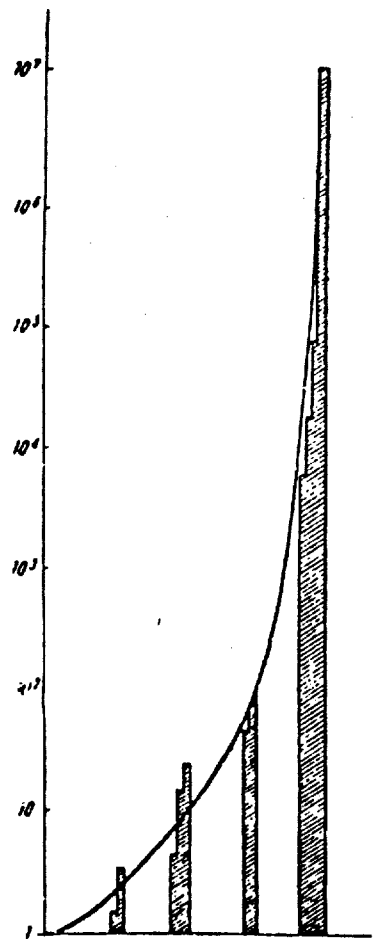
Ability to multiply in the tissues of a certain species of animals is one of the most characteristic features of the pathogenic microorganisms.

As the investigations of V. F. Sosova (150, 152) indicated, nonpathogenic coliform bacteria, injected subcutaneously in healthy rabbits and in rabbits at the beginning of radiation sickness, are not able to develop. However, during the period of acute radiation sickness the same coliform bacterium has the ability to multiply intensely, i.e. it behaves like a pathogen.

From Fig. 1 it can be seen clearly, that the infection of rabbits with a culture of coliform bacteria at a dose of 200 mil. bacteria (according to the optical standard) within 3-5 days following a lethal X-irradiation (1100 r) leads to the accumulation of several thousand times more germs in the cutaneous inflammatory foci than in nonirradiated animals or animals irradiated but infected within the first day following exposure. The number of microbes (calculated per 1 gr. of tissue) was determined by means of seeding of dilute batches of the focal tissues within 48 hours after infection.

The data on other variations of the experiments of V. F. Sosova (152) in which a 10-fold smaller dose of the intestinal coliform bacteria (20 mil.) was used for the subcutaneous infection verify the possibility of intense multiplication of nonpathogenic microbes in the tissues of an irradiated host. After 48 hours following infection, the number of microbes in 1 gr of the tissue of the inflammatory focus of each of the irradiated rabbits was 10-100 times larger than the number injected, which is approximately equal to the number of coliform bacteria at identical inflammation times, but after infection with 200 mil. microbes. At the same time the mass of microbes in the inflammatory foci of a nonirradiated animal decreases significantly (Table 4).

The paper of Smith and Wood (305) indicates, that after intramuscular infection of irradiated mice with pneumococci, the number of living microbes in the injection sites exceeded the number of microbes in the control animals by several thousand times (Fig. 2).



2 hrs. 1 day 2 days 3-5 days
Infection time following exposure.

Fig. 1. Increase in the number of intestinal coliform bacteria in cutaneous inflammatory foci of irradiated rabbits (1100 r) dependent on the time of the intracutaneous infection following a total-body X-irradiation.

The histograms show data on the number of microbes in irradiated rabbits. The figures at the left side show how many times the number of microbes in the cutaneous focus of an exposed rabbit is larger than the mean number of germs in the foci of nine control animals.

Table 4

Data verifying the multiplication of nonpathogenic coliform bacteria in the tissues of exposed rabbits after intracutaneous infection within 3 days following irradiation (1100 r).

Rabbit's number	Characteristics	Doses of microbes in 0.2 ml. used for infection	Number of living microbes in 1 g of tissue of the inflammatory focus within 48 hours following infection.
1989	Irradiated	2×10^7 2×10^8	2.0×10^9 4.4×10^9
1990	"	2×10^7 2×10^8	2.2×10^8 1.8×10^9
1953	Nonirradiated	2×10^7 2×10^8	1.0×10^4 8.0×10^4

An increased content of microbes in inflamed tissues of irradiated animals was observed also after infection with staphylococci (139), petrusal coliform bacteria (153), and influenza virus (145). As was mentioned previously, B. G. Avetikian and A. G. Artemova (1) have found more microbes in the inflamed appendix of irradiated mice, than in a similar focus of autoinfection of animals not subjected to irradiation.

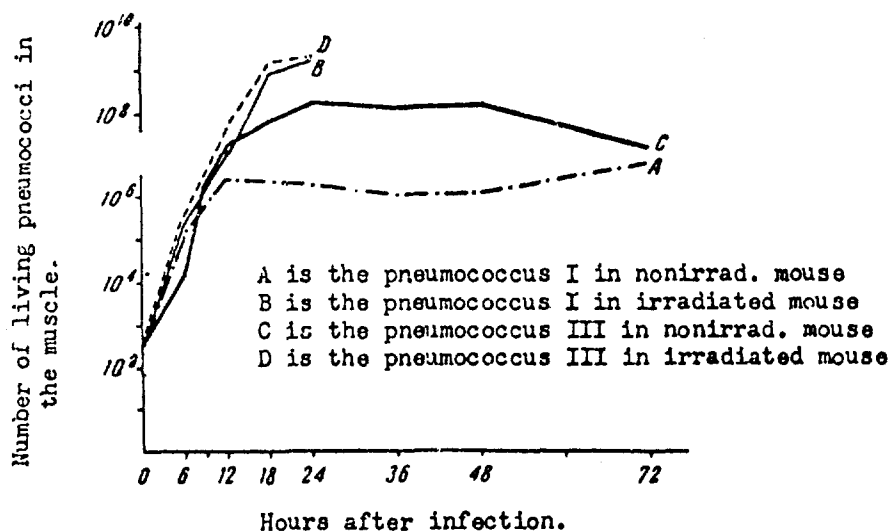


Fig. 2. Change in the number of pneumococci of types I and III in infected leg muscles of nonirradiated and irradiated mice (according to the data of Smith and Wood (305)).

Thus, the data presented indicate, that the properties of bacteria in an irradiated organism change. New biochemical characteristics appear in microbes, and the stability to some agents (antibiotics, bactericidal stain) increases, as does the virulence and reproductive power.

These new properties in microbes are induced by the effect of the macroorganism which has been changed by the action of the penetrating radiation, and in particular, by damage to the mechanisms of natural immunity.

Basically the damage to natural defenses leads to an increased permeability of the mucosa of the intestines and respiratory tracts to microbes. The number of cells, which carry out phagocytosis decreases, the barrier function of lymph nodes, becomes disturbed bactericidal efficiency of blood serum and bactericidal function of the body covers decreases, trophic and metabolic processes in tissues change, and ability to produce antibodies is disturbed. Some of these changes can create favorable conditions for invasion of the autoflora by germs, others may stimulate a further multiplication and spreading of the microbes in the organism.

Consequently, in studying the reasons for the development of autoinfection, in radiation sickness one has to take into account the facts of changes in the macroorganism as well as in the microbes--commensals--under these conditions.

The analysis of data on the consequences of atomic bombs dropped by the Americans on the cities of Japan in 1945, and data of numerous tests with experimental irradiation of animals showed that angina, pneumonia, enteritis, ulcerous colitis, sepsis, and other infection complications develop.

The study of bacterial cultures from the blood and organs of irradiated humans and animals (219, 227, 274, 265 and others) showed that the infections appearing in radiation sickness are consequences of autoinfection by microbes--commensals--which are the ordinary dwellers of the upper respiratory tracts, cutaneous covers, and for the main part, of the intestines.

Of microbes dwelling in the intestines of animals different species of intestinal coliform bacteria, the representatives of the Salmonella group, blue pus bacteria, different Proteus strains, yeast, enterococci, micrococci, more rarely anaerobes, were found in tissues; hemolytic streptococcus, green streptococcus, white and golden staphylococcus, diphtheroids were also detected among the representatives of the flora of the mucous and cutaneous membranes.

This summary of the species of microbes found in the tissues of irradiated animals allows one to set up a connection between the infectious complications in radiation sickness and the automicroflora of an animal, and the paper of Bradner, Bernstein and McCarthy (193) removes all doubts in this problem.

Three kinds of microorganisms: Proteus mirabilis, Paracolon bacrum, Pseudomonas aeruginosa, separated by these authors from the blood of irradiated mice and studied by the serological method, proved to be identical by their antigenic structure to the kinds of bacteria which are found in the intestines of the same animals before irradiation.

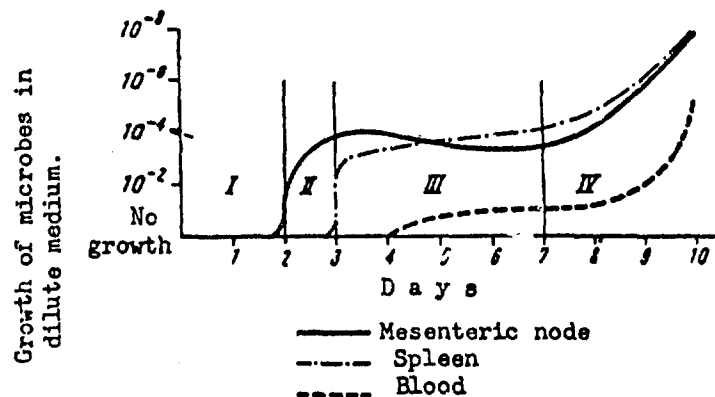


Fig. 3. Dynamics of change in the number of microbes in the tissues of rats at different times following exposure to 600 r.

The role of the intestines, with their microflora, as a source of autoinfection was confirmed also by the experiments of R. V. Petrov (125), in which the rats were killed at daily intervals after irradiation (600 r) in groups of five animals to obtain simultaneously cultures from the mesenterial nodes, the spleen and the blood. The observation showed that

microbes which penetrate the intestines gradually spread throughout the organism. Thus within two days following irradiation, the microbes could be separated only from mesenteric nodes; somewhat later, after three days, from the spleen; and only 4-5 days following exposure, the microbes were detected in blood.

The stage of seeding by microbes of the tissues investigated increased until the 10th day, the death time of the animals (Fig. 3).

On the basis of these data R. V. Petrov has suggested four periods in the development of autoinfection in an irradiated organism:

- 1) period of sterility--lasts through one day after irradiation; there are no microbes in the organs;
- 2) period of seeding of the regional lymph nodes--lasts 2 to 3 days after irradiation; microbes can be found only in lymph nodes;
- 3) the period of a relative compensation of reticulo-endothelial system; the fixing capacity of the organs of reticulo-endothelial system is still well preserved, and in consequence bacteria from blood can be separated rarely and only in small numbers; it covers time from the 3rd to the 7th day;
- 4) the period of decompensation of defense mechanisms is characterized by a sharp increase in the number of microbes in organs and blood; this precedes death of the animals.

It should be noted, that these observations were carried out on one species of animals only (rats) using a dose of 600 r. At other radiation doses and in other species of animals the periods indicated might have other starting times and different durations.

The possibility of the infection of an irradiated organism by bacteria dwelling on mucosae of respiratory tracts is shown in the paper of A. E. Ivanov and V. F. Sosova (55). According to their observations, intratracheal infection of exposed rabbits (800-1100 r) led to the penetration by pathogenic (pneumococcus of type I) as well as by nonpathogenic (pneumococcus of type III, coliform bacterium) cultures of microbes in the blood circulation. At the same time in the blood of nonirradiated animals, one could detect only pneumococcus of the type III pathogenic for this species of animals.

To determine the degree of susceptibility of exposed organisms to infection, numerous investigators have undertaken studies on experimental infection of different species of animals by using all known methods of infection. Nonpathogenic and pathogenic microorganisms have been tested in the experiments: the intestinal, typhoid, dysenteric, tubercular, diphtherial, pertussal bacteria, Bacillus anthracis, microbes of the Salmonella group, blue-green rods, the rod of proteus, B. perfringens, avirulent plague rod, leptospirosis, viruses of influenza, smallpox, trypanosoma and a series of other microbes, viruses and protozoans (69, 139, 140, 145, 152, 153, 163, 200, 203, 205, 212, 224, 229, 230, 236, 237, 240, 247, 255, 260, 261, 262, 269, 271, 293, 294, 295, 297, 299, 303, 304.)

The results of these investigations lead to the conclusion, that in animals subjected to median lethal radiation doses, resistance to infections decreases, with the result that an accelerated seeding of the organs and blood with microbes, a shortening of the life span and a lower survival rate are observed.

The data of these papers allow one to draw three conclusions, of practical importance.

1. The irradiated organism becomes more susceptible to infections induced not only by pathogenic microbes, but also by microbes of the commensal type.

2. Decreased resistance to infectious agents increases with the development of radiation sickness and it is especially pronounced during the period of its acute course.

3. Infection complications can be a direct cause of death in radiation sickness.

The investigations of prof. I. A. Pigalev and B. B. Moroz (129), which showed that resistance to infection decreases not only due to external radiation, but also due to the penetration of radioactive substances in the organism, are of a special interest. When mice subjected to polonium or radiothorium were infected with pneumococci, sepsis developed rapidly and the animals died in larger numbers than irradiated only or infected only with this microorganism.

The determination of the important role of the germ factor in radiation sickness, naturally, leads one to hope that antimicrobial methods will assist in preventing and treating infection complications and thus increase the efficiency of the therapy of radiation sickness.

The use of different antibiotics separately and in combination--penicillin, streptomycin, terramycin, aureomycin (148, 157, 218, 219, 220, 221, 227, 228, 233, 235, 247, 261, 264, 266, 302, 324) and others decrease the seeding of microbes from blood, prolong the life span of the irradiated animals and increase their survival rate. The best results were obtained by M. A. Tumanian and Z. V. Shevtsova (164), who used a mixture of penicillin streptomycin and levomycetin in their experiments with irradiated apes.

The favorable effect of antibiotics confirmed the concept of the importance of the role of the microbe factor in radiation injuries. At the same time it was shown clearly, that the microbial factor is not the only one (as was assumed by several authors at one time), which results in death to the exposed organism.

The possibilities of decreasing the number of unfavorable end results in radiation sickness due to infectious complications are not exhausted as yet. Further study of the immunobiological reactivity of the organisms injured by radiation and detection of efficient schemes for the use of antibiotics will widen these possibilities.

Chapter 2

State of Natural Immunity in an Irradiated Organism.

The development of autoinfection in an irradiated organism described in the first chapter is connected not only with the acquiring of new biological properties of the microbes of the autoflora, but chiefly with disturbances in the immunological reactivity of the irradiated organism; the present chapter of this book will be dedicated to a detailed account of these disturbances.

I. I. Mechnikov¹⁾ emphasized the fact that nonsusceptibility to infections is a complex phenomenon, which depends on many factors.

It is well-known, that the intensity of the natural immunity depends on the physiological state of all systems of the organism. The integration of these systems, their dependence on nervous and endocrine regulation, and the effect of the environmental factors contribute to the complexity of the mechanism of the resistance of the organism to the microbes. On the basis of examination of the literature data and experiments, many authors in their papers have proven the dependence of the state of nonsusceptibility on the level of total reactivity of the organism, functional state of the nervous system (3, 49, 10 and others), endocrine regulation (15, 103, 28, 29 and others), food rich in protein and vitamins (61, 114, 12, 226, 306), and seasonal and climatic factors (60, 161, 162 and others).

This summary could be continued by indicating the papers that show correlation between the phenomena of immunity and the functions of different systems and organs and the effects of various drugs, other factors of the environment and so on, on immunity. But the present chapter aims at solution of the problem not of the nature of immunity as a biological factor, but of its alteration by such an environmental factor as ionizing radiation. We are interested only in the effect of substantial radiation doses which cause radiation sickness.

At present many reviews on the problems of the pathogenesis of radiation sickness are published in the native and foreign literature; from these it can be seen that during this sickness the physiological state of all systems of the organism changes. Consequently, one may assume a priori, that the state of natural immunity will also be altered to a large extent. However, the published experimental papers on studies of the immune state following irradiation have a general shortcoming: absence of a correlated investigation if not at all, then at least of the more important mechanisms of immunity together with a clinico-physiological investigation of the pathogenesis of radiation sickness. The

1) I. I. Mechnikov, Immunity Problems, Ed. by Ac. of Sc., USSR, 1951.

investigators usually studied some one mechanism of resistance dependent on the dose and more rarely on the phase of the radiation response. And even if investigation of several mechanisms was carried out, then each study was done on different groups of animals. Therefore in the present review we are compelled to describe the change in the various mechanisms of nonsusceptibility individually, and in our conclusions we shall attempt to give the scheme of the total complex of changes dependent on the phase of radiation sickness. For the same reason, we are not able to confirm our concept of the causes of the disturbances that occur in the mechanism of nonsusceptibility, by data based on facts. We do not know of any papers devoted to experimental clarification of the effect of nervous and endocrine regulation, as altered by radiation sickness, on the processes of immunity, disturbances in metabolism, enzyme systems and oxygen saturation of tissues. It is possible to state one's opinions on these disturbances as original causes for change in the immunity only on the basis of a comparative analysis.

Thus, let us proceed to the description of the state of various immunity factors in irradiated animals.

The first barriers which limit the distribution of causes of infectious sicknesses and microbes of the autoflora in the organism are the mucosae and skin. In a healthy organism they are nonpenetrable for commensals and for a majority of the pathogenic microbes. But various pathologic processes can change their permeability. Radiation increases the permeability of such a vitally important barrier, as the mucosa of the gastrointestinal tract to microbes and toxins which results in the seeding of the organism with conditionally pathogenic bacteria (192, 67, 158). In irradiated animals even the permeability of vascular walls (111, 112, 90 and others) and ophthalmic and cutaneous barriers (68) has been found to be increased.

But the barrier function of the tegmina of the organism is limited not only by the permeability function (or, more correctly, nonpermeability for bacteria). It is known, that excretions and secretions of skin and mucosa may exert a bactericidal effect.

In the literature accessible to us we failed to find experiments on bactericidal properties of covers of irradiated animals, except for the investigations of the authors of this book.

N. N. Klemparskaya has worked out a method for the determination of the bactericidal properties of skin. It is as follows: a dilute portion of the broth culture of coliform bacterium is transferred to skin (shaved or free of hair cover). Then imprints of skin are taken on glass plates with Endo medium after different time periods, as for instance, 10-15-30 and 45 minutes. Colony counts after incubation of the plates at 37° for 24 hours enables one to evaluate the decrease in the number of living bacteria during the observation period. By means of this technique N. N. Klemparskaya first studied the bactericidal activity of the skin of exposed animals (80). The effect of radiation at a dose of 400, 800 and 1100 r on the bactericidal activity of the skin of the belly and of ears of rabbits was investigated (126 tests on 17 animals were carried out). Thus, N. N. Klemparskaya has established, that after irradiation a decrease in the first stage of bactericidal activity (within 15 min. after smearing) is observed, which is first shown by the skin of belly (on the 1st or 2nd day), and later by the skin of the ear (within 3-5 days).

The appearance of a «local leucopenia» (i.e. decrease in the number of leucocytes in blood taken from the cutaneous vessels of the belly) in the presence of a normal or increased number of leucocytes in blood taken from

the ear, could be observed parallel to the change in the bactericidal properties. Generally, in the skin of the belly, the depression of bactericidal properties (Fig. 4) as well as the regional leukopenia were more prominent, which, obviously could be attributed to the reflex effect of the injured abdominal organs, especially the intestines.

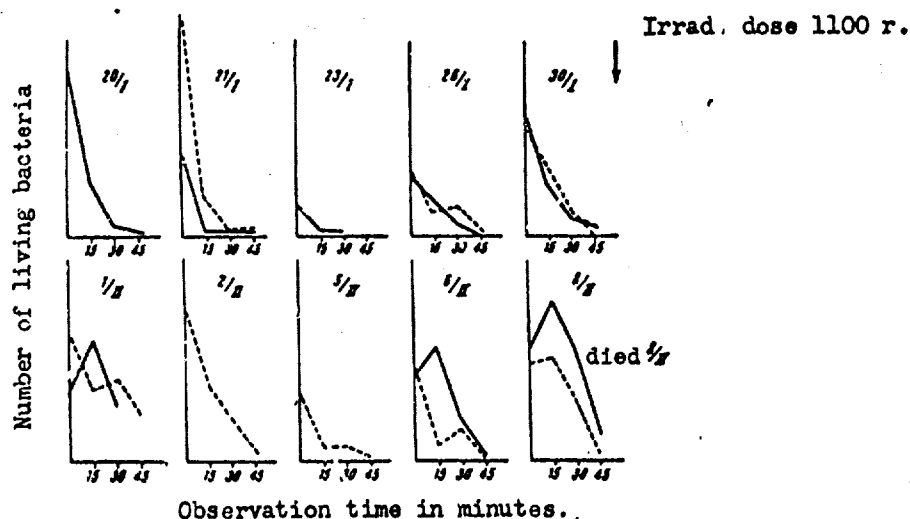


Fig. 4. Graphic representation of the results of investigations on bactericidal properties of the skin of a rabbit exposed to a dose of 1100 r.

The solid line designates the number of living bacteria on the skin of the belly, the broken line: the number of living bacteria on the skin of the ear.

O. G. Alekseyeva with A. A. Kanarevskaya investigated the bactericidal properties of skin of the lateral body surface in five dogs exposed to 600 r. The authors noted, that within 1-1½ hours following irradiation, an increase in the bactericidal properties of the skin in four dogs was observed (within 15 minutes, 10-15% more bacteria died than before irradiation). At the climax of the clinical syndrome of radiation sickness a pronounced depression of bactericidal activity appeared. On the last days before death a slight increase in bactericidal properties of the skin of the irradiated dogs was observed, which failed to attain the initial (preirradiation) level in three animals.

R. V. Petrov in his studies of the bactericidal properties of the skin of the lateral surface of the body in 19 dogs exposed to definitely lethal doses of 1630-3000-3830-5970 r, noted, that the higher the bactericidal activity of the skin of dogs before irradiation, the longer they lived after the exposure to lethal doses. The increase in the life span of these dogs was equal to 1-3 days. These tests proved once more, that the bactericidal properties of skin reflect the effect of the general reactivity of the organism.

A co-worker in our laboratory--O. R. Nemirovich-Danchenko--in her studies of the bactericidal properties of the skin of dogs treated with a dose of polonium of 0.1 mC per 1 kg weight, observed a depression of these properties during the period of development of radiation sickness. This depression was more pronounced in untreated animals.

Thus, our experiments allow one to conclude, that during the period of development of the clinical syndrome of radiation sickness a decrease in the bactericidal activity of the skin and especially of the skin of the belly appears. During the primary reaction to radiation, when stimulation of the functional activity of the nervous system takes place, an increase in metabolism and stimulation of the hematopoietic organs are observed; one can detect also the activation of bactericidal properties of skin. Sometimes, on the days immediately before death activation of the bactericidal properties of the skin of the animal also takes place, although more frequently there is a sharp depression of the bactericidal activity. The climax of radiation sickness is characterized by a more or less pronounced depression of the bactericidal properties of skin towards non-adapted microbes (coliform bacterium). The results of the studies of the microflora of the skin of dogs exposed to 600 r (see experiments of O. G. Alekseyeva in I chapter) indicate a depression of the bactericidal properties of skin also towards its autoflora.

In the discussions of the bactericidal properties of tegmina it is proper also to mention the state of the bacteriolytic activity of saliva. Saliva continuously washes the mucosa of the oral cavity, mouth and tonsils and thus stimulates the purification of the latter from microbes. Besides the mechanical purification which is brought about not only by the flow of the liquid, but also by the absorption of bacteria by the epithelial cells and their phagocytosis by leucocytes,--saliva also possesses bacteriolytic properties. These properties are characteristic of saliva because of the presence of the enzyme lysozyme.

In the literature accessible to us we did not find experiments on the investigation of the lysozyme content in the saliva of animals with acute radiation sickness. But a mention is made in the literature of the decrease in the lysozyme content in the tissues of irradiated animals (189). This leads us to assume, that its amount in saliva has altered.

In the determination of the amount of lysozyme in saliva, only the data of O. G. Alekseyeva are at our disposal. The saliva was taken from a fistula of the parotid gland of four dogs with chronic radiation sickness. Over a period of 6-7 months the dogs received daily (except for Sundays) with their food 1 μ C per 1 kg of weight the radioactive isotope Sr⁹⁰. The experiments are not yet completed, but some preliminary results can be presented. An increase in the amount of lysozyme (by 1-4 dilutions) within 3-4 months after the beginning of treatment was observed in two dogs, which according to E. N. Klimova, had the strong type of higher nervous activity. However, within a month following the end of the treatment a tendency to a decrease in lysozyme amount was exhibited. The results in two dogs with a weak type of higher nervous activity were not identical: parallel to the depression of the reflex activity a significant decrease in the titres of lysozyme during the entire observation period took place in one dog. The changes in the second dog occurred corresponding to the type of changes in dogs with a strong type of higher nervous activity, except that the activation phase appeared later (within six months following the beginning of treatment).

Therefore, even during chronic radiation sickness, there is a perverted production of lysozyme by salivary glands; considering this, there is a foundation for assuming a disturbance of this process during acute radiation sickness.

Thus, after irradiation the first barrier--the body skin--becomes permeable not only for pathogenic, but even for conditionally pathogenic microbes. The microbes penetrate into the lymph spaces and can be taken to

the next barrier by the flow of lymph--to the lymph nodes. But in radiation sickness, the barrier function of lymph nodes is greatly disturbed. The depression of the barrier functions was proven convincingly by P. N. Kiselev and co-workers (67, 71) and the experiment of B. G. Avetikian and A. G. Artemova (1).

After overcoming the barrier mechanisms, the microbes get into the blood stream. Here various bacteriostatic and bactericidal substances affect them and the microbes are subjected to phagocytosis.

A normal serum has the property of destroying some microbes. A series of authors have indicated the decrease in this capability in irradiated animals. Some of them investigated the complement titres, others followed the bactericidal effect of serum on a suspension of microbes in in vitro tests. The depression of the capability of the blood serum to destroy microbes took place not only at lethal radiation doses (76, 280), but also at sublethal ones (188, 259).

The data in the literature on the time of appearance of this depression are contradictory: some of them show a decrease in the lytic properties already by 24 hours, others only after 4-5 days following irradiation. But all authors agree on the restoration of bactericidal properties of blood and high titres of complement during the reparation period, if the experimental animal recovers.

In 1954, a report was published, showing that Pillemer and co-workers (276, 277) have separated a new serum protein--properdin--, which possesses a pronounced bactericidal property, and was successfully used by the authors in the therapy of radiation sickness. Its therapeutic use was based on the data on the decrease in its quantity in irradiated animals: 25-35 units were found in normal serum of rats, 4-6 units from the second day following exposure to 500 r, and less than a unit during the period from the 7th to the 17th day.

In addition, blood contains specific antibodies formed as a result of active immunization. There is a vast literature on the effect of radiation on the production of specific antibodies and it has been summarized many times in reviews (313, 314, 158), to which we refer our readers. But here we shall only mention, that the production of specific antibodies is significantly inhibited during the period of clinically observed acute radiation sickness.

The question arises: what is the mechanism of depression of the production of normal and specific antibodies in radiation sickness? This question is complex, and undoubtedly there is no clear answer to it as yet. Some authors point out the significance of the damage to the organs rich in lymphoid elements (spleen, appendix), others ascribe significance to the granulocytopenia, the third group blame primarily the disturbances in the immunochemical properties of serum proteins (245, 313, 128, 314).

As is known, the destruction of microbes in blood may be due not only to the presence of normal and specific antibodies in serum, but also to the phagocytic activity of its cells.

Studies of the phagocytic properties of blood leucocytes in in vitro tests and more rarely in in vivo tests with leucocytes in peritoneal exudate were carried out by many investigators and were started long ago. The majority of investigators recorded a depression of the phagocytic activity of leucocytes. Several authors indicated, that this depression is displayed only on the 2nd through the 7th day following irradiation. The following papers discuss the

depression of the phagocytic activity of leucocytes: Schwienhorst (292), Schonig (289), Rosselet and Sarian (284), Wilkinson (326), V. V. Demidas (41), P. H. Kiselev, V. N. Sivertseva and P. A. Buzin (76), Fishman and Shechmeister (214) and others. In 1956, a paper was published by Donaldson, Marcus, Ko Gui and Percins (210) who were the first to pay great attention to the second phase of phagocytosis--intracellular digestion. They proved, that on the 6th to the 11th day or from the 6th to the 26th day (depending on the radiation dose) of radiation sickness, the intracellular digestion in mice, which received an intraperitoneal injection of fowl erythrocytes following exposure to 350-450 r, ceased almost completely. The disturbance of the intracellular digestion was attributed by the authors to a change in phagocytes, not to the change in antibodies (opsonins). Our investigations also show, that the phagocytic activity of leucocytes fails to decrease immediately after irradiation.

In their studies of the phagocytic activity of blood neutrophils toward a living culture of *Staph. aureus* in *in vitro* tests (O. G. Alekseyeva and A. A. Kanarevskaya) showed, that within the first day after irradiation of dogs with a dose of 600 r an activation phase may appear. The authors have found the activation phase in four out of five dogs. But during advanced radiation sickness (from the 3rd through the 8th-10th day) all dogs displayed a phase of depression. In two dogs it was weak and the phagocytic index remained rather high. However, we believe, that the phagocytic index in irradiated animals cannot serve as an indicator for the intensity of phagocytic reaction. Of course, during the climax of radiation sickness, due to the damage to hematopoietic organs, a significant decrease in the microphage reserve is noted. Therefore, the intensity of phagocytic reaction should be determined from the absolute number of phagocytosing leucocytes and phagocytosed cocci in 1 mm³ of blood. The increase in the phagocytic index during the climax of radiation sickness, which has been observed by many investigators, should be considered a compensation reaction of the organism. But it cannot be always absolute. Returning to the investigations of O. G. Alekseyeva and A. A. Kanarevskaya, one should point out, that the increase in the phagocytic index in two dogs, in which this index during the climax of the sickness was rather high, could not compensate for the decrease in the phagocyte reserve and that the absolute values were very low: in one dog (Malyshka) it decreased 2-3 times, in another (Starichok) even 5-60 times in comparison with the initial level.

The depression of the phagocytic activity of blood leucocytes on the 3rd-15th day following exposure to 600 r (i.e. over the climax period of radiation sickness) and a sharper decrease in the absolute values were confirmed by O. G. Alekseyeva, and by subsequent experiments on 11 dogs. During the terminal period, phagocytosis practically dropped to zero in both the first experiments as well as in the second.

It is known, that the phagocytic activity of leucocytes increases substantially after a specific immunization. But it never attains such a level in irradiated, vaccinated animals during the climax of radiation sickness, as compared with nonirradiated, immunized animals. We can refer to the investigations of O. G. Alekseyeva concerning phagocytosis *in vivo* of actively immunized animals with regard to diphtheria (5) and typhoid bacilli (6).

Our investigations (O. G. Alekseyeva, (5)) showed that if the development of radiation sickness is induced by exposure to a definitely lethal dose, the depression of phagocytic reaction occurs even in animals with a species immunity. The experiments were performed on 77 white mice and 48 rats, which, as is known, possess a species nonsusceptibility to diphtheria and respond to the intraperitoneal injection of diphtheria

bacteria with an intense phagocytic reaction. The mice were exposed to an x-ray dose of LD75/14,¹⁾ but the rats were exposed to a dose of 3000 r of x-rays from a cobalt source with a strength of 93.5C. The studies of phagocytosis after an intraperitoneal infection in mice were carried out on the 5th day following irradiation, but in rats--on the 2nd day.

The irradiated animals responded with a significantly less pronounced local inflammatory cellular reaction, decreased phagocytic activity (2-3 times lower in mice and 18-19 times lower in rats in comparison with nonirradiated mice), and with delayed intracellular digestion by 9-18 hours, which led to an intense multiplication of bacteria in the abdominal cavity.

How could the decrease of phagocytic reaction of leucocytes after irradiation be explained? It is known, that the state of phagocytosis depends on many factors: on the functional state of the nervous system (134) and endocrine regulation, a temperature factor, osmotic pressure, concentration of hydrogen ions (quoted from Menkin (263)), leucotoxin--a substance which stimulates the diapedesis of leucocytes--, on the presence of catabolins of nucleoproteids, histamine, hyaluronidase (257), on the presence of special antibodies preparing the microbes for phagocytosis (opsonins, tropins, and the active system of Pollack and Victor (279)), and a series of other factors.

Not all by far of the listed factors affecting phagocytosis in irradiated animals have been investigated. There are only a few papers in the literature dedicated to studies of special factors affecting phagocytosis. Shechmeister and Fishman (298) in tests on rats and rabbits showed, that by exposing them to a dose of 500 r and higher the migration of leucocytes decreases markedly by the 2nd day. Thus, not only is the production of phagocytes depressed, but also their mobility decreases. Savitskii (286) detected substances which damaged leucocytes, and which during the first 15 hours following irradiation increased the adhesive index approximately 5-6 times. In another paper, Fishman and Shechmeister (214) reported, that extracts of leucocytes taken on the 3rd day of radiation sickness did not possess bactericidal properties, which were found by them in leucocytes of nonirradiated animals or in leucocytes taken within a day after irradiation. One may assume that irradiated animals have antibodies against leucocytes in their serum, similar to those detected by Finch, Ross and Ebanh (213) in the serum of animals suffering from various blood diseases. Such an assumption might be based on the fact, that the antibodies discovered by the authors disturbed the processes of migration, diapedesis, taxis and digestive capability of leucocytes causing their lysis, agglutination and vacuolization. Also, the phagocytic reaction of animals suffering from abnormal blood circulation was changed, similarly to that in animals suffering from radiation injuries. If the consequences are similar, then it is very probable, that one of the causes for their appearance is identical as well. If disturbances in the nervous--endocrine regulation and metabolism are also considered, then the reason for disturbances in the phagocytic reaction in radiation sickness becomes clearer.

Thus, the microbes which get into the blood, are damaged to a lesser degree due to the changed properties of the plasma and blood cells. However, this question has not been clarified as yet. It is known, that the reticulo-endothelial system plays an important role in the struggle of the organism with microbes.

Is the defensive function still preserved in radiation sickness?

1) Radiation dose which killed 75% of the animals within 14 days.

Numerous researchers have tried to solve this problem. They succeeded in finding that in various animals with acute radiation sickness and in humans after X-ray therapy, the ingestive capacity of the cells of the reticulo-endothelial system decreases with regard to intravenously introduced dyes (108, 206, 176); it decreases also with respect to different bacteria (315), solutions of salts labelled with radioactive phosphorus or gold (222, 283, 258), and even with respect to iron released by hemolyzed erythrocytes (143).

However, several authors failed to note any disturbances in the absorptive function of the reticulo-endothelial system, although they used radiation doses similar to those of the previous authors (196, 275).

Such a contradiction becomes more understandable if one becomes familiar with the paper of Gordon, Cooper and Miller (229). These authors injected pneumococcus (type A) into the ear vein of rabbits exposed to 800 r, and then counted the number of bacteria in the blood. They succeeded in establishing, that the rate of blood purification in irradiated animals does not change much during the first hours. Bacteria vanish from blood, and then appear again.

These authors conclude that the reticulo-endothelial system is capable of removing for a prolonged period of time bacteria circulating in the blood, but it is not capable of retaining and destroying them. It is appropriate here to mention once more the paper of Donaldson, Marcus, Ko Gui and Perkins (210) on the decrease in the digestive capacity of phagocytes.

Consequently, one may assume, that even if the absorbing function of the reticulo-endothelium under certain experimental conditions remained unimpaired, nevertheless, the captured microbes will not be digested and may give a secondary bacteriemia. It is understandable, that if a nonliving agent is used to test for phagocytic activity, then some investigators were not able to note the depression of the defensive role of the reticulo-endothelial system. An incorrect conclusion could be reached also on the use of germs, if blood was examined only during the first hours following infection.

The intensity of the absorbing capacity of the reticulo-endothelial system depends not only on purely biological factors but also on physicochemical process, on the ability of cells to adsorb the object to be phagocytized. The investigations of O. G. Alekseyeva showed, that this process is impaired in irradiated animals. The experiments were carried out on rabbits exposed to 800 r. The adsorptive properties of many tissues with respect to a living culture of *Staph. aureus* were studied. It was established, that parallel to the decrease in the adsorptive properties of lymph node tissue and of the mucosa of the small intestine during the period of primary reaction and the climax of radiation sickness, the adsorption of microbes by the cells of liver and kidneys, and by the 10th-14th day also of the spleen, may increase.

But the antimicrobial factors in the internal organs do not limit themselves to phagocytosis, they can also produce lysozyme, release bactericidal substances, and, finally, they possess a special form of immunity displayed in their unresponsiveness to the effect of bacterial toxins (the 3rd immunity factor of Kravchenko and Galanova (89).

How does radiation affect these mechanisms causing nonsusceptibility to microbes?

According to Bernardini (189), the amount of lysozyme in lung and spleen (only these two organs were investigated) of rats decreases markedly

after irradiation. The author attributes great significance to the direct effect of radiation on the enzyme lysozyme. We cannot agree with such a viewpoint.

In 1931 Antonioli (184) reported, that the tissues of guinea pigs exposed to 750 r exert a smaller bactericidal effect on the microbes which are uncommon in the intestine, than the tissues of nonirradiated guinea pigs.

One of the authors of this book (N. N. Klemparskaya (79)) has studied in detail the state of bactericidal properties in the organs of irradiated animals. She studied the bacteriostatic properties of spleen, kidneys, lungs, liver, duodenum and small intestines (in the latter the contents and minced tissue were studied separately) toward coliform bacteria, *Staph. aureus* and autoflora. Organs were taken from rabbits irradiated with doses of 400, 800 and 1100 r, from mice exposed to 750 r, from rats exposed to 600 r and guinea pigs exposed to 500 r. The tissues of organs of nonirradiated animals possess bacteriostatic properties to an insignificant degree. After irradiation, especially if exposed to a lethal dose, the bacteriostatic properties are more pronounced. It was found also that bacteriostatic substances are thermolabile. It was noticed, that the increase in bacteriostatic properties of the tissues and contents of small intestines was often the most pronounced. The clearest results were obtained from animals, that died after treatment and not from sacrificed animals. Thus, in 10 nonirradiated rabbits neither the contents of the intestine nor its wall possessed the bacteriostatic property, but the wall of the small intestine of rabbits exposed to 1100 r markedly inhibited the growth of coliform bacteria in 16 out of 35 cases and the intestinal content did the same in 26 out of 34 cases. These observations indicated that it is necessary to determine the time of appearance of these changes, what initiates them and how they cope with the increased multiplication of bacteria in the intestine. To solve these problems, special experiments on mice and rabbits were carried out, which resulted in the finding, that bacteriostatic action is not connected with a direct effect of X-rays, but depends on processes which develop during the days following irradiation. Irradiation of intestines as well as other organs causes disturbances in the natural mechanisms concerned with the depression of multiplication of bacteria, which leads to an increase in their number. The number of bacteria increases on tegmina as well as in the body cavities which are in communication with the environment (among them also the intestine), from which the microbes penetrate into the internal organs.

With developing radiation sickness, degeneration of cellular elements in many organs begins. Obviously during this process intracellular bacteriostatic substances are released, which is manifested in in vitro experiments in the investigations of the tissues of irradiated animals. However, such an appearance of bacteriostatic substances cannot play a defensive role, because accumulation and spreading of large numbers of bacteria in the organism occurred up to this time. Since the largest mass of damaged cells may be found in the intestine (during the terminal period almost a complete disintegration of the intestinal epithelium has been observed), the bacteriostatic activity in the in vitro experiments was displayed mostly in the tissues of this organ.

The investigations of a co-worker in our laboratory, O. R. Nemirovich-Darchenko, also indicate damage to the normal bacteriostatic properties of the intestinal excretions, which occurs before the beginning of the mass autolysis of the epithelium. She has studied the bacteriostatic properties of the feces of dogs treated with a dose of polonium of 0.1 mC/kg. The author found, that the depression of this antimicrobial factor appears on the 4th-5th day following injury.

The change in bacteriostatic properties of feces during radiation sickness was revealed by O. G. Alekseyeva in her tests on chronic poisoning of dogs with Sr⁹⁰. Four dogs received daily, except for Sundays and holidays, 0.02 μ C/kg of radioactive strontium with food. From the first through the 11th month (the observation time) a decrease periodically alternating with a sharp activation in the bacteriostatic properties of feces was observed, and the abrupt changes in these properties were accompanied by a change in the bacterial picture of the intestines.

V. V. Vasil'evskaya (113) has discovered the fact that in acute radiation sickness caused by polonium poisoning, the << tissue >> immunity is affected also--the unresponsiveness of the smooth muscles of the small intestine disappears.

The presented facts make it clear that in radiation sickness, the environment of microbes in the organism changes significantly. Due to the depression of natural immunity in the irradiated organism, conditions appear which stimulate the development of infectious complications. How will the affected organism react to these germ foci? Does the powerful defensive reaction of the organism--inflammation-- suffer in this injury too?

In 1934 I. P. Mishchenko (109) in reviewing the experiments of his co-workers indicated, that irradiation with massive doses stimulates the severity of inflammation. V. G. Garshin and his co-workers M. M. Bol'shakova, M. A. Zakhar'evskaya and V. V. Osinskaya (22, 24, 25, 47, 118) over a number of years have studied the inflammatory response to the injection of sterile diatomaceous earth or turpentine in oil. Basically their work was directed to clarification of the mechanism of roentgenotherapy of inflammatory processes. But in order to clarify the effect of massive radiation doses, total-body irradiation was used in many tests by the authors. It was found by them that the intensity of formation of the << leucocytic embankment >> is depressed, the formation of granulations decreases and the fibroblasts are damaged. Later I. M. Neiman and A. Ya. Sinai (115) have established the fact, that on the 7th-16th day after the creation of a focal inflammation by the injection of paraffin in mice exposed to 50-200 r, a depression of the histiocytic reaction was observed. V. V. Shikhodyrov (178) studied the character of changes in the inflammatory reaction to the subcutaneous injection of celloidin in rats in relation to radiation dose. He showed not only depression of the inflammatory reaction, but also the appearance of a hemorrhagic reaction following irradiation with median lethal and sublethal doses.

But all the investigators mentioned studied not infectious but aseptic inflammation. We are interested in the character of the inflammatory reaction to the microbic agent. We failed to find such experiments in the accessible literature. The only exceptions are the investigations of V. F. Sosova (152).

Our investigations were started in 1951. The focal infection was induced by the method of intracutaneous injection of 1-day-old suspensions of different microbes: coliform bacteria and more rarely staphylococcus, *B. perfringens*, streptococcus, pneumococcus and blue-green pus bacteria.

The inflammatory reactions of irradiated animals to the injection of different microbes, in addition to the individual peculiarities, had common symptoms which distinguished them from the response of nonirradiated animals. These symptoms were: the appearance of tissue necrosis, hemorrhages in the inflammatory focus and generalization of the infection. The changes mentioned took place during the climax of radiation sickness induced by lethal and sub-

lethal doses and to varying degrees they were exhibited by various species of laboratory animals, but rabbits displayed them most clearly (Fig. 5).



Fig. 5. Cutaneous focus of infection of nonirradiated (a) and irradiated (b) rabbits (1100 r) after 48 hours following infection with coliform bacteria. The infection was carried out within three days after irradiation.

In case of recovery, the response of the animal returned to normal (Fig. 6).

In order to clarify the reasons for the appearance of a necrotic-hemorrhagic character in the inflammation and those for the development of bacteriemia after a subcutaneous injection of relatively small doses of microbes-saprophytes (200 mil. organisms), special experiments were carried out. In tests using shielding of skin sections, we have established the fact that the unusual reaction to the microbe agent depends on the total effect of radiation on the organism.

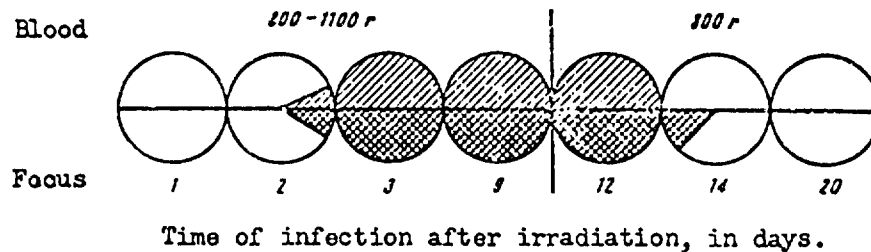


Fig. 6. The inflammatory character and bacteriemia of rabbits infected at different times after irradiation. The upper non-crosshatched semicircles indicate the absence of bacteriemia; the crosshatched semicircles show the presence of bacteriemia. The bottom semicircles show the ordinary inflammatory reaction, the crosshatched--the necrotic--hemorrhagic inflammation.

Great attention was paid to studies of the number of microbes in the focal infection. These experiments were carried out on rabbits exposed to 1100 r, that received at different times after exposure a subcutaneous injection of coliform bacteria or *Staph. aureus*. Then within 48 hours after infection the focus was excised and the number of viable bacteria per 1 g of tissue was determined. Fig. 1 (see 1st Chapter) showed a diagram of the mean values

reflecting the accumulation of microbes in inflammatory foci at different periods of radiation sickness. One can see that during the latent period, the number of microbes in the inflammatory foci of irradiated animals exceeds that of control animals only by several multiples of 10. But at the climax of radiation sickness, the accumulation of bacteria proceeds rapidly, and it exceeds the corresponding indices in the control group by several thousand and hundred thousand times.

In order to determine the time when this change in reactivity of the organism appears, a group of rabbits received an intracutaneous injection of coliform bacteria in 8 sites simultaneously (7×10^7 living bacteria per dose) and then within 3, 6, 9, 12 and 48 hours following infection the inflammatory foci were excised. The data from rabbits that were subjected to experiment within 17 hours after irradiation did not differ from that of the control animals. But the data from rabbits infected within 3 days following irradiation, differ significantly from the controls. (Fig. 7). While the number of germs in the inflammatory foci of nonirradiated animals gradually decreased, and by 48 hours reached a concentration of several thousandths per 1 g of focal tissue, in exposed rabbits a huge number of microbes, amounting to several times ten or a hundred million microbes per 1 g of focal tissue on the third day of radiation sickness, was maintained during the entire experimental period.

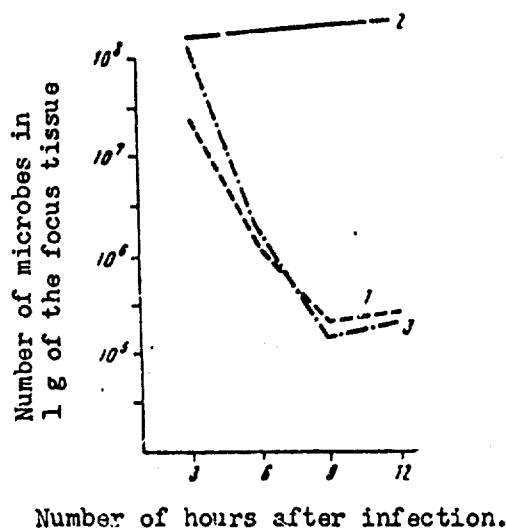


Fig. 7. Changes in the number of microbes in cutaneous foci within the first 12 hours following infection.

1-infection within 17 hours following exposure to 1100 r; 2-infection within three days following irradiation; 3-control group.

How can the fact be explained that during the climax of radiation sickness the number of microbes per 1 g at all times after infection remains within limits, close or almost identical with the injected dose? A decrease in focal microbes occurs definitely, because bacteremia develops, but at the same time the dimensions of the focus increase (by 48 hours after infection it amounts to 3.5-6g). Consequently, this can be explained only by the fact that the injected microbes are not only preserved, but they even multiply intensely.

On the basis of the investigations of the number of microbes-saprophytes in inflammatory foci (V. F. Sosova) and in accordance with a more intense accumulation of pathogenic microbes in the infection site, which was noted during the examination of the smears-imprints (O. G. Alekseyeva, (5 and 6)) or

in the determination of the number of microbes (R. V. Potrov, see Chapter V, Table 24), and also on the basis of the data in the literature (153, 1), one may assume, that the altered inflammatory reaction in radiation sickness depends not only on disturbances in the reactivity of the organism, but, undoubtedly on the increase in the dose of the infection agent as well.

The depression of the intensity of immunity of animals during radiation sickness results in a decreased resistance to conditionally pathogenic microbes (see the data of Chapter 1) and even more to pathogenic infectious agents and their toxins. In the mechanism of decrease in resistance to microbes, not only are the depression of bactericidal humoral factors and phagocytic reaction of importance, but also the significant increase in biological effect of increasing dose of the infectious agent, due to a more intense accumulation of microbes and a change in their biological properties in the tissues of an irradiated organism.

On closing the brief review of the literature and our own data on the studies of the state of different factors of natural immunity we think it is expedient to give a total summarizing scheme of these changes in relation to the phase of radiation sickness.

During the period of the primary reaction (i.e. during the first hours following irradiation) and partly during the latent period (i.e. within 1-2 days after irradiation) the intensity of the majority of immunological processes either does not change, or even can be activated. It is possible that some reactions (for instance, the adhesive ability of leucocytes) become disturbed soon after first hours, but this does not lead to a decrease in immunity, altogether. Nevertheless in animals infected with microbes at this time, or poisoned with bacterial toxins, the developing infection or intoxication may take a more severe course than in nonirradiated animals. This is because the infection process is prolonged and covers the following periods of radiation sickness.

To some degree in the latent period and always during the climax of the sickness, depression of the natural nonsusceptibility of the organism to microbes appears, and the intensity of all mechanisms of the immunity decreases. Is this decrease uniform? Does the depression of all mechanisms of immunity take place immediately in an animal? To answer this question, one should check the intensity of all defense reactions in an animal at one time. But as we wrote at the beginning of the chapter, the investigators usually studied individual mechanisms of immunity on different groups of animals. In evaluating our results we should note that the depression of any reaction ordinarily takes place only in part of the animals. Very rarely a function proves to be decreased in 100% of the animals investigated and at all times.

This observation indicates, that even at the climax of radiation sickness the organism maintains some degree of compensating ability. It should be said that in the case of lethal radiation sickness this compensating ability can be relative: i.e. an increased intensity of any mechanism of immunity cannot compensate the lesions in other mechanisms.

However, the presence of compensation reactions deserves attention and requires study, because understanding of their nature will assist in finding effective therapeutic methods.

The presence of compensation is confirmed also by our experiments in studies of the immunological reactivity of dogs exposed to 600 r (O. G. Alekseyeva and A. A. Kanarevskaya, 1955).

The results of investigations of immunobiological reactivity of 3 dogs before irradiation and on the 5th day, i.e. at the climax of radiation sickness, are presented in Table 5.

At this period all mechanisms of the natural immunity of the dog Starichok that were investigated proved to be inhibited, and this was accompanied also by a change in the cutaneous microflora toward an increase in the number of bacteria and in the percentage of stocks that breakdown mannite.

On the fifth day following irradiation, in the presence of a sharp depression of bactericidal properties of skin and some disturbance in the content of the cutaneous microflora, one could observe in the dog Malyshka a pronounced activation of the phagocytic reaction. We intend to consider this activation as the manifestation of compensation. However the compensation was relative and could not completely stop the decrease in the neutrophil reserve. In consequence, although the phagocytic index increased ten times, nevertheless fewer cocci were phagocytized, than before the irradiation.

Table 5

Investigation of several immunological reactions in dogs before irradiation and on the fifth day of radiation sickness (radiation dose 600 r).

Kind of reaction.	Starichok		Malyshka		Damka	
	before	after	before	after	before	after
	irradiation		irradiation		irradiation	
Bactericidal activity of skin (% of dead coliform bacteria within 15 min. after placing them on skin)	89	46	88	5	83	94
Phagocytic reaction of blood neutrophils percentage of phagocytosis	14.6	2	10	48	15.8	9
Phagocytic index.	0.5	0.02	0.5	5.1	0.82	1.53
Abs. number of phagocytized cocci in 1 mm ³ of blood	67 000	700	4 000	2700	3 000	500
Microflora of skin: the number of bacteria per 12 cm ² of skin	164	400	403	510	215	30
Percentage of stocks breaking down mannite	7.8	25	1	15	17.3	44

Phenomena of relative compensation were also observed in the dog Damka, but they were exhibited in a different manner. Some activation of the bactericidal properties of skin evidently stimulated a decrease in the number of bacteria, but did not prevent an increase in the percentage of stocks that degrade mannite. But the increase in the phagocytic index because of more efficient capture of cocci by any one phagocytizing neutrophil failed to compensate the decrease in the phagocytic reserve.

Thus, the period of the climax of radiation sickness, despite the fact, that some compensating ability is maintained, is nevertheless characterized by a depression of natural immunity. This depression is sharply pronounced during the terminal stage.

If recovery from radiation sickness occurs, a gradual restoration of the disturbed mechanisms of resistances has been observed; restoration of individual immunity factors usually does not proceed simultaneously. The instability and shift of the phases of depression and excitement may take place for a prolonged time. It should be noted that the state of immunity during the remote consequences of radiation sickness has not been studied sufficiently as yet.

Since each period of radiation sickness is characterized by a definite state of natural immunity, then, consequently, the immunological technique may be applied, for the determination of the reactivity of the exposed organism.

Is it possible to assume, that the changes in immunity in radiation sickness are specific only for this given sickness? We have already expressed the idea that the character of these changes is specific for any period of the radiation process. But one cannot assume that the changes are specific for radiation sickness. The mechanisms of natural immunity are themselves non-specific, and, consequently, changes in them cannot be specific. Depression of the phagocytic reaction is described in different inflammatory processes (166), anginas (37), diseases of internal organs (27) and so on. A decrease in the titers of lysozyme in saliva has been observed during some local diseases of the nasopharynx (94), and typhus (107). Depression of the formation of serum complement has been described during diabetes, uremia, thyroidectomy, acute yellow atrophy of the liver (quoted from 11), typhus (11), rheumatism of Baillot (spelling?) (43) and so on. Our own experiments carried out on animals with a severe form of benzene intoxication (V. F. Sosova, (152)) verify the possibility of appearance of the characteristic inflammatory reaction during other pathologic processes. The necrotic-hemorrhagic reaction to an intracutaneous injection of microbes might be observed also in rabbits with severe benzene poisoning, and a huge number of microbes (hundred thousand times more than that of controls) accumulate in inflammatory foci; generalization of the infection (bacteriemia) takes place.

Consequently, nothing is known of any specific immunological reactions in radiation sickness at present, but there is a type of change in the entire complex of mechanisms of natural immunity, which is characteristic for the phases of radiation sickness. Only in this sense one may speak of the specificity of disturbances in immunological reactivity after irradiation.

Chapter 3

Induced Immunization in Radiation Effect. Effect of Ionizing Radiation on the Development and Preservation of Active Immunity.

The data on spreading of the microbes of autoflora in the internal organs of an irradiated organism presented in the preceding sections, and data on disturbances in many factors of natural immunity, indicate the importance of the investigation of the peculiarities of immunobiological reactivity under the influence of ionizing radiation.

The vast use of atomic energy in industry, agriculture and medicine makes it necessary to study the capacity for development of full active immunity in organisms, which are being subjected to the effects of irradiation, because the great significance of the problem in epidemiologic practice is obvious.

The investigation of the possibility of active immunization in irradiated animals and humans was carried out by many investigators, and at present there are many papers published, of which reviews can be found in the native as well as in the foreign literature (129, 159, 312).

A substantial disturbance of immunogenesis was found, when antigen was injected in an irradiated organism; preservation of sufficient resistance was established, when irradiated animals were immunized before exposure.

The inhibiting effect of ionizing radiation on the formation of active immunity is expressed almost identically after the effect of various sources of external and internal radiation (α -, β -, and γ -radiation). There are data (232), that local irradiation, if it is carried out at the site of the intracutaneous injection of antigen before the immunization, also decreases the production of antibodies to a large extent.

However, many questions connected with the investigation of peculiarities of the active immunization of irradiated organisms, require further studies.

Thus, for instance, the effect of irradiation on the active development of resistance in the organism to infection with living germs has been investigated inadequately. In the majority of papers the changes in antibody titer, not tests of resistance to infection, are the indicators of immunological reactivity. Although the formation of antibodies indicates the reaction of the organism after the injection of antigen, all the same,

It was established by I. I. Mechnikov (106), N. F. Gamalei (21) and other authors (45, 131, 64), that their number does not allow one to estimate the actual nonsusceptibility of a vaccinated organism to viruses, which is determined by a multiple complex of cellular and humoral defensive factors. It is known, for instance, that the highly effective protective antigens of the Siberian plague microbes do not initiate the appearance of antibodies at all.

Likewise, there is no relation between antibodies and the tissue immunity described by A. T. Kravchenko and N. V. Galanova (89).

One may expect a more precise evaluation of the state of the active defenses of an organism against the infectious agent in vitro from the phagocytotic reaction, which directly demonstrates damage to the microorganisms. The data presented above indicate not only a significant lesion of this important natural defense factor after exposure, but it also shows depression of the specific activation of this process due to the effect of immunization.

However, investigation of individual factors in the effect of the organism on microbes in vitro cannot, of course, replace evaluation of the stability of the entire organism, which becomes possible only in experiments in which the infection is carried out with living germs. It is of interest, that there are data already on the non-correspondence between a low-order stability of irradiated animals to test by infection and the presence of significant numbers of antibodies (64, 65).

Unfortunately, there are only a few authors in radiobiology who use the method of infection with living pathogenic microbes in order to determine the degree of immunity (5, 6, 234, 165), and, therefore, further studies of this problem on experimental models of different infections are of great theoretical and practical value.

The role of individual organs and systems in the development of active immunity is another question important for the understanding of the peculiarities of immunogenesis in irradiated organisms and many features of the pathogenesis of radiation injuries.

There are a few investigations in this region, and they consisted basically of protection from a direct effect of radiation on the spleen or appendix, which led to an increased survival of animals and improvement of immunogenesis (295).

By means of tests involving the transfer of cells from an organism that received an antigenic stimulus into another which was not subjected to immunization, efforts were made to establish the important role of other tissue elements.

Thus, for instance, Harris and co-workers (236) illustrate the important role of lymph nodes in antibody formation. The transfer of regional lymph nodes from an immunized animal (taken within three days following the injection of antigen) into an irradiated rabbit enabled one to obtain antibody titers that were much higher than those in control animals which were only irradiated. If the rabbits was irradiated not before but after the transplantation of the glands, then the production of antibodies decreased to a large extent.

Interesting data are reported by Jaroslow and Taliaferro (244). Adding of extracts or crushed suspension of tissues (spleen, yeast, epithel-

ioma, but not those of kidneys or muscles) allows one to attain antibody formation in irradiated animals, although more slowly and to a lesser degree than in nonirradiated rabbits. Separate injections of the suspensions of tissues and antigens were ineffective.

Similar data on the increased antigenic effect of dysenteric bacteria in irradiated rabbits, by adding a suspension of washed cells of the spleen or lymph nodes of a rabbit to them with contact *in vitro* at 37° during 30 minutes, are reported by Harris T, Harris S. and Farber (239).

It should be noted, that the stimulation of the antigen effect is attained by means of homologous, not heterologous tissue, which is a weak stimulant of the organism. This fact indicates the necessity of careful studies of the response of an irradiated organism to homologous tissue components.

Little is known about the sensitivity of different stages of the process of immunogenesis to the effect of radiation and about the substance of this effect. It is not known what is more damaged--the process of acceptance of an antigen stimulation or the following response to it. It is completely unknown, which phases of this response are the most sensitive and so on.

It is known only, that irradiation during the period of the first 2-3 days after the injection of antigen leads to a significant decrease in immunogenesis. This fact, which is recorded in the papers of different authors (209, 312, 159), requires study, in order to understand the nature of the effect of radiation on the immunological reactivity of the organism.

The problem of the importance of the site of injection of the antigen in increasing the efficiency of immunization after irradiation has not been clarified as yet.

We do not know of any papers in this direction, either native or foreign, although in the region of the general immunology numerous data are accumulated, which indicate the important role of a correct selection of route and site of injection of antigen in order to attain an intense immunity (36, 44, 84 and others).

There is an almost complete lack of experiments on postvaccinal reactions in irradiated organisms and on the investigation of the possibility of revaccination after irradiation in the presence of the basic immunity produced before irradiation. And finally, very little is known on the problem to what extent the changes in immunological reactivity observed during radiation sickness are specific, that is, for the effect of ionizing radiation.

In our investigations of the effect of radiation on the development of active immunity in animals we used evaluation of the degree of non-susceptibility by means of infection of the vaccinated animals with a lethal dose of the culture of a living microbe. Antityphoid immunization, with the testing of intensity of immunity by means of an intraperitoneal injection of a suspension of a daily agar culture of typhoid bacteria, was used as an experimental model for the investigation of this problem.

Although under natural conditions white mice do not have typhoid, their infection with this microbe causes distinctly pronounced phenomena of toxicoinfection with spreading of the injected bacteria in the internal or-

gans and their constant presence in blood; therefore this model, as it is known, is widely used in laboratories for the investigation of many problems connected with studies of the efficiency of active immunization. One thousand six hundred twenty-one mice weighing 18-20g were used in these experiments. The vaccinated and control animals were maintained in jars in groups of 6-10 and they obtained ordinary food (milk, oats, bread).

More frequently the injection of 1 Dcl (100% lethal dose-LD100) (sometimes 2 Dcl) of microbes was used for infection of mice. We did not use a larger quantity of Dcl, because such an increase is connected with an increase in the number of the injected microbe bodies, i.e. with the increase in the dose of the injected endotoxin, which leads not to the development of toxicoinfection, but to the loss of the animals from intoxication. (For instance, according to the Reed and Mench method, which is used by many authors, the number of the injected Dcl amounts to eight, but the results are computed totally from the death rate from one to eight Dcl). The existing methods of antityphoid immunization create basically antiinfectious immunity (it is known, that the attaining of an antiendotoxic immunity presents temporarily great difficulties), and the testing of its intensity by means of an acute poisoning with endotoxin scarcely could be correct. After the injection of 1 Dcl of typhoid bacteria the mice were observed over a period of three days, because those which survived this time usually lived further. Typhoid monovaccine, heated at 56° during an hour, taken from stock No 4446, which was used also for the infection of mice in order to check the efficiency of the vaccination, in our experiments was used as the inoculative preparation. One absolute lethal intraperitoneal dose of this culture, injected in a volume of 0.5 ml of the physiologic saline solution, amounted to 125 mil. of microbe bodies of a daily agar culture.

In part of our experiments tetravaccine prepared in the Gamaleya Central Institute of Experimental Medicine was used as the antigen.

Injection of 400 mil. microbe bodies of the vaccine in a volume of 0.5 ml of physiologic saline solution was used for a single intraperitoneal immunization, but in subcutaneous inoculations the injection of 200 mil. for reimmunizations and 400 mil. for a single one were used. We have tested chiefly the effect of a single injection of antigen in the indicated doses, by which, using intraperitoneal inoculation, sufficiently intense immunity could be attained to provide resistance to 1-2 Dcl of microbes, within 7-10 days. Frequent immunization under conditions of alternating reactivity of the irradiated organism is inconvenient, because its completion as well as the data of the efficiency tests extend widely beyond the limits of the period of radiation sickness and do not allow estimates of the intensity of immunity at different stages. It is known that a single immunization is used in general immunobiology in order to attain the most correct evaluation of the quality of the vaccine (102). A single injection of antigen enables one to determine the difference in the development of active immunity in control and irradiated animals even during the course of radiation sickness.

To investigate the effect of radiation on the maintenance of existing active immunity and to determine the efficiency of inoculations after irradiation, the immunization and irradiation was carried out in different combinations (Table 6) and at different periods of radiation sickness.

Testing of intensity of immunity by means of infection was carried out within seven days following immunization, i.e. after the shortest time period in which the presence of immunity would be estimated during the radiation sickness, which took place at this time. In order to study the

significance of the site of application of antigen, the immunization was achieved by different methods of injection of the inoculative substance. The subcutaneous method, which is widely used in the inoculation practice, intraperitoneal method, used in the majority of experimental works, and cutaneous vaccination according to the technique worked out by us (50 mg of dried, powdered vaccine was transferred to a scarified skin; the skin injury was achieved by means of emery paper) were tested, taking into account the results of a series of authors on the high efficiency of intracutaneous and cutaneous inoculating methods (8, 52, 83) (the experiments of N. N. Klemparskaya). The radiation effect was achieved by a total-body X-irradiation with the apparatus RUM: voltage 180 kV, current 15 mA, distance 50 cm, dose rate 18-24 r/min; filters 0.5 mm Cu + 1 mm Al, the total dose 300 r (in part of tests the dose was 100-200 r). In one series of tests the irradiation of mice was carried out with a dose of 367r of γ -rays from a cobalt source with an activity of 234 C at a distance of 35 cm.

This one series deals with the studies of radiation effect on a formed, active immunity attained as a result of an intraperitoneal injection of vaccine 21 days before irradiation (the tests of R. V. Petrov).

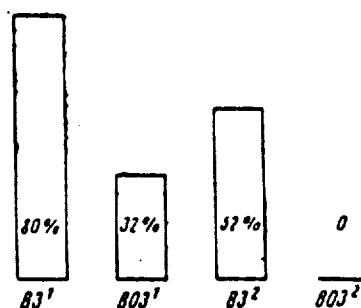


Fig. 8. Effect of radiation on the intensity of active immunity to the causative agent of typhoid in white mice.

- B3¹ - vaccination and infection with 1 Dlm (minimal lethal dose);
 B03¹ - vaccination, irradiation and infection with 1 Dlm;
 B3² - vaccination and infection with 2 Dlm;
 B03² - vaccination, irradiation and infection with 2 Dlm.

As Fig. 8 shows, irradiation failed to lead to a complete disappearance of immunity, but caused a significant decrease (the survival rate of irradiated animals was 32% in comparison with 80% in control mice). This decrease was especially sharply pronounced after infection with 2 Dcl; there were no survivors in the group of irradiated animals.

Analogous data were obtained (N. N. Klemparskaya) also in the X-irradiation of immunized animals.

Table 6 (See following page).

The survival rate of mice that received subcutaneous injection of the vaccine after their infection with 1 Dcl, was 51.3%, but that of animals irradiated after vaccination was found when tested on the second week of radiation sickness to be equal to 33.3%.

Table 4

Efficiency of various methods of active immunization of white mice before and after irradiation. Results of immunity tests by intraperitoneal inoculation of Del.

Immunization Method	Number of inoculations	Inoculation dose in r	Inoculated before or after irradiation	Day of radiation sickness on which inoculation took place.	Day of radiation sickness on which infection took place.	irradiated, inoculated		nonirradiated, only inoculated		survival rate, %	mice, total	nonirradiated, died	survival rate, %						
						mice, total	died	mice, total	died										
Subcutaneous	3	300	before	--	First week	80	45	47.2	39	19	51.3	20	20	0					
					Second week	12	8	33.3	--	--	--	--	--	--	--				
	Single	300	before	after	First week	First	53	16	71.7	46	11	76.0	25	23	8				
						Second	62	17	72.7	17	2	88.2	11	10	9				
						Third	99	65	24.2	12	2	83.3	14	14	0				
						Fourth	50	22	55.0	--	--	--	8	8	0				
						First	24	9	62.4	12	1	51.7	3	3	0				
						Second	28	14	50.0	16	7	56.2	5	5	0				
						Third	12	8	33.3	--	--	--	5	5	0				
						Fourth	10	3	70.0	--	--	--	3	3	0				
Intraperitoneal	Single	100	after	Within a month	First week	32	21	37.7	--	--	--	8	8	0					
					Second	20	2	90.0	--	--	--	10	10	0					
					Third	15	6	60.0	9	2	77.7	7	7	0					
					Fourth	18	16	11.1	20	8	60.0	4	4	0					
					Single	300	before	after	Second week	First	19	17	11.1	10	1	90.0	4	4	0
										Second	14	1	92.8	17	2	88.2	6	6	0
										Third	14	1	92.8	17	2	88.2	6	6	0
										Fourth	14	1	92.8	17	2	88.2	6	6	0
										First	14	1	92.8	17	2	88.2	6	6	0
										Second	14	1	92.8	17	2	88.2	6	6	0

A greater depression of immunogenesis was observed if the inoculations were carried out after X-irradiation. The stage of sickness depended on radiation dose and period of radiation sickness.

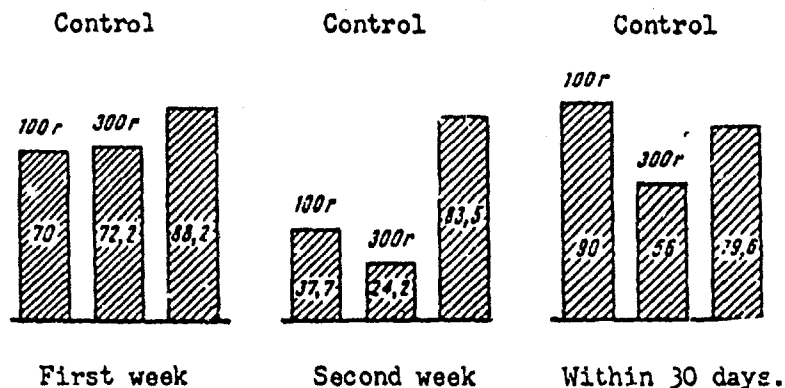


Fig. 9. Immunization efficiency in mice at different periods after irradiation with a dose of 100-300 r (the percentage of survival is given). Intraperitoneal vaccination was carried out.

Fig. 9 shows a significant decrease in the immunization efficiency at a definite period after irradiation and intraperitoneal injection of antigen. One can see, that this decrease is especially pronounced in immunization in the second week, i.e. during the peak of radiation sickness. The capacity to develop active immunity is restored within 30 days following radiation. The higher the radiation dose, the slower the restoration.

The total data of the experiments on efficiency of immunization by different methods at different periods after irradiation are presented, as mentioned before, in Table 6. Two common mechanisms, confirmed at present, by numerous papers published should be mentioned: the active immunity formed up to the moment of irradiation is also maintained after it, decreasing markedly during the course of radiation sickness. But if the inoculation is carried out on irradiated animals, then the development of nonsusceptibility to infection with living microorganisms is highly depressed. Simultaneously with the present investigations, studies of active immunity on the typhoid infection model were carried out by M. A. Tumanian and A. V. Izvekova (165), who used infection with 1-8 Dcl according to the Reed and Mench method. It is of interest, that depression of immunogenesis is manifested differently if antigen is injected in different regions of the body. Immunization by means of intraperitoneal injection of vaccine in the experiments of N. N. Klemparskaya gave rather intense immunity on the first week following inoculation and on the first day after irradiation (the survival rate after the injection of 1 Dcl was 70-72%).

This method allows one to immunize animals at the time of the so-called radiosensitive phase (1st-2nd day). Such a fact has great value not in revealing the presence of any special sensitivity in a certain phase of immunogenesis in general, but in showing how important are studies of the conditions for reception of antigen.

As the presented data show, the lower the radiation dose, the more rapidly occurs the restoration of immunobiological reactivity in mice after a month following irradiation (100-300 r). Thus, for instance, intraperitoneal immunization on the 3rd-4th week after exposure to 300 r resulted

... survival rate of 56%, but the inoculations of animals exposed to a dose of 100 r gave a survival rate of 90%.

Thus, the capacity of the irradiated organism to develop active immunity depends on the radiation dose, on the period of radiation sickness, on the relationship between the time of immunization, radiation effect, and the site of antigen injection. Obviously the intensity of immunity produced before irradiation as well as that attained by means of inoculation of the irradiated organism will be the lowest at the period of the pronounced clinical syndrome of radiation sickness.

Moreover, immunization at this time is dangerous and contraindicated, because it may cause serious harm. Unfortunately, this problem, although it has a great practical value, is not clarified by the data in the literature.

Shechmeister, Bond and Swift (294) have described an increase in the number of cases of bacteriemia in mice irradiated and vaccinated with killed culture of plague bacteria as compared with that of animals irradiated only.

Some experimental data of N. N. Klemparskaya are given below, which are illustrated in Fig. 10 and Table 7. Judging by the number of mice that died from immunization alone, it may be concluded that the irradiated organism becomes extremely sensitive to the toxic effect of the vaccine. Thus, for instance, if a single injection of typhoid monovaccine, prepared by the author, caused death to 5-7% of the animals that were healthy before inoculation, then the percentage mortality of animals on the first week of radiation sickness increased to 10.6, and on the second week to 83.4, and on the second week to 83.4%, and on the second week to 83.4%. These data indicate the necessity of paying great attention to studies of reactivity to vaccination in irradiated organisms, in order to obtain certain values and contraindications for attaining active immunization of the organisms, which had, at any stage, contact with ionizing radiation.

It is well known, that the reaction of the organism to the injection of antigens can alter markedly dependent on the presence of preliminary contact with the same antigen. Despite the absence of data on the nature of this phenomenon at present, preliminary immunization is widely used in immunological practice in order to attain a higher vaccination efficiency.

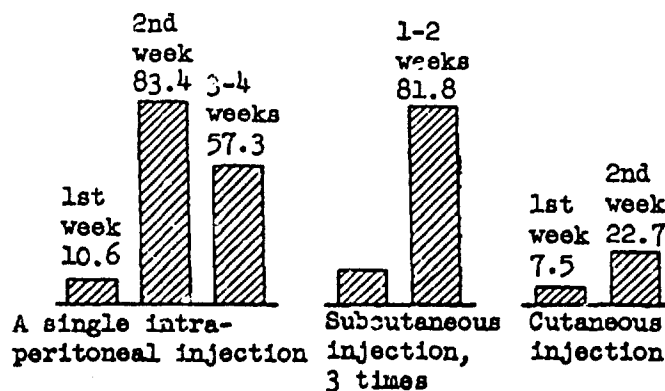


Fig. 10. Percentage mortality of mice after inoculations at different periods of radiation sickness using different methods for the injection of typhoid vaccine.

There are only a few data in the radiobiological literature on the efficiency of revaccination of irradiated organisms, and at the period of the performance of this experiment (1955) these data referred only to the studies of the production of antibodies.

I. A. Pigalev (129) in his laboratory has obtained data which indicate the absence of production of immune bodies after revaccination with diphtheria anatoxin in rabbits, that received an injection of LD_{50/60} of polonium.

A. F. Kosov (87), on the contrary, observed a sound formation of agglutinins in rabbits that received a revaccination with living tularemia bacteria at 6-7 months following their exposure to 800 r. Such a difference may be explained by differences in the nature of the effect as well as by differences in the periods chosen by the authors for the determination of antibody titers.

Table 7

Reactivity of mice to different vaccination methods before and after irradiation

Vaccination method	Before and after exposure to 300r; the number of inoculations	Day of radiation sickness on which inoculated.	Death rate of animals after inoculations.		
			Total number of mice	Number of deaths	Death rate, %
Subcutaneous injection, 3 times	Before irradiation	---	194	30	15.4
	After irradiation	From the 1st through the 15th day	55	45	81.8
Intraperitoneal injection	Before irradiation: single injection three times	---	106	27	25.4
		---	35	21	60.0
		---	141	48	34.0
	After irradiation (single)	First day	93	10	10.6
		Second week Third-fourth week	85 87	71 50	83.4 57.3
Cutaneous	Before irradiation	---	23	2	11.7
	After irradiation	First through third day	63	5	7.5
		Second week	22	5	22.7
Only Irradiated (300 r)			65	5	7.0

Dose of polonium whose injection caused death to 50% of animals over the period of 60 days.

In the experiments presented here, primary immunization as well as revaccination were carried out after irradiation. Reaction of the irradiated organism and efficiency of revaccination, if a preparatory injection of antigen before radiation were used which, as was indicated, significantly increases the efficiency of subsequent inoculations,--still remained in the dark.

In our opinion, such a possibility of increase in efficiency of immunization has to be tested in radiobiological experiments. To clarify the question of a possible increase in efficiency of antityphoid immunization of irradiated animals by means of a preliminary immunization before exposure, three series of experiments were carried out by N. N. Klemparskaya; the results are presented in Tables 8, 9 and 10.

In the first series of experiments (Table 8), the efficiency of revaccination after irradiation, by different methods at various periods during radiation sickness was determined. The data obtained were compared with the results of single immunization of irradiated animals, which were obtained previously and presented in Table 6. If the survival rate of irradiated mice that received an intraperitoneal inoculation, on the second week of radiation sickness previously was 24.2%, then in the experiment in which such an inoculation was revaccination, the survival rate increased to 62.4%. In this series there was no simultaneous immunization of irradiated mice without a preliminary preparation by injecting antigen, therefore in the following experiments the efficiency of immunization of nonirradiated (vaccinated and revaccinated) mice and that of similar groups of irradiated animals were investigated simultaneously. The experimental results are presented in Tables 9 and 10 according to two different methods of immunization.

Comparison of the survival rates of vaccinated and revaccinated irradiated mice shows a remarkable increase in the efficiency of immunization in the presence of a preliminary (before exposure) contact with antigen.

Thus, for instance, revaccination increases the survival rate of mice inoculated and infected after exposure: using the intraperitoneal method it increases from 38.8 to 81.8%, and using the subcutaneous injection it increases from 35.4 to 82.2%.

It is important to take into consideration that after revaccination, the toxic effect of the vaccine on the organism and the number of animals whose death was caused by revaccination (before infection) decreases.

The data obtained indicate the direction of further investigations with regard to the possibilities of effective immunization of irradiated organisms. Different antigens in different schedules of primary and secondary immunization have to be tested, and investigation of the characteristics of this phenomenon must be carried out in order to understand its nature.

What is the factor on which this favorable effect of preliminary contact of organism with antigen before irradiation depends, which secures the success of the subsequent immunization?

Two important factors have to be considered here. One of them was well known to immunologists previously, but the other one (although individual facts were reported in different papers) drew our attention only during the performance of the present experiments.

An increased sensitivity of the organism to the perception of antigen stimulation and an increased intensity of the response after a preliminary contact

Table 8

Single injection	Vaccination time before exposure, in days	Testing of immunity before revaccination	Rad. dose in r.	Period of radiation sickness in which revaccination took place.	Efficiency of revaccination of mice after irradiation (vaccination was carried out before exposure). Testing of immunity to 1 Dcl of living culture by intra-peritoneal injection											
					irradiated		nonirradiated (control)									
					total number of mice	Survival rate in %	total number of mice	Survival rate in %								
Intraperitoneally	7-12	Almost absent: 1 mice out of 5 survives or all of them die	300	1st-3rd day 2nd week 3rd-4th week	39	9	41	8	16	16	81.7	16	16	--		
					16	6	6	6	6	76.8	6	6	62.4	6	6	--
					12	1	1	1	1	91.6	1	1	1	1	100	1
Subcutaneously	8	The same	300	3rd day 14th day 30th day	10	3	24	--	12	12	100	12	12	--		
					6	4	8	8	8	33.3	8	8	100.0	8	8	20
Outaneously	6	The same	300	2nd week	30	26	10	2	10	80	10	8	20			

antigen is the first factor. There are data in the published literature on the determination of the optimal conditions under which such a repeated immunization could be used (the duration of the intervals, doses of antigen and the like, are studied) (51, 169, 100). The possibility of practical use of revaccination in order to attain a complete, active immunity to different infectious diseases has been established.

It may be assumed that the increased sensitivity to the given antigen in animals vaccinated preliminarily is maintained and that the revaccination carried out on exposed animals proves to be more effective.

Table 9

Efficiency of vaccination and revaccination of irradiated and control mice that received an intraperitoneal inoculation of 1 Dcl.

Kind of immunization	Group of mice	No of experiment	Infection of mice exposed to a 300 r dose within						
			2-3 weeks			4-6 weeks			
			total number of mice	Number of deaths	survival rate in %	total number of mice	Number of deaths	survival rate in %	
Revaccination ¹⁾	Irradiated	1	10	5		12	3		
		2	12	2		10	1		
		3	10	1		5	0		
		4	12	0		8	1		
	T o t a l.....			44	8	81.8	35	5	85.7
	Control (nonirrad.)	1	10	0		13	2		
		2	6	0		5	0		
		3	10	0		6	0		
		4	10	0		8	0		
	T o t a l.....			36	0	100	32	2	93.7
Primary immunization	Irradiated	1	10	4		10	3		
		2	8	6		8	2		
		3	10	6		6	1		
		4	8	6		8	1		
	T o t a l.....			36	22	38.8	32	7	78.1
	Control (nonirrad.)	1	10	0		12	0		
		2	8	0		7	0		
		3	12	0		6	0		
		4	10	0		5	0		
	T o t a l.....			40	0	100	30	0	100

¹⁾ The primary vaccination was carried out 15-20 days before irradiation.

However, in addition to this circumstance, other phenomena which we discovered only during the observation of vaccinated and irradiated animals, have to be considered as well.

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Table 10

Kind of immunization	Group of mice	No. of exper.	Infection after exposure to 300 r within													
			1 week			2-3 weeks			4-6 weeks			Survival rate in %				
			Total number of mice	Number of deaths	Survival rate in %	Total number of mice	Number of deaths	Survival rate in %	Total number of mice	Number of deaths	Survival rate in %					
Revaccination 1)	Irradiated	1	9	2			6	4			6	--	1			
		2	10	1			10	2			8	1	2			
		3	12	3			10	4			8	4	2			
		4	8	1			--	4			--	--	--			
		5	6	1			--	--			--	--	--			
	Total	45	8	82.2	34	14	58.7	34	5	85.3						
Primary Immunization	Control (nonirradiated)	1	10	1			6	--			10	--	1			
		2	8	2			10	1			10	1	2			
		3	12	1			10	--			8	2	--			
		4	8	1			10	2			6	--	--			
		5	6	--			10	--			6	--	--			
	Total	44	5	88.6	36	3	91.6	34	3	91.1						
Revaccination 2)	Irradiated	1	10	4			10	7			8	3	3			
		2	8	6			8	5			10	5	5			
		3	10	6			12	9			12	5	5			
		4	8	6			8	3			6	2	2			
		5	12	9			6	2			--	--	--			
	Total	48	31	35.4	44	26	40.8	36	15	58.3						
Primary Immunization	Control (nonirradiated)	1	10	4			10	7			10	3	3			
		2	10	5			10	8			10	8	2			
		3	8	2			7	2			7	2	2			
		4	7	2			7	2			12	6	6			
		5	6	2			6	2			8	2	2			
	Total	41	15	63.4	45	15	62.2	45	15	62.2						

1) The primary vaccination was carried out 15-20 days before irradiation.
 2) After the infection all surviving mice became severely ill.

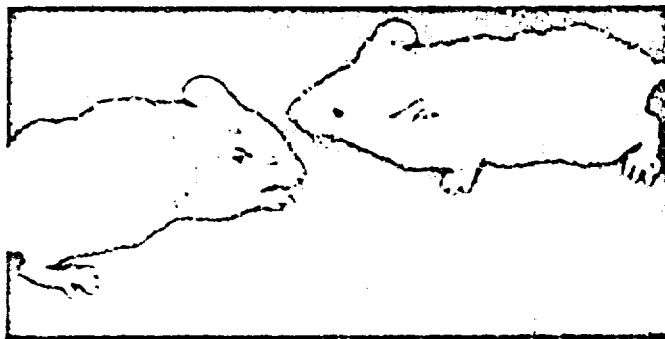


Fig. 11. Appearance of mice exposed to 400 r on the 12th day of radiation sickness.

The mouse to the right was immunized with typhoid vaccine two weeks before irradiation; the mouse to the left was not subjected to immunization.

We mean to nonspecific favorable effect of the injection of the substances of the vaccine and that of inflammation caused by it, on the course of radiation sickness.

The papers of I. A. Pigalev and his co-workers contain a note that in animals that received injections of radioactive substances, or in which septic inflammatory foci (176) were created, or that were subjected to active immunization with typhoid vaccine and tetanus toxoid, the survival rate increases.

Our attention was drawn by the great difference between the general condition of mice that were vaccinated before irradiation in order to prevent an attack of typhoid, and mice which did not get such an immunization (the experiments of N. N. Klemparskaya). Fig. 11 shows two mice exposed to an identical dose (400 r). The picture was taken on the 12th day of radiation sickness. To the left is the control mouse; she is sluggish, sick, with ruffled hair; she failed to receive the preliminary immunization. To the right is the mouse that before irradiation was inoculated once with typhoid bacteria; her appearance is comparable to that of healthy mice.

N. N. Klemparskaya set up experiments in order to study the effect of active immunization on the course of radiation sickness. Observation was made of the changes in weight and in death rate of mice, that received the vaccine 14, 7 and 3 days before irradiation and of those with no such preparation. Three hundred forty-nine mice weighing 18-20g were taken for the experiment. The inoculated and control animals were exposed simultaneously to a dose of 400-450r under the conditions described above.

A great difference in weight change of the mice inoculated preliminarily and the noninoculated mice, that were irradiated, was observed. While the weight of mice irradiated only dropped continuously, an increase was observed in the inoculated. The animals grow, appeared healthy and had good appetites. Their death rate was 8-12 times lower than that of mice which failed to receive a preliminary inoculation.

These facts deserve close attention and investigation, because they indicate an additional important effect of active immunization (in addition to protection against the infection), which can be very useful for persons working under the conditions of radiation exposure. The observations presented agree

well with the data of other co-workers of our laboratory. Thus, V. F. Sosova in her experiments with rabbits has found that a fourfold intravenous injection of the vaccine of coliform bacteria, paratyphoid Brod and Flexner dysentery rod before the exposure to 800 r doubles the survival rate of the animals, retards the development of leukopenia and accelerates the restoration of leucocytes. On the contrary, the injection of the same vaccines after irradiation led to a severe general reaction and deterioration in the condition of the rabbits. O. R. Nemirovich-Danchenko has observed the favorable influence of a preliminary immunization on mice that received polonium at a dose of 0.1 mC/kg. A decrease in the body weight of injected animals by 10% was observed, and, all of them died by the 17th day (their mean life span was 14 days), but mice that also received tetravaccine once or twice lived till the 37th day (average 24 days), did not lose weight, and even increased it by 6-7%. G. M. L'vitsyna reports an increase in the survival rate of guinea pigs exposed to a 200 r X-ray dose, and inoculated with the BTsZh intracutaneously two weeks before irradiation. Eleven out of 12 inoculated guinea pigs survived, while in the noninoculated group only two out of nine pigs survived. All these data are consistent with the facts presented in the paper of D. A. Kaulen (64). The author has observed a lower death rate in guinea pigs that received diphtheria anatoxin and were exposed to 350 r.

Consequently, investigation of active immunization in exposed organisms provides data not only for practical conclusions on the increase in its efficiency in order to protect the body from infectious diseases, but also assists in interpreting the pathogenesis of radiation sickness and disturbances in the reactivity of the organism characteristic of the sickness.

A theoretical investigation of the causes of such a favorable effect of active immunization on the course of radiation sickness is of interest too. One could assume that immunization and radiation affect the same physiological systems of the organism, and if the stimulation from the antigens has been perceived then, obviously, these systems become refractory and do not react to the effect of radiation. Such facts are described in immunology (50, 223 and others) with reference to a reciprocal effect of two injected antigens. This assumption explains another phenomenon too, namely, the disturbance in immunogenesis in cases, in which the exposure precedes immunization, which under such conditions becomes ineffective.

Thus, we have obtained data (by using living bacteria injections for the evaluation of the intensity of immunity), which indicate the importance of many conditions for the efficiency of immunization: relationships between the times of irradiation and immunization, the radiation dose and the period of radiation sickness. We consider, that special attention has to be paid to further study of the dependence of the efficiency of immunization on the selection of the site of injection of the antigen. The fact of a significant increase in the toxic effect of the inoculation substance after exposure requires attention as well.

Despite a significant depression of immunogenesis after irradiation, a rather intense immunity was successfully attained. At the same time the harmful effect of radiation was decreased by means of a preliminary contact of the organism with the antigen before irradiation.

Consequently, a definite course for further experimental investigations has been set. These studies must find effective and practically accessible methods which would help to increase the immunobiological reactivity of the irradiated organism.

On closing we must discuss the question of the specificity of the changes in immunobiological reactivity with respect to the effect of ionizing radiation.

It is well known that anything that depresses the vital activity of the organism, such as starving, overheating or cooling, exhaustion, traumas, intoxication, infectious diseases, or tumors, decrease the ability of the organism to develop active immunity. The effect of definite radiation doses is referable to the same depressing factors.

A general impression forms that there is no particular specificity in the character of the effect of radiation on immunogenesis. However, with a more careful examination of this question several peculiarities can be found, characteristic of the effect of radiation.

The damaging effect of radiation on immunogenesis is intensely pronounced and follows the irradiation directly, even when the general condition of the organism remains good. This disturbance in the capacity for active immunization, which appears early, evidently, is connected with an impediment in the perception of antigen stimulation.

In the reactions to all the other factors listed above, the disturbance in immunogenesis is the consequence of the development of a severe condition in the organism, i.e. it is because of the disturbance in the possible reaction to the antigenic stimulation. Depression of immunogenesis in this case appears not from the beginning of the reaction to any of these factors, but when serious pathologic changes develop in the organism. Consequently, the effect of radiation on immunogenesis may be specific not with respect to the end result, but with respect to its effect on a definite stage of immunogenesis and the character of this effect.

Thus, studies of radiation effect on active immunity may assist a deeper understanding of the stages of immunogenesis.

Further investigations in this region have to assist in obtaining new theoretical information and working out of practical measures for increasing the quality of active immunization in organisms subjected to the effects of ionizing radiation.

State of Passive Immunity in Irradiated Animals.

As seen from the above discussion, studies with respect to the effect of radiation on active immunity were begun a long time ago. But the state of passive immunity under the conditions of irradiation was neglected. The researchers ignored this question completely, unjustly. Meanwhile the vast use of the serotherapy and seroprophylaxis in the practical activities of the sanitation officers does not allow one to leave it unsolved.

The first report that we found in the literature accessible to us concerning the effect of radiation on passive immunity, goes back to 1944. These are the experiments of Naiman (271), which indicated, that if rats are infected with trypanosomes together with the injection of a specific serum within an hour following their exposure to 300-500 r, the efficiency fails to change. The author explains the almost complete absence of radiation effects on serotherapy by the fact that during this period (primary reaction) the macrophage system of animals has not yet changed, and it participates in the defense of the organism.

If the research work is presented in chronological sequence, then the experiments of one of the authors of this book (O. G. Alekseyeva) have to be mentioned. The experiments were started in 1948.

At the beginning of the experiments of O. G. Alekseyeva (5) only the investigations of Naiman were published. During the experimentation time two more papers appeared.

The investigations of Adler and Shechmeister (182) showed that after intraperitoneal injection of the toxin of the causative agent of gas gangrene (*Clostridium septicum*) on the 7th day following total body exposure to a 350 r dose, the LD_{50} decreased twofold as compared with the nonirradiated animals. Thus, the LD_{50} of nonirradiated mice was equal to 0.15 ml of the toxin, but was only 0.07 ml. in irradiated mice. Based on these data the authors assumed, that for protecting against a dose with a greater toxic effect more antitoxic serum is required. The experiments confirmed this assumption. The PD_{50} (prophylactic dose) of serum for nonirradiated mice amounted to 5.71×10^{-4} ml., but it increased to 1.97×10^{-3} ml. for irradiated mice.

The authors of the second paper Hale and Stoner (234) continued the investigations of the effect of radiation on the preventive effect of antitoxic serum after the animals were poisoned with toxins. They observed a depressing effect of radiation, at a dose of 750 r of γ -rays of a cobalt source, on the preventive properties of the antitetanus serum in mice poisoned by the toxin.

The other part of investigations of these authors are of great interest to us. These are the data with respect to a complete depression of the protective effect of serum in mice infected with pneumococci (type III) three days before the exposure to a dose of 625 r. First, these investigations indicate that the passive immunity created earlier, might become depressed under the effect of radiation. Second, the conditions of the experiment are more natural, because infection with a culture, not poisoning with a toxin, is used.

These two concepts are the basis of our own investigations (O. G. Alekseyeva, (5)).

1. To test the intensity of antidiphtheria immunity, even in cases in which antitoxin serums are used, the infection must be done with a living culture, not by poisoning with exotoxin. The method of experimental infection of animals with microbes monopathogenic for humans is rather artificial and there is no reason to complicate the conditions by a more unnatural effect: by a single injection of a large dose of toxin. Besides there is a vast literature indicating that immunity to diphtheria depends not only on the presence of antitoxins, but also on antibacterial factors of defense.

2. The reaction of antitoxic serums does not limit itself to the creation of an antitoxic immunity, but it depends much on the condition of the antibacterial defense mechanisms of the organism and assists their stimulation.

In the first series of experiments we investigated the possibility of creating passive immunity during the period of development of the clinical syndrome of radiation injury. Guinea pigs were exposed to X-irradiation at an $LD_{50/30}$ dose¹⁾, on the 4th-6th day they received the injection of 5000 AE anti-diphtheria serum and simultaneously or after 1-1½ hours they were infected sub-

1) Radiation dose which caused death of 50% of animals during 30 days.

In seven out of twelve control (nonirradiated) animals local purulent inflammatory reactions developed, but they recovered by the end of the observation period (one month). The remaining five animals endured the infection with no clinical phenomena. Two out of seven experimental guinea pigs (irradiated) died with symptoms of a developing diphtheria infection, four were sick and recovered and only one guinea pig failed to display any pathologic symptoms. To clarify the significance of some antibacterial mechanisms in the depression of passive immunity observed in irradiated animals, tests were made which aimed at studies of the intensity of the phagocytic in vivo reaction and the course of the inflammatory reaction to intracutaneous infection with the culture.

The study of phagocytosis was carried out on irradiated guinea pigs that were passively immunized according to the same scheme by means of an intraperitoneal infection with 3 bil. microbes. After 1, 2, 3, 4, 5, 6, 9, 12, 18, 24, 48, 72 and 120 hours, puncture samples extracted from the abdominal cavity were investigated microscopically.

The irradiated animals showed a later appearance of phagocytosis in the exudate (after 3-5 hours) as compared with nonirradiated control guinea pigs (after 1-3 hours), lower intensity (8 times lower), shorter duration of the phase of increase (up to 6 hours after infection; the observation was continued to 24-48 hours) and a more rapid cessation of this process (phagocytes were observed to disappear within 48 hours, while in control guinea pigs they were present after 72 hours). In consequence the sterilization of the abdominal cavity infected with diphtheria bacteria was retarded to the 5th day and more, while in nonirradiated animals it occurred within 18 hours and never later than two days.

The study of the inflammatory reaction to intracutaneous infection with 100 mil. of microbes in passively immunized animals was first carried out on guinea pigs. The irradiation and immunization conditions were as was indicated previously. Observation over four days failed to note any difference between the local reaction in irradiated (11 guinea pigs) and nonirradiated (7 pigs) animals. By forestalling objections, it should be noted that D. R. Kaulen (64) could not detect difference in the course of the intracutaneous reaction to the diphtheria toxin between irradiated and nonirradiated, passively immunized, animals. These observations allow one to conclude that it is not so much that depression of passive immunity after irradiation is pronounced, as that the macroscopic form of the inflammatory reaction to intracutaneous injection of diphtheria bacteria (or toxin) is changed, or that intracutaneous reactions in general do not reflect the intensities of passive immunity. But, perhaps, a more detailed examination could detect the difference?

Aiming at this goal, experiments were set up on rabbits. The scheme was identical to that for guinea pigs, but the intracutaneous reaction was initiated simultaneously on five sections of the body, and then after 1, 3, 6, 12 and 24 hours the inflammatory loci were excised for histological investigation. On the fifth day following exposure to a sublethal dose of X-rays, 7500 AE of serum, and, after 1½ hours, 100 mil. of microbes per sample, were injected.

The nonirradiated rabbit displayed an intense inflammatory reaction with edema, hyperemia and local leucocytosis. Bacteria were not observed in slices of the inflammatory loci by six hours following infection. The scars after biopsies healed by primary intention.

The intensity of edema, hyperemia and especially that of local leucocytosis was less pronounced. The bacteria were determined in cuts at all observation times. The scars after biopsy became suppurated and healed by secondary intention.

Thus, the experiments performed provide a conclusion that on the 5th day of radiation sickness, even after the injection of increased doses of antitoxic antidiphtheria serum, the organism is not capable of mobilizing antimicrobial defense mechanisms. But certainly the antitoxic mechanisms play a great role in antidiphtheria immunity. What happens to them? How will immunity develop after the injection of minimal serum doses? Our experiments did not clarify these questions, but they allowed one to ascertain whether resistance to diphtheria bacteria in irradiated animals is maintained after the injection of serum. However, they failed to discover all the mechanisms of this phenomenon.

After the completion of our work in the laboratory of V. L. Troitskii, D. R. Kaulen (64) carried out studies of antitoxic mechanisms of antidiphtheria immunity by injecting 10 AE to guinea pigs at different times after exposure to 150 r. Within 48 hours following the injection of serum the animals received 30 Dlm of diphtheria toxin each. Under such an experimental arrangement, almost all nonirradiated guinea pigs survived, and 0.16 AE of antitoxin was found in their blood. Almost all irradiated animals (the experiment was arranged within 1-7 days after exposure) died, in spite of the fact that their blood also contained 0.16 AE each. In a special series of experiments it was found, that after passive immunization the irradiated and control animals contain antitoxin in identical titers.

The author comes to a conclusion similar to ours: the state of reactivity of the organism, not the amount of antitoxin, plays the chief role in the development of passive immunity in irradiated animals. But how is this to be coordinated with the data of Adler and Sheckmeister (1.82) with respect to the dependence of efficiency of passive immunity on increased sensitivity of the organism to toxin? The point is, that there is no contradiction. The dynamics of formation of passive antibodies do not change or change insignificantly, which was confirmed also by the investigations of Hollinsworth (242), Bukantz, Dixon and Darmin (195). But the biological effect of serum as the stimulant of defense mechanisms decreases, because the biological effect of the toxin dose increases because of the increased sensitivity of the irradiated organism. At the same time the organism injured by radiation sickness reacts less to the injection of a serum dose, effective previously. This is completely understandable, because the mechanisms of natural nonsusceptibility to microbes are damaged (see Chapter 2). However, the concept of passive immunity as an active process of the macroorganism, in which all mechanisms of nonsusceptibility to microbes participate, lately is shared by the overwhelming majority of investigators; in our opinion, this concept does not require any references from the literature, the more so since the experiments with irradiated animals once more confirm this statement.

In the second series of experiments we worked at clarification of the problem of the effect of radiation on the formed passive immunity. The experiments were carried out on guinea pigs, immunized with antitoxic purified and concentrated serum of diaenzyme -2 and diaenzyme - 3 at doses from 800 to 5000 AE. After 3-4 hours, part of the animals were exposed to X-rays on the RUM-1 apparatus at doses of 500, 300, 195 and 125. Thereafter at different times of the radiation process the animals were infected subcutaneously with living virulent culture (stock No 144, gravis type). Weight, leucocyte count, body temperature, state of the local inflammatory reaction, and general behavior of animals were observed; the development of diphtheria paralysis was taken into account. The

pathomorphological picture was described in dead animals, and cultures from blood of heart, liver, spleen, kidneys and lungs were made. Film preparations and smears--imprints of the subcutaneous cellular tissue from the infection site--were prepared. Altogether 218 guinea pigs (including the controls) were used. In 18 of them the phagocytic reaction and the seeding of internal organs during the intraperitoneal infection were studied.

One may see from Table 11 that the survival rate depends on all three effects (immune, radiation and infecting). At lethal radiation doses regardless of the dose of infection there are almost no survivors, despite the fact that the doses of serum are completely effective for nonirradiated guinea pigs. Thus, in this case the loss of the animals is due to the radiation effect.

Table 11

Effect of radiation on the survival of passively immunized guinea pigs after their infection with diphtheria culture.

Dose of serum in AE	Radiation dose in r	Day after irradiation on which infection occurred.	Dose of infection in Dlm	Irradiated		Nonirradiated	
				Total	Died--were sick-recovered	Total	Died--were sick-recovered
5 000	500	Immediately and after 1 day	2	8	8-0-0	8	0-0-8
1000-800	300-195	Immediately	<1	14	12-2-0	6	0-2-4
800	125	Immediately		14	7-5-2	7	0-2-5
1 000		After 3 days	2	14	9-3-2	6	1-0-5
5 000		After 7 days	2	13	3-7-3	8	1-3-4
5 000		After 12 days	2	14	10-3-1	7	4-3-0

But if a sublethal radiation dose and infection with 2 Dlm are used, then the depression of passive immunity depends on the phase of the immune and radiation processes.

During the first three days after the injection of serum the immunity of nonirradiated animals is rather intense.

The number of nonsurvivors among the irradiated guinea pigs was highest in the groups in which injection of the culture was carried out on the third day following irradiation, as compared with infection done simultaneously with irradiation; i.e. in the present case we see an example of the fact that against the background of an intense immunity the death rate depends on the phase of radiation sickness. Well, in fact, in a simultaneous infection the first <<meeting>> of the organism with a microbe occurs against the background of a nondepressed reactivity, and the <<breakdown>> of immune mechanisms begins only in the process of the development of infection. But if the infection is carried out on the third day of radiation sickness, the microbe meets an altered organism from the very beginning.

Infection on the 7th day of radiation sickness was endured by experimental animals relatively easily, at any rate better, than by the guinea pigs of the preceding experimental groups, although in nonirradiated animals

at this period the serum is already less effective. Evidently, this may be explained by the fact that on the 7th day at a dose of 125 r the phase of repair of radiation sickness begins. The activation of compensating mechanisms of the organism made the fight against the microbes more effective, although the comparison with the corresponding control, nonirradiated, group indicates, that a full effect of serum was not reached in irradiated animals.

Infection on the 12th day induces the highest death rate, but here is another cause of death. In this case the mortality depends on the phase of the immune process. The results obtained in the control group indicate a sharp decrease in the stage of passive immunity at this time.

The results of studies of the clinical features of the pathological process, and especially analysis of the cause of death of the experimental animals, indicate, that it is not always correct to speak of a decrease in passive immunity. The irradiated animals develop such a complicated symptom complex that often it is hard to decide whether development of infection due to the depression of the defensive effect of serum took place, or the immunity was high, infection was absent, and therefore, radiation sickness was the only cause of death. In Table 12 analysis of the character of this process is attempted in dead animals. This table shows that only 22% of irradiated animals die of the typical diphtheria infection accompanied by sepsis, whereas this is usually observed in the group in which the passive immunity was very weak and in the control group. More frequently (49%) the combination of diphtheria infection with radiation sickness takes place. In part of the guinea pigs (20%) no symptoms of infection could be seen. If exposed to 500 r, then the animals die only of radiation sickness, because the infection does not develop in them.

Table 12 (See following page).

The absence of the infection process in these animals may be explained in two ways: either passive immunity is maintained and is effective, or even at its depression, infection does not develop in consequence of the unresponsiveness of the organism exposed to a lethal dose. But after exposure to 125 r, radiation sickness is provoked by the infection, because this radiation dose is not lethal for normal animals.

And, finally, death could result from endogenous sepsis explained by autoinfection, which develops at late times in animals weakened by the preceding pathologic processes.

To clarify the mechanism of the decrease in the effect of serum prophylaxis of diphtheria, experiments were carried out in order to study the course of the phagocytic reaction in vivo and the seeding of the organs after subcutaneous infection. The technique of the arrangement of the experiment was similar to that of studies of the survival after the exposure to 125 r.

Within 3 days, the irradiated animals develop on the infection site vast infiltrates with a central necrosis. Necrosis failed to develop in non-irradiated guinea pigs, and the slight infiltrates are resolved completely by the 7th day after infection.

It is seen from Table 13 (in which average data are presented) and the microphotographs of film preparations (Fig. 12 A and B), that the phagocytic reaction of irradiated animals proved to be very depressed; this led to a more massive accumulation of diphtheria bacteria in the infection site.

Table 12

Dependence of the causes of death on radiation sickness, infection and state of passive immunity.

Dose of serum in AE	Radiation dose in r	Day after irradiation on which infection occurred	Infection dose in Dlm	Total number of animals	Number of deaths	Number of animals	Radiation sickness days after the infection	Radiation sickness + diphtheria infection		Diphtheria seris number of animals	days after infection	Endogenous infection after recovery from radiation and infection.
								number of animals	days after the infection			
5000	500	Immediately and after 1 day	2	6	8	7	8-11	1	5	--	--	--
1000-800	300-195	Immediately	41	14	12	3	8-14	10	8-14	1	2	15
800		Immediately		14	7			4	8-14	--	--	--
1000		After 3 days		14	9			7	8-9	1	1	21
5000	125	After 7 days	2	13	3			2	9-20	--	--	20
5000		After 12 days		14	10			--	--	9	1-14	30
5000-800	500-125	Immediately after 12 days (total)	2-1	78	19 (6%)	20 (26%)	8-11	24 (49%)	5-20	11 (22.4%)	1-14	15-30 (8.2%)
5000-800	--	---	2-1	35	6 (17%)	--	--	--	--	5	3-35	24

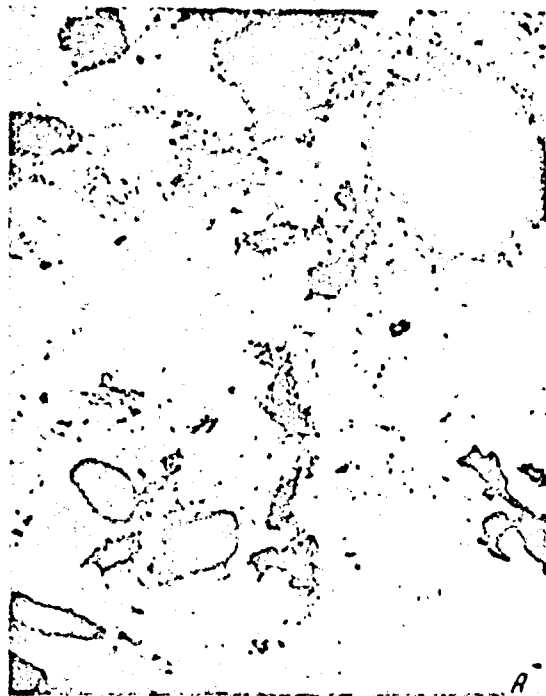


Fig. 12A. Film preparation of subcutaneous cellular tissue from the infection site of a passively immunized irradiated guinea pig 21 hours after infection.

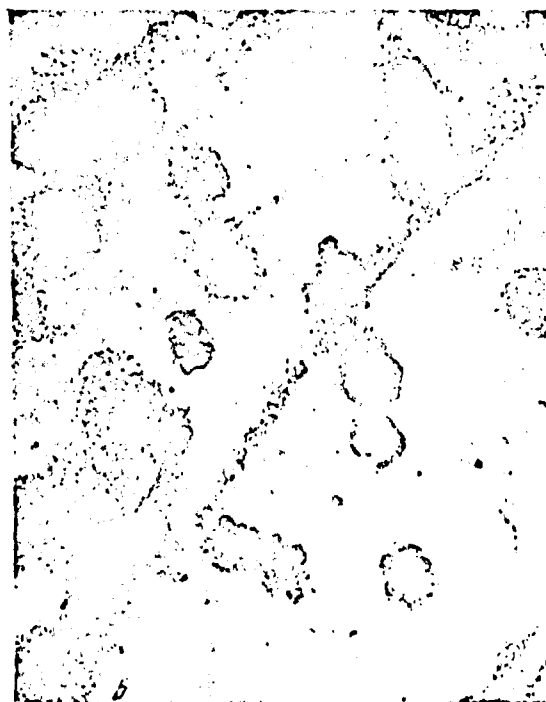


Fig. 12B. Film preparation of subcutaneous cellular tissue from the infection site of passively immunized nonirradiated guinea pig within 21 hours after infection.

Table 13

Course of the inflammatory reaction in passively immunized guinea pigs after spontaneous infection with 2 Dlm within 3-7 days following exposure to a dose of 125 r.

Factor	irradiated			nonirradiated		
	days after infection			days after infection		
	21 hrs.	3 days	7 days	21 hrs.	3 days	7 days
Appearance of inflam. reaction.	Sharp edemas, hemorrhages	Infiltrate 5x x 5 cm, necrosis in the center	Infiltrate 5x x 5 cm, necrosis	Slight edema	Infiltrate 1 x 1 cm	Traces of infiltrate
Number of phagocytizing leucocytes in 10 visual fields. 1)	12	26	2	15	53	200
Number of free nonphagocytized bacteria in 10 visual fields.	130	90	50	10	70	40

1) average data of 10 animals.

Examination of the results of the analysis of organs for the presence of diphtheria bacteria showed some tendency to seeding of these with bacteria in irradiated animals (Table 14), however, the extent of bacteriemia in both groups was identical.

Table 14

Distribution of diphtheria bacteria in the organs of passively immunized guinea pigs after exposure to 125 r.

Animal group	Total number of culture from each organ	Number of positive results			
		blood from heart	liver	spleen	kidney
Irradiated	9	2	6	4	2
Nonirradiated	9	2	5	2	5

One can find bacteria in the kidneys of nonirradiated animals more frequently, which might indicate their excretion from the organism in urine. In irradiated guinea pigs the bacteria more frequently are retained in liver and spleen. But these conclusions can be only hypothetical, because there are only a few observation data.

We examined the effect of infection on the end result of radiation sickness, but, perhaps, the preliminary injection of serum also affects it. To solve this problem, special experiments were set up. After exposure to a dose of 195-300 r, 9 noninfected guinea pigs died and only one survived. After a preliminary injection of 800-1000 AE of antidiphtheria serum identical

doses killed only four out of eight animals. Graham J. and R. (231), and also Stroud, Bruce and Summers (309) have reported on the favorable effect of preliminarily injected serums and plasmas on the course of radiation sickness of different animals.

In summarizing the results of the experiments of the second series it should be noted, that even in cases in which animals with a firm, formed passive immunity are irradiated after infection, a severe pathologic process can develop, frequently with a lethal end result.

Several papers on studies of the effect of radiation on passive immunity, chiefly after the injection of the serum at a time which was very close to the radiation time, were published last year.

Only P. N. Kiselev and E. V. Karpova (75) have studied the effect of antitoxic serums at different periods of radiation sickness. But the faults of many papers were characteristic of their investigations--the efficiency of the immunity was not checked after infection with the culture, but by poisoning with the toxin. The authors showed on a huge number of animals (2000 mice), that neutralization of the effect of the specific toxin requires 2-3½ times more antitetanus or antigangrene serum if it is injected 24 hours before the poisoning with toxin, and within 24 hours, 6, 14 or 17 days after irradiation with 438-500 r. By the 30th-40th day of radiation injury the efficiency of passive immunization is restored, which agrees completely with the data of these authors on the normalization at this time of the reaction of the organism to toxin (74).

Thus, P. N. Kiselev and E. V. Karpova revealed some degree of decrease in the efficiency of serum prophylaxis even on the first day of radiation injury. G. N. Kryzhanovskii and N. N. Lebedeva (92) using a model of local tetanus intoxication (only intoxication, not infection!) failed to note changes in the effect of serum prophylaxis within the first day following exposure of rats to a dose of 400 r. However their experimental conditions were quite different--the radiation dose was lower (rats are more radioresistant, than mice),--and the toxins and serums were injected directly after irradiation. The therapeutic effect of serum under these experimental conditions did not change.

The experiments of R. V. Petrov (124) performed with the goal of studying the effect of radiation on antitetanus and antigangrene serum therapy have to be recognized as more methodically correct.

In his investigations R. V. Petrov did not use poisoning with toxin, but infection with corresponding microbes. The state of immunity was studied by means of infection only within the first day following irradiation.

Antitetanus immunity was studied on 200 mice. Irradiation was carried out with a dose of 367 r of γ -rays from a cobalt source with a power of 234 C. The infection was done with 0.1 ml. of daily culture (dilution 1:50), mixed in an equal amount with 10% of calcium chloride, 10 hours after irradiation. Immediately after infection part of animals received antitetanus serum of diaenzyme--3 at doses of 125 and 375 AE. The radiation dose was sublethal (all 50 mice survived), all control (for infection) animals died on the 6th day (Fig. 13).

The injection of 125 AE of serum in nonirradiated mice prevented death in 32% of the animals, but if the mice were exposed preliminarily to 367 r, then 26% survived, and their mean life span became shorter by 2-3 days. A question arises, whether the depression of passive immunity can be

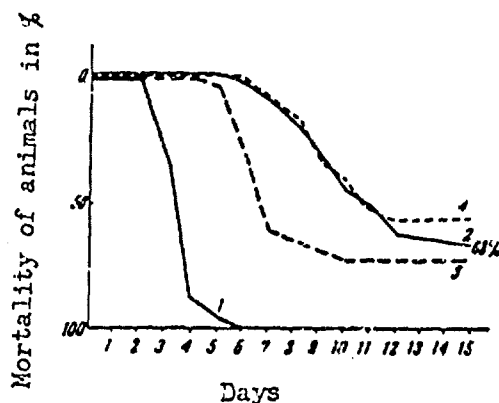


Fig. 13. Effect of radiation on the efficiency of serum prophylaxis of tetanus infection in mice.
 1-infection control; 2-control of the protective effect of a dose of serum;
 3-irradiation and infection by using one dose of serum; 4-irradiation and infection by using three doses of serum.

compensated. All investigations of the preceding authors showed convincingly that even at a significant depression of the passive immunity in the organism compensating possibilities are preserved. This conclusion may be made if only on the basis that part of the animals always maintain immunity, but the study of phagocytic and local reaction in passively immunized animals also displays a nonidentical degree of depression in various individuals. If the natural compensating possibilities are maintained in an irradiated organism, then one may expect more effect from the use of special methods which increase the specific resistance. An increased serum dose could be one of those methods. Adler and Shechmeister (182) have proven that this is an effective method in the case of poisoning with toxin. We also succeeded in showing its value after infection with the culture (Fig. 13). Thus, the irradiated mice, that received a triple dose of serum (375 AE) survived in 43% of cases, i.e. the percentage was even higher than that of nonirradiated mice that received one dose of serum.

These experiments allow one to conclude, that if the animals are infected with a culture of tetanus viruses, then the therapeutic effect of the serum decreases with the first days following irradiation.

The depression of efficiency of serum in poisoning with tetanus toxin within the first days of radiation injury was noted also by P. N. Kiselev and E. V. Karpova (75), that in poisoning with diphtheria toxin by D. R. Kaulen (74), in the infection with diphtheria bacteria by O. G. Alekseyeva (5). Why is serum ineffective in these cases, although infection occurred at the period at which the mechanisms of natural immunity had not yet broken down? Evidently, the fact is that a prolonged time covering also the beginning of the period of development of radiation sickness, i.e. the period, at which the mechanisms of immunity are subjected to significant damages, is necessary for stopping the infections indicated.

According to such a viewpoint one would expect that in case of the development of infection which is stopped by the injection of therapeutic serum during 2-3 days, there will be no decrease in the efficiency of its action under the given experimental conditions. This fact, evidently, explains the results obtained by Naiman (271) with trypanosomiasis.

Our own data (R. V. Petrov, (124)) confirm this conclusion. The efficiency of therapeutic use of 160 AE of antigangrene serum (diaenzyme-3) was studied on 55 guinea pigs by infecting the animals intraperitoneally with 0.6 ml. of daily culture of *B. perfringens* mixed with 10% of calcium chloride within 10 hours following exposure to 367 r. One may see in Fig. 14 that by the 4th day, 100% of the control (on infection) animals were dead; they displayed typical phenomena of gas gangrene. If the infected animals received a specific serum, then over the period from the 8th through the 14th day, 20-30% died of the second pus-producing infection. But if the guinea pigs were irradiated preliminarily, then over the period mentioned above, 90% of the animals died having typical symptoms of radiation sickness. One fails to note any of the clinical syndrome of gas gangrene in them. Consequently, the serum is effective, but since the radiation dose is lethal, the animals surviving the infection die of radiation injury (at identical times and in identical percentage to those of the control animals after irradiation only).

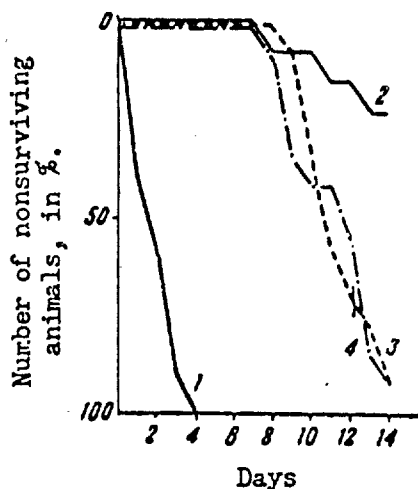


Fig. 14. Effect of radiation on the efficiency of serum prophylaxis of gas gangrene in guinea pigs.

1-control of infection; 2-control of the effect of serum; 3-control of irradiation; 4-irradiation and infection with the injection of serum.

On completion of the data with respect to studies on the effect of radiation on passive immunity one more experiment has to be mentioned.

B. N. Sofronov (153) on a model of focal pertussis infection has studied the effect of exposure to 400 r on the efficiency of serum therapy and serum prophylaxis. The author succeeded in detecting the fact that increased numbers of bacteria can be found in lungs of irradiated animals more frequently in comparison with the serum controls. However, the efficiency of serums in these animals decreased slightly, and the number of microbes in the lungs always was much lower than that in normal mice that did not receive serum. An insignificant depression of the efficiency of immunization after irradiation is entirely understandable, because the serum was injected either four hours before irradiation, or within twenty days after it (the phase of reparations).

Thus, in summarizing all investigations presented it should be noted that in using serum prophylaxis or serum therapy of the infections of irradiated animals, or in the case of a supposed irradiation, one might

expect a decrease in the efficiency of these measures. However an increased serum dose is still capable of compensating the harmful effect of radiation injury. Besides this, compensation of the decreased effect of serum therapy and serum prophylaxis can be achieved by using other therapeutic means, for instance, antibiotics (see experiment of R. V. Petrov in Chapter 5).

What determines the depression of the effect after the injection of antitoxic and antimicrobial serums in irradiated animals? It follows from the analysis of our own and the literature data, that this depends on the change in reactivity of the organism, on depression of natural immunity and on increase in the number of microbes in an irradiated organism. The time of circulation in blood and the rate of excretion of the antibodies evidently do not change (195 and 242).

On closing it should be said, that the state of passive immunity in radiation sickness is a rather poorly studied chapter of the immunology of an irradiated organism and it requires further detailed investigation.

Chapter 4

Peculiarities in Allergic Reactivity of Irradiated Organisms.

Determination of the peculiarities of the allergic reactivity of an irradiated organism, the least studied section of radiobiology, requires further investigations, analysis of the data obtained and theoretical generalizations.

The existing data concerning this question represent only the beginning of work in this region, which, in our opinion, is one of the most important in relation to the understanding of the nature of radiation injuries.

The difficulties in the investigation of the peculiarities of the allergic reactivity of an irradiated organism lie not so much in the region of accumulation of factual material, but in understanding of the nature of these processes and explanation of their role in radiation sickness.

The great importance of allergic phenomena is confirmed by the data on the clinical study of radiation injuries.

Thus, for instance, from a detailed description of the clinical syndrome of sickness induced by ionizing radiation it is apparent, that many of the symptoms are very similar to the phenomena characteristic of allergic states: decreased body temperature or fever, a decreased complement titer, leucopenia, delayed coagulability of blood, appearance of hemorrhages on skin and in internal organs, disturbances in the function of the gastrointestinal tract, the presence of a latent period and so on.

In addition to this, it is well known that many therapeutic means effective in the cure of radiation sickness are referable to desensitization therapy.

All these data forced many authors to assume a probable role of allergic phenomena in the pathogenesis of radiation sickness. However, there was no uniform opinion on the question of the nature of the sensitizing agent causing this state. Some assumed the possibility of sensitization of the organism with the bacteria of autoflora, which accumulate greatly in an irradiated organism (32), others suggested sensitization by the proteins of foodstuffs absorbed in unchanged form due to an increased permeability of the intestines (67), a third group pointed out the possibility of autosensitization by the disintegration products of tissues (Cronkite, 207). In fact, Cronkite considers the latter process scarcely probable, since it is known that allergy is connected with the formation of antibodies, but in an irradiated organism this process is damaged to a large extent. However, all these assumptions were expressed on the basis of theoretical discussions and were not supported by experimental data. Thus, there was a necessity for experimental solution of

any questions connected with allergy in irradiated organisms. Since it is obvious that allergic phenomena take place in the development of radiation sickness, then one may assume, that the investigation of allergy problems in this sickness is valuable for understanding of its pathogenesis and etiology. Investigation of the problems of pathogenesis of radiation sickness is the central problem of radiobiology, because understanding of nature of the processes determining the development of the sickness will permit one to justify the use of the corresponding therapeutic and prophylactic measures, and it will enable one to work out diagnostic methods for recognition of the sickness even in the absence of expressed clinical symptoms.

Besides its great theoretical value, the study of the allergic reactivity also has clinical importance. The problems of complications after the injection of serums (serum sickness and anaphylactic shock), the possibility of diagnosis of infectious diseases from cutaneous and eye allergy tests, the peculiarities of the development of idiosyncrasies to food and therapeutic substances and the like, are connected with allergies. Almost none of these phenomena are studied under the effects of ionizing radiations, and closing of this gap is an urgent problem of our scientists.

An irradiated organism continuously interacts with various heterologous sensitizing agents, as for instance with foodstuffs and microorganisms of the autoflora and environment. It was natural to assume that an increased absorption of such allergens in the presence of an increased permeability of the intestines of an animal with radiation sickness (67) can play a role in the development of allergic symptoms in this sickness. However, many facts contradict these assumptions and make it doubtful that food and bacterial allergens participate as compulsory pathogenic agents in the development of radiation sickness, but at the same time they do not deny their possible aggravating additional effect on the course of the basic pathology.

It is well known that the accumulation of large masses of bacteria in the cavities and tissues of an irradiated organism does not occur on the first days, but chiefly at the end of the first week (at a total-body exposure to lethal doses). Consequently, the development of primary changes--the reaction of the organism during the latent period and at the beginning of the sickness--cannot be attributed to bacteria, whose accumulation and distribution in the organism, evidently, is not the cause, but a consequence (and complication) of radiation sickness. It has been found, that development of typical radiation sickness and death of an organism may occur with neither bacteriemia nor accumulation of bacteria under the effect of high radiation doses (274). And finally, the unsuccessful efforts at immunization against the bacteria of autoflora (301), the experiments of V. F. Sosova (150), who revealed a decrease in the reaction (not an increase, as in the case of sensitization) of irradiated rabbits to the injection of a suspension of killed coliform bacteria under the skin, and also the impossibility of passive transfer of characteristic peculiarities of the reactivity of an irradiated organism allow one to conclude, that bacteria are not the allergens which determine the characteristic allergic manifestations of radiation sickness.

Only one paper (P. N. Kiselev) reports on foodstuffs. Guinea pigs received an oral administration of horse serum after exposure to 1-2HED¹⁾ of X-rays. The checking of the development of sensitization was carried out in in vitro tests with slices of intestines and uterus by addition of the same serum. The author obtained contraction of slices of these organs in response

1) HED is the cutaneous erythematous dose, a unit, which was used in previous papers; it is equal to 500-600 r.

to the addition of horse serum and he evaluated it as an indicator of sensitization. However, he failed to carry out the controls which were so important in these interesting experiments. Lack of controls made the conclusions unreliable. It was necessary to prove the specificity of sensitization by adding other heterogeneous and homologous protein extracts.

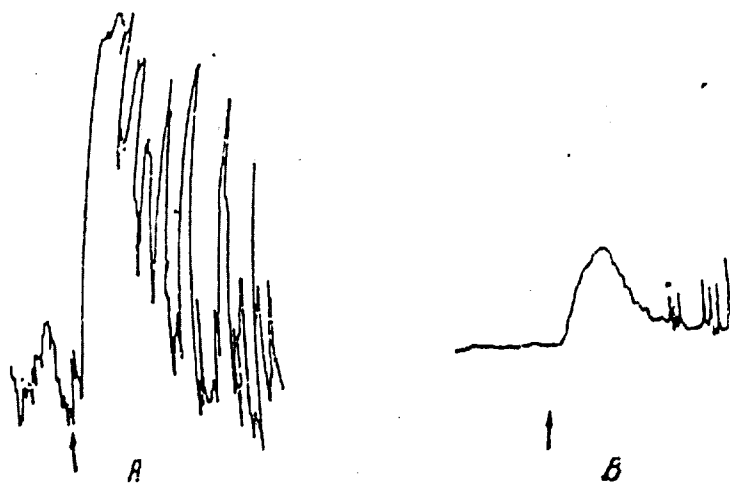


Fig. 15. Reaction of isolated intestine of irradiated (A) and nonirradiated (B) guinea pigs to horse serum (arrows show the time of injection of serum).

The experiments of our co-worker G. M. L'vitsina indicated that the reaction described did not reflect sensitization to horse protein, because it was found that the organs of irradiated animals in *in vitro* tests always react to by contraction to heterogeneous as well as homologous proteins. Obviously, the state of increased sensitivity to disintegration products of autotissues which develops in an irradiated organism is accompanied by a nonspecific reaction to heterogeneous proteins.

Examples of reaction of the intestines of irradiated (the 7th day following an exposure to 500 r of X rays) and healthy guinea pigs to the addition of an extract from intestines or liver of the same pig, followed by the extract of the intestines of a healthy pig or horse serum to the liquid which washes the preparation, are shown in Figures 15, 16 and 17.

In contrast to the intestines of a nonirradiated guinea pig the tissues of an irradiated animal display a hyperergic reaction to heterogeneous and their own protein products.

It is known that the character of the food exerts great influence on the course of radiation sickness and on the development of allergic processes (65). However, it is not an etiologic factor, because radiation sickness is reproduced independent of the character of the foodstuffs introduced in the organism, and it may develop even under conditions of complete starvation. As was found by Allen (183), starving even increases the radiosensitivity of animals to local and total-body irradiation.

All these data do not permit assumptions of absorption of heterogeneous food proteins and microorganisms as causes for development of allergic phenomena in radiation sickness.

What is the nature of the allergens that determine the allergic reactions in radiation sickness? In addition to the similarity between symptoms of the allergic states and radiation sickness listed by us, serious

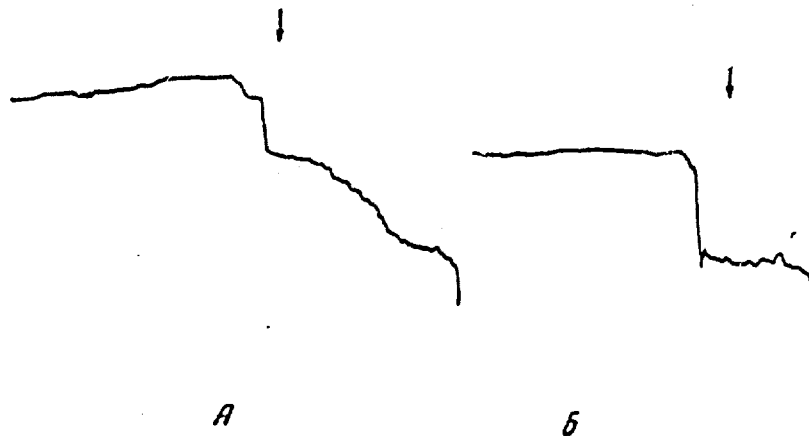


Fig. 16. Reaction of the isolated intestine of a nonirradiated guinea pig to the liver extract of another nonirradiated pig (A) and to the extract of its own liver (B) (the time of injection is denoted by arrows).



Fig. 17. Reaction of the isolated intestine of an irradiated guinea pig to the extract of its own liver (the time of injection is denoted by an arrow).

attention must be paid to a phenomenon, study of whose nature could assist the investigation of the nature of the allergens that play an important role in the effect of ionizing radiation.

A peculiar change in the character of the inflammatory focus (attained by intracutaneous injection of a suspension of living culture of coliform bacteria) in rabbits exposed to X-irradiation at a dose of 1100 r has been described by V. F. Sosova. Instead of the slight hyperemic infiltrate characteristic of the control animals, the formation of vast infiltrate with necrosis and a compact hemorrhagic site reminiscent of the manifestation of the Schwartzman phenomenon has been observed. This means that a hyperergic reaction develops. The injection of killed bacteria fails to display such a picture; it induces a response even smaller than that of the control group. If this hyperergic reaction were the result of a developing increased sensitivity to the autofloral bacteria, then this allergy would be displayed also after the injection of killed microbes and their disintegration products.

Thus, in this case it was not the presence of the microbe that was important, but the fact of its action on the tissues of an organism, which results in death and destruction the cellular elements. The formation of decomposition products of tissues in the inflammatory focus could be the

<< decisive >> factor, and in the presence of an increased sensitivity to tissue products it could explain the development of a local hemorrhagic reaction.

It is of interest that such a reaction develops only after infection at a certain period (not sooner than within 3 days) of radiation sickness, which might be explained by the necessity of a definite period of time for the development of autosensitization. It is well known, that antibodies to denatured protein in irradiated animals first appear at this time (72 and 110).

The necrotic-hemorrhagic reaction to the injection of autoflora bacteria, described by V. F. Sosova, is connected with a definite segment of the body (side surfaces of the body) and it fails to show in ear tests. Similar zonal manifestations of allergic reactions of the skin that covers the injured organ, are described by E. B. Halfen (168) in brucellosis.

In her search for allergens suitable for the manifestation and maintenance of a state of homoallergy N. N. Klemparskaya has carried out experiments on rabbits by injecting the suspensions of homologous tissues in nonirradiated and irradiated animals. These experiments showed that the state of increased sensitivity and hyperergic local reaction with necrosis and hemorrhagia can be initiated by repeated injection of these substances in healthy animals. In addition to this it developed that homosensitization changes the sensitivity of animals to ionizing radiation.

Thus, for instance, a single cutaneous injection of a suspension of mucosal tissue of the small intestine or liver of a rabbit fails to cause any local changes, except for a mild hyperemia and slight diffuse edema, which disappear within 24 hours. After repeated injections of homologous tissue, if injected in sites unusual for its presence, as for instance, into skin, the appearance of necrosis and hemorrhages on the site of injection, decrease in weight of the animal and disturbances in the functions of intestines are observed.

The injection of suspensions of homologous tissue into irradiated animals caused accelerated mortality; death occurs within 12-18 hours after the injection of tissues on the 2nd-3rd day following exposure to X-rays at a dose of 1100 r; usually this radiation dose causes death on the 6th-8th day. It should be noted that the amount of tissue products and their absorption rate, evidently, are of importance. Thus, for instance, the injection of living bacteria into the skin of irradiated rabbits initiates a slow, gradual appearance of a small quantity of tissue decomposition products, sufficient for the development of local hemorrhagic reaction, but it does not cause (as it has been observed after injections of tissue suspensions) more rapid death of the irradiated rabbits. These data reveal the importance of thorough studies of the value of auto- and homosensitization in the pathogenesis of radiation sickness.

Autosensitization, as is known, is understood to involve the sensitizing effect of tissue proteins of the same organism. The possibility of such an effect became obvious only because of the experiments and teachings of I. I. Mechnikov (106), which showed, that under definite conditions the substances of the organism itself can be antigens and cause the appearance of antibodies, cytotoxins, which exert a destructive effect on tissue and function of an organ, and from which immunizing substances were obtained. Isospermotoxins and autonephrotoxins were described by him. Simultaneously the cytotoxins, obtained by means of heteroimmunization, i.e. immunization by the tissues of animals of different species, leucocytotoxins, cytotoxins to cerebral tissue, spermocytotoxins and others were studied by I. I. Mechnikov

in detail. It has been found that these antibodies specific to a certain tissue possess a capacity for disturbing significantly the function of definite organs and for causing death of animals. Thus, the investigations of I. I. Mechnikov provided the foundation for studies of the cytotoxin effect of antibodies, and showed that the capability of an organism to produce specific antibodies developed during the process of evolution. The production of antibodies, as a rule, is defensive in character, but under certain conditions (when the tissues of organism are antigens) the antibodies turn from a defensive to an opposite role and become a cause of serious disturbances in the function of organs and even of death of the organism.

Investigation of heterotoxins obtained by the immunization of animals by tissues of another species stimulated further development of the knowledge with respect to the reaction to antibodies in the tissues of an organism. It developed, that under certain conditions of exposure at low doses, cytotoxins may exert a therapeutic effect on many physiological processes (16). Numerous experiments which we do not intend to discuss in the present paper, are dedicated to investigation of this problem and to testing of the therapeutic cytotoxin serum of A. A. Bogomolets. However, the studies of autotoxins after I. I. Mechnikov proceeded very slowly and interest in them has been restored only lately, in connection with many experiments which showed that absorption of the decomposition products of intrinsic tissues (for instance, in trauma, breaking up of a tumor, necrosis of tissues because of bacteria, effect of fetus and so on) can cause severe pathologic states in the organism. At the 13th All-Union Conference of microbiologists, epidemiologists, hygienists and infectionists, in reports of many scientists, serious attention was paid to this problem. Many interesting data were presented which showed a fruitful creative development of the work of I. I. Mechnikov in the region of auto-allergy in our country. N. N. Zhukov-Verezhnikov (46) reported creation of a new medical-biological pathway in the science of << noninfectious immunology >>, which includes the problems of immunology in mutability of organisms, embryogenesis, immunohematology, immunology of tissues (transplantation questions) and blood compatibility, immunology of radiation sickness and burns.

Investigation of these various problems, which have great theoretical and practical value, led to establishment of a series of new methods and required the working out of a detailed classification of the antigens of cells and tissues of the organism, which has been reported.

V. I. Ioffe (59) emphasizes the importance of clinical immunology, which permits studies of pathogenesis of the sickness and reactivity of the organism at different periods during the course of the sickness (nonspecific immunology). The report of P. N. Kosiakov (88) was dedicated to the questions of noninfectious immunity in connection with studies of compatibility of tissues, immunobiological relationships between the mother and fetus, and effects of tumors and other factors on the organism.

One of the conditions for the appearance of the autosensitizing reaction against substances of the same organism proved to be the decomposition of tissues caused by different reasons; it leads to a partial denaturation of proteins and absorption of the mass of substances in blood stream, where proteins of the given organs do not enter. It has been found, that even with no significant denaturation, repeated injections of homologous cerebral tissue cause encephalomyelitis in guinea pigs, rats, dogs, mice and monkeys (268, 273, 249, 254, 316, 322 and others). These data assist in explaining the appearance of pathologic phenomena of the nervous system which sometimes complicated antirabies vaccinatioous (101, 86). After a single subcutaneous injection of

homologous testicular suspension or sperm into guinea pigs and rats, aspermia was attained with atrophy of testicular tissue (215). Homologous extract of cardiac muscle injected subcutaneously in the experiments of Muth (270) induced the development of myocarditis, and the injection of homologous kidney tissue (199) led to the development of glomerulonephritis. Data of the experiments of V. R. Khesin (170) and I. S. Ginzburg (26) verify the important role of autoallergy in sicknesses submitted to surgical treatment.

Vascular reactions determined by autosensitization of an organism in pregnancy are described by P. D. Gorizontov and D. S. Shliapin (34).

Questions connected with the studies of immunohematologic reactions, concerning which a vast literature is presented in the review of Dausset (208), arouses special interest. It was established that autoantibodies to the formed elements of blood are present in many sicknesses of hematopoietic organs (anemia, leukopenia, thrombocytopenia).

Tests on animals and even observations on humans showed that the injection of serums containing such antibodies initiates the development of corresponding changes in the blood picture.

Summarized data on numerous experimental works in the area of auto-sensitization are presented in several review articles (142, 318, 285). Clinicians have paid serious attention to the questions of autoallergy in the pathology of internal sicknesses, also (155, 250, 321, 282).

The phenomena of autoallergy which appear as a result of reaction between physical factors and the organism are as yet insufficiently studied. We know only the experiment of Karady (248), which showed that even such a factor as heating of the intrinsic serum of animals to 56-58°, or cooling it to -5° for 1½-2 minutes, creates a state of sensitization after its injection into guinea pigs. Repeated injection of such a serum within 2-3 weeks leads to the development of shock (in the absence of << cross >> reactions to the injection of an improperly prepared serum). Such data can explain the appearance of nettle rash in humans and allergic disturbances after the cooling of their bodies. The effect of radiant energy, as sunlight, ultraviolet rays and ionizing radiation, also pertain to physical factors. However, there are only a few experimental works devoted to studies of the autosensitizing effect of these factors. Allergies caused by the injection of other heterogeneous proteins in irradiated animals have been studied much better.

There are several methods for solution of the problem concerning the presence or absence of autosensitizing effect of ionizing radiation. Since the process of allergenicity is connected with the interaction between antigens and antibodies, it should be found, first, whether there are any antigenic peculiarities in the tissues of irradiated organisms, and, second, whether there appear antibodies to the tissues of the same organism. On the basis of the published data both questions can be answered in the affirmative. The appearance of antigenic peculiarities in the tissues of an irradiated organism, and production of antibodies in it, which react with denatured (by any method) proteins of the organism have been established, too. The establishment of these important facts required the use of new, unique investigational methods.

R. V. Petrov and L. I. Il'in (126) used the method of anaphylaxis with desensitization according to L. A. Zil'ber for the studies of the antigenic peculiarities of the tissues of normal and irradiated animals. As is known, the antigenic differences of tissues in tumor growth, during the process of embryonic and individual development and during chronic inflammatory illnesses were detected by this method.

The authors sensitized guinea pigs by preparations of cellular nuclei, mitochondria, microsomes and structureless cytoplasm of liver and mucosa of small intestines of normal rats and rats exposed to 2000-2500 r 2-3 days following irradiation, i.e. in the terminal period of radiation sickness. The preparations were made by Down's method on Soviet ASL-1 and ASL-2 separators. The tests for presence of sensitization and desensitization were carried out on a homologous preparation with 21-30 days. A cross check of guinea pigs by antigens (by different fractions, which contain tissue structures) of normal and irradiated rats showed that the nuclei of the cells of liver and mucosa of the intestines of irradiated rats contain less antigenic complexes, than those of normal animals. On the other hand, antigens present in healthy rats has been found in mitochondria, microsomes and cytoplasm obtained from the tissues. The presence of a new antigenic quality is recorded also in spleen, bone marrow and blood of exposed animals.

Data disclosing the character of changes observed in liver and in intestinal mucous membrane can be presented as an illustration (Table 15).

Table 15

Changes in the antigenic properties of cellular microstructures of liver and intestinal mucosa of irradiated white rats.

Microstructure of the cell.	Appearance of a new antigenic property		Loss of part of normal antigens	
	liver	intestines	liver	intestines
Nuclei	-	-	+	+
Mitochondria	-	+	+	+
Microsomes	+	+	+	-
Structureless cytoplasm	+	+	-	-

L. A. Zil'ber, G. M. Frank, A. D. Snozhko (54) and V. A. Artamonova have described the change in antigenic properties of liver and spleen of irradiated rabbits, established by the same method by using salt extracts of organs and nucleoproteid fraction. The report confirms the studies of R. V. Petrov and L. I. Il'ina, which had established the possibility of changes in the antigenic properties of tissues of an irradiated organism.

The presence of new antigenic properties in the tissues of the irradiated organism is shown also by another method--the complement-fixation reaction.

By means of immunization of rabbits with liver tissues of normal and irradiated rats exposed to 2000 r (the liver was extracted after 5, 24 and 72 hours following irradiation), serums were obtained, which were used for setting up direct and cross complement-fixation reactions with extracts of liver tissue as antigens. The results obtained are presented in Table 16 (R. V. Petrov and L. I. Il'ina).

The insignificant differences in antigenic properties, expressed in a different stage of hemolysis, can be seen from the liver tissue taken after five hours following irradiation.

A distinct difference in the degree of complement fixation and in serum titer has been disclosed in liver tissue taken within three days after exposure. Thus, for instance, serum of rabbit No 57 reacts with the homo-

Table 16

Titers of complement-fixing antibodies of rabbit serums against the liver tissue of irradiated (2000 r) and normal white rats.

Antigens (aqueous salt extracts)	Antiserums					
	to normal liver rabbit No 28	to liver taken after 3 days following ir- radiation rabbit No 18	to liver taken after ir- radiation rabbit No 57	to liver taken after a day following ir- radiation rabbit No 19	to liver taken after 5 hours following ir- radiation rabbit No 26	to liver taken after 5 hours following ir- radiation rabbit No 53
Normal liver	1:320	1:320	1:10	1:160	1:80	1:20
Liver taken after 3 days following irradiation	1:160	1:1280	1:80	--	--	--
Liver taken after 1 day following irradiation	1:320	1:640	--	1:160	--	--
Liver taken 5 hours after irradiation	1:320	1:640	--	--	1:80	1:40

Note: The titer of the serum was considered to be the greatest dilution of serum which gave the complement-fixation reaction not less than by two crosses.

cross antigen (liver extract of an irradiated rat) in a dilution of 1:80 with a similar antigen from the liver of a healthy rat, only in dilution 1:10, and not for +++ , but for ++.

Thus, the presence of antigenic peculiarities in the tissue of irradiated organisms admits the appearance of a new property which makes the tissues of that organism heterogeneous and active in the antigenic sense. The use of the anaphylactic method with desensitization and the complement-fixation reaction makes it possible to establish changes in the antigenic content of tissues and the time of their appearance, but it does not allow one to solve the problem with respect to their nature, which requires further investigation. The decrease in antigenic complexes may be the result of decomposition of the tissue, and the appearance of new antigens in such an organ as liver, which is a barrier for many substances proceeding along the portal system from the intestines, could indicate the adsorption of cellular decomposition products from other organs. The clarification of these peculiarities, and also the study of their specificity in radiation pathology requires further investigation.

The development of the autosensitization process due to radiation effects as an effective hypothesis may be presented as follows: from the first moment of external irradiation (and continuously during the prolonged effect of radioactive substances) destruction of cells occurs in many organs, and due to the change in permeability the absorption of the products of cellular decay in the bloodstream takes place.

Their interaction with the chemoreceptors of vessels is, evidently, the initial sensitizing stimulus, whose intensity is determined by the quantity of these products, i.e. by the radiation dose.

Obviously, the sensitizing effect of tissue products is exerted even with a slight degree of denaturation, which occurs during the destruction of cells, because the displayed differences in antigenic content of organs of healthy and irradiated animals at this period are only slightly apparent; they form mainly during the period of the climax of radiation sickness.

It would be important to catch the first phase of disintegration of tissues after irradiation, and absorption of the proteins of the organs by the bloodstream.

With this goal R. V. Petrov and L. I. Il'ina set up experiments using radioactive labeling (S^{35}) of protein compounds. A sulphur-labeled (S^{35}) solution of methionine was injected subcutaneously in white rats, and after two days part of the animals were killed in order to determine the level of inclusion of the radioactive methionine in the proteins of different organs. Afterwards the animals were subjected to X-irradiation at a dose of 600 r. After 6, 24 and 72 hours following irradiation, the groups of irradiated and nonirradiated animals were killed and in these also the radioactivity of the tissues was determined. Each time four rats were killed. In the comparison of the radioactivity of tissues in irradiated and non-irradiated rats killed at identical times, redistribution of the label was detected in irradiated animals (Fig. 18). Within six hours the amount of labeled proteins in bone marrow, mucosae of the small intestine, and testes decreased, but in liver and spleen the radioactivity increased. At one day and three days the label kept accumulating in liver; its excretion took place in other organs. Such a redistribution is possible only through the bloodstream.

One may assume only, that after this primary effect of tissue products, further development of the autosensitization process begins. On

the 3rd day the appearance of antibodies can be established in blood and organs, which give a positive complement-fixation reaction with denatured proteins of the corresponding organism.

The appearance of such antibodies cannot be indifferent for a normal functioning of a series of organs and systems, because the interaction between the cytotoxin antibodies and cellular elements also changes the permeability of cellular membranes and the intensity of metabolism. Probably, many pathologic phenomena in radiation sickness may depend on a similar influence of autoantibodies.

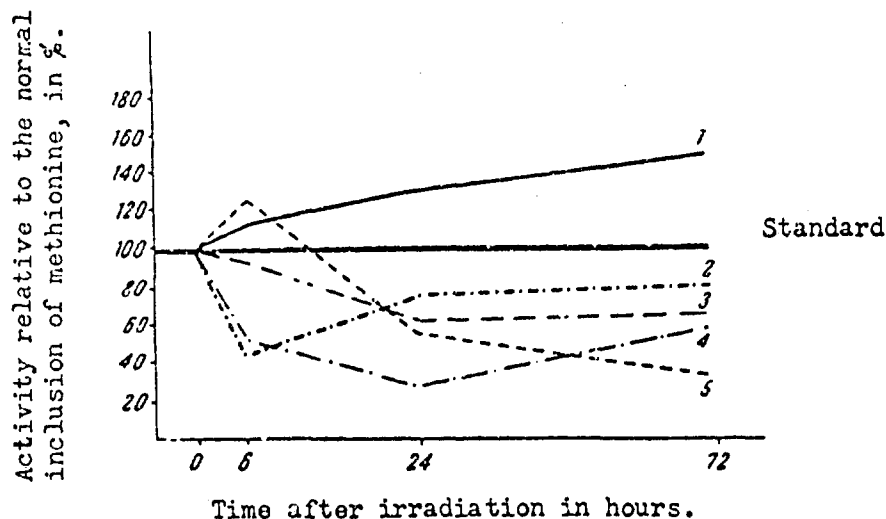


Fig 18. Redistribution of the label (S^{35}) of methionine in protein in the organism of white rats after irradiation. In the reaction were used: 1-liver; 2-bone marrow; 3-testes; 4-mucosa of intestines; 5-spleen.

Of course, further accumulation of data in this region could essentially change the presented scheme; however, in the present form it seems to be useful for the evaluation of individual experimental facts in relation to a definite interpretation. On the basis of these concepts one feels, that it is important to study not only the mechanism of the primary interaction between the products of tissue disintegration and the receptor apparatus of vessels and tissues, but also to investigate in detail the biological effect of the antibodies forming in radiation sickness.

The appearance of antibodies against the autotissue substances was investigated mainly by the method of the complement-fixation reaction. Tissue extracts containing protein, denatured by freezing and thawing; by heating or by alcohol were used as antigens. The experiments of I. P. Mishchenko and M. M. Fomenko (110), P. N. Kiselev, P. A. Buzini, V. A. Semina (72) have established the fact that a positive complement-fixation reaction with such an antigen in animals exposed to radiation appears from the 3rd-4th day of radiation sickness. A similar reaction occurs also in burns and, as the investigations of P. N. Kiselev with co-workers (72) showed, also in any damage to the tissues, for instance after the injection of microbes, turpentine injections, autohemotherapy, ultraviolet irradiations and so on. The antibodies are nonspecific to the intrinsic denatured proteins (because serums taken from animals with different reactions are completely adsorbed by one and the same denatured homologous antigen) and they are preserved in the organism to four months.

P. N. Kiselev, F. A. Buzini and K. I. Nikitina (73) have found, that the titer of antibodies to the homologous protein increases in animals after fractional irradiation simultaneously with increased resistance of the animal to repeated irradiation. Thus, the data obtained indicated that irradiation, like many destructive effects, denatures the proteins of the organism; as a result a reaction appears, namely the formation of antibodies specific to the denatured substances of tissues. At present it is impossible to judge the role of these antibodies in the pathogenesis of radiation injury or their functional influence on the tissues of a given organism by the data mentioned above; meanwhile studies of this problem are of a great interest. If any antibodies appear in an irradiated organism, then they should exert an effect on the vital activity of cells and tissues, because even I. I. Mechnikov indicated, that cytotoxic antibodies change significantly the function of many organs and systems. However, one fails to discover this effect by means of the complement-fixation reaction. There are two methods for its detection: either to study the in vivo effect of blood transfusion from irradiated into nonirradiated animals, or to observe the in vitro effect of blood (serum) on the cells of different tissues of the same organism.

It has been found that the blood of irradiated animals, especially that taken from specific body areas, contains hypotensive substances (33), i.e. acquires a new biological activity.

We have not seen papers discussing the in vitro effect of humoral products on intrinsic cellular elements of an irradiated organism, except for the investigations of B. N. Tarusov (156) with respect to the presence of hemolytic products in the organs after irradiation relatively to erythrocytes of healthy animals.

To study this question the method of Freund and Kammer¹⁾ which they suggested for the determination of cancerolytic properties of serum, was used by N. N. Klemparskaya. In this reaction the decrease in tumor cells in the serum of the sick is determined. The numbers of cells before and after maintenance for one hour in this medium at 37° are compared.

The reaction of Freund and Kammer permits evaluation of the degree of preservation of a normal lytic (with regard to tumor cells) activity of blood serum, which sharply decreases in cancerous organisms.

The principle of evaluation of the lytic activity of serum, on the basis of determination of the disintegration of cells, was used in the experiments of N. N. Klemparskaya not for studies of dissolution of pathologic (tumor) cellular elements, but for investigation of the effect of extracts of organs and blood on the intrinsic cells of the same organism. For this purpose a method of preparation of individual components for the cytolytic reaction were worked out by N. N. Klemparskaya, who has studied the conditions under which it could be carried out and computed. By means of this reaction the cytolytic activity of extracts from organs and blood serum of control and irradiated animals was investigated at different periods of radiation sickness in 51 mice, 27 guinea pigs, 74 rats, 14 dogs and 22 rabbits. Cells of liver, spleen, kidneys and intestinal mucosa and leucocytes were the objects of lysis. It was found that the cytolytic activity of blood plasma and of extracts from intestines, bone marrow, spleen and kidneys increases with developing radiation sickness; agents appear which are lytic not only to the

¹⁾ Quoted from A. A. Bogomolets.

cells of organs (as for instance, liver), but also to leucocytes, and the appearance of leucocytolysins precedes the development of leukopenia. The nature of these lytic agents requires further study, but even now a connection between their appearance and immunization by the products of cellular disintegration can be established, since the appearance of lytic agents can be noted not only in the irradiated animals but also in healthy organisms only after injection of homologous tissue of intestines and spleen into guinea pigs and rats. If these cytolytins prove to be antibodies, they are different from bacteriolysins, because they do not lose their function after inactivation at 56° over the course of 30 minutes like tissue antibodies, described by Spar, Bale, Wolff and Goodland (307). The dynamics of the appearance of cytolytins in irradiated rats and dogs, and also in animals sensitized by homologous tissues, are presented in Fig. 19, 20 and 21.

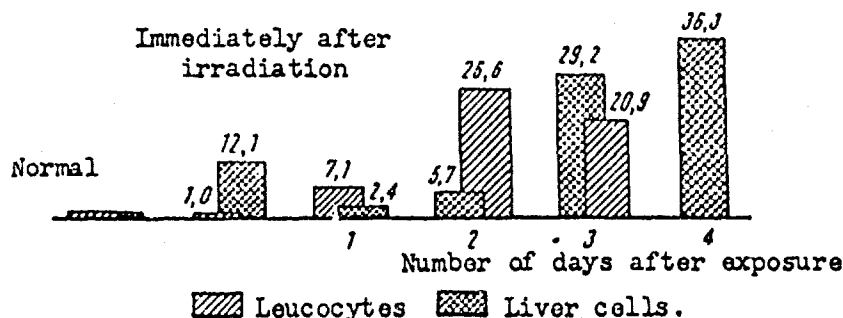


Fig. 19. Increase in the number of sharply positive reactions with liver cells and leucocytes, in per cent of the total number of reactions, in rats at different periods of radiation sickness.

Thus, on the basis of the data presented above one may judge of the presence of changes in an irradiated organism which are of importance for understanding the pathogenesis of radiation sickness: the character of cellular antigens changes and humoral products appear capable of reacting in the complement-fixation reaction with denatured protein of the same species, and also dissolving the cells of organs and leucocytes of the same animal. These data indicate the necessity to study questions of pathogenesis of radiation sickness by immunologic methods and the close connection between the phenomena appearing after exposure and the change in the immunobiological reactivity of the organism.

Analysis of the mechanism of action of therapeutic methods renders great help in the study of etiology of many sicknesses. Explanation of the reasons for effectiveness of these treatments must be an imperative clause in the testing of any theory treating of the nature of the basic changes in radiation sickness. Therefore, study of the efficacy of various means of desensitizing therapy is one of the methods for investigation of the role of autoallergy in the pathogenesis of radiation sickness. As is known, different methods are used in the treatment of radiation injuries (95), many of which share a common property--the capability for desensitization. This fact, in our opinion, deserves great attention as proof of the allergic character of many processes taking place in radiation sickness. On the basis of the observations of N. N. Klemparskaya, two additional desensitizing methods for the treatment of radiation sickness can be added to those known already; by means of these a certain therapeutic effect was attained in the experiments on rabbits.

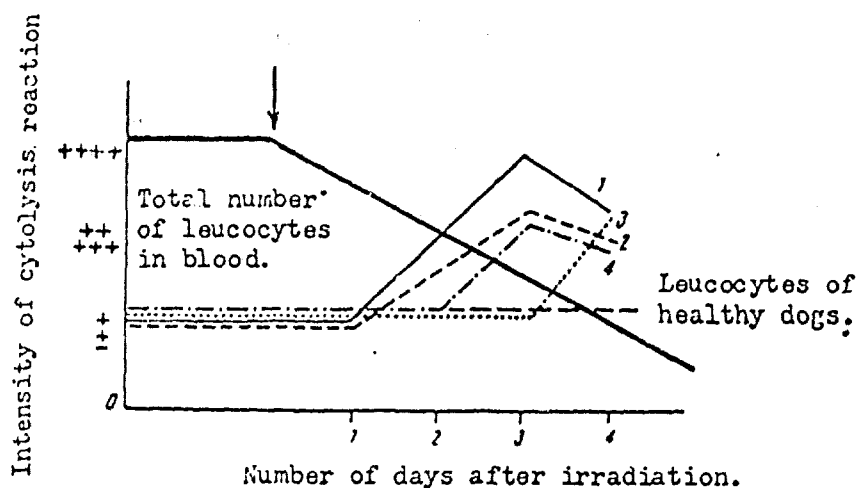


Fig. 20. Change in the number of leucocytes, and dynamics of appearance of leucocytines in dogs exposed to 600 r.

Used in the reaction: 1-leucocytes + blood of nontreated animals; 2-leucocytes of nonirradiated animals + blood of treated; 3-leucocytes of treated animals + blood of nontreated; 4-leucocytes + blood of treated animals, arrow shows the time of irradiation.

The first one is a 5% antipyrene solution which was used on the basis of the report of Mitina (quoted from I. A. Chernaya and co-workers (174)), who attained complete desensitization by means of endocardial injection of antipyrene to guinea pigs before decisive injection of heterologous protein. Since the endocardial injection is an intervention which is merely endured by irradiated animals, after much research it was replaced by intravenous injections. It was found, that the therapeutic effect is preserved after a decrease from 5 to 1.5% in the concentration of antipyrene solutions at the beginning of the treatment even at more than 48 hours after irradiation. By means of daily injections of antipyrene solution with other effects excluded an increase in the survival rate from 33.3 to 56.2% was obtained in rabbits exposed to X-irradiation at a dose of 800 r. The other desensitizing method used by N. N. Klemparskaya, based on the instructions of E. I. Gudkova and P. P. Sakharov (38) with respect to the desensitizing effect of novocaine solutions proved to be more effective. After a series of failures in using one and two per cent solutions, a good therapeutic effect was attained using daily injections of a mixture of 10 ml of 0.25% novocaine solution and 10 ml of 40% glucose solution based on the recommendation of R. Vartapetov and P. Zhuchenko (18), who used a mixture of these solutions for the treatment of some illnesses of pregnancy). Seven out of nine rabbits exposed to 1000 r survived, while all nine nontreated control animals died. A good therapeutic effect of weak novocaine solutions was noted simultaneously by M. N. Livanov.

Finally, there is one more possible way for studying the role of allergy in the pathogenesis of radiation sickness: efforts to induce several phenomena characteristic of the given sickness by means of experimental sensitization of animals by substances of tissues of the same species, i.e. homosensitization.

Efforts were made by N. N. Klemparskaya to attain some changes characteristic of radiation sickness by means of homosensitization; but

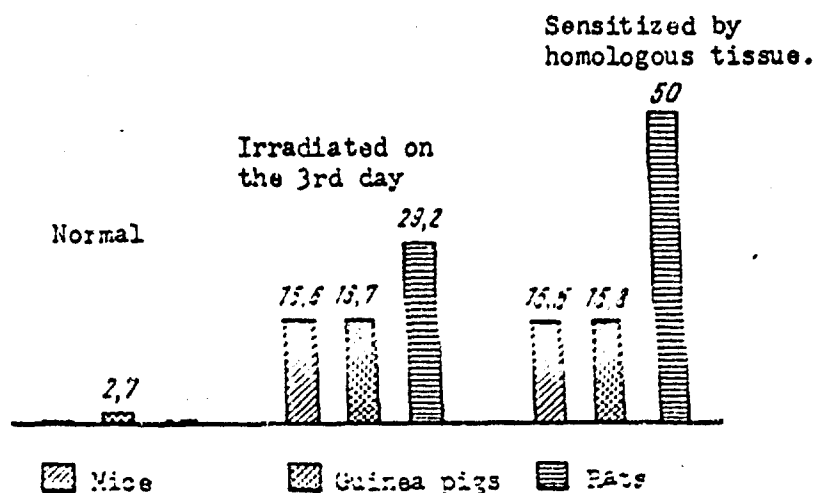


Fig. 21. Comparison of intensities of the cytolytic effect of the extracts of organs on the cells of homologous liver tissue of nonirradiated, irradiated and sensitized tissues of animals (the percentage of sharply positive reactions of the total number of samples is given).

she took note of the difficulties connected with the accomplishment of this experiment (81). These difficulties are a complete absence of data on the necessary amount of tissue and its qualitative state, of the route and number of its injections (it is evident from the absorption of the disintegration products of irradiated tissues, that this process is continuous and prolonged, and this is hard to reproduce using an experimental injection) and finally of the character and origin of tissue products.

All these differences between experimental sensitization and the process initiated by irradiation do not explain the development of pathologic processes in animals for all cases. However, by means of homosensitization, pathologic states having several symptoms rather similar to those of radiation sickness were achieved in many animals.

It should be noted that the process of autosensitization develops immediately after the primary injury of tissues by radiation energy, and therefore, by the experimental injection of a prepared tissue suspension, of course, it is impossible to restore such phenomena, as epilation or cataract development, which depend on a direct injury of tissues of the given organs by radiation.

In the performance of the experiments it should be noted that allergen characteristics of tissue proteins of different organs are not identical. Thus, for instance, N. A. Gorbunova and G. M. Tsykina (30) have revealed a difference in anaphylactogenic properties (in an experiment on heteroallergy) even in the serum proteins of blood. In addition to the qualitative peculiarities of tissue proteins their quantity (i.e. mass of the given tissue) and the stage of vascularization of an organ, upon which depends the rate of absorption of the products of tissue disintegration, must be of great importance as well. In our opinion, the tissue of the mucous membrane of the small intestines, richly provided with blood vessels and the lumen of intestines ordinarily containing a great quantity partially autolyzed disintegration products of cells, from which, as known, the intestinal enzymes develop (179, 135, 136). In 1907 L. B. Popel'skii (133) showed that the contents of the small intestine are highly toxic if injected

into the vein of animals of the same species. Obviously there is no absorption of indigenous proteins of autolyzed intestinal epithelium in a healthy organism, but it becomes possible after damage to the permeability of the intestinal wall caused by ionizing radiation.

It is generally known, that during the clinical course of radiation sickness the symptoms of damage to function of the gastrointestinal tract are well expressed. Histological investigations showed the presence of necrotization and sealing of epithelium, vacuolization, swelling and disintegration of the nuclei of cells of mucosae and submucosae, formation of erosions, ulcers and hemorrhages (90).

N. N. Klemparskaya (79) in studying not only slices, but preparations--imprints--stained according to the method of Romanovskii-Ginz, made simultaneously from different organs of the same irradiated rabbit, has established the greatest destructive changes to be in the mucosae of the small intestine. Complete destruction of the epithelial cells was observed--cytolysis with the formation of amorphous masses staining blue--, at the peak of radiation sickness and in nonsurviving animals, while the structure and typical color of the cells of other organs (kidneys, liver, spleen) were preserved, although the vacuolization of nuclei and swelling of protoplasm were noted.

This vast cellular disintegration occurs during the appearance of diarrhea, fever and rich seeding of organs with bacteria of intestinal auto-flora against the background of a significant increase in the number of bacteria in the intestinal contents (123). The presence of lysis of a large number of cells of the mucosa of intestines, as the observations of N. N. Klemparskaya (80) indicated, is indicated by a substantial increase in the bactericidal activity of the intestinal contents before death of the animal, which at this terminal period does not have any protective value for the organism. Increase in the bactericidal activity of the intestinal contents of rabbits that died on the 6th-7th day following exposure to 1100 r, and of mice exposed to 500-750 r, in contrast to normal animals, shows complete bacteriostasis of the autoflora in bacterial cultures on a mixture of agar with this content.

The functional changes in the activity of the cutaneous tegmina and in the blood content of the vessels of the stomach region, discovered by this author, prove the important role of damage to the organs of abdominal cavity in the pathogenesis of radiation sickness (80).

An early decrease in the bactericidal function of the skin of the belly and appearance of leukopenia was established by N. N. Klemparskaya in the vessels of this region, while even leucocytosis was observed in the peripheral vessels of other body regions. These changes could be the consequence of segmentary reflex effects of the pathologically changed organs of abdominal cavity. The presence of local leucocytosis in the cutaneous vessels over an inflammatory locus is a phenomenon well known to internists and surgeons (17, 93, 63).

In the given case there is no leucocytosis, but regional leukopenia, dependent, evidently, on severe damage to the organs of the abdominal cavity, not inflammatory but rather degenerative in character.

Thus, a series of facts confirms the important role of injury of the intestinal tract in radiation sickness, which results in the development of the corresponding clinical syndrome; zonal disturbances in the physiological functions appear and the process of autosensitization develops, explained by

the absorption of intestinal content which usually does not get into the bloodstream in the indigenous form and in large quantities.

Subcutaneous injection of 10 ml. of suspension of intestinal tissue.

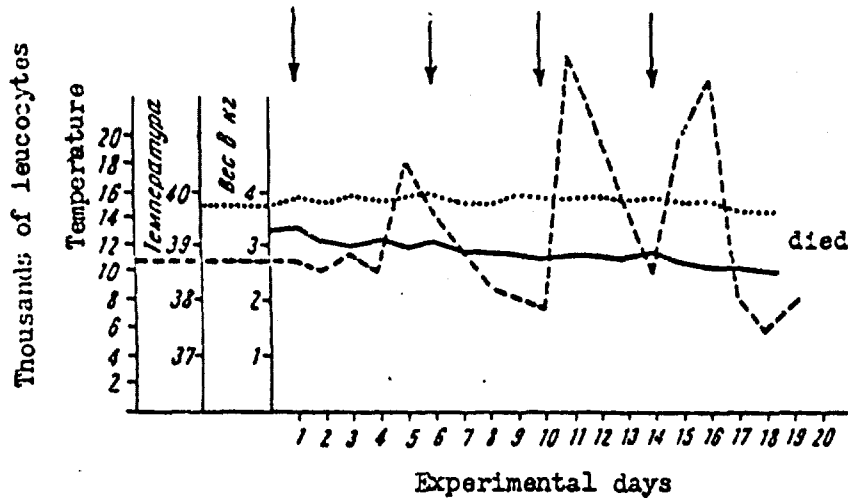


Fig. 22. Changes in some indices of the condition of a rabbit after a series of subcutaneous injections of homologous intestinal tissue; arrows show the time of injection of the tissue.

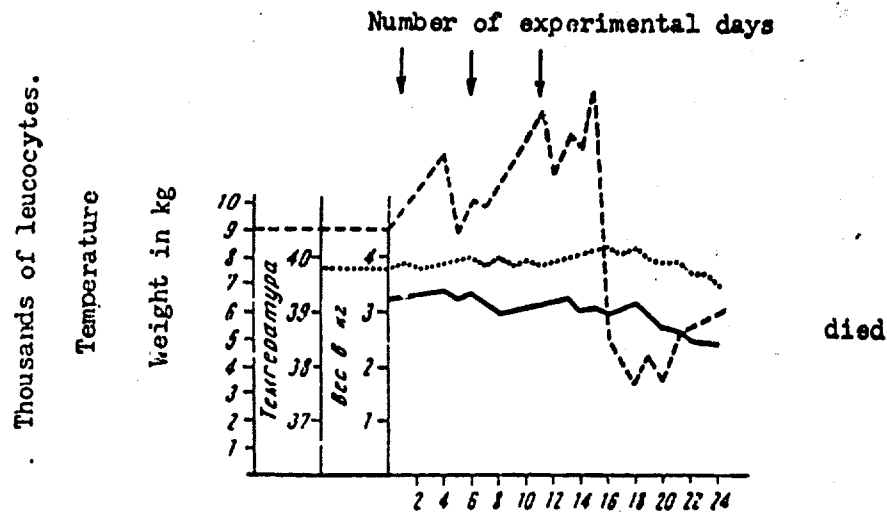
Of course, in the development of the total symptomatic complex of radiation sickness many other factors must be taken into account also: destructive changes in many organs, the mutual neuro-humoral effect of a series of physiological systems, and first of all the nervous system. However, in our opinion, injury of intestinal tract by radiation plays one of the chief roles in the development of subsequent changes in the organism. M. N. Pobedinskii (132) indicates, that of all variations of local irradiations only the irradiation of stomach results in the development of radiation sickness.

Studies of the decisive role of changes in the structure of tissue antigens in the development of autosensitization of irradiated animals are very important.

Final clarification of this problem first of all requires studies on the specificity of these changes with respect to radiation, because it is well known that differences in the antigenic structure of tissues, made apparent by the method of anaphylaxis with desensitization, are displayed in many sicknesses and even at different ages of animals.

Development of significant changes in tissue antigens with developing radiation sickness, especially shortly before death of the animal, is shown in the investigations of R. V. Petrov and L. I. Il'ina (126). It is possible, that these changes are consequences, and not causes of the primary processes caused by radiation.

Disturbances in the permeability of vessels and tissues are of great importance in the development of autosensitization in radiation sickness. A special importance of this factor in the development of allergy in general is reported by M. A. Skvortsov (144); he considers the increase in



0.1 ml of suspension of the intestinal tissue injected intracutaneously in ears and tail of a rabbit:

Fig. 23. Changes in several indices of the condition of a rabbit after many intracutaneous injections of homologous intestinal tissue; the arrows show the injection time.

permeability to be one of the basic factors that determine both the possibility of sensitization and the occurrence of the << resolving >> contact of allergen with tissue antibodies. Under the conditions of damaged permeability of the vessels of an irradiated organism disintegration products of cells in the indigenous form are absorbed by the bloodstream. Obviously the changes in the state of proteins associated with tissue damage and the presence of some peculiarities in the antigens of various organs are sufficient for the sensitizing effect.

Let us remember that in the investigations of I. I. Mechnikov and the series of authors mentioned above (249, 254, 268, 316, 322) nephritis, myocarditis and encephalitis were attained by injection of minced tissue of healthy organisms.

To study the biological effect of the injection of homologous tissue of irradiated and healthy animals N. N. Klemparskaya has carried out experiments on 96 rabbits, 43 guinea pigs and 462 mice.

Freshly prepared 10-25% suspensions of tissues of normal and irradiated animals of identical species were injected by different methods intravenously, subcutaneously, intramuscularly and intracutaneously.

Tissues of the small intestinal mucosae, liver, spleen and encephalon were used. The greatest number of experiments was carried out with intestinal tissue.

The data obtained confirmed the report of L. B. Popel'skii (133) with respect to the toxicity of the intestinal contents of healthy animals, and new facts can be established which characterize the biological effect of the tissues of this organ. The injection of an extract or suspension of homologous tissues of the small intestines of healthy and irradiated rabbits induces the development of leukopenia, appearance of fever on the 5th-8th

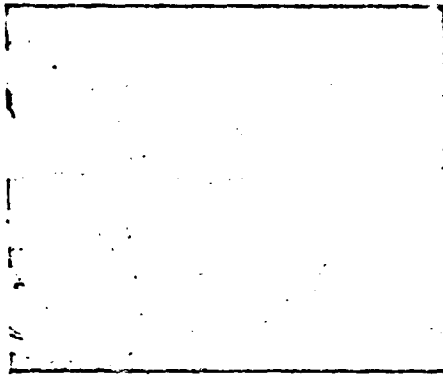


Fig. 24.A. Trophic ulcers on the soles of hind feet of rabbits after 4-6 weeks following injection of homologous intestinal tissue.

day, changing into hypothermia before death, weight loss and appearance of hemorrhages in lungs and intestinal tract in healthy animals.

To illustrate the changes in the condition of animals after injection of homologous tissues, Fig. 22 and 23 show the dynamics of the changes in weight, temperature and leucocyte count. Cultures from organs of nonsurviving animals oftener remain sterile or give growth to intestinal autoflora, and sometimes to white staphylococcus.

Finally in part of the animals the development of symmetrically located trophic ulcers on the soles of both hind feet of rabbits was observed after a prolonged period of time following the injection of tissues.

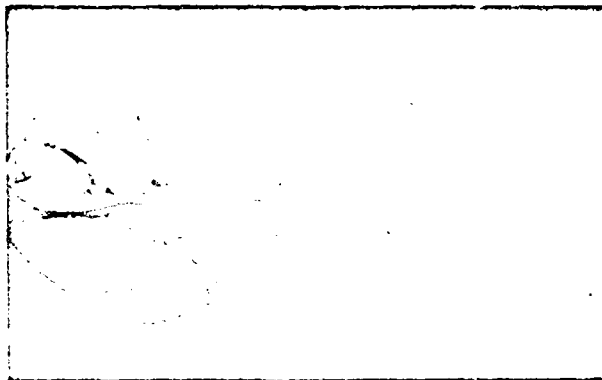


Fig. 24 B. Trophic ulcers on the soles of hind feet of rabbits after 4-6 weeks following the injection of cellular fractions of the mucosae of small intestines.

The appearance of these ulcers (Fig. 24) is very similar to trophic ulcers on the feet of rabbits after single local irradiation of one of the legs; the ulcers have been described by P. D. Gorizontov (33).

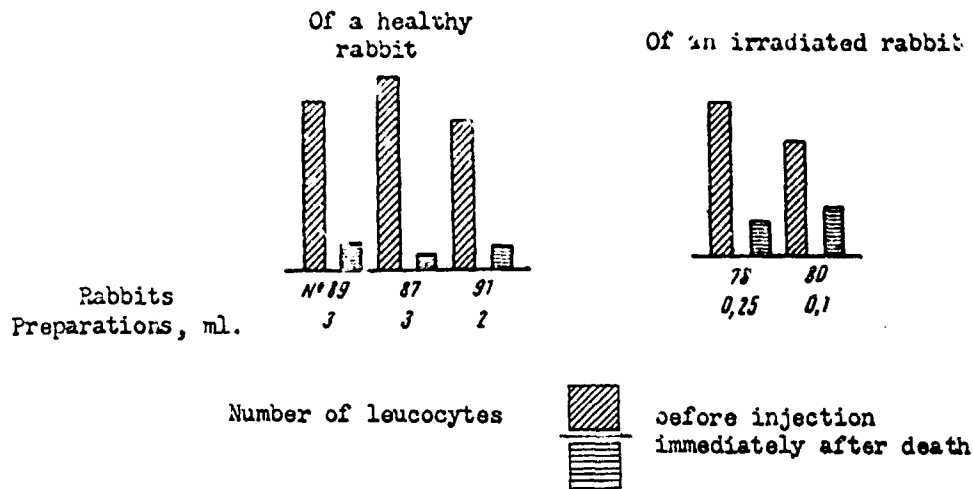


Fig. 25. Change in leucocyte count after intravenous injection of mitochondria of the intestinal mucosae of healthy and irradiated animals.

In addition to the direct effect of homologous tissue fractions the authors have also recorded the appearance of pathologic processes at a remote period after the time of injection.

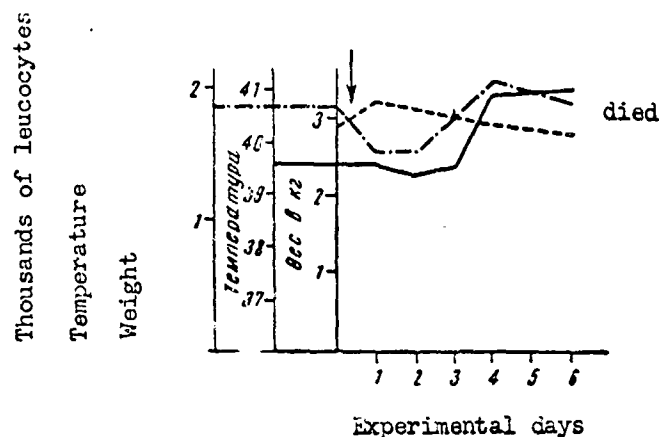


Fig. 26. Effect of intravenous injection of 10.5 mg of the mitochondrial fraction in healthy rabbit; the arrow shows the moment of injection.

After intravenous injection of 10 mg of a fraction of mitochondria of the mucosae of homologous intestines in four healthy rabbits the development of fever (40-41°) on the 4th and 6th day and loss of weight were observed in all of them; two of them died on the sixth and eleventh day with hemorrhages in the lungs necrosis of the mucous membrane of the duodenum and the beginning of the small intestines. The cultures from internal organs were sterile (Fig. 26); white staphylococcus was separated from kidneys of one out of four rabbits taken.

Such occurrences were not observed in rabbits that received an injection of the mitochondrial fraction of irradiated animals, probably

because the amount of this preparation was much smaller (2.5 mg of this fraction was a lethal dose).

All these clinical symptoms and the development of autoinfection are very similar to the changes observed in radiation injuries.

The injection of liver suspension initiated these symptoms much less frequently and only in animals most sensitive to allergy--guinea pigs. Allergenicity of the substance taken from healthy animals in these experiments was higher, than that from the irradiated animals. An impression may form, that the disintegration of tissue and depolymerization of complex compounds induced by radiation lead even to impoverishment of the tissue with respect to antigenic substances. This contradicts the assumption of the appearance of special toxins in the tissues of an irradiated organism.

In order to carry out detailed studies of the changes in biological effects of tissues of an irradiated organism, N. N. Klemparskaya, R. V. Petrov and L. I. Il'ina have observed the effect of different fractions of the tissue of the mucosa of small intestines and the liver (of nuclei, mitochondria and cytoplasm) obtained by the method of Claude (201) and Dounce (211) on healthy animals.

One hundred and fifty rabbits were used for the studies of the character and nature of the biological effects of these substances.

These experiments revealed a capacity for a very active biological effect expressed in the development of shock directly after intravenous injection of definite numbers of cells of the mitochondrial fraction of mucosa of the small intestines. The mitochondrial fraction, containing 10 mg of the preparation per 1 ml of rabbits irradiated with 1000 r of X-rays or Co^{60}

γ -rays was more active within 6-24 hours after irradiation, than the preparation of identical concentration taken from healthy animals. After intravenous injection of 2.5 mg (or 0.25 ml) of the preparation of irradiated animals and 25-30 mg (or 2.5-3 ml) of mitochondria of the intestinal mucosa of healthy rabbits, rapidly occurring death from shock was observed in a healthy rabbit--recipient (within 30 seconds to 1½ minutes). Asthma and shaky gait appeared, the animal fell on its side, intense spasm developed, excretion of urine was observed and the animal died. Sharply pronounced leukopenia was always discovered after death in blood taken from the heart, as it can be seen from Fig. 25.

There were no macroscopic changes in the organs, except for hyperemia of lungs and small intestines.

As seen from the data presented, the shock-like effect of mitochondria from irradiated rabbits was much greater than that from healthy ones, although the character of the induced changes was identical. Probably this as well as the qualitative changes may be explained by a presence of a large quantity of the products of depolymerization of tissue substances, which accumulate during the centrifugation of this fraction.

Such a manifestation of biological effect was observed only after the intravenous injection of the given fraction and was absent after injections of equal and much larger quantities intraperitoneally, intracutaneously, intramuscularly or subcutaneously.

Of all those preparations of the tissues of liver and small intestinal mucosae, after intravenous injection of 10 mg of preparation by protein content death was caused only by the fraction containing intestinal mitochondria. Preparations of liver cells and fractions of nuclei and cyto-

plasm of intestines in these quantities were well endured by rabbits and neither caused death nor the development of leukopenia.

Formation of trophic ulcers on both hind feet of the rabbit within 34 days after a single intravenous injection of the preparation of nuclei of the intestinal mucosa of irradiated rabbits in one of the manifestations of a delayed effect of sensitization by the fractions of homologous tissues (Fig. 24B). Ulcers from the injection of whole suspensions of intestinal tissue (see Fig. 24A) and ulcers which developed as a result of local irradiation of one of the legs are similar to those described above (33).

Thus, in experiments dealing with the injection of suspensions of indigenous tissue and of individual microstructures of cells it has been found that the intestinal substances have an active biological effect on an animal of the same species.

How can it be, with no radiation effect and with an inevitable destruction of cells in the intestines no autosensitization develops?

Evidently, the amount of all the products and the rate of their absorption by the bloodstream are of great importance, because it has been established that normal homologous and even heterologous serum has a detoxicating effect on the shock-producing capacity of the mitochondrial fraction of intestines under the compulsory condition of contact for an hour at 37° has been established. Simple mixing of serum with the mitochondrial preparation did not hinder the development of shock.

The important role of autosensitization of the organism in the development of radiation injuries is confirmed not only by the facts presented above with respect to the development of pathologic conditions after injection of tissue products, but also by the fact that homosensitization makes the organism especially sensitive to radiation. Against the background of an existing increased sensitivity to the tissue products, radiation by causing damage to the cells and by changing permeability becomes a « decisive » factor, which affects the organism more severely than radiation with no sensitization. In the experiments of N. N. Klemparskaya only one out of 16 rabbits exposed to X-irradiation of 800 r died of shock after exposure, but 6 out of 7 preliminarily sensitized with the tissue of rabbit's intestines (2 weeks to 5 months) died during the first four hours.

During the first five days seven out of twenty mice exposed to a dose of 600 r died, but ten out of twelve mice sensitized with the tissue of homologous intestines died over this period. However, if the injection of tissue occurs after irradiation even with sublethal radiation doses, then it becomes a « decisive » factor, and animals die after amounts of tissue products, which are well tolerated by healthy animals. Thus, for instance, five out of six guinea pigs irradiated with 50 r died after injection of liver tissue, but only one out of eight nonirradiated animals died. Consequently, both the injection of tissues and irradiation can be mutually aggravating factors.

This circumstance has great practical value, because it explains why an organism undergoing some process connected with absorption of tissue disintegration products, for instance, trauma, inflammatory infection locus, pregnancy and so on, usually responds severely to ionizing radiation.

In light of these data, evaluation of the role of infectious processes in an irradiated organism is of special interest. One must take into account that in addition to the effect of toxic products of metabolism of the microbe cell itself, in inflammatory processes of microbial etiology, the breakdown of tissues of the organism and formation of « endogenous

factors >> take place, which, according to the judgment of Kh. Kh. Planeliyes (130) is of great significance in the pathogenesis of infectious diseases. This circumstance is especially important against the background of an auto-sensitized (for instance, after irradiation) organism in which the appearance of the products of tissue disintegration plays a <<decisive >> role and aggravates the course of the basic process of autosensitization.

On the other hand, the presence of the sensitization process in the organism determines the severe course of the local inflammatory process. As O. A. Kirilenko (66) showed, in the infection site of an organism sensitized by heterologous protein, as in the process in irradiated animal, many more bacteria accumulate than in a normal organism, and the process is more severe in character. In our opinion, together with a series of other causes, the severe course of infectious processes in irradiated organisms depends on this mutual effect of autosensitization and infectious damage (connected with local formation of tissue disintegration products).

The existing literature data refer to the effect not of homo-, but heterosensitization on the course of infections. All authors who have studied this problem come to one and the same conclusion, that the infection with microbes of animals sensitized by heterologous protein leads to a more severe course of radiation sickness as compared with the control animals. D. F. Zsimbalist and N. A. Emel'yanova (171) have reported an increased mortality rate in sensitized guinea pigs (more than four times), if infected with diphtheria bacteria. Eighty five per cent of these animals had bacteriemia, and their infection dose was significantly lower than that of nonsensitized animals.

L. L. Andyba (62) has studied the course of diseases caused by pneumococci, streptococci, viruses of encephalomyelitis and intoxication by diphtheria toxin. The importance of using a definite amount of sensitizer—horse serum—and a dose of microorganisms has been reported. In certain combinations of these two factors a significant aggravation of the infectious process in sensitized animals has been attained. The author presents a review of foreign papers reporting on the severe course of streptococcus and staphylococcus infections in sensitized rabbits and dogs. However, we failed to find published investigations with respect to the question of the effect of autoallergy on the course of infections.

The experiments of N. N. Klemparskaya performed on 24 rabbits and 36 guinea pigs indicated that experimental intracutaneous injection of living culture of coliform bacteria into nonirradiated animals sensitized with homologous tissues leads to the development of more severe local inflammatory changes in comparison with the control animals: the dimensions of infiltration areas increase, and necrosis and hemorrhages appear. The spreading of the injected microbes into internal organs occurs in these animals, and in many cases it is followed by their death, which was never observed after intracutaneous injection of coliform bacteria in healthy guinea pigs and rabbits. Table 17 is presented as an illustration; it shows, that single and repeated injections of homologous tissue cause a decrease in the weight of animals and increase in their sensitivity to the intracutaneous infection, which is manifested both by an increase in the intensity of local inflammatory symptoms and by death after infection. The specific peculiarities of the effect of homo-sensitization by various tissues on the course of infections require further studies, but one can see from the data presented, that the effect is not identical: the aggravation was higher in guinea pigs sensitized by intestinal tissues as compared with the sensitization by liver tissue. The sensitization was carried out 6-10 days before the injection of the decisive dose.

Table 17.

Effect of homologous sensitization on the course of experimental infection.

Species	No in- suc- cession	Sensitization	Inoculum used for infect- ing with col- iform bacteria	Maximal dimensions (cm) and character of inflammatory locus	Loss of weight during the per- iod of sensiti- zation	Compli- cations	Course of the infec- tion	Organs in which coliform bacteria were found
Guinea Pigs	1	Single, 0.5 ml of 30% suspension of intestines of a healthy Guinea pig.		Diffused infil- tration	60	--	Died within 24 hrs.	Heart, spleen, kidneys.
	2			Dense infiltration 2 x 2, dark center	90	--	Died within 48 hrs.	Heart, spleen, kidneys
	3	Single, 0.5 ml of 30% suspension of liver of a healthy Guinea pig.	2.5 bil.	Diffuse dense infil- tration, necrosis 1 x 0.5	15	--	Alive	
	4			2.5 x 1.5; necrosis 1 x 1	10	--	"	"
	5	Control		Infiltration 2 x x 2.5	0 (+ 40)	--	"	"
	6	Control		Infiltration 2 x x 3	0 (+ 15)	--	"	"
Rabbits	1	Single, 0.5 ml of 25% sus- pension of intestines of a healthy rabbit	100 mil.	Infiltration 3 x x 2	55	--	"	"
	2			Infiltration 4 x 2; hemorrhage 2 x 1	40	--	"	"
	3	Control		Infiltration 3 x x 3.5; petechia	0 (+ 30)	--	"	"
	4	4 times within 10 days, mixture of suspension of liver tissues and intestines	2.5 bil.	Infiltration 4 x x 4; hemorrhages	87	--	Trophic ulcers on both hind legs	"
	5			Infiltration 3 x 3; necrosis	0 (+ 45)	--	"	"
	6	Control		Infiltrate 2 x 1.5		--	"	"

The external appearance of cutaneous inflammatory loci of two rabbits—control (A) and experimental (B), sensitized by the suspension of the intestinal tissue of a rabbit, is presented in Fig. 27.

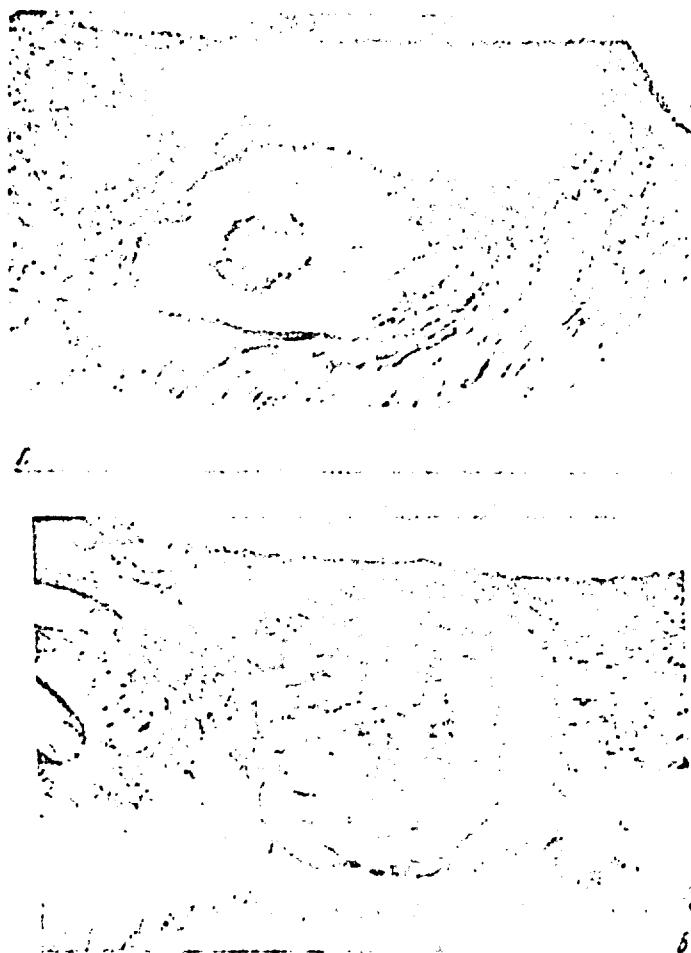


Fig. 27. External appearance of inflammatory locus in rabbits within 24 hours after the injection of 10 bil. of coliform bacteria. A—in control rabbit, B—in a rabbit sensitized 25 days before injection of the intestinal tissue. Hemorrhages of large dimensions and edema extending down to the lower area of the stomach can be seen in the experimental rabbit.

Much larger dimensions of infiltration area, and hemorrhages, can be observed in the experimental animal.

The aggravation of the experimental infection in homosensitized animals was demonstrated especially clearly in the experiments of N. N. Klem-parskaya, R. V. Petrov and L. I. Il'ina by the injection of various rabbit tissues containing various microstructures of cells: nuclei, mitochondria and cytoplasm, obtained from healthy as well as irradiated animals, into rabbits. The rabbits received an intravenous injection of 10 mg of the fractions indicated (except for the mitochondrial fraction of intestines of an irradiated rabbit; it was injected intravenously in the amount of 1-2 mg, because a higher dose caused death of an animal; or 10 mg were injected, but by another route) and after 13-35 days, intracutaneous infection with a living one-day-old culture of coliform bacteria (1 bil. microbes) ^{was carried out} The data obtained are presented in Table 18.

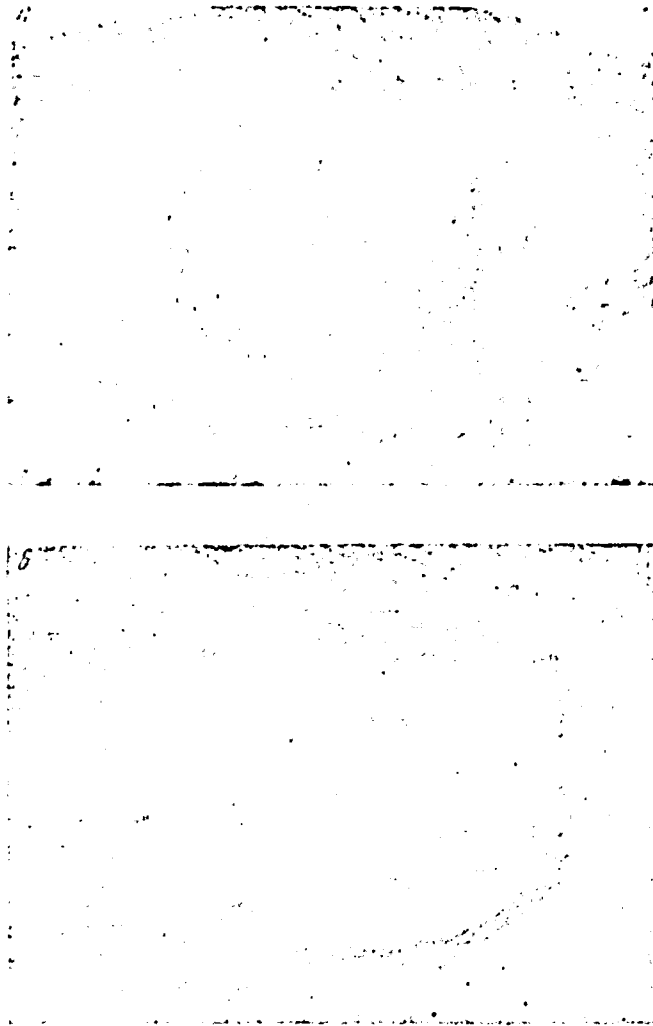


Fig. 28. External appearance of inflammatory focus 24 hours after intracutaneous injection of 1 bil. of coliform bacteria. A-rabbit No 83 (control); B-rabbit No 61 sensitized 35 days and once more 13 days before infection by intravenous injection of cytoplasmic fraction of intestines of an irradiated rabbit.

A slight infiltration with a moderate hyperemia from 3 to 5 cm. in diameter formed on the site of injection of two control, nonsensitized rabbits. The size of infiltration areas in nine rabbits that received intravenous injections of tissue fractions of healthy animals also were within these limits, except for two rabbits that received injections of preparations of cytoplasm and mitochondria of intestinal mucosa. However, these animals had neither necrosis nor hemorrhages.

The course of the local inflammatory process was much more severe in rabbits sensitized by various fractions containing microstructures of cells of irradiated animals.

Necrosis on the site of injection of coliform bacteria, and intensely expressed hemorrhages, appeared in six out of nine rabbits. The external appearance of these foci reminded very much of the necrotic-hemorrhagic reaction to the intracutaneous injection of coliform bacteria in irradiated rabbits described by V. F. Sosova (Fig. 28). Consequently, the sensitizing effect, with regard to damage to the tissues by microbes, was more conspicuous

Table 18

Effect of sensitization by different tissue fractions of normal and irradiated rabbits on the intensity of the local inflammatory reaction.

Organs of rabbit	Normal or irradiated with a dose of 1000 r.	Fraction	No. of the sensitized rabbit	Intensity of the local inflammatory reaction after an intracutaneous injection of	
				1 bil. of coliform bacteria culture	0.1 ml. of turpentine
Liver	Normal	Cytoplasm	54	+	Not checked
			48	+	
	Irradiated	Mitochondria	55	++	
			51	++	
		Cytoplasm	60	+++	
			66	++++	
Mitochondria	65	+++			
	68	++++			
Intestine	Normal	Cytoplasm	56	+++	Not injected
			57	+	
		Mitochondria	71	++	
			59	+	
	Irradiated	Cytoplasm	77	+++	
			61	++++	
		Mitochondria ¹⁾	76	++++	
			62	+++	
			70	++++	
			67	++++	
Control rabbits with no sensitization			83	++	Not injected
			97	+	

Note: Designation of the intensity of the reaction from the diameter of the infiltrate: + infiltration area to 3 cm; ++ infiltration area from 3 to 5 cm.; +++ infiltration area above 5 cm; ++++ infiltration area above 5 cm, presence of necrosis and hemorrhages.

1) Since this fraction of mitochondria after intravenous injection causes death by shock, rabbit No 70 received intraperitoneal and rabbit No 67 intramuscular injections.

in tissue fractions obtained from irradiated animals, which is a proof for the presence of qualitative changes in cellular substances induced by radiation.

The action of microorganisms is not necessary for the development of a necrotic-hemorrhagic reaction on the skin, because (see Table 18) the injection of 0.1 ml. of turpentine gives the same effect. Evidently the formation of products which result from the disintegration of tissue cells, and which are the « decisive » factors in homosensitization, is important. Thus, one may assume that one of the causes of hemorrhagic necrosis on the site of the injection of bacteria in irradiated animals, evidently is the formation of disintegration products of the tissue in the inflammatory focus in conjunction with the state of autosensitization developing at this time.

Aggravation of infectious processes in autosensitized organisms may depend not only on local development of necrosis and hemorrhages connected with allergic reactions to tissue disintegration products, but also on another condition, that of unresponsiveness to antigens of the antigenic stimulus, which also is determined by the effect of tissue disintegration products, and consequently, connected with depression of the immunobiological reaction to the spread of microbes.

Local cutaneous test (of the type of Pirke reaction) is one of the methods used for development of sensitization of an organism to some allergen; therefore, one could try to develop a state of allergy to the substances of different tissues in irradiated animals by means of intracutaneous tests. For this purpose the suggestions of a series of authors (66, 323, 216) with respect to achievement of more clearcut results in local allergic reactions after the injection of allergen in special zones of the organism [eyes, upper lip (labial reaction) and testes] must be taken into account. Actually, the injection of a 20% suspensions of fresh homologous tissues in the skin of the lateral side surface of the body or extremities of irradiated animals failed to give us an opportunity to observe local allergic reaction, and only the use of the zones indicated namely skin of the upper lip, permitted observation of a clearly expressed necrotic-hemorrhagic reaction with edema; these were local injections of suspensions of tissues in experiments on irradiated mice and guinea pigs, carried out by N. N. Klemparskaya in cooperation with N. A. Kraevskii and V. V. Shikhodyrov. A similar labial reaction appears in mice only on the 3rd day, and in guinea pigs on the 7th day following exposure to lethal doses of X-rays. Of four kinds of allergens checked--extracts of homologous tissues, suspensions of bacteria, sterile milk and horse serum--positive reactions were obtained only to the injection of the extract of small intestines of irradiated (rarely of healthy) mice.

A positive test could be seen within 18-24 hours from the development of edema which covered not only the region of the lip on the injection side, but spread to the neck and head, and also from the appearance of hemorrhage. Microscopic preparations showed edema, necrosis, hemorrhages and absence of any cellular infiltration, i.e. a reaction of the hyperergic type.

Such a positive labial test can be achieved by the injection of extracts of homologous tissues only at a definite period of radiation sickness, indicated above. The hyperergic reaction fails to appear in mice if this substance is injected within the first two days, 24 hours or later following irradiation (dose of 600 r) (evaluation was made by visual examination and analysis of microscopical preparations) These data indicate, that the state of increased sensitivity to the substances of homologous tissues requires a definite time for its development, whose duration, in the examples provided, agreed with the latent period. Simultaneously with the examination of the irradiated animals, the same substances were always injected into healthy mice and guinea pigs. External examination did not show any local reaction. Focal cellular infiltration was observed in histological preparations.

Positive labial tests could be achieved by other means than the injection of prepared extracts of homologous tissues of irradiated animals. If the formation of disintegration products of cells can be caused by changing the osmotic pressure, for instance by injection of distilled water, then at a definite period of radiation sickness, hemorrhage and necrosis appear in irradiated animals. Positive cutaneous hemorrhagic reactions after the labial injection of distilled water in mice irradiated with a dose of 600 r (injection done on the 3rd day) were obtained by N. N. Klemparskaya, N. A. Kraevskii and V. V. Shikhodyrov; the same were obtained in experiments with dogs subjected to an identical treatment (intracutaneous injections in the belly beginning with the 10th day after irradiation) by N. N. Klemparskaya

and N. V. Raeva. A simultaneous injection of physiologic saline solution in these animals did not cause any local reactions. In the experiments with dogs it was found that the capacity to react to intracutaneous injections of distilled water by the formation of hemorrhage is preserved in surviving animals over several months. It is well known, that if a certain reaction to some antigen—allergen develops in the organism, then the perception of other antigenic (allergenic) stimulations decreases significantly (233). Such an unresponsiveness in sensitized animals with respect to diphtheria anatoxin was established by P. F. Zdrovskii (48) and confirmed later by I. E. Alatyrtseva and S. A. Usmanova (4), who in addition to this reported that sensitized rats acquire greater sensitivity to diphtheria toxin.

A. A. Klimentova (82) has established a significant decrease in the production of typhus agglutinins under the conditions of vaccination and revaccination after a primary sensitization by horse serum.

Depression of the reaction to the immunizing stimulus which develops after the appearance of autoallergy, is probably one of the factors explaining the severity of the course of infectious diseases in sensitized organisms, in which the defensive mechanisms are not satisfactorily mobilized. The unresponsiveness of a sensitized organism may be displayed to the perception not only of antigenic, but, evidently, also of allergenic stimuli. Of course, there are not sufficient experimental data in this field at present. It is necessary to clarify the effect exerted by the development of the sensitization process on the perception of repeated allergenic stimuli. That such an effect exists can be judged by the data presented in this monograph, which show that the capacity for immunogenesis and allergic reactions after irradiation decreases substantially.

It may be assumed, that the autosensitization which develops due to irradiation is the cause, which makes the organism refractory to the perception of other antigenic stimulations. It is not our task to review the literature concerning the depression of immunogenesis after irradiation. A series of articles may be recommended to those who wish to become familiar with this problem (I. A. Pigalev (129), V. I. Troitskii, O. V. Chakhava and N. A. Kozlova (159), W. H. Taliaferro and L. G. Taliaferro (312) and others).

It should be noted only that the significant decrease in immunogenesis in immunization after irradiation not only contradicts, as Cronkite (207) thinks, the hypothesis of autosensitization, but proves to be one of the convincing confirmations (on the basis of data presented above, on the depression of immunogenesis in any sensitization process).

There are many less data concerning the course of allergic reactions in irradiated organisms, than on the change in immunogenesis. All of them indicate a depression of allergic reactivity during this response. Thus, for instance, Becker (187) and later V. F. Sosova (151) recorded a depression of the Schwartzman phenomenon in experiments on irradiated animals. V. F. Sosova reports in her work that there are several phases in the development of Schwartzman's phenomenon after exposure to a dose of 1100 r: first, during the first 25 hours, an intravenous injection of filtrate of bacterial culture produces a severe general reaction followed by death of the rabbits. After 48 hours following exposure to a dose of 1100 r, the phenomenon is expressed as usual (the reactivity of the organism has not yet changed), but on the 3rd-5th day after irradiation it is completely depressed and the organism becomes unresponsive to the injection of killed bacteria and some chemical substances. Consequently, the depression of the Schwartzman phenomenon coincides with the time of appearance of tissue antibodies and with the change in sensitivity of the organism to the injection of extracts of homologous tissues and large quantities of living microbes (hyperergic

reaction). These data agree well with the concept that the development of the autosensitization process (after irradiation with lethal doses beginning with the 3rd day after irradiation) decreases the capacity of the organism to react to other stimulants. It is generally known, that any process of sensitization changes the function of the nervous system, causing the development of an inhibiting process in its centers (which occurs also in radiation sickness).

A decrease in allergic reactivity in irradiated animals is displayed in different phenomena of the allergic state.

In 1913 Heinrich (241) showed a decrease in the intensity of anaphylactic reactions in irradiated guinea pigs. This was confirmed by Capolli (198) who showed additionally, that in vitro irradiation of the serum of sensitized animals decreases its allergenic activity. V. A. Samtsov and A. A. Gorodetskii (138), and later Yu. N. Sokolov (147) described the depression of the Arthus phenomenon in irradiated rabbits, Liebersohn and Shimanko (quoted from Yu. N. Sokolov), and also Lernox (252) have described the decrease in intensity of the manifestation of cutaneous tests by tuberculin. The data relative to the effect of radiation on the occurrence of anaphylactic reactions in sensitization before irradiation are contradictory.

Z. I. Poluboyarinova has observed a depression of the shock reaction in guinea pigs sensitized before injection of radioactive substances (radon, radiothorium) over a period from the 3rd to the 7th day after treatment, with restoration of allergic reactivity on the 14th day.

However, Stoner and Hale (308) reported an increase in anaphylactic shock reactions in mice sensitized by γ -rays before irradiation, especially over the period from the 2nd-7th day of radiation sickness. Probably this discrepancy is connected with a difference in experimental conditions and in the character of the radiations.

Evidently, many problems of allergic reactivity of the organism under the effect of ionizing radiation require further study. There is a little information on the development of infectious allergies under these conditions.

It is known, that a series of allergic reactions found a vast use in diagnostics of infectious diseases (reactions of Pirke, Birne, maleic test, reaction to tularin and so on).

The only publications which we know of at present (147, 252) indicate a great change in the manifestations of allergic reactivity of an irradiated organism to bacterial allergens.

A detailed investigation of the change in reaction of an irradiated organism to the injection of allergens (tuberculosis, brucellosis and tularemia bacteria), where sensitization of animals was accomplished by injection of living vaccines 3 days and 2 weeks before, and within 1, 3 and 7 days after irradiation were carried out by G. M. L'vitsyna in our laboratory. The experiments were 350 guinea pigs and 10 rabbits; the guinea pigs were exposed to doses of 100, 200 and 500 r of X-rays or by injecting each with 0.03 mC of polonium. The rabbits were exposed to 800 r.

Irradiated rabbits sensitized both before and after treatment reacted to the injection of tuberculin in a manner identical to that of the control animals, when they were examined on the 3rd day of radiation sickness. How-

over, in a test (Mantoux reaction) on the 7th day after irradiation always gave a more pronounced inflammatory reaction than in nonirradiated animals sensitized by BCG vaccine. This amplification of the allergic reactivity of the guinea pigs increased with increasing radiation dose.

At first we were astonished by these data with respect to depression of allergic reactions to heterologous protein in irradiated animals, which did not at first agree the data presented in the literature above, astonished us. A detailed investigation of the discrepancy indicated the important role of species and the value of nonspecific responsiveness of the tissues of irradiated guinea pigs even to such an insignificant trauma, as that produced by allergen during its contact with the tissues of a sensitized organism.

The previously described strengthening of allergic reactivity in irradiated guinea pigs, as indicated by many confirmatory experiments, was actually nonspecific and was observed in pigs at this period after injection of a series of other allergens (for instance, brucellin) and even such substances, as distilled water (which causes local formation of disintegration products of tissues) and meat-infusion broth. This nonspecific inflammatory reaction to the injection of allergens has great practical significance, because it is obvious, that such widely used allergic reactions as Pirke, Burne and the like, cannot have diagnostic value for irradiated organisms.

It is of interest, that during an internal—not external—irradiation G. M. L'vitsyna has observed not amplification, but, on the contrary, depression of the allergic reactivity to bacterial allergens and absence of nonspecific reactions. This difference in the effect of a single external irradiation and prolonged continuous effect of the incorporated substances can be explained, for instance, by difference in the duration of irradiations of both kinds. In fact, after repeated external irradiation (50 r daily to a total dose of 500 r) not amplification but depression of allergic reactions to tuberculin in sensitized guinea pigs was observed. It was found that, besides this, species is of great significance: a single external irradiation of rabbits leads to a complete depression of allergic reaction to tuberculin (in animals sensitized with BCG vaccine before irradiation) with nonspecific reactions absent. The data of G. M. L'vitsyna concerning the appearance of positive reactions to allergens of tuberculosis, brucellosis and tularemia bacteria in animals that received injections of heterologous and homologous liver and intestinal tissue, and in guinea pigs with cutaneous inflammatory loci which appeared after injection of coliform bacteria are very interesting. Evidently, the diverse processes which lead to disintegration of cells and absorption of tissue products, can change the course of allergic tests with bacterial allergens and lead to the appearance of nonspecific reactions. These data require intent attention in order to evaluate properly the data of allergic tests in organisms that have had contact with ionizing radiation.

A question arises: to what degree are these changes in allergic reactivity specific for the effect of ionizing radiation?

It is known, that the depression of the allergic reactivity has not been observed only after irradiation. It has been observed also after the injection of cortisone (272) on a model of anaphylactic shock and the Schwartzman phenomenon (287, 291), in pregnancy (85) and in the development of avitaminoses (65).

Unresponsiveness to tuberculin after injection of yperite preparation has been established in rabbits injected with BCG vaccine (204). These data allow one to conclude, that the development of pathologic processes af-

ter radiation treatment are based on phenomena common to other conditions of the organism connected with a change in its reactivity, differing in its intensity and rate of development due to the peculiarities of the appearance of radiation injuries.

The theory on the leading role of autoallergy in the pathogenesis of radiation sickness could also serve for investigation of the effect of various methods used for prophylaxis and therapy of radiation sickness, because many methods used for this purpose prove to be capable of affecting allergic processes. Thus, for instance, some of them can prevent the body's perception of allergenic stimuli, others depress the development of the process of sensitization which already had begun and so on.

From this point of view, in our opinion, effective therapeutic and prophylactic means can be separated into three large groups according to their effect on the process of autosensitization.

All prophylactic methods whose use is effective only if they are injected several hours or minutes before irradiation may be attributed to the first group.

Substances rather different in their chemical composition (for instance, sulphur-containing organic and inorganic preparations, cyanides and the like) refer to them. In addition to their efficiency in prophylactic use in the struggle against radiation injuries, the presence of a capacity to depress the function of chemoreceptors of vessels, which, as known, has a great significance in the perception of allergic and antigenic stimulation, is a common property of all these substances (167, 13, 14, 146). Against the background of change in chemoreception under the effects of these preparations before irradiation, there evidently occurs a weakening of the toxic and sensitizing effect of the disintegration products of tissue absorbed by the blood stream after irradiation, which results in decreased severity of radiation sickness. The degree of correspondence of the effect of protective substances on chemoreception to the prophylactic effect in radiation sickness is to be studied in detail. If such a correspondence were constantly present, then prophylactic preparations could be selected before they are checked on animals under the condition of radiation treatment; the evaluation of their effect on chemoreception could be carried out in an acute experiment, which is a cheaper and quicker method. Then the effect of the chosen effective substances on survival rate could be tested in radiation sickness.

The second and third group would include agents which exert an effect when used at different periods following external irradiation or against the background of a continuing internal effect of radioactive substances.

Their separation into two groups, in our opinion, is based on different modes of action of these agents on the process of sensitization.

Injection of preparations of heterologous and homologous tissue, injection of antibiotics and blood transfusion can be assigned to the second group.

Each of these effects separately, as known from corresponding literature data, is capable of causing a state of sensitization in the organism, which, evidently, as a reaction to a stronger stimulus, competitively depresses the development of autoallergy.

Effects which decrease the total allergic reactivity of an organism can be grouped together in the third group.

Thus, for instance, special food regimes, vitamin therapy, anesthetic and narcotic agents, anoxia, decrease in environmental temperature and the like (65) exert such effects.

These peculiarities of agents of the three groups mentioned have to be taken into account in the search for new ways to combat radiation sickness.

The theory of the autoallergic nature of radiation sickness can provide data for the basic questions which are the criteria, as B. N. Tarusov (156) indicates, of the accuracy of the theory of pathogenesis of radiation injuries; this theory may explain, why a comparatively small energy effect induces severe pathologic processes and why there is a latent period after which the development of the sickness develops progressively.

Thus, the importance of autoallergy process in the development of radiation sickness is confirmed by many facts: the presence of clinical features characteristic of allergic sicknesses, the efficacy of desensitizing therapy, peculiarities of the change in immunobiological reactivity of the irradiated organism (changes in other allergic states are analogous), changes in antigenic properties of tissue proteins; and experiments on homosensitization, which resulted in some pathologic manifestations characteristic of radiation sickness, in nonirradiated animals (leukopenia, loss of weight, fever followed by hypothermia, and appearance of hemorrhages in internal organs).

Studies of these theoretical problems on the nature of the basic processes in the organism in radiation sickness has great practical value, because they indicate ways to search for effective means to combat this sickness, assist in understanding its causes; and detection of means that affect the depressed immunobiological reactivity of an irradiated organism, makes it possible to evaluate the allergic tests under such conditions and to use corresponding agents to fight the developing autoinfection.

A detailed study of the peculiarities of the allergic reactivity of an irradiated organism requires further investigations in order to solve a series of theoretical and practical problems. Even the data obtained in this direction indicate the great significance of this section of radiobiology.

The investigation of the nature of the autoallergic processes will provide a possibility not only of affecting the response of the organism to various allergens, but also of affecting the basic cause of radiation sickness in order to prevent it and to cure.

Chapter 5

Infectious Diseases in Radiation Sickness.

The data presented in the preceding chapters indicated changes in immunity which appear after the treatment of the organism with ionizing radiation. Due to this, the interaction with the normal microflora of the body is destroyed and at certain radiation doses (median lethal and lethal) the development of endogenic infection usually is observed in an irradiated organism. The sensitivity of irradiated animals to infection with microbe-commensals increases sharply, and the infection usually ends with the generalization of the infection.

If after irradiation an endogenic infection develops because of conditionally pathogenic commensals and if the organism becomes extremely sensitive to infection with them, then, naturally, it will be more sensitive also to infection with pathogenic bacteria—stimulants of infectious diseases.

Our numerous experiments and those known from the literature concerning the infection of irradiated animals by the agents of infectious diseases serve as confirmation; among the latter B. perfringens, B. Breslau, B. tetani, C. diphtherial, Staphylococcus aureus, hemolytic streptococcus, the agents of leptospirosis, malaria, Trypanosoma infection, a series of viruses and so on, can be mentioned. Let us discuss some of them.

An increased sensitivity of irradiated animals to the agent of gas gangrene is revealed in experiments on rabbits (V. B. Sosova), guinea pigs and white mice (R. V. Petrov).

Intracutaneous infection of rabbits with a culture of B. perfringens which normally is never followed by a necrotic reaction with gas formation and bacteremia, leads to the development of such symptoms after infection of animals on the 3rd day following X-irradiation at a dose of 1100 r. If after 18 hours after injection of microbes the inflammatory locus of control animals represents a hyperemic infiltration with dimensions of 3-5 x 5-6 cm, then in irradiated animals, intense hemorrhages and black spots of necrosis on the skin extend over several multiples of ten centimeters, covering the entire belly and breast region, and, on the site of injection, crepitation (i.e. gas formation) was reported.

The infection of a contaminated cutaneous-muscular wound of normal guinea pigs with 0.1 ml of B. perfringens culture leads to death in 40% of animals. However, if the pigs are as a preliminary exposed to a dose of 200 r, then under identical conditions, death from gas gangrene has been observed in 100% of the cases (Table 19).

Table 19

Results of infection of wounds with the agent of gas gangrene in irradiated and control guinea pigs.

Group of guinea pigs	Number of animals	Dose of culture in ml	X-ray dose in r	Number of nonsurviving animals on days following irradiation and infection.						Number of non-survivors within 10 days in %
				1st	3rd	4th	6th	7th	10th	
Infected control	10	0.1	--	--	1	1	1	1	--	40
Irrad. and infected	10	0.1	100	--	2	3	--	--	--	50
Irrad. and infected	10	0.1	200	2	4	1	--	2	1	100
Irradiated control	10	--	200	--	--	--	--	--	?	20

Analogous data are obtained using γ -rays for irradiation.

The mortality of white mice from gas gangrene after intramuscular injection of a minimal lethal dose of the infectious agent on the 5th-7th day after irradiation exceeds by 3-5 times the death rate of intact animals.

The increase in the sensitivity of white mice to oral infection with *B. Breslau* after 2 days following treatment with γ -rays at a dose of 367 r is illustrated in Fig. 29 (the experiments of R. V. Petrov and A. F. Kosov). From Fig. 29 it can be seen that the mortality of nonirradiated infected animals is four times lower than in the control group. Analogous data are obtained after infection of mice irradiated previously (Table 20). At the same time the increase in sensitivity to infection with *B. Breslau* after irradiation does not depend on the stage of the initial susceptibility of mice to the given microbe. Gowen and Zelle (230) have revealed this in their experiments on various strains of mice with mouse typhus; several strains of mice were characterized by high susceptibility to *B. Breslau*, others by low susceptibility one. After two weeks following γ -irradiation at a dose of 400-600 r the sensitivity to infection increased in all animals.

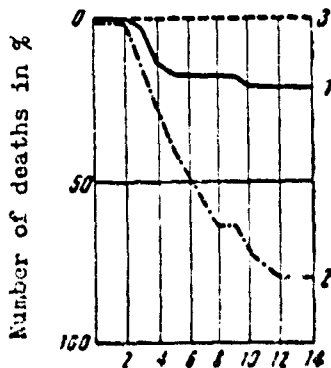


Fig. 29. Effect of irradiation on the mortality of white mice from infection with *B. Breslau* regardless of strain. 1-infected control; 2-irradiated and infected; 3-irradiated control of the irradiation.

Table 20

Results of enteric infection of irradiated and control white mice with B. Breslau culture after two days following irradiation (experiments of A. F. Kosov).

Group of mice	Number of mice	Dose of X-rays in r	Dose of microbe culture in ml.	Number of nonsurviving mice over 30 day period	Death rate in %
Infected control	39	--	3-6	8	20.0
Irradiated and infected	28	300	3-6	18	64.0
Irradiated control	28	300	--	1	3.5

In this relation the tests with experimental leptospirosis are very significant (R. V. Petrov).

It is known that mature guinea pigs and especially white mice are extremely resistant to infection with leptospire. In our experiments, in which parenteral injection of the leptospire culture « Rat Ramenka »¹⁾ was carried out in mature rabbits and white mice, there was not even one case of death in nonirradiated animals (8 rabbits and 60 mice). At the same time nine out of ten rabbits infected after irradiation died, and 26 out of 60 mice died in irradiated control groups, (Table 21).

We failed to observe death in normal white mice using L. icterohaemorrhagiae²⁾ (see Table 21) for infection; guinea pigs died in 30% of the cases, and two out of three infected young rabbits also died. An infection of irradiated animals analogous in every way always led to death from leptospirosis in all mice, guinea pigs and young rabbits. These experiments will be described below in detail. It must be pointed out here that diagnosis of death from leptospirosis is based not only on the fact that the death rate was extremely low in the animal groups subjected to a single radiation treatment, but also on the bacteriological and pathologic-anatomical examination of animal cadavers. The bacteriological investigations (culture and microscopy) of kidneys and blood) showed the presence of leptospirosis in tissues. Pathologic-anatomy and histological investigations, carried out by V. V. Shikhodyrov, showed the presence of morphological changes characteristic of leptospirosis.

1) The strain of leptospire « Rat Ramenka » was obtained from prof. A. A. Varfolomeyeva in Mechnikov's Institute, for which we express our profound gratitude to her. In its antigenic properties this strain is very close to L. canicola and it is pathogenic for guinea pigs.

2) We consider it our pleasant duty to express our gratitude to prof. S. Ya. Liubashenko for the gift of the strain of L. icterohaemorrhagiae No 542. This strain serologically is identical to the Weil strain and it is pathogenic for puppies, guinea pigs and young rabbits.

Table 21

Results of infection of irradiated and control animals with the agent of leptospirosis

Species of animals	Group of animals	Number of animals	Dose of X-rays in r	Number of hours between irradiation and infection	Strain of leptospire	Number of nonsurviving animals	Number of days between irradiation and death
Rabbits	Infected controls	8	--	--	K ¹	0	--
	Irradiated and infected	4	600	2-3	K	3	8-13
		2	600	48	K	2	9
		4	500	24	K	4	10-13
	Irradiated controls	5	600	--	--	1	9
4		500	--	--	1	28	
White mice	Infected controls	60	--	--	K	0	--
		10	--	--	I ²	0	--
	Irradiated and infected	40	350	2-3	K	9	4-30
		20	400	2-3	K	17	3-26
		10	400	96	I	10	6-30
	Irradiated controls	20	350	--	--	2	6-11
		30	400	--	--	13	3-29
Guinea pigs	Infected controls	10	--	--	I	3	19-60
	Irradiated and infected	10	200	2-3	I	10	9-10
	Irradiated controls	10	200	--	--	3	12-15

- 1) << Rat Ramenka >> strain of leptospire.
- 2) *L. icterohaemorrhagiae* leptospire.

All these experiments as well as experiments with the agents of tetanus (R. V. Petrov (124)) and diphtheria (O. G. Alekseyeva (5)), which verify an increased sensitivity of irradiated animals to infection with the agents of infectious diseases pathogenic for them, agree with the literature data entirely. Although other authors in their papers fail to describe experiments in which the irradiated animals were infected with the agents of leptospirosis, gas gangrene, diphtheria, tetanus, all publications indicate the mechanism described. It is displayed in the infection of guinea pigs with the agent of tuberculosis (269), white mice with pneumococci and streptococci (205, 294), white rats with the agents of trypanosoma infection (271), chickens and white mice with the plasmodium of malaria (311, 300) and so on.

The experiments of Shechmeister and Adler (296) are of special interest. They showed increased sensitivity of irradiated white mice not

only to induced infection with the bacteria of pseudotuberculosis, but also to natural infection of uninfected animals by infected ones that were placed in contact with them. After the infected animals had been kept together with the nonirradiated ones, no death was observed among the latter over the course of three weeks. Under the same conditions pseudotuberculosis caused death to 22% of animals that were exposed to 200 r, and to 44-70% of animals irradiated with 350 r. Pseudotuberculosis was diagnosed on the basis of clinical, bacteriological and serological methods.

Experiments on infection of irradiated animals with pathogenic viruses must be discussed separately. As is known, in order to be reproduced the virus must be included in metabolism of the cell (137, 154, 53), which is strongly damaged after irradiation (181, 93, 58). But can a change in metabolism delay the reproduction of a virus and, due to this fact, decrease the susceptibility of an irradiated organism to virus infections? A series of investigations gives a negative answer to this question.

Back in 1939 Clemmesen (202) declared that intracutaneous infection of irradiated rabbits (300-700 r) with the soft fibroma virus leads to development of a tumor which is larger than in control animals, and to a more prolonged its manifestation in the organism.

Much higher morbidity and mortality in irradiated (350 r) mice in comparison with the controls after infection with ectromelia following contact with sick animals were observed by us (R. V. Petrov).

Beutler and Gezon (191); A. A. Smorodintsev (145), V. N. Sivertseva (141), O. P. Peterson and others (121) showed decreased resistance of irradiated white mice and rats to influenza virus.

Sensitivity to infection with rickettsia also increases after radiation treatment (319, 225).

Thus, ionizing radiation increases the sensitivity of the organism to infection with agents of infectious diseases.

How soon does this state appear after irradiation: immediately or after some time?

Different investigators indicate different answers to this question depending mainly on the character of the infectious process used in the experiments.

It was shown in preceding chapters that the state of natural immunological reactivity of the organism after treatment with ionizing radiation does not change immediately. Despite the fact that increased permeability of tissues (68, 70) and regional decrease in bactericidal activity of skin (N. N. Klemparskaya (36)) have been observed on the first day, general resistance to the infectious agent still persists at this time.

There are no cases of bacteremia among irradiated animals till the third day, but the inflammatory reactions during the first and second day after irradiation proceed in a manner identical to those of control animals.

Evidently, almost complete compensation of the developing damages to immunity factors occurs during the first two days, and only after this time further depression and decompensation begins. By the third day bact-

eremia develops, and the inflammatory reactions are characterized by the predominance of hemorrhagic and necrotic components with a sharp depression of proliferation.

Based on these facts one may imagine that the sensitivity of animals to infection with pathogenic microbes will not change during the first day after irradiation, if the infection process is stopped on the 1st-2nd day, i.e. before the period of << breakdown >> of immunity. Experiments show the accuracy of this assumption.

After intramuscular infection of intact white mice with sublethal doses of the agent of gas gangrene mixed with calcium chloride, part of the animals died within 1-2 days; the animals surviving this period lived, but their infection loci became demarcated and did not progress. In consequence, from our viewpoint, the sensitivity of the mice to infection with the agent of gas gangrene on the first day following irradiation is unchanged.

Fig. 30 shows experimental results (R. V. Petrov (124)) on clarification of the period of increased sensitivity of irradiated white mice to infection with *B. perfringens* No 243. Six hundred animals were irradiated with Co^{60} γ -rays at a dose of 439 r; such an irradiation causes death to 3-4% of mice. On the day of irradiation and then after 2, 4 and so on till the 20th day following, 50 experimental animals at a time were subjected to infection. At the same time a group of nonirradiated mice was infected, of which 10-20% died.

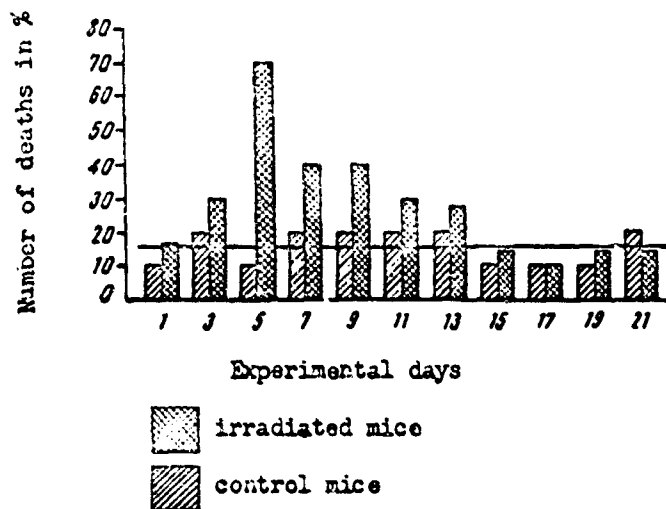


Fig. 30. Mortality of white mice from gas gangrene after infection at different times following irradiation.

The percentage mortality of irradiated mice was different depending on the time which passed between irradiation and infection. If the infection was carried out on the day of irradiation, the mortality rate of irradiated animals was within the limits of the mean percentage of deaths of the controls (the horizontal line on Fig. 30). An increase in mortality due to infection was observed on the 3rd-to the 13th experimental day; its maximum was on the 5th day following irradiation.

An increased sensitivity to B. perfringens failed to show in experiments on guinea pigs on the first day following injection by the method described.

Experiments with intraperitoneal infection of white mice with Staphylococcus aureus are analogous in the respects indicated. In this case the result of interaction between macro- and microorganisms also is resolved during the first 2-3 days. Animals that survive three days after infection survive in the majority of cases. The infection of intact mice led to death of 45% of the animals in our experiments (R. V. Petrov). The irradiated animals died approximately in the same percentage (48%), if infection was carried out on the day of exposure to 450 r. If the mice were infected within four days after radiation treatment, 96% died (Table 22).

Table 22

Results of intraperitoneal infection of white mice by Staphylococcus aureus
No 209

Group of mice	Number of mice	Irrad. dose in r.	Infection dose in bil. of microbes	Number of nonsurviving animals on days following infection			Total number of nonsurvivors	Percentage of nonsurvivors within 3 days after infection
				1st	2nd	3rd		
Infected controls	40	--	2.5	5	11	2	18	45
Infected on the irradiation day	50	450	2.5	4	19	1	24	48
Infected after 4 days after irradiation	50	450	2.5	4	2	2	48	96
Irradiation controls	20	450	--	0	2	3	5	25

If the times of the increase in sensitivity to infection in the experiments reported were established on the basis of mortality of animals, then verification is found in the paper of Clapper and co-workers (200) by the times of appearance of generalization of the focal infectious process. White mice were exposed to 350 r of X-rays and received a subcutaneous sublethal dose of pneumococcus type III. By means of cultures of blood from the tail the rate of appearance of bacteremia was determined.

When infection was carried out immediately after irradiation, the times of appearance of bacteria in blood of experimental and control animals were identical. If the infection was carried out within three days after radiation treatment, then bacteremia in the experimental group developed sooner and was more conspicuous.

In the experiments of V. F. Sosova (see Chapter 1) a model of the focal infectious process was produced by means of subcutaneous infection of

rabbits and dogs by conditionally pathogenic bacteria. Such a process was characteristically stopped very rapidly and within 24-48 hours demarcated by a zone of proliferative inflammation. Owing to this, in animals infected within two days following irradiation, a quick demarcation of the focus developed and the course of the latter did not differ from the control. However the infection on the third day led to the development of necrotic-hemorrhagic inflammation, progressive multiplication of microbes in the focus and generalization of the infection.

Thus, it is characteristic of rapidly proceeding infectious processes, when the result of interaction between macro- and microorganisms is decided over the first two days, that the sensitivity of animals to infection is unchanged on the irradiation day¹⁾. It increases and is distinct from the 3rd day, i.e. in infection during the period of the «breakdown» of immunity.

By this, of course, we do not predetermine the end result of interaction between any irradiated animal, and, let us say, the agent of gas gangrene after transfer to a wound on the day of radiation treatment. The experiments described, including also those with the agent of gas gangrene, show only a model of a rapidly progressing infectious process which is stopped before the period of decompensation of immunity. It is natural that the duration of the infectious process caused by B. perfringens in different species of animals, and also with different doses of the infectant, may be different. Also the period between the time of irradiation and the damage to immunity might differ. It also depends on species and on radiation dose.

The problem of sensitivity to infection during early periods after radiation treatment is solved only in a general form: if the infectious process is stopped during the period of compensation of immunity, i.e. if the end result of macro- and microorganisms is decided before the «breakdown» period, then infection at early times will not display increased sensitivity of macroorganisms.

The matter is different in infection of irradiated animals with infectious agents which have a prolonged course and cover the period of decompensation of immunity even in infections carried out simultaneously with irradiation. For illustration of this one must turn to Table 21, from which it can be seen that infection of rabbits, white mice and guinea pigs with the agent of leptospirosis not only after 2-4 days following radiation treatment, but even by 2-3 hours leads to a lethal course of leptospirosis, while either no deaths or a low mortality were observed in control animals.

Experiments on infection of white mice with live culture of B. tatarum (R. V. Petrov (124)) show the same thing: irradiated animals die of tatarus earlier and in a higher percentage than the controls, if infected during the first hours after exposure to ionizing radiation.

An analogous phenomenon has been observed in tests with experimental malaria in irradiated chickens (300), with trypanosoma infection in white rats (271), with petrusal infection in mice (153), and so on.

¹⁾ It should be remembered, that all this is correct only with regard to median lethal radiation doses. Sensitivity to infection at superlethal doses increases much earlier. We have observed it with gas gangrene in guinea pigs exposed to 2000 r (R. V. Petrov).

An impression is forming that the sensitivity of irradiated animals with respect to the infection by agents of the listed infectious diseases increases immediately after irradiation. The reason for this, as it appears to us, is that these infectious processes because of the duration of their courses cover the period of «breakdown» of immunity. In fact, the course of experimental malaria and trypanosoma infection is measured by weeks. Death due to leptospirosis takes place on the 7th-15th day after infection and later. In our tetanus experiments animals died within 6-10 days.

If it is true that the duration of the infectious process determines the times of increase in sensitivity of an irradiated organism to infection, then two following mechanisms should be observed.

First, sensitivity to infection with the same infectious agent immediately after irradiation should be different, if in one case the infection method leads to the development of a rapidly progressing process, but in the other case to the lingering process.

Second, radiation treatment must aggravate the long continuing infectious process also in the case in which irradiation of the animal is carried out after infection. The first as well as the second phenomena actually take place.

The first is shown in our experiments on gas gangrene (S. V. F. trov). We saw that infection of white mice and guinea pigs with a culture of the agent mixed with calcium chloride led to the development of the acute process which either caused death of the animal or was abolished within two days. The sensitivity of irradiated and nonirradiated animals to infection during the first day after irradiation is identical. If the second infection method is used—in the wound, which leads to development of a more prolonged process, then increased mortality of irradiated animals takes place even after infection during the first day following irradiation (see Table 19 and Fig. 30).

An analogous phenomenon can be observed in comparing the papers of G. N. Kryzhanovskii and N. I. Lebedeva (92) with the data presented above. These authors came to a substantiative conclusion, that irradiation of rats (400 r) 10-12 minutes before the infection of toxin does not aggravate the course of tetanus intoxication. In our experiments, however, tetanus was more severe in mice irradiated immediately before infection.

This contradiction may be easily explained if one keeps in mind that we used the culture of *B. tetani* and the infection led to an infectious process of relatively long duration (6-10 days); however, G. N. Kryzhanovskii and N. I. Lebedeva induced tetanus with a single injection of toxin which caused death of animals within 3-3½ days. However if toxin is used in doses that lead to the development of a prolonged process, then the experimental results agree with ours (74).

The second mechanism is proven by experiments of many investigators. Tests with experimental malaria infection in chickens (311) showed that exposure of chickens to 500 r after 1, 3, 12 and 16 days following infection shortens their life span and increases parasitism.

The results of the experiments of B. N. Sofronova (153) performed on the model of experimental petrusal infection also confirm the facts mentioned above. Irradiation of mice within three days after infection led to aggravation of the infectious process.

Irradiation of white mice with X-rays at a dose of 300 r 1 and 12 days following infection with *B. Breslau* also led to aggravation of the course of infection, indicated by increased mortality of experimental animals (Table 23) and greater weight loss.

Table 23

Effect of radiation on mortality rate of white mice after enteral ^{infections} with *B. Breslau*

Group of mice	Number of animals	Infection dose in mil. of microbes	Radiation dose in r	Day of death after infection				Number of deaths	Percent- age mortality
				1st-6th	7th-12th	13th-18th	19th-24th		
Infected controls	56	12.50	--	5	10	4	4	23	33
	19	6.25	--	0	2	2	0	4	
	20	3.06	--	0	3	1	0	4	
Irradiated controls	28	--	300	0	0	1	0	1	3
Infected and irradiated after 1 day	12	12.50	300	3	4	1	0	8	53
	14	6.25	300	0	6	1	0	7	
	10	3.06	300	1	1	2	0	4	
Infected and irradiated after 12 days	46	10.00	300	-	-	-	-	19	42

Experiments with irradiation at remote times after infection verify the activation of latent infection induced by radiation, the transition of chronic forms to acute, and the possibility of appearance of relapses of infectious diseases after irradiation. This problem is extremely interesting from the theoretical viewpoint and important from the practical view point. The answers cannot be identical for different infections because of the peculiarities in their pathogenesis and in the character of immunities.

It could be assumed a priori, that irradiation will not lead to the activation of such an infection, as gas gangrene, despite the presence of living microbes in tissues, because it fails to produce conditions necessary for the development of gas gangrene. These conditions consist of the presence of necrotic tissues, which cannot be created by irradiation alone. This assumption was entirely confirmed by experiments carried out on white rats (R. V. Petrov). The animals received intramuscular infections with sublethal doses of *B. perfringens*. After 5-6 hours following infection, the site of injection developed edema, which covered the main part of the hip, and crepitation due to the appearance of gas. However, within a day the infection site was demarcated and did not spread. After 3-4 days it was apparent only as a slight consolidation, from which in all cases pure cultures of the agent of gas gangrene could be separated. Irradiation of these rats with sublethal doses of ionizing radiation did not exert a visible influence on the infection site, and the gas gangrene did not develop.

We also failed to induce activation of latent leptospirosis infection in white mice (R. V. Petrov). The infection of these animals with

the agent of leptospirosis leads to a short (3-5 days) leptospiremia which ceases after accumulation of specific agglutinins and lysins in blood. After the leptospiremic period leptospire disappear from all internal organs except the kidneys. Large numbers of antibodies are accumulated in blood (their titer is equal to 1:100 000 and more).

After 3-4 weeks no clinical symptoms of infection are displayed; the infectious agents can be found only in the kidneys. Treatment of the animals with X-rays (400 r) at this period does not cause intensification of leptospirosis.

In this case evidently, there was an infection which could not be activated after irradiation because of the character of the immunity. It is known (9, 19), that accumulation of agglutinins and lysins in blood play a great role in immunity, while radiation, as we saw previously (see Chapter 3), does not exert an essential effect on the number of antibodies formed and circulating.

However, the activating effect of radiation on the latent infectious process is manifested in experiments with a series of other infections. Thus, for instance, irradiation of white mice that were recovering from mouse typhus or that had been carriers of the agent for long periods led to activation of the infection process. The experiments were arranged in the following way: White mice were infected per os with B. Breslau culture at a dose of 12.5 mil. microbe bodies. Such an infection caused death in 40% of the animals. The majority died within the first two weeks. After a month following infection the surviving mice (28 animals) were subjected to X-irradiation at a dose of 300 r. All mice died; in most of the cases B. Breslau were cultured from the internal organs.

In order to check whether activation of the latent infection actually occurs and mortality could be ^{explained} otherwise by a decrease in resistance of the convalescent mice to irradiation, an experiment with infection of animals was carried out; this infection did not show clinical symptoms. For this purpose an amount of B. Breslau was passed by cannula into the stomach which did not kill any mice. Over a course of one month all 38 animals were clinically healthy and gained 4-5 g each. After the mice were checked to determine whether they were carrying the agent (the culture was separated from 2 out of 3), the animals were exposed to X-irradiation at 300 r. Nine out of 35 mice, i.e. 26%, died during 16 days. After such an irradiation 3-5% of animals die of radiation sickness. Activation of the disease as a cause of death was confirmed by the detection of B. Breslau in mesenterial nodes, liver and spleen of nonsurvivors.

However, on the basis of these and similar experiments it is not possible to affirm categorically that the activation of an existing infection always occurs after irradiation. Such experiments do not exclude the possibility of infection of animals from each other after irradiation, when the sensitivity to infection is increased, and release of microbes takes place. Due to this fact a new or repeated sickness, appearing as the result of a new infection, may be considered as activation of the infectious process.

An original solution of this difficulty was found in the paper of Shechmeister and Adler (296). They experimented with mice of the R-line, which are characterized by capacity to develop infection and spontaneous appearance of pseudotuberculosis. Under the usual conditions, the number of deaths from this infection varied from 0.5 to 4% within 12 weeks. If the mice were exposed to X-irradiation at a dose of 350 r or 250 r, then the mortality from pseudotuberculosis increased to 40-69% within 4-10 weeks.

To clarify whether activation of latent infection plays a role in this increase in death rate, or whether the spreading of pseudotuberculosis is due to an increased susceptibility in irradiated animals maintained together, a second experiment was performed by Shechmeister and Adler: one hundred-and-ten irradiated mice were separated into two equal groups; the first one was placed in a common cage. Animals of the second group were placed after irradiation in glass jars, isolated from each other, two mice in each. Thus, a postradiation infection was not possible in the second group. Regardless of this, the death rate during the first three weeks was identical: 20% of mice died of pseudotuberculosis over this time. With the 4th week a difference in mortality between the two groups was observed. While it continued to increase in the first group and reached 60% in the 8th week, in the second group there were almost no deaths after the 3rd week. This difference may be explained by spreading of pseudotuberculosis among irradiated animals of the first group, which was impossible in the second group. Activation of the latent or «subclinical» form is the only reason for increasing mortality from the indicated infection in mice maintained separately.

In connection with this, the data of Schmitt and Thierfelder (288) are of interest. They have observed the development of herpes zoster within 1-73 days after cessation of roentgenotherapy in tumor and other diseases.

It is known that the virus of herpes can always be found in the latent state in the human body and that it causes sickness when the defensive powers of the organism are weakened (53). In this case X-irradiation was the factor which activated the latent infection. Relapse of experimental malaria was observed by Taliaferro and co-workers (311) in chickens irradiated after 20-27, 39 or 72 days following infection.

Activation of latent dysentery after radiation treatment has been observed in monkeys by V. L. Trotskii and M. A. Humanian (157). Thus, the effect of ionizing radiation on the one hand determines an increased sensitivity of the organism to infection with the agents of infectious diseases, and on the other, may lead to the activation of a series of latent or chronically progressing infections.

How long is the duration of the period of decreased resistance of an organism after irradiation with regard to the pathogenic microbes? Inasmuch as the damaging effect of radiation on immunity is the basis of this phenomenon, the duration of this period should depend not only on radiation dose, species and individual sensitivity, but also on the peculiarities of the infectious process and immunity. However, when the effect of radiation on immunity was discussed, we did not touch on the peculiarities during different infections; this affects the duration of period of increased sensitivity of the irradiated organism to the infection with any agent, because various damaged factors of immunity are not restored simultaneously.

There are some examples of variations in the duration of the period indicated depending on the infection but under otherwise identical conditions.

Normal resistance of irradiated (433 r of γ -rays) white mice to intramuscular infection with the agent of gas gangrene is restored within 15 days (see Fig. 30), but increased sensitivity of mice irradiated with a dose of X-rays of equivalent biological effectiveness (350 r) to aerogenic infection with hemolytic streptococcus has been observed during 12 days (292). After similar irradiation the recovery of sensitivity of animals to S. enteritidis takes place by the 30th day (297), but does not occur to Leptospirae even within 10 weeks (R. V. Petrov). Infection of mice with

L. icterohaemorrhagiae killed 14 out of 16 animals within 72 days following irradiation while there were no deaths among the infected controls. Over this period of time the mice did not die of radiation sickness.

Thus, one and the same species of animals exposed to identical doses of ionizing radiation, and in the last three examples at identical dose rates (20-25 r per minute), manifests an increased sensitivity to various infections over the course of different time periods.

Increased sensitivity of irradiated animals to the agents of infectious diseases under the effect of radiation is accompanied by the appearance of peculiarities in the course of these diseases. To characterize these peculiarities, the characteristic features of so-called local focal infections have to be discussed.

We wrote in Chapter 2, that the unusual character of the inflammatory reactions in radiation sickness is manifested in a depression of the development, and sometimes even in a complete absence of the cellular component of inflammatory foci. This was illustrated with the experiments on intracutaneous infection of irradiated rabbits with coliform bacteria and several other microbes. This is confirmed by the data of many experiments published (115, 90, 91, 177, 178), and also by observations of humans affected by ionizing radiation (325, 253).

The results of observations of focal changes appearing after infection of irradiated guinea pigs with the agent of gas gangrene (R. V. Petrov (124)) are given below.

As is known (39), the peculiarities of the local manifestations of gas gangrene infection show in the development of serous-alterative process (edema, necrosis) and gas formation.

In the progressing gas gangrene, edema spreads extremely rapidly, the necrosis of tissues becomes more extensive, and gas is seen far beyond the limits of the infection site. Productive-infiltrating processes have not been observed. Development of the latter is associated only with the beginning of recovery. The infection locus is demarcated by the zone of productive inflammation, and this infiltrate prevents further extension of the anaerobic process, which becomes benign in character. I. V. Davydovskii (39) reports, that a nonspecific inflammatory process which follows the anaerobic specific process is a turning point to convalescence and it verifies objectively the beginning of recovery.

In our experiments on the infection of normal guinea pigs with the culture of B. perfringens, such a turning point started usually within 2-3 days. Under the same conditions, the infiltration which demarcates the infection locus did not appear. As we saw it (Table 19), 100% of the guinea pigs exposed to 200 r died of gas gangrene, while in control animals only 40% died.

Evidently, the depression of the development of productive inflammation is the cause of the second peculiarity in the local manifestations of anaerobic gangrene in irradiated animals. This peculiarity is characterized by a conspicuous edema and necrosis of tissues, and also by an abundant accumulation and vast extension of gas as compared with the control animals.

The third peculiarity is the fact that the changes indicated develop more rapidly in irradiated animals than in nonirradiated. X-ray pictures

illustrate these facts. The unusual character of the local infectious processes is revealed also in the investigation of many infectious agents.

In addition to the data of the first two chapters, the results of computation of the agents of gas gangrene in lg of the focal tissue of irradiated and intact guinea pigs can be presented (R. V. Petrov). For the purpose of calculation, the tissue homogenate was inoculated in a plate with Wilson-Blair agar, whose surface was covered with meat-infusion agar. It can be seen from Table 24, that accumulation of significantly larger numbers of microbes occurs in the infectious foci of irradiated guinea pigs, than that in controls. The difference is especially marked-several thousand fold-if the animal is infected with a sublethal dose of the agent (25 mil. of microbe bodies by the optical standard). However, if a conditionally lethal dose (150 mil. of microbe bodies) is used for infection, then in the foci of irradiated guinea pigs the accumulation of microbes is 1.7 times larger than in the control animals.

Table 24

Number of microbes (in thousands) in lg of the injured tissue of irradiated and control guinea pigs during gas gangrene infection.

Group of guinea pigs	After one day following infection with 25 mil. of microbe bodies.		After 5 hours following infection with a dose of 150 mil. of microbe bodies.		After a day following infection at a dose of 150 mil. microbe bodies.	
	inflam. focus in muscle (from hip)	inguinal cellular tissue	inflammation focus in muscle (from hip)	inguinal cellular tissue	inflammation focus in muscle (from hip)	inguinal cellular tissue
Nonirradiated	63	4.9	280 000	3	1 500 000	35 000
Irradiated with a dose of 350 r four days before infection	210 000	1 400	490 000	51	2 700 000	33 000

Note: Each number is the arithmetical mean of the results of examination of three guinea pigs.

One can see from Table 24, that the multiplication of microbes on the periphery of the focus in irradiated animals occurs more intensely than in controls: within five hours after the injection of 150 mil. of bacteria into the femoral muscles the number of microbes in the region of inguinal cellular tissue exceeds by 17 times that of nonirradiated animals. This difference disappeared within 24 hours, evidently, due to the use of a high infection dose, which caused death of all animals by the beginning of the second day. This assumption is confirmed by the fact that after infection with a sublethal dose of microbes (25 mil.), the number of microbes in the inguinal cellular tissue of irradiated animals after a day following infection was several hundred times higher than in the control animals.

A significant accumulation of microbes in the infectious foci of irradiated animals was observed by us also during experimental diphtheria infection in guinea pigs (O. G. Alekseyeva). The examination of smears-

imprints from the foci of experimental and control animals showed a markedly higher number of microbes in the former.

The experiments of B. N. Sofronov (153) concerning experimental tetanus infection in irradiated white mice also agree with our data.

Analogous results were obtained by V. N. Sivertseva (140) in the study of a focal staphylococcus infection, and by A. A. Smorodintsev (145) in irradiated mice infected with influenza virus.

The accumulation of large numbers of microbes in the infectious foci of irradiated animals is accompanied by an unfailing absorption of the microbes by blood. This peculiarity is described by us in Chapter 1. The experiments of B. N. Sofronov, B. N. Sivertseva, P. N. Kiselev (68, 70), Brooks (194) and others mentioned above, can be presented as sources from literature indicating this accumulation. All the authors listed have recorded an early generalization of the focal infection typical for irradiated animals even in those cases in which it never appears under normal conditions.

The observations of Brooks are especially indicative; they illustrate the generalization of wound infection caused by pathogenic β -hemolytic streptococcus. The experiments were performed on dogs exposed to X-irradiation at a dose of 100 r. Immediately after irradiation, a burn was made, which covered 20% of the body surface; the burn was produced by applying a plate, heated to 60°, to the skin. The wound surface, which appeared within several days beginning with the 3rd-4th day was spontaneously complicated by β -streptococcus infection. In 30 out of 40 dogs the β -streptococcus was detected in blood, and septicemia was observed in all cases after the appearance of the agent in the wound. Streptococci in the wound and blood were serologically identical. There was no generalization of the β -streptococcus infection in 10 animals, and they survived.

In the control (nonirradiated) group of dogs the β -streptococcus complication on the wound surface was not accompanied by the penetration of cocci into blood. The mortality in this group of animals was six times lower than among the irradiated.

Thus, the predominance of alterative inflammation components over the proliferative, accumulation of large quantities of microbes in the focus, and generalization of the infection are characteristic of the focal processes in irradiated animals.

The general symptoms of infection diseases, appearing under the conditions of radiation damage to the organism, also have essential peculiarities. The most distinctive peculiarity which has been observed continuously and has been described over the course of many years, was reported in this book. Higher mortality of irradiated animals after infection with any agent is this special feature.

Is this only the result of a more severe course of infection, or does the infectious disease in turn aggravate the radiation sickness, and the combination of two pathologic processes affecting each other provide a higher mortality?

It is hard to imagine two pathologic processes proceeding simultaneously in an organism without affecting each other. It is even more difficult to demarcate the symptoms of each process and to find out which one was the cause of death. This may be easily decided only under certain conditions, for instance, when radiation sickness has not yet developed, but the in-

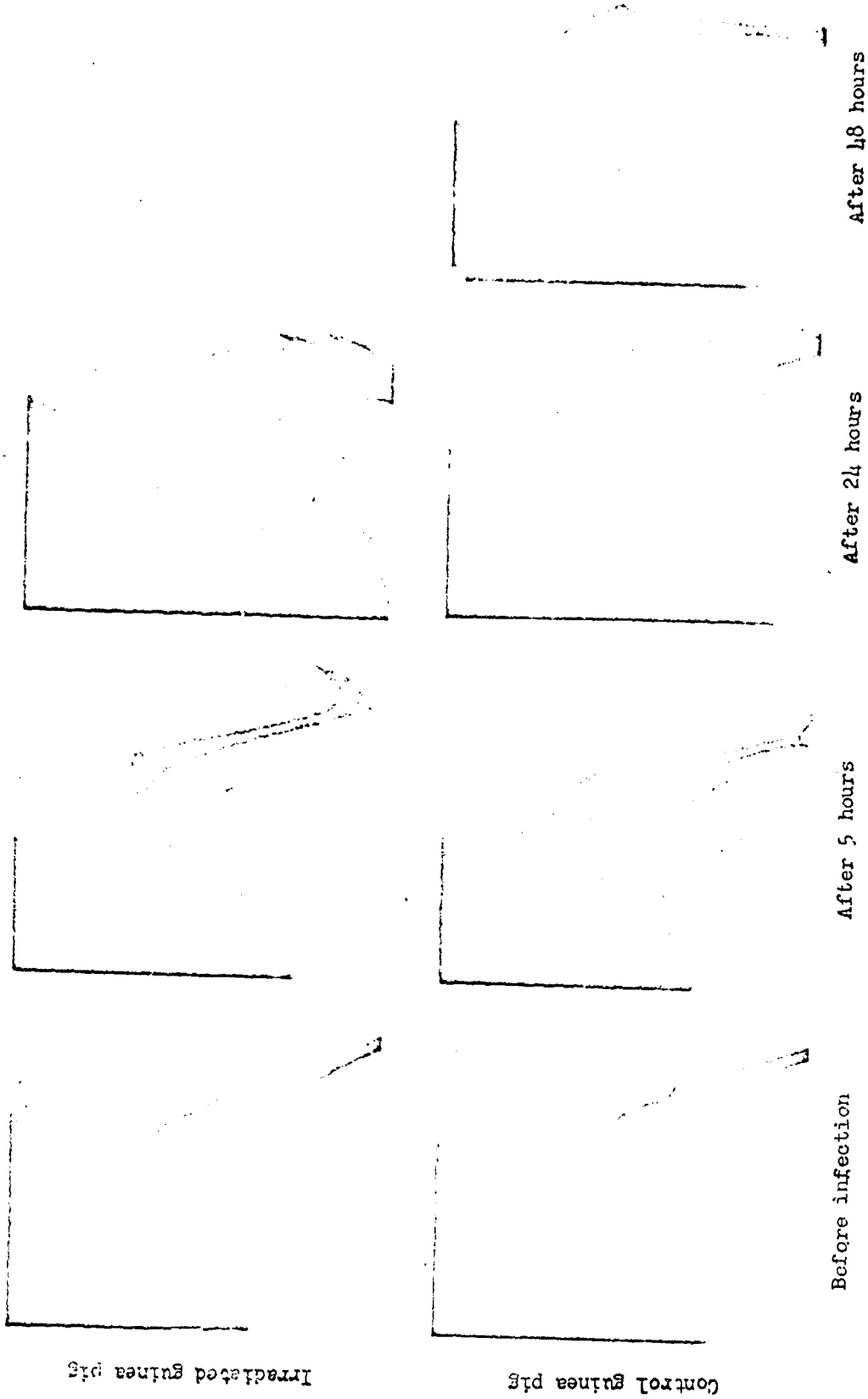


Fig. 31. Change in the X-ray picture in gas gangrene in irradiated and control guinea pigs. The pictures are taken before infection and 5, 24 and 48 hours following it. The control pig survived for 2 days, the experimental for one day.

Irradiated guinea pig

Control guinea pig

fection is proceeding rapidly and exhibits distinct external symptoms. Thus, if on the third day after irradiation guinea pigs are infected with the agents of gas gangrene infection, then they die on the fourth day displaying clearly pronounced gas gangrene. At this time the development of radiation sickness is only beginning, and deaths have not been observed before the 7th-8th day. In such an arrangement of the experiment, one can see clearly that radiation treatment aggravates the course of gas gangrene infection and that the latter is the cause of death. A more severe course of several infections is manifested as clearly, if the animals are infected at remote times after irradiation. We have observed this in the case of experimental icteric leptospirosis in white mice infected after 40 and 72 days following radiation treatment, when acute radiation sickness was already over.

Under other conditions two pathologic processes interlace more tightly, but their combination is not an arithmetical sum; it is more complex. In many cases not only may aggravation or perversion of either manifestation of the disease be observed, as we saw in the example of focal infections, but also a peculiar << extinction >> of one process by the other has been recorded.

It is found by Moroz (cited by I. A. Pigalev (129)), that tetanus intoxication aggravates radiation sickness in white rats caused by injection of polonium at a dose of 0.1 mC per 1 kg of weight. At this dose of the radioactive substance acute radiation sickness developed and 100% of animals died within 30 days. After a simultaneous injection of a lethal dose of tetanus toxin all animals died over the period from the 9th to the 17th day, while the toxin alone caused death of rats on the 5th-8th day. In experimental animals the picture of tetanus intoxication was slightly pronounced, but general tetanus in many cases did not develop at all. Thus, in combining two pathologic processes intensification of radiation injury and weakening of tetanus intoxication were observed simultaneously.

Very interesting examples of the mutual effect of radiation injury and infection were observed by O. G. Alekseyeva in experiments relative to infection of irradiated guinea pigs with the agent of diphtheria. It has been noted, that if two pathologic processes are combined, the strongest one prevails. The weaker process comes forward as supplementary, aggravating the other. If two pathologic processes of approximately equal force meet, then a sharp mutually aggravating effect has been observed (for a description of the experiments see Chapter 3).

At the International Conference on Peaceful Uses of Atomic Energy in Geneva in 1955 I. A. Pigalev (129) put the question relative to the mutual effect of radiation injury and infection. In our book the attention of the reader is drawn to the indicated interaction because the peculiarities of the general manifestations of infectious diseases, which are under the condition of radiation effect, can be discussed only from such a viewpoint.

What are those peculiarities? In which sections of the pathologic process are they manifested?

In fact all manifestations of infection in an irradiated organism differ from those in an intact one. Even in comparing the appearance of animals infected after irradiation with the controls they often show a more severe state. Especially clearly is this displayed in small laboratory animals, in particular in white mice, infected with different bacteria (B. Breslau, agents of leptospirosis, pus-producing cocci and so on). While the control (irradiated only and infected only) animals are still completely healthy in their appearance, the experimental animals are in an evidently sick condition: their hair loses luster, the animals become unclean and

inactive, they sit with fur ruffled. The difference in their appearance persists during the entire experiment.

Great changes in weight indices are typical of experimental animals. For instance, rabbits that were infected with the agent of leptospirosis at one day following irradiation (500 r), lost 380-590 g of weight within 10 days, while animals irradiated only or infected only lost not more than 70 g (R. V. Petrov). In a group weighing of white mice infected with B. Breslau (experiments of A. F. Kosova), an analogous phenomenon was recorded.

Temperature reactions in infectious diseases of irradiated animals are also of interest. As is known, fever is one of the main general symptoms of infectious disease (57). It is known also, that radiation injuries often are accompanied by an increased body temperature, and one of the frequent causes of an increased temperature is a developing infection (31). Therefore, the experiments (R. V. Petrov) in which an earlier hyperthermia is registered in experimental animals in comparison with the controls, do not cause surprise. Thus, for instance, an increase in temperature to 40° and higher was observed in guinea pigs infected with L. icterohaemorrhagiae from the 8th to the 15th day following infection. Under identical conditions an increased temperature was observed in animals infected after irradiation (200 r) over the period from the 5th-8th day. The animals died on the 9th-12th day, but, despite the presence of clearly exhibited infection (jaundice, leptospiremia), by the 8th day the body temperature was normal or dropped below normal figures. After the infection of cutaneous-muscular wounds in guinea pigs with a culture of B. perfringens (R. V. Petrov (124)), peculiarities of the temperature reaction in irradiated animals-absence of a prolonged increase in temperature, typical for normal guinea pigs- was recorded. The infection in irradiated animals led to a temporary rise in temperature, which on the second day dropped below normal; gas gangrene, as indicated above, had a more vigorous course.

The observations of rabbits that were injected with coliform bacteria at different times after irradiation (V. F. Sosova (152)) showed, that the temperature reaction may be absent during the period of marked manifestation of radiation sickness, even during the generalization of the infection. The absence of hyperthermia has been observed in many tests with experimental diphtheria infection in irradiated guinea pigs (O. G. Alekseyeva).

The explanation of this phenomenon may be found in the change in general reactivity of the organism in radiation sickness (33, 97, 129) and also in the disturbance in thermoregulation (31, 20). The latter does not develop immediately after irradiation. The temperature reaction to the injection of pyrogenal in dogs exposed to 400-600 r decreases from the 3rd-4th day. The injection of pyrogenic vaccine at 24 hours following exposure to ionizing radiation (1500-1000 r) causes a smaller rise of the body temperature than in the control group. When it was injected after 48 hours, a decrease in the temperature can even be observed, i.e. a perverted reaction occurs. The disturbance in thermoregulation, obviously, is based on a change in the functional state of the vegetative centers of diencephalon (97).

A similar change in reactivity is revealed also by the leucocyte counts in blood of animals infected after irradiation. It is known that many infectious processes are accompanied by leukocytosis. The infectious leucocytosis in irradiated animals develops only at early times after radiation treatment, and it changes rapidly into leukopenia, typical of radiation sickness. Thus, an intracutaneous infection of guinea pigs with staphylococci within the first two days after X-irradiation at a dose of 100-500 r (experiments of G. A. Shekatile) in 33% of cases leads to the development of leukocytosis. This leukocytosis is temporary, but all the same it is there. The organism reacts

normally to the infectious agents. However, if this infection occurs later, when the increase in the number of leucocytes can be observed only in 18% of the cases, and it cannot be called leucocytosis: there is only a slightly excessive number of white cells in blood after infection as compared with their number before it. After an infection of guinea pigs on the 3rd-10th day after irradiation with 500 r there was no consistent rise in the number of leucocytes. Several absolute numbers, which illustrate these facts, are presented in Table 25.

Table 25

Increase in the number of leucocytes in blood of irradiated guinea pigs within 24-48 hours after intracutaneous infection with a culture of Staphylococcus albus

No. of guinea pig	Day after irradiation on which infection occurred.	Radiation dose in r			
		100	200	300	500
1	1-2nd	4700-11800 ¹	4800-11900	3550-5350	2250-4500
2		5400-13350	2800-4550	2150-4000	
1	3-10th	2450-5000	1700-3200	850-1000	No cases of increased leucocyte count.
2		1800-3700	2700-5500	800-1200	
1	Were not irradiated			9200-15750	
2				8500-14900	

1) The first number indicates the number of leucocytes in 1mm^3 of blood before infection, the second-the maximal increase after infection.

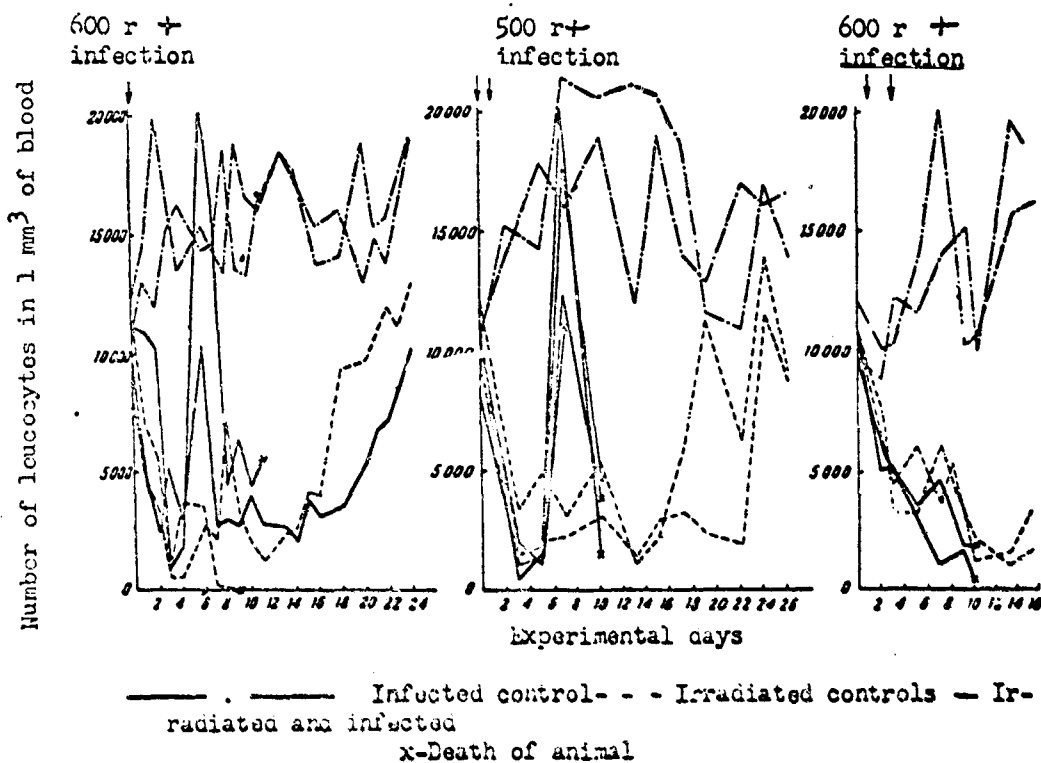


Fig. 32. Change in the leucocyte count in the blood of irradiated and control rabbits in leptospirosis.

Fig. 32 shows the results of computation of the number of leucocytes in the blood of rabbits infected with the agent of leptospirosis at different times after irradiation (R. V. Petrov). One may see, that leptospirosis in nonirradiated animals is accompanied by leucocytosis (15000-20000 cells in 1 mm^3 of blood). Leucocytosis develops also if infection is carried out on the day of radiation exposure or within a day afterward. However, infection within two days following irradiation does not lead to an increased number of leucocytes in blood; their number decreases parallel to that in the irradiated control animals.

Thus, if infection is carried out on the first two days after irradiation, then the infectious process leads to the development of leucocytosis. If the infection is done later, then the microbe does not induce reaction in the form of leucocytosis typical for it. Only a somewhat more profound leukopenia than in radiation sickness which is not complicated by infection, has been observed.

These observations have been confirmed by the experiments of many investigators (194, 271).

Endogenic infectious complications, regularly developing during the << leukopenic period >> (76, 77), also do not lead to the development of leucocytosis; the animals die with a profound leukopenia present.

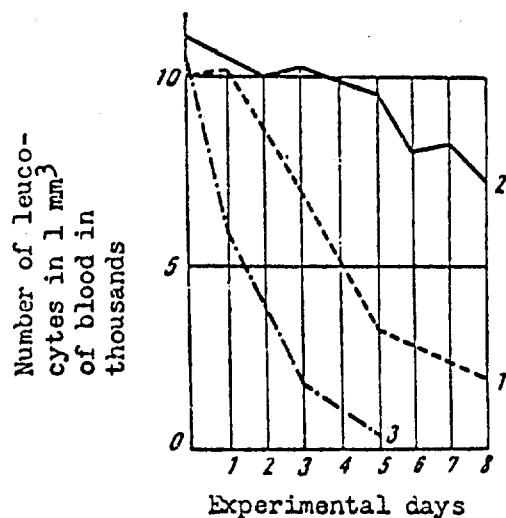


Fig. 33. Change in the number of leucocytes in the blood of guinea pigs during gas gangrene.
1-Irradiated controls; 2-infected controls; 3-infected and irradiated.

It is easy to imagine that in the case in which the two pathologic processes-the infectious and radiation lead to leukopenia, they exert a summed effect. Similar phenomenon could be seen (R. V. Petrov) in the example of experimental gas gangrene in guinea pigs. After infection of the wound with the agent of this disease, development of a slight leukopenia has always been observed (7000-8000 leucocytes in 1 mm^3 of blood, while the normal level is 10000 - 11000). Fig. 33 shows the dynamics of the change in the number of leucocytes in the blood of guinea pigs that were infected only, irradiated only and infected after irradiation. In the latter case leukopenia is characterized by special severity, and it develops decidedly more rapidly, than in the first two cases. The data presented verify the peculiarities of manifestation

of several symptoms common for many pathologic processes (change in weight and body temperature, and reactions of white blood cells).

Is the special character of the course of infections during radiation injuries restricted to this, or does it extend also over the specific features of infectious processes, which separate them in a special group of diseases (the presence of a microbe-agent, its spreading and multiplication in the organism, accumulation of immune bodies and others)?

In characterizing focal infectious processes in irradiated animals we said that accumulation of substantially greater number of bacteria, than is the control group, is typical for them. A series of experiments show, that this is typical also for generalized infectious processes.

An intraperitoneal infection of nonirradiated rats with Trypanosoma lewisi (271) in the amount of 10000-15 000 leads to a phenomenon, in which at the climax of the sickness (on the 10th day) the number of parasites in blood amounts to 200 000-700 000 in 1 mm³. If animals infected at one hour after irradiation (300-500 r) are examined at an identical time, then 1-2 mil. of parasites, i.e. 3-5 times more than in nonirradiated, have been found in 1 mm³ of blood.

If on the day of exposure to a sublethal dose of X-rays (500 r), chickens are infected with plasmodia of bird malaria, then the same regularity has been observed: the number of the parasites in the blood of irradiated chickens exceeds twofold that in controls (18100 to 8 800 computed per 10000 erythrocytes).

Mouse typhus in irradiated white mice proceeds with more pronounced infection of the internal organs and blood (150), than in the control animals. Intravenous injection of Proteus in mice irradiated with 400-500 r (240) leads to a sharp increase in blood, while the number of microbes disseminated by blood of nonirradiated animals decreases sharply.

The facts presented enable one to assume, that accumulation of large quantities of microbes in the tissues proves to be a typical feature of most of the infectious processes in an organism injured by ionizing radiation. Exceptions to this concept must be evaluated correctly. Thus, for instance, Rigdon and Rudisell (281), later Singer (300) under defined experimental conditions discovered a lower parasitemia in irradiated white mice infected with Pl. Berghei. Singer has revealed, that irradiation at one day or 20 minutes before the infection of mice leads to the development of a less pronounced parasitemia, than in nonirradiated animals. An identical radiation dose used within a day or on the 3rd day following infection guarantees almost complete disappearance of the parasites from blood by the 7th through the 11th day; temporarily (by the 5th day) their number increases 3-4 times. However, if the irradiation is carried out on the 7th day after infection then variations in the number of erythrocytes in either direction are insignificant.

Taking into consideration the peculiarities of the mutual effect of two pathologic processes, Singer interprets this unusual response on the basis of two phenomena. Pronounced tropism of Pl. Berghei to young erythrocytes and the absence of capacity to use mature forms of mouse erythrocytes are the first concepts. A quick damage-within a day after irradiation- to erythrocytes (217, 42, 56) is the second concept. By comparing the two concepts one may understand the lower level of parasitemia in mice irradiated a day before infection: it is caused by the smaller number of young forms of erythrocytes in the blood of irradiated mice.

If the irradiation is carried out on the 3rd-7th day after infection, then interaction of at least three main factors, which define the course of the combined injury, occurs: stimulated hematopoiesis which takes place due to the beginning of the infectious process, its depression under the effect of radiation, and damage to the immune mechanisms. Depression of immunity allows a quick multiplication of parasites. However, this situation cannot persist long, because the stimulation of erythropoiesis is replaced by depression, and, consequently, by a decrease in the number of young erythrocytes. Increase in the number of the blood parasites is replaced by decrease. Singer reports, that the stimulation of hematopoiesis which precedes irradiation, imposed a pattern on the course of radiation sickness in that its symptoms were less pronounced. Thus, the meeting of two pathologic processes mutually affects their manifestations.

In the description of the peculiarities of focal infectious processes in irradiated animals, it was pointed out that an early generalization of infections, even in cases in which it never appears in the controls, is typical.

Is this peculiarity common also for infectious diseases, for which spreading of the agent in the whole organism is an absolutely constant pathogenetic link?

Experiments ascertain this question positively (247, 200, 140). The most descriptive results are obtained by V. N. Sivertseva (140) in her experiments with enteric infection of white mice with B. Breslau. After the infection of intact mice the bacteria in mesenteric nodes were first revealed on the 5th day, and in liver and spleen on the 8th day. In animals irradiated (470 r) 2 days before infection, the bacteria in mesenteric nodes, liver, spleen and blood were found as early as the 2nd day.

The same paper demonstrates one more peculiarity of infectious diseases in irradiated animals—decrease in the natural bactericidal activity of the organism toward the agent. Although B. Breslau in the liver and spleen of control mice are discovered only up to the 15th day after infection, in irradiated they are found up to the 30th day.

The experiment of V. N. Sivertseva completely agrees with analogous observations of the co-worker of our laboratory A. F. Kosov.

In experiments with leptospirosis infection in irradiated mice (R. V. Petrov), data are obtained which confirm the regularity described above.

It is known, that leptospirosis is characterized by the presence of a leptospiremic phase which persists for 4-5 days, and by a following dissemination of leptospirilla in the organs; the organisms can be found in kidneys for a prolonged time—up to several months (40, 19, 117). In order to trace those phases in irradiated animals, white mice were killed at different times after infection (2-7 animals at a time) and blood cultures, emulsions of liver and kidneys were made. The tissues of liver and kidneys were emulsified by means of a special apparatus for sterile crushing of the organs (R. V. Petrov (122)). The cultures were incubated for not less than a month.

In Table 26 are shown the results of examinations of white mice at different times after intraperitoneal injection of the «Rat Ramenka» culture within four days following irradiation. Table 26 shows that after the infection of normal white mice, leptospiremia was observed during the first four days, but after the infection of irradiated mice it could be seen significantly longer (7-13 days). In the kidneys of control animals leptospirilla were detected during the first five days, thereafter over the period between the 38th-90th day, and then only on the 220th day. In irradiated mice the agents were de-

ected during the first 16 days. The second period of manifestation of leptospirilla does not persist till the 90th day, but till the 190th day. Examination after 220 days also showed the presence of leptospirilla in kidneys.

It should be noted that we as well as other investigators (35) have observed temporary disappearance of leptospirilla from all tissues examined. However, in irradiated animals these periods were either significantly shorter (from the 6th through the 25th day in control animals and from the 17th through the 25th day in experimental), or were absent (141-190 days).

Table 26

Discovery of leptospirilla in the tissues of white mice at different times after infection.

Group of Animals	Days after infection																					
		1	2	3	4	5	6	7	9	10	11	12	13	14	16	17-25	38	70	90	141	190	220
Control of infection	Blood	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Liver	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Kidney	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+
Irradiation (350 r) and infection	Blood	+	+	+	+	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
	Liver	+	+	+	+	+	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-
	Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+

Symbols: + means that growth of leptospirilla is revealed in the tissue culture; - means that the tissue culture is negative.

A slower disappearance from an irradiated organism of the agents of infection is verified by the experiments of O. G. Alekseyeva on infection of guinea pigs with diphtheria. After an intraperitoneal injection of 2 bil. diphtheria bacteria in guinea pigs that received antitoxic serum, the abdominal cavity becomes sterile within 24-48 hours, while in the animals that were irradiated five days before infection the microbes can be found in the cavity for five days and longer.

Thus, in infectious processes of irradiated animals a longer period of spreading of microbes in the organism, and also a longer period of excretion and natural bactericidal activity was observed.

It was indicated above that in focal infectious processes of irradiated animals, alterative phenomena prevail in the inflammation picture and proliferative components are sharply depressed or are entirely absent. There is a basis for assuming that inflammatory changes in generalized infections are distinguished by the same unusual characteristics. Unfortunately, we failed to find investigations in detail on this question in literature. Let us present the observations known to us.

N. A. Krayevskii (90-91) repeatedly has indicated the unusual character of inflammatory changes in autoinfectious complications during radiation sickness, which are accompanied by dissemination of microbes in the internal organs. Histological investigation of tissues indicates that the islets of bacterial accumulation, and also the necrotic foci are not surrounded by an inflammatory zone.

In macroscopic investigations of mesenteric lymph nodes in mice infected with mouse typhus, V. N. Sivertseva (140) has discovered an increase

in the nodes of the intact animals, and absence of such a reaction in irradiated animals. The experiments of P. N. Kiselev (70) also show the unusual character of the reaction of lymph nodes after radiation treatment; they indicate lower fixing capacity of these nodes relative to pathogenic B. Breslau.

We failed to find data in the literature on the questions of the peculiarities of inflammatory reactions in generalized infections of irradiated animals as well as data on the formation of antibodies in the dynamics of the infectious process after radiation treatment. The domestic and foreign literature is rich in descriptions of the effect of radiation on the production of antibodies, but in all these experiments the effect of ionizing radiation on the formation of antibodies was studied in immunization, not during the course of an infectious disease (see Chapter 3). In connection with this, we think it to be expedient to report in detail on our investigations of the number of antibodies during experimental leptospirosis in animals exposed to X-irradiation (R. V. Petrov).

In addition to the peculiarities of the accumulation of agglutinins, data will be presented which characterize the spreading of leptospirilla in organism. This is necessary because the disappearance of the stimulant from blood during leptospirosis usually coincides with the appearance of antibodies in it (19), and, evidently, is dependent on it, because the mechanism of immunity to this infection is basically connected with the accumulation of immune bodies (9). However, this has not been proven by experiments, in which the depression of the production of antibodies was induced—depression, which leads to a corresponding prolongation of period of circulation of leptospirilla in blood; therefore investigation of the formation of antibodies during the process of leptospirosis infection in an irradiated organism, and parallel studies of the spreading of leptospirilla in it, are of special interest.

The experiments were carried out on rabbits weighing 1.5-3 kg and guinea pigs weighing 200-250 r. The animals were exposed to sublethal doses of X-rays: rabbits to 500-600 r, guinea pigs to 200 r. The infection was carried out with a strain of the agent of « Rat Ramenka » leptospirosis. The rabbits were infected intravenously, guinea pigs intraperitoneally. The infection resulted in development of a latent infection with no lethal outcome in nonirradiated animals.

Analysis of the results (Table 27) showed depression of the formation of antibodies in irradiated rabbits.

The depression was slight in the group of animals infected during the first hours after irradiation. The beginning of the formation of antibodies in these lagged 1-2 days in comparison with the control group. Leptospirilla were found in blood during 6-8 days, while in the control group leptospiremia persisted for 3-5 days.

If the rabbits were infected within a day following irradiation, then a sharp depression of the production of antibodies was observed: the agglutinin titer in blood of irradiated animals was 1:40-1:1600, at the same time in the control group it was 1:100000 to 1:1600000. The beginning of the formation of antibodies was 2-3 days later. Leptospiremia lasted for 9-10 days.

Infection of rabbits within two days after irradiation did not result in appearance of antibodies at all. The leptospirilla may be found in blood till death.

Table 27

Results of infection of rabbits with the stimulant of leptospirosis «Rat Ramenka».

Group of animals	No of the rabbit	Weight of rabbit in g	Radiation dose in r	Hours after irradiation when infections occurred	Duration of leptospiremia from the infection time (in days)	Maximal agglutinin titer in blood	Day after irradiation on which death occurred
Infected controls	2	2 620			5	1620 000	Survived
	5	3 170			4	640 000	"
	7	2 900			4	800 000	"
	8	2 200			4	1600 000	"
	29	2 800			1	800 000	"
	30	2 900			3	400 000	"
	43	1 610			3	800 000	"
	44	1 530			3	1600 000	"
Irradiated and infected	1	2 390	600		8	80 000	13th
	3	2 930	600	2-3	7	128 000	8th
	6	2 800	600		6	1280 000	Survived
	10	2 470	600		6	1600 000	13th
	39	1 860	500		10	1 600	13th
	40	1 530	500	24	10	320	13th
	41	1 620	500		10	800	13th
	42	1 750	500		9	40	10th
	25	2 810	600	48	7	0	9th
	26	2 580	600		7	0	9th
Irradiated Controls	1	2 600	600				Survived
	9	2 500	600				"
	11	2 400	600				9th
	27	2 590	600				Survived
	28	2 580	600				"
	35	1 550	500				"
	36	1 590	500				28th
	37	1 610	500				Survived
	38	1 300	500				"

Fig. 34 shows the dynamics of the formation of antibodies and the duration of leptospiremia in rabbits of the control and the experimental groups.

The experiments on guinea pigs showed the same mechanism. For the purpose of illustration the results of infection of two irradiated and two normal animals are presented in Table 28.

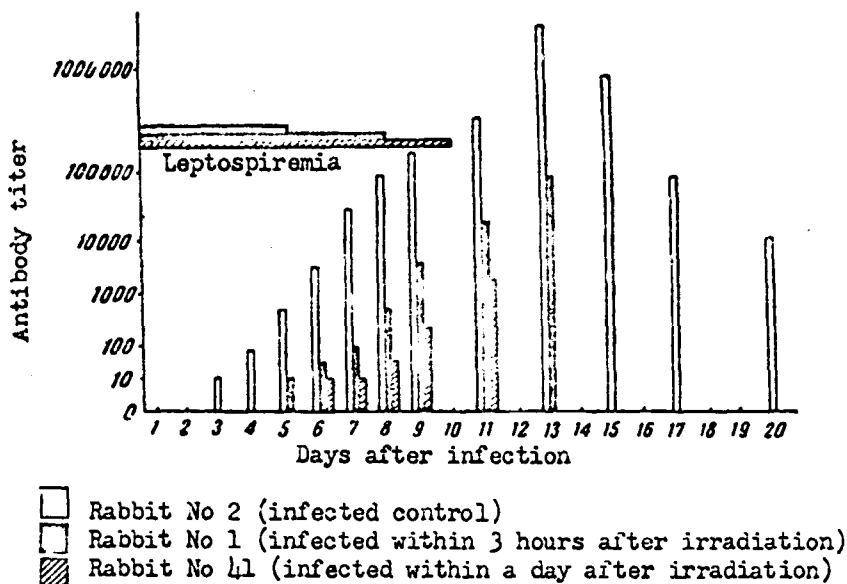


Fig. 34. Formation of antibodies and duration of leptospiremia in rabbits infected with the agent of leptospirosis

Table 28

Results of infection of guinea pigs with the agent of leptospirosis « Rat Ramenka 77 ».

Group of animals	No. of guinea pig	Height of guinea pig in g	Radiation dose in r	Hours after irradiation when infection occurred	Duration of leptospiremia in days	Agglutinin titers on days on which infection occurred.					
						4th	7th	9th	11th	14th	17th
Infected controls	13	250			4	50	1350	4000	4000	36000	36000
	14	230			4	50	1350	1350	1350	1350	36000
Irradiated and infected	5	270	200	48	8	0	50	150	450	Died	
	6	240	200	48	8	0	0	150	Died		

The data presented permit one to draw two conclusions.

First, the depression and deceleration of the process of antibody formation during experimental leptospirosis in irradiated animals are accompanied by corresponding prolongation of the period of circulation of leptospirilla in blood. This fact, in our opinion, is important not only as one of the factors explaining the more severe course of leptospirosis in irradiated animals. It indicates the greater role of specific antibodies in the pathogenesis of leptospirosis, and, in particular, in the mechanism of liberation from leptospirilla.

Second, the formation of antibodies in irradiated animals during the process of infectious sickness is subjected to the same mechanism, which are

established for immunization of the animals at different times after radiation treatment: absence of change or a slight depression of antibody production after the injection of antigen simultaneously with irradiation, and sharp or absolute depression of antibody formation after the injection of antigen within 1-2 days (see Chapter 3).

In describing the depression of antibody formation in radiation sickness, the problem of the diagnostic value of determination of antibodies in blood during any infectious disease of an irradiated animal cannot be overlooked. Taking into consideration that the infection in such an organism can proceed without the appearance of immune bodies in blood, reactions of the Vidal type under the conditions of radiation injury might lose any diagnostic value.

In Chapter IV we reported the peculiarities of allergic reactions in irradiated animals. It was noted that their specificity decreases and non-specific allergy to the substances of various bacteria even appears. It is natural that this fact also lessens the value of allergic tests as diagnostic means.

The diagnostics of infection in radiation injury on the basis of clinical features also can be puzzling, inasmuch as the clinical manifestations of an infectious sickness, as was shown in this Chapter, distinguish themselves by their atypical character.

All this permits us to assume that only the isolation of the culture of a specific agent can be considered as an absolutely reliable diagnostic sign of infection in an irradiated organism. However even this may in a series of cases be difficult due to the dissemination of the microbes of autoflora into the tissues in radiation sickness (see Chapter 1). The last circumstance requires obligatory use of elective culture media.

Before completing this Chapter, it is necessary to answer two more questions which are important from both the theoretical and the practical viewpoint.

The first can be formulated as follows: since ionizing radiation sharply destroys the mechanism of nonsusceptibility, which leads to an increased sensitivity of the organism to pathogenic bacteria and to a severe, usually lethal, course of infections, can an irradiated organism not be affected by an infection, which is not characteristic of this species? We did not find special investigations dedicated to this problem, but several data in literature and our own studies permit one to answer this question negatively, temporarily. Kolmer and co-workers (251) have announced, that they failed to overcome an inherited nonsusceptibility of rabbits, guinea pigs, rats and fitchews to the virus of poliomyelitis, despite the fact that the animals were subjected to irradiation twice-before and after infection. The radiation doses were equal to 100-250 r each. At the 12th Conference of Microbiologists, O. P. Peterson and co-workers (121) reported analogous data relative to preservation of the inherited nonsusceptibility of guinea pigs to the virus of yellow fever after irradiation. Our efforts to cause diphtherial (O. G. Alekseyeva) or anthracic (R. V. Petrov) infectious processes in irradiated white rats failed; they were infected with the bacteria of diphtheria and Siberian plague. The species nonsusceptibility of animals was preserved.

An analogous conclusion can be drawn also on the basis of an experiment, which aimed at the creation of a «*jar epidemic*» of leptospirosis among irradiated white mice (R. V. Petrov). Transfer of mice infected with the agent of leptospirosis (strain «*Rat Ramenka*») to healthy

animals did not lead to the development of epidemics among the animals in contact. This fact was confirmed by negative results in the cultures of organs of mice killed at different times, and also by the absence of specific antibodies in the blood. This is understandable, because mature white mice possess a high natural resistance to leptospirosis, and, as we saw previously, even an induced infection of white mice with the culture of leptospirilla leads to the development of a latent infectious process only. After the induced infection, a clinically apparent infection with lethal end results developed in irradiated mice, therefore, one might expect it to be possible to cause epidemics in a jar of irradiated animals by placing infected mice among them. However, prolonged observation of these mice indicated that they as well as the intact mice, failed to become infected with leptospirosis, i.e. despite irradiation (350 r), the natural nonsusceptibility was preserved. Of course, we realize the relative nature of the results of this experiment, since we do not know whether the conditions were favorable for infection of healthy mice by the sick and, if they were, what was the infecting dose. However, in the perspective of the experiments presented above, this experiment also could be evaluated as the verification of the preservation of species' nonsusceptibility in radiation sickness.

In all probability, diseases not characteristic of the species, cannot appear after radiation treatment, which, apparently, depresses all factors of immunity (phagocytosis, formation of antibodies and so on). If this is correct, then the experiments on irradiated animals demonstrate once more that specific nonsusceptibility is dependent not only on phagocytosis and other universally accepted factors of immunity, but also on the biochemical composition of the tissues and on the type of metabolism. The latter, evidently, creates conditions which are inadequate for the interaction between the two species, i.e. for the appearance of an infectious disease.

From the practical point of view the question concerning the efficiency of antimicrobial therapy of infectious diseases under conditions of radiation injury is rather important.

The data of Chapter 1, which discuss methods for combatting endogenic infection in radiation sickness, show the definite efficiency of many specific antimicrobial means, among which antibiotics occupy the leading place. This alone permits one to assume, that in exogenic infectious diseases of irradiated organisms, the specific antimicrobial means vary in efficiency. Illustrative facts are obtained in experiments with different diseases. Let us discuss some of them.

Treatment of white mice with penicillin begun 24 hours after intramuscular infection with 100 Dlm of hemolytic streptococcus leads to the recovery of animals and to the destruction of the microorganisms, even at the site of injection, within 48 hours. A similar experiment on animals exposed to a dose of 450 r one day before infection showed high efficiency of the therapy: the experimental animals were dying parallel to the irradiated control animals, i.e. of radiation sickness, not of streptococcus sepsis. However, at the injection site the living streptococcus was preserved for a long time. The effect of biomycotherapy in a similar experiment, judging by the survival rate, turned out to be two times smaller than that in irradiated mice (247).

The use of penicillin for the therapy of infectious complication of the wound surface in irradiated dogs decreased their mortality from 73 to 19%; the mortality in the control (nonirradiated) group was equal to 12% (Brooks and co-workers (194)).

Treatment of experimental petrusal infection in irradiated white mice showed a decreased therapeutic effect of immune serum and biomyacin using them separately (153).

Experiments on studies of efficiency of specific therapy of experimental gas gangrene in guinea pigs exposed to 200 r of X-rays were carried out by R. V. Petrov. Gas gangrene was initiated by intramuscular injection of an absolutely lethal dose of *B. perfringens* mixed with calcium chloride. A clinically pronounced infection developed within 3-4 hours, and the animals died within 1-3 days.

A combination of specific antitoxin serum with penicillin was used for therapy. Such a combination, according to data in the literature (99, 320), is the most effective. In our experiments the medical treatment began within 1-3 hours after infection. Penicillin at a dose of 1-2 tolerance units per 1 kg of animal weight was injected directly into the focus of infection. Serum (0.2-0.4 tolerance units per 1 kg of weight) was injected in the muscles of the other (healthy) hip. At one hour after infection the indicated preparations were injected once. In the case in which therapy began within 3 hours after infection, injection of the preparations was repeated after one day at an identical dose.

The experimental results are presented in Table 29. The mortality rate was evaluated over a seven days' period. Such an observation period was selected for two reasons. First, usually no deaths were observed in animals irradiated only, at this period (at this radiation dose guinea pigs die after the seventh day of radiation sickness). Second, when this infection method is used, the animals die of gas gangrene during the first three days. The majority of animals die on the 1st-2nd day. In guinea pigs that survived seven days due to the medical treatment, the focus of infection is demarcated by an infiltration zone and becomes encapsulated; in these cases we failed to observe death of animals of gas gangrene at later times. The results obtained agree with the data in literature (2).

Local manifestations of gas gangrene infection were evaluated by us according to the following scale: \square - the presence of crepitation, i.e. accumulation of gas in the tissue; $++$ the presence of edema and infiltration exceeding the limits of the hip; $+$ the presence of infiltration covering the hip; $+$ the infiltration not covering the whole hip, its diameter not exceeding 1 cm.

One sees from Table 29 that the efficiency of the therapy of experimental gas gangrene in guinea pigs is different and depends first of all on the time that passed between the infection and beginning of therapy. Thus, while the combination of serum and penicillin within one hour after infection resulted in recovery of all guinea pigs, a similar therapy started within three hours after infection, was effective in 13 out of 15 animals. Therapy of experimental gas gangrene with penicillin or serum applied separately is also effective, if the preparations are injected earlier.

A more severe course of experimental gas gangrene and lower efficiency of therapy with penicillin only or serum only, is characteristic of irradiated guinea pigs.

Table 29

Effect of radiation on efficiency of the therapy of gas gangrene in Guinea pigs.

Group of animals	Therapy within one hour following infection				Therapy within three hours following infection				Local manifestation by the 7th day in survivors	
	number of animals	local manifestations of infection within a 7 day period following infection	non-survivors within a 7 day period in nonsurvivors	local manifestation of infection in nonsurvivors	local manifestation by the 7th day in survivors	number of animals	local manifestations of infection within a day following infection	non-survivors within a 7 day period of infection in non-survivors		local manifestation by the 7th day in survivors
Infected controls	15	+++Γ	15	+++Γ		5	+++Γ	5	+++Γ	
Irradiated + infected	10	+++Γ	10	+++Γ		5	+++Γ	5	+++Γ	
Infected + serum	20	+++Γ	3	+++Γ	++	4	+++Γ	2	+++Γ	++
Irradiated, infected + serum	10	+++Γ	4	+++Γ	++	3	+++Γ	1	+++Γ	++
Infected + penicillin	10	+++	0	+++	++	15	+++Γ	2	+++Γ	++
Irradiated + infected + penicillin	10	+++Γ	2	+++Γ	++	10	+++Γ	1	+++Γ	++
Infected, serum + penicillin	10	+	0	+	-	5	+++Γ	0	+++Γ	++
Irradiated + infected + serum + penicillin	10	++	0	++	+	10	+++Γ	1	+++Γ	++
Irradiated controls	10		0			5		0		

Note: The radiation dose was 200 r.

On the contrary, the combination of serum with penicillin is equally effective against gas infection in both irradiated¹⁾ and nonirradiated animals.

Obviously, the use of a single antitoxic (serum) or antibacterial (penicillin) agent in a nonirradiated organism is effective in halting the infectious process. The same method used in the case of gas gangrene in irradiated animals proves to be less effective, and only the combination of both means leads to the desirable result. The explanation for this phenomenon, evidently, is similar to the explanation for a higher sensitivity of irradiated animals to infectious agents, i.e. depression of the immunity factors (leukopoiesis, phagocytic activity, formation of antibodies and so on).

The data presented indicate that if an infectious disease begins in the irradiated organism, then the therapeutic effect is highest in cases in which therapy is directed simultaneously against all pathogenetic links of the infection.

On closing this section one is tempted to answer the following questions: are the indicated peculiarities of the course of infectious diseases specific only for radiation injury of an organism or do such perversions appear in different pathologic states characterized by profound damages to metabolism? Many published papers showing the peculiarities, similar to the described ones of infectious processes during starvation, avitaminosis, disturbances in the activity of endocrine glands, acid-base equilibrium, different poisonings, etc. give the answers to this question.

During alimentary dystrophy, for instance, the sensitivity to the agents of infectious increases, and even such microbes, as the fecal alkalization agent or Bacterium Morgani frequently proves to be pathogenic for humans (127). The formation of antibodies after immunization and during the course of the infectious process is sharply depressed in protein deficiency (127, 197).

Numerous experiments of A. M. Kirchenstein (78) show the peculiarities of disturbances in immunity and in the course of infectious diseases during avitaminosis. The similarity of these disturbances with those of radiation sickness is obvious. The sensitivity to bacteria and their toxins in scurvy increases by several multiples of ten. Infectious diseases (staphylococcus infection in guinea pigs, pneumonia in humans) proceed in a more severe form and frequently with no leucocytosis or increased temperature. The phagocytic activity of leucocytes is decreased, and the function of the reticulo-endothelial system is depressed which is demonstrated, in particular, in the weakening of the capacity of regional lymph nodes to retain the bacteria spreading from the infection focus. The formation of antibodies is depressed, and complement titer in blood decreases. An analogous phenomenon has been observed in deficiency of other vitamins. Thus, latent psittacosis infection of parrots which fails to give clinical features, is transformed into a lethal disease, if vitamin B₁ is excluded from their food (273). Micholitz and Clausen (quoted from A. M. Kirchenstein (78)) have observed, that rachitic children are more susceptible to infections and in particular to whooping cough, than children who obtain D vitamin in food. Predisposition to infections has also been observed in A-avitaminosis.

1) The fact should be emphasized, that the infection of animals was carried out within the first three hours following irradiation. There is a foundation for assuming that after the infection at later times, the therapy of gas gangrene will be less effective.

Disturbances in hormone balance also increase the sensitivity of the organism to infections. This fact was emphasized even in the papers of I. I. Mechnikov (106). Experiments show an increased sensitivity of animals to the agents of many infections after the injection of cortisone (290, 267). The injection of testosterone in mice increases the multiplication of influenza virus in lungs (246).

A decrease in phagocytic activity, the capacity to produce antibodies, weakening of bactericidal properties of the organism and decrease in the resistance to infection have been observed in experimental acidosis (192, 310). Poisoning of white mice with malonate decreases their resistance to S. typhi marium (190). V. F. Sosova has reported on analogous changes in inflammatory foci in animals poisoned with benzene during radiation sickness.

The data presented permit one to affirm, that the described peculiarities of infectious processes under conditions of radiation injury are not specific or characteristic only of radiation sickness. They are the result of a profound damage to immunity and reactivity of the organism, which may occur not only in response to ionizing radiation, but also in other pathologic states. However, in hypovitaminosis, hormonal disturbances, starvation and so on, immunological reactivity can be preserved at a high level for a prolonged time, and in many cases its changes are not so profound or do not involve all factors of immunity. In radiation sickness, the disturbance in immunobiological reactivity takes place rapidly, comprises all factors of immunity and is manifested sharply. Therefore when an infectious disease appears after radiation exposure the peculiarities of interaction between the macro- and microorganisms, to which this chapter is dedicated, will certainly be observed.

C o n c l u s i o n s

Our data presented in the present paper refer to all basic disciplines by which the peculiarities of interrelations between microbes and an irradiated organism are characterized.

Data on changes in the properties of microbes and dynamics of their spreading in an irradiated organism are given. The basic peculiarities of immunobiological reactivity and the character of allergic reactions are demonstrated. What is the significance of the data obtained for theoretical studies of radiation sickness and for practical development of measures for combatting infections in irradiated organisms?

In the field of studies of autoinfection in radiation sickness, the data indicate that under the effect of the irradiated organism the biological properties of its microbe-autoflora change significantly, the virulence of microbes and their resistance to antibiotics increase and, the bacterial count both in inflammatory foci and in body cavities inhabited by microorganisms increase by several hundred thousand times. However, this change in properties and increase in the amount of the microbe mass fails to occur immediately after irradiation. During the first 2-3 days the organism still possesses sufficient possibilities for stopping the infectious processes, and its tissues are not infected with bacteria of the autoflora. The observations of the dynamics of development of autoinfection showed the presence of high defensive functions in many organs, in which noticeable damage begins (at lethal radiation doses) only from the 4th-5th day following radiation. Consequently, the use of powerful bacteriostatic agents and antitoxic preparation by the first day after irradiation can entirely prevent (as shown in the model of gas gangrene infection) the development of infectious processes.

According to the data in the literature and on new experimental models (leptospirosis, gas gangrene) aggravation of the course of infections has been shown in irradiated animals. This is manifested in an increased mortality rate and in the significantly larger size of local inflammatory processes with accumulation of huge numbers of the microorganisms in tissues. Depression of antibody formation, increased duration of the retention of pathogenic microbes in the organism and prolonged excretion has been noted. The character of the temperature and leucocytic reaction typical of the given infection changes, too.

The course of infectious diseases, whose duration covers the phase of clinically displayed radiation sickness, changes especially sharply. However if the infectious disease is of a short duration, (which happens rarely) and the infection occurs on the first day following irradiation, then the course of the disease is almost unchanged in comparison with the nonirradiated animals.

Thus, an infected and irradiated organism appears to be a more dangerous source of infection, than an infected but not irradiated; however it

is much more difficult to reveal the cause of this due to the mild or non-typical course of the disease and the unreliability of the general serologic and allergic reactions (because after irradiation the production of antibodies and development of allergy changes). Therefore, the laboratory investigational methods which permit the detection of pathogenic microbes in blood and excretions of the organism become very significant. The sanitary-hygienic measures which prevent the possibility of carrying the infection to other organisms are also very important. Not only have the facts of a different infection course in irradiated organisms been stated by us, but also efforts were made to find therapeutic means for these processes. It has been shown, that the combination of antitoxic serum and antibiotics can be useful and may lead to recovery from an infectious disease (gas gangrene) of animals subjected to lethal radiation doses.

Profound disturbances in the immunobiological reactivity of the organism lie at the basis of phenomena which characterize the change in infectious processes after irradiation. It is known that the reactivity of an organism depends on many factors-functional state of the nervous system, endocrine regulation, food rich in protein and vitamins, state of metabolism, activity of many enzyme systems, and oxygen supply. The effect of seasonal and climatic factors are of a great value, too.

The importance of the indicated factors in the formation of immunity in an irradiated organism has hardly been investigated at all. The basic attention of the authors of the published works was dedicated mainly to the study of the change in the production of antibodies and in the intensity of phagocytosis together with the evaluation of the resistance to infection with living microbes or to the injection of exotoxins.

In addition to the characteristics of antibodies, phagocytosis and resistance of infected irradiated animals to living microorganisms, other manifestations of defensive functions of an organism are also clarified in our book: the bactericidal and bacteriostatic activity of the tegmina, organs and their excretions, the adsorption capacity of cells and tissues, the production of lysozyme and the determination of the phagocytic activity not only from *in vitro* samples, but also by injecting living pathogenic microbes in the irradiated organism. All these multiform manifestations of the capacity of the organism to destroy microbes are not damaged immediately after irradiation and not simultaneously. Also the depression is not continuous, but in phases usually coinciding with the times of exacerbation of the clinical symptoms of the irradiated organism. It has been noted that injuries to the defense reactions frequently are arranged in zones, and they are displayed nonuniformly in various body sections, probably, in connection with the differing severity of injury of individual organs and systems by radiation.

The use of several tests for the detection of early disturbances in natural immunity in irradiated organisms may have practical significance (as for instance, determination of the bactericidal activity of skin, amount of lysozyme in saliva, phagocytic reaction, content of microflora) in order to diagnose early the presence of pathologic effects of radiation on the organism.

Observation of the state of natural immunity permits one to detect changes in the reactivity of the organism under the effect of therapeutic treatments, and may help in the evaluation of the efficiency of the medical treatment.

As is known, the presence of an induced immunity in the organism exerts the strongest specific defensive effect against infection. In this book, for evaluation of the state of active and passive immunity in irradi-

iated organisms, not only are the reactions of immunity used, but also infection with living bacteria (agents of diphtheria, typhoid, gas gangrene and tetanus). According to the data from the literature and from our experiments, depression of immunobiological reactivity after irradiation takes place, especially during the period of the clinical syndrome of radiation sickness. Besides this, it was found that depression of active immunity is displayed differently after different routes of injection of antigens, and it can be compensated to a large extent by preliminary immunization before irradiation. Revaccination after irradiation increases the survival rate of infected animals subjected to irradiation in comparison with vaccination alone. At the same time, another useful effect of preliminary (before irradiation) vaccination can be mentioned: the vaccinated animals tolerated radiation sickness significantly better and survived to a larger extent. Consequently, in addition to its important specific prophylactic effect, vaccination before irradiation may have a practical value for the abatement of the intensity of radiation injury. However, if an irradiated organism is infected, then it was observed, that irradiation not only decreases the efficiency of vaccination, but increases the severity of the postvaccination reaction to a large extent. Taking these data into consideration, great attention should be paid to the discovery of indications and contraindications for parenteral vaccination in humans subjected to the effect of ionizing radiation.

The presence of a decrease in the efficiency of passive immunization in irradiated animals verifies the importance of the role of active response of the organism to the injection of therapeutic serum. This active response to already-formed antibodies, obviously, is strongly depressed after treatment with ionizing radiation. However, despite the serious changes in the reactivity of the organism, the efficiency of serum therapy was successfully increased by increasing the serum dose and combining its injection with the use of antibiotics.

It is shown that in irradiated organisms not only does the efficiency of active and passive immunization decrease, but also allergic reactions, which appear due to the injection of heterologous protein (Schwartzman phenomenon, anaphylactic shock, cutaneous tests with bacterial allergens), are depressed. Intensification of the cutaneous reactions to bacterial allergens and appearance of intensely displayed nonspecific reactions are observed in guinea pigs, which makes it impossible to use cutaneous tests in guinea pigs for evaluation of the infection of the organism with corresponding infectious agent.

Thus, the character of the reaction of an organism to the injection of substances of antigenic nature changes substantially after treatment with ionizing radiation. Development of a state of sensitization to the disintegration products of the intrinsic tissues proves to be one of the reasons for this change.

It is well known, that if a reaction to some antigenic stimulant appears in an organism, then perception of the effect of other antigenic agents decreases sharply. Investigation of the problems of autoallergy of an irradiated organism has great theoretical and practical significance. The facts relative to the change in antigenic properties of tissues, biological effect of homosensitization, detection of cytolysins and redistribution of the tissue proteins after irradiation, labial tests on the extracts of homologous tissues and so on, mark only the beginning of investigations in this field.

Change in the reactivity of the organism to bacterial after homosensitization deserves attention: after the infection of sensitized rabbits and guinea pigs a type of necrotic-hemorrhagic reaction identical to that in irradiated animals has been observed. The animals sensitized with tissue

substances of the same species of organisms acquire an increased sensitivity to the effect of radiation, which may explain why in trauma, pregnancy or infection (i.e. during processes connected with tissue sensitization) the organisms are affected by radiation more severely than the healthy ones. The studies on allergies to tissues have great theoretical value, because they pertain to the field of studies of the pathogenesis of radiation sickness. Obviously, the solution of this problem is of great practical importance in order to discover pathways for prophylaxis and therapy of radiation injuries.

Many of the reported changes in the reactivity of an irradiated organism may be found also in other sicknesses and therefore, apparently, cannot be considered as specific for radiation sickness. However the combination of all changes, their significant intensity against the background of the general state of the organism, which frequently is still good, are characteristic of radiation injuries. The presence of compensating reactions makes it necessary for one who uses immunobiological tests for the evaluation of the state of the irradiated organism not to confine himself to any one indication, but always to use the data of several reactions.

The study of interrelations of microbes and the irradiated organism proves to be one of the most interesting sections of medical radiology, and has great theoretical and practical value. Further accumulation of data in this field will permit one to detect means for successfully combatting the infection developing in irradiated organism.

Literature cited.

1. Avetikian B. G. and Artemova A. G., Medical Radiology, 1956, No 4, p. 35.
2. Ageyev A. K., Theses of Reports on All-Union Conf. of Pathologoanatomists June 4-9, 1954, L., 1954, p. 86.
3. Ado A. D., Antigens as Extreme Stimulants of Nervous System, M., 1952.
4. Alatyrtseva I. E. and Usmanova S. A., ZhMEI., 1955, No 11, p. 87.
5. Alekseyeva O. G., Experimental Study on Effect of Radiation Damage to the Resistance of the Organism to Diphtheria Bacteria, M., 1954.
6. Alekseyeva O. G., Herald of Roentgenology and Radiology, 1957, No 1, p. 15.
7. Alekseyeva O. G. and Domshlak M. P., Quoted from Leshkovich L. I., Zh MEI, 1958, No 2, p. 34.
8. Alisov P. A., Naval Physician, 1944, ed. 4, p. 49.
9. Aristovskii V. M., Minkevich I. E. and Frid S. M., Textbook of Medical Microbiology, Medgiz, 1949.
10. Bagirova L. E., Data to the Characteristics of Effect of Nervous System on Process of Production of Antibodies and Opsoning-Phagocytic Activity of Leucocytes, Baku, 1953.
11. Berliand A. S. and Bronshtein R. M., Clinical Medicine, 1934, v. 12, No 5, p. 734.
12. Blumberg M. Ya., Treatise of the Microbiology Institute of Ac. of Sc. of Latvia SSR, 1953, No 2, p. 113.
13. Bogatskaya L. N. and Kogan Yu. S., Bulletin of Experim. Biology and Medicine, 1953, No 11, p. 11.
14. Bogatskaya L. N. and Kogan Yu. S., Bulletin of Experim. Biology and Medicine, 1953, No 12, p. 49.
15. Bogomolets A. A. Crisis of Endocrinology, M., 1927.
16. Bogomolets A. A., Manual in Pathologic Physiology, Ac. Sc., USSR, 1940.
17. Boidyk R. I., Diagnostic Value of Comparative (Local) Leucocytosis in Some Internal Sickesses, Vinnytsa, 1952.
18. Vartapetov R. and Zhuchenko P., Medical Worker, 1954, No 102 (1326), Dec. 21.
19. Varfolomeyeva A. A., Leptospirosis in Human, 1949.
20. Volokhova N. A., Medical Radiology, 1956, No 4, p. 25.
21. Gamaleya N. F., Infection and Immunity, Medgiz, 1939.
22. Garshin V. G., Herald of Roentgenology and Radiology, 1938, v. 19, p. 346.
23. Garshin V. G., Alimentary Dystrophy in Blocked Leningrad, Medgiz, 1947.
24. Garshin V. G., Bol'shakova M. M. and Osinskaya V. V., Herald of Roentgen. a. Rad., 1935, 14, p. 371.
25. Garshin V. G., Zakhar'evskaya M. A. a. Osinskaya V. V., Herald of Roentgen. a. Radiology, 1936, 18, p. 412.
26. Ginzburg I. S., Symposium of Sc. Papers on Oncology and Associated Fields, Baker, 1939, ed. 2, p 25.
27. Gogochkina A. A., Phagocytic Reactivity of Blood Leucocytes in Some Internal Sickesses, Gor'kii, 1953.
28. Goncharova V. I., On Some Physiological Factors of Nonspecific Individual Resistance to Bacterial Infections, M., 1947.

29. Goncharova V. I., Bulletin of Experim. Biology and Medicine, 1954, 37, No 5, p. 33.
30. Gorbunova N. A. and Tsyypina G. M., in the book: Scientific Experim. of the Co-workers of Stalinabad Federal Medical Institute, 1954, p. 55.
31. Gorizontov P. D. In the book: Biological Effect of Radiation and Clinical Features of Radiation Sickness, Medgiz, 1954, p. 107.
32. Gorizontov P. D., Record of Pathology, 1955, No 4, p. 3.
33. Gorizontov P. D. In the book: Radiation Medicine, Medgiz, 1955, p. 80.
34. Gorizontov P. D. and Shliapin D. S., Record of Pathology, 1948, No 3, p. 13.
35. Gorshanova E. N., Zh MEI, 1956, No 8, p. 67.
36. Gromov A. S., ZhMEI, 1956, No 8, p. 107.
37. Gudkova E. I., Treatise of the Federal Sc.-Investigational Institute of Ear, Throat and Nose in 1952, M., 1953, p. 92.
38. Gudkova E. I. and Sakharov P. F., Bulletin of Experim. Biology and Medicine, 1954, No 12, p. 48.
39. Davydovskii I. V., Pathologic Anatomy and Pathogenesis of Human Diseases, 1, Medgiz, 1956.
40. Dal' M. K., Toka-Revich K. N., Tushinskii M. D., Volkova E. A. a. Figurina M. M., Infectious Leptospirosis Jaundice, Medgiz, 1944.
41. Demidas V. V., Theses of Reports on All-Union Conference in Medical Radiology. Section of Experimental Radiology, Medgiz, 1956, p. 98.
42. Egorov A. P. In the book: Biological Effect of Radiation and Clinical Features of Radiation Sickness, Medgiz, 1954, p. 154.
43. Egorov B. A. and Yakubovich Z. A., Clinical Medicine, 1934, 12, ed. 4, p. 600.
44. Zheltenkov A. I. a. Anokhina S. V., Treatise of the Institute «Microbe»>, 1, Saratov, 1951, p. 134.
45. Zhdanov V. M., Herald of Ac. of Sc., USSR, 1951, No 3, p. 12.
46. Zhukov-Verezhnikov N. N., Theses of Reports on the 13th All-Union Conference of Hygienists, Epidemiologists, Microbiologists a. Infectionists, L., 1956, p. 55.
47. Zakhar'evskaya M. A., Herald of Roentgen a. Radiology, 1938, 20, p. 313.
48. Zdrodovskii P. F., Treatise of the 3rd Session of Ac. of Sc. of the USSR, M., 1947, p. 143.
49. Zdrodovskii P. F., Problem of Reactivity in the Teaching on Infection a. Immunity, Medgiz, 1950.
50. Zdrodovskii P. F., Zh MEI, 1953, No 5, p. 6.
51. Zdrodovskii P. F., Theses of Reports of the 9th Session of Ac. of Sc. of USSR, March 7-12, 1955, M., p. 68.
52. Zelikina M. A. a. Pavlova E. G., Record of Biological Sciences, 1934, 25, ser. B., ed. 3, p. 676.
53. Zil'ber L. A., Science on Viruses, Medgiz, 1956.
54. Zil'ber L. A., Frank G. M., Snezhko A. D. a. Artamonova V. A., Medical Radiology, 1956, No 2, p. 17.
55. Ivanov A. E. a. Sosova V. F., Quoted from No 129.
56. Ivanov I. I., Balabukha V. S., Romantsev E. F. and Fedorova T. A., Metabolism in Radiation Sickness, Medgiz, 1956.
57. Ivashentsov G. A., Tushinskii M. D., Bashenin V. A., a. Danilevich M. G., Course of Acute Infectious Diseases, Medgiz, 1951.
58. Il'ina L. I., Blokhina V. D. a. Uspenskaya M. S., Medical Radiology, 1957, No 4, p. 23.
59. Ioffe V. I., Theses of Rep. on the 13th All-Union Conf. of Hygienists, Epid., Microbiologists and Infect., L., 1956, b. 2, p. 53.
60. Kabanova E. A., Effect of Factors of External Treatment on Resistance of Animals After Infection with Flexner Dysentery, M., 1951.
61. Kagan G. Ya., Nutrition Problems, 1953, 12, No 5, p. 42.
62. Kandyba L. L., In the book: Allergy Ed. by Ac. of Sc., USSR, Kiev, 1938, p. 282.
63. Kassil' G. N., Proceedings of Ac. of Sc. of USSR, 1953, 92, No 4, p. 863.
64. Kaulen D. R., Effect of X-Irradiation on Antitoxic Antidiphtheria Immunity, Diss., M., 1956.

65. Kemmerer G., Allergic Diathesis and Allergy Sicknesses, Biomedgiz, 1936.
66. Kirilenko O. A., Zh MEI, 1956, No 4, p. 78.
67. Kiselev P. N., Herald of Roentgenology a. Radiology, 1940, 22, p. 38.
68. Kiselev P. N., Effect of X-rays on Permeability and Barrier Functions of the Organism Tissues, Diss., L., 1950.
69. Kiselev P. N., Herald of Roentgenology a. Radiology, 1953, No 6, p. 8.
70. Kiselev P. N. In the book: Biological Effect of Ionizing Radiation, Dosimetry a. Use of Radioactive Substances for Therapeutic Purposes, Medgiz, 1954, p. 5.
71. Kiselev P. N. In the book: Radiology Problems, Medgiz, 1956, p. 210.
72. Kiselev P. N., Buzini P. A. a. Semina V. A., Herald of Roentgenology a. Radiology, 1955, No 3, p. 3.
73. Kiselev P. N., Buzini P. A. a. Nikitina K. I., Medical Radiology, 1956, No 1, p. 43.
74. Kiselev P. N. a. Karpova E. V., Medical Radiology, No 2, p. 23.
75. Kiselev P. N. a. Karpova E. V., Medical Radiology, 1956, No 4, p. 31.
76. Kiselev P. N., Sivertseva V. N. and Buzin P. A., Zh MEI, 1955, No 12; p. 54.
77. Kiselev P. N., Sivertseva V. N. a. Karpova E. V., Theses of Rep. on the 13th Conf. of Microbiologists, Epidemiologists, Hygienists a. Infectionists, L., 1956, b. 2, p. 62.
78. Kirchenstein A. M., Microbiology a. Immunology Problems, M., 1954.
79. Klemparskaya N. N., Treatise of All-Union Confer. in Medical Radiology, M., 1957, p. 153.
80. Klemparskaya N. N.; quoted from No 129.
81. Klemparskaya N. N., Bulletin of Exper. Biology a. Medicine, 1956, No 5, p. 22.
82. Klementova A. A., Zh MEI., 1949, No 7, p. 7.
83. Kliukhin S. a. Vygodchikov G., Hygiene a. Epidemiology, 1925, 1, p. 69.
84. Konovalov E. V. a. Solov'eva T. A., Bulletin of Experim. Biology a. Medicine, 1953, No 12, p. 52.
85. Kornienko I. A., Theses of Rep. on the Conference of Young Scientists, ed. by Ac. of Sc., USSR, M., 1956.
86. Korotkevich V. I., Theses of Rep. on Scientific Interinstitutional Conference on the Problem << Basis of Immunity >> . 1954, p. 5.
87. Kosov A. F., quoted from No 129.
88. Kosiakov P. N., Theses of Rep. of the 13th All-Union Confer. of Microbiol., Hygienists, Epidemiologists a. Infectionists, L., 1956, b. 2, p. 58.
89. Kravchenko A. T. a. Galanova N. V., Third Factor of Acquired Immunity, ed. by Ac. of Sc., USSR., M., 1948.
90. Kraevskii N. A., in the book: Biological Effect of Radiation and Clinical Features of Radiation Sickness Medgiz, 1954, p. 170.
91. Kraevskii N. A., In the book: Radiation Medicine, Medgiz, 1955, p. 257.
92. Kryzhanovskii G. N. a. Lebedeva N. I., Medical Radiology, 1956, No 3, p. 59.
93. Kuzin A. M. a. Budinova E. V., Proceedings of Ac. of Sc., USSR., 1953, v. 91, No 5, p. 1183.
94. Kuroedova I. Ya, in the book: On Physiological a. Pathologic Properties of the Excretion from Nose Cavity, Khar'kov, 1938.
95. Kurshakov N. A., Clinical Medicine, 1955, v. 33, No 6, p. 12.
96. Lebedeva K. M., ZhMEI., 1954, No 8, p. 88.
97. Lebedinskii A. V., In the book: Effect of Radiation on Organism. Ed. by Ac. of Sc. of USSR., 1955. p. 43.
98. Levitskaya A. A., Clinical Medicine, 1953, v. 31, No 2, p. 71.
99. L'vov A. N., Gas Infection, Medgiz, 1946.
100. Magerramov B. M., Treatise of Ac. of Med. Sc. of USSR., M., 1954, p. 24.
101. Man'kovskii N. B. a. Bracheva Z. N., Physiolog. Zhurnal, 1956, v. 2, No 1, p. 40.
102. Markovich A. V. a. Vorob'ev A. A., Bulletin of Experim. Biology a. Medicine, 1955, No 5, p. 53.
103. Medvedeva N. B., Experimental Endocrinology, Kiev, 1946.

104. Medne K. K., Treatise of Microbiology Institute of Ac. of Sc. of Latvia SSR, 1955, No 3, p. 133.
105. Meisel M. N., in the book: Effect of Radiation on Organism. Rep. on Geneva Conference. Ed. by Ac. of Sc. of USSR, 1955, p. 78.
106. Mechnikov I. I., Nonsusceptibility in Infectious Diseases, Medgiz, 1947.
107. Mironov S. G. a. Khalipskaya E., Treatise of Khar'kov Medical Institute. Jubileum Symposium, Khar'kov, 1935.
108. Mishchenko I. P., Herald of Roentgenology a. Radiology, 1929, v. 4, ed. 1-6, p. 496.
109. Mishchenko I. P. In the book: Roentgenology and Oncology. Khar'kov, 1934.
110. Mishchenko I. P. a. Fomenko M. M., Herald of Roentgenology a. Radiology, v 13, 1934, ed. 5, p. 327.
111. Mogil'nitskii B. N. a. Brummstein M. S., Record of Pathology, 1946, v. 13, ed. 3.
112. Mogil'nitskii B. N., Goldberg A. F. a. Shekhonin V. P. In the book: Roentgenology Problems. Treatise of the Central Inst. of Roentgen. a. Radiology, 1952, v. 7, p. 268.
113. Moroz B. B. a. Vasil'evskaya V. V., Proceedings of All-Union Confer. on Medical Radiology. Section of Experim. Radiology, Medgiz, 1956, p. 102.
114. Muceniece A. Ya., Treatise of Microbiology Inst. of Ac. of Sc. of Latvia SSR., 1953, v. 2, p. 103.
115. Neiman I. M. a. Sinai A. Ya., Bulletin of Experim. Biology and Medicine, 1947, v. 24, ed. 3, p. 164.
116. Nemirovich-Danchenko O. R., /Klemparskaya N. N., Alekseyeva O. G., Sosova V. F., Theses of Rep. on Confer. in Central Sc.-Investigational X-ray-Radiological Institute, Dedicated to the 40th Anniversary of the Great October Social. Revolution, L., 1957, p. 70-71.
117. Wikiforova A. S., Zh MEI, 1952, No 6, p. 66.
118. Osinskaya V. V., Herald of Roentgenology a. Radiology, 1938, v. 20, p. 296.
119. Perets L. G., Significance of Normal Microflora for Human Organism, Medgiz, 1955.
120. Perets L. G. a. Mostova R. S., Herald of Roentgenology a. Radiology, 1933, v. 12, ed. 3, p. 113.
121. Peterson O. P., Lozhkina A. N., Kozlova I. A. a. Sklianskaya E. I., Theses of Rep. of the 13th Confer. of Microbiologists, Epidemiologists, Hygienists a. Infectionists, L., 1956, book 2, p. 101.
122. Petrov R. V., Laboratory Work, 1955, No 1, p. 22.
123. Petrov R. V., Advanced Biology, 1957, v. 14, No 4, p. 82.
124. Petrov R. V., Medical Radiology, 1957, No 2, p. 61.
125. Petrov R. V., Herald of Roentgenology a. Radiology, 1957, No 1, p. 3.
126. Petrov R. V. a. Il'ina L. I., Bulletin of Experim. Biology a. Medicine, 1956, No 4, p. 59.
127. Petriaev E. D., Zh. M. E. I., 1947, No 10, p. 65.
128. Pigalev I. A., in the book: Biological Effect of Radiation a. Clinical Features of Rad. Sickness, Medgiz, 1954, p. 76.
129. Pigalev I. A., in the book: Effect of Radiation on Organism. Reports of Soviet Delegation on International Conference for Peaceful Use of Atomic Energy, Ac. of Med. Sc., USSR, Geneva, 1955, p. 157.
130. Planel'yes Kh. Kh., Zh MEI, 1955, No 2, p. 90.
131. Pletsityi D. F., Soviet Medicine, 1951, No 11, p. 5.
132. Pobedinskii M. N., Radiation Complications in X-Ray and Radiotherapy, Medgiz, 1954.
133. Popel'skii L. B., Russian Physician, 1907, No 45, p. 1549.
134. Puchkov N. V. In the book: Modern Problems of General Pathology a. Medicine, M., 1950.
135. Razumov M. I., Nutritional Problems, 1952, v. 11, No 4, p. 18.
136. Razumov M. I., Nutritional Problems, 1953, v. 12, ed. 3, p. 33.
137. Ryzhkov V., Zh MEI., 1947, No 11, p. 33.

138. Samtsov V. A. a. Gorodetskii A. A., Record of Pathologic Anatomy and Pathologic Physiology, 1936, v. 2, ed. 4, p. 47.
139. Sivertseva V. N., Effect of Preliminary Irradiation of Organism on the Course of Infectious Processes, Diss., L., 1955.
140. Sivertseva V. N., Medical Radiology, 1956, No 3, p. 52.
141. Sivertseva V. N., Herald of Roentgenology a. Radiology, 1956, No 5, p. 3.
142. Sigal M. Z., Advanced Biology, 1952, v. 33, ed. 3, p. 409.
143. Sipovskii P. V., Herald of Roentgenology a. Radiology, 1936, v. 16, ed. 5, p. 373.
144. Skvortsov M. A., Pathologic Anatomy of the Most Frequent Diseases of Children, Medgiz, 1946.
145. Smorodintsev A. A., Theses of Reports on the Conference of Young Scientists Concerning Problems of Medical Radiology, L., 1955, p. 21.
146. Smorodintseva G. I., Theses of Reports on the Conference of Young Scientists Concerning Problems of Medical Radiology, L. 1955, p. 30.
147. Sokolov Yu. N., Treatise of Molotov's Central X-Ray and Radiology Institute, M., 1941, v. 5, p. 103.
148. Sosova V. F., Quoted from Duplishchevci A. P., Medical Radiology, 1956, No 2, p. 46.
149. Sosova V. F., Ivanova A. E., Medical Radiology, 1956, No 6, p. 24.
150. Sosova V. F., Treatise of All-Union Conference on Medical Radiology, M., 1957, p. 160.
151. Sosova V. F., Quoted from No 79.
152. Sosova V. F., Some Peculiarities of the Infection Process in Radiation Sickness, Diss., M., 1956.
153. Sofronov B. N., Medical Radiology, 1956, No 2, p. 33.
154. Sukhov K. S., in the book: Against Reactionary Mendelism-Morganism, 1950, p. 124.
155. Tareyev E. M., Soviet Medicine, 1955, No 3, p. 3.
156. Tarusov B. N., Principles of Biological Effect of Radioactivity, Medgiz, 1954.
157. Troitskii V. L. and Tumanian M. A., quoted from No 129.
158. Troitskii V. L. and Tumanian M. A., Transcaucasian Radiological Conference. Theses of Reports. Tiflis, 1955, p. 42.
159. Troitskii V. L., Chakhava O. V. and Kozlova N. A., Medical Radiology, 1956, No 1, p. 49.
160. Trop I. E., Theses of Reports of Scientific Conference of Omsk Epidemiology a. Microbiology Institute, Omsk, 1952, p. 7.
161. Trop I. E., Treatise of Omsk Federal Sc.-Investig. Institute of Epidemiology, Microbiology an Hygiene, Omsk, 1954, No 2, p. 169.
162. Trop I. E., Treatise of Omsk Federal Sc.-Investig. Institute of Epidemiology, Microbiology an Hygiene, Omsk, 1954, No 2, p. 187.
163. Tumanian M. A., Herald of Roentgenology a. Radiology, 1955, No 3, p. 9.
164. Tumanian M. A. and Shevtsova Z. V., Medical Radiology, 1956, No 2, p. 41.
165. Tumanian M. A. a. Izvekova A. V., Medical Radiology, 1956, No 1, p. 59.
166. Firsova F. P., Change in Phagocytic Reaction of Blood of Sick in Inflammatory Processes and Due to the Pathogenetic Therapy, Diss., M., 1952.
167. Frolova M. A., in the book: The Best Scientific Exper. of Aspirants of Medical Higher Educational Institutions and Sc.-Investigat. Institutes, Medgiz, 1951, p. 115.
168. Khalfen (Halfen) E. B., Bulletin of Experimental Biology and Medicine, 1951, v. 31, ed. 1, p. 23.
169. Khaliapina (Haliapina) K. G., Treatise of Ac. of Med. Sc., USSR, Medgiz, 1954, p. 7.
170. Khesin (Hesin) V. R., Treatise of the 24th All-Union Confer. of Surgeons of People's Commisariat of Health a. Welfare of USSR, Medgiz, 1939, p. 56.
171. Tsimbalist (Cimbalist) D. F. a. Emelianova N. A., Symposium of Reports and Annotations of Kazakh's Federal Medical Institute from 1932-1952. Alma-Ata, 1954, p. 35.

172. Chekatilo G. A., Theses of Reports on the Conference of Young Scientists concerning Problems of Medical Radiology, L., 1955, p. 20.
173. Chekatilo G. A., Theses of Reports on All-Union Conference Concerning Medical Radiology. Section of Experimental Radiology, Medgiz, 1956, p. 103.
174. Chernaya L. A., Samodelkina E. N. and Kaplina Z. P., Zh MZI, 1954, No 8, p. 53.
175. Chernorutskii V. M., Alimentary Dystrophy in Blocked Leningrad, M., 1947.
176. Chuchukalo A. I., Theses of Reports on the 1st All-Union Conf. concerning Medical Radiology. Section of Experimental Radiology, Medgiz, 1956, p. 91.
177. Shikhodyrov V. V., Theses of Reports on Conference Dedicated to the 250th Anniversary of City Leningrad, 1957, p. 14.
178. Shikhodyrov V. V., Theses of Reports on All-Union Conference Concerning Medical Radiology. Medgiz, 1956, p. 94.
179. Shlygin T. K., Advanced Biology, 1952, v. 33, ed. 1, p. 14.
180. Abernathy R. J. Clin. Investing, 1951, v. 30, N. 6, p. 626.
181. Abrams R. Arch. Biochem., 1951, v. 30, p. 90.
182. Adler F. L., Shechmeister I. L. Proc. Soc. Exp. Biol. Med., 1952, v. 80, N. 4, p. 660.
183. Allen F. Am. J. Roentgenol., 1955, v. 73, N. 1, p. 70.
184. Antonioli G. M. Strahlentherapie, 1931, Bd. 41, S. 496.
185. Arnold. J. Hygiene, 1929, v. 29, p. 82.
186. Baldi A. Riv. di malarial., 1950, v. 29, p. 349.
187. Becker R. M. Proc. Soc. Exp. Biol. Med., 1948, v. 69, N. 2, p. 247.
188. Bell C. G. Feder. Proc., 1952, v. 11, N. 1, p. 462.
189. Bernardini A. Boll. Soc. ital. biol. Sper., 1954, v. 30, N. 7, p. 1073.
190. Berri L. J., Mitchell R. B. Science, 1953, v. 118, N. 3057, p. 140.
191. Beutler E., Gezon H. M. J. Immunol., 1952, v. 68, N. 3, p. 227.
192. Bonnano A. Riv. Radiologica e Fisica med., 1933, v. 3, p. 403.
193. Bradner W. T., Bernstein S. E., McCarthy R. E. Proc. Soc. Exp. Biol. Med., 1955, v. 89, N. 1, p. 107.
194. Brooks J. W., Evans E. F., Ham W. T. a. Raidd J. D. Ann. surg., 1952, v. 136, N. 3, p. 533.
195. Bukantz S. C., Dixon F. J., Dammin G. J. J. Clin. Investigation, 1950, v. 29, p. 800.
196. Callaway J. L., Kerby G. R. Arch. Dermat. Syph., 1951, v. 63, p. 200.
197. Cannon P. R., Chase W. E. a. Wissler R. W. J. Immunol., 1943, v. 47, N. 2, p. 133.
198. Capelli. Rad. med., 1930, v. 5, XVII/II, p. 1300.
199. Cavelti P. A., Gavelti E. S. Arch. pathol., 1945, v. 40, N. 3, p. 163.
200. Clapper W. E., Roberts J. E., Meade G. H. Proc. Soc. Exp. Biol. Med., 1954, v. 86, N. 3, p. 420.
201. Claude A. J. Exp. Med., 1946, v. 84, N. 1, p. 51.
202. Clemmesen J. Am. J. Cancer, 1939, v. 35, p. 378.
203. Clemmesen J., Andersen E. K. Acta path. et microbiol., Scandinav., 1948, v. 25, N. 5.
204. Cohen S., Mokychie W. J. Infect. Dis., 1954, v. 94, N. 1, p. 39.
205. Corper H. J. and Chovey P. J. Infect. Dis., 1920, v. 27, p. 491.
206. Coute E. Radiology, 1936, v. 16, N. 3, p. 416.
207. Cronkite E., In the book: Radioactive Decay and Medicine. Publ. house for International Literature, M., 1954, p. 154.
208. Dausset Y. Le sang, 1954, N. 7, p. 683.
209. Dixen F., Bukantz S., Talmage D., Dammin G. Am. J. Pathol., 1951, v. 27, N. 4, p. 679.
210. Donaldson D., Marcus S., Ko Gyi K., Percins E. J. Immunol., 1956, v. 76, N. 3, p. 192.
211. Dource A. L. J. Biol. Chem., 1943, v. 147, p. 689.
212. Ely I. O. a. Ross M. H. Neutron effects on animals, Baltimore, 1947.
213. Finch S., Ross Y., Ebangh F. J. Lab. a. Clin. Med., 1953, v. 42, N. 4, p. 555.

214. Fischman M., Shechmeister I. L. Biol. abstracts, Sect. B., 1955, v. 29, N. 11.
215. Freund I., Lipton M. M., Thompson G. E. Proc. Soc. Exp. Biol. Med., 1954, v. 87, N. 2, p. 408.
216. Freund I., Stone I. J. Immunol., 1956, v. 72, N. 2, p. 138.
217. Furth J., Andrews G., Storey R. H. a. Wish L. Blood, 1952, v. 7, N. 1, p. 94.
218. Furth F. W., Coulter M. P. a. Howland J. W. Am. J. Pathology, 1952, v. 28, N. 1, p. 25.
219. Furth F. W., Coulter M. P. a. Howland J. W. Am. J. Pathology, 1952, v. 28, N. 2, p. 171.
220. Furth F. W., Coulter M. P. a. Howland J. W. Am. J. Pathology, 1952, v. 28, N. 2, p. 185.
221. Furth F. W., Coulter M. P., Miller R., Howland J. W., Swicher S. J. Lab. a. Clin. Med., 1953, v. 41, N. 6, p. 918.
222. Gabrieli E. R., Auskaps A. A., Yale J. Biol. a. Med., 1953, v. 26, N. 2, p. 159.
223. Gunter C. Bull. org. mond. Sante, 1955, v. 13, N. 3, p. 409.
224. DeGara P. F., Furth J. J. Immunol., 1945, v. 50, p. 255.
225. Geaz J. a. Davis D. Trans. Roy. Soc. Trop. Med. a. Hyg., 1942, v. 36, p. 1.
226. Ginchi G., Scuro L. A., Sorice F., Fidanza A. Riv. Inst. sieroterap. ital., 1953, v. 28, N. 4, p. 281.
227. Gonchery L., Marston R., Smith W. W. Am. J. Physiol., 1953, v. 172, N. 2, p. 359.
228. Gordon L. E., Miller C. Ph. a. Hahne H. G. Proc. Soc. Exp. Biol. Med., 1953, v. 83, N. 1, p. 85.
229. Gordon L. E., Cooper D. B., Miller C. P. Proc. Soc. Exp. Biol. Med., 1955, v. 89, N. 4, p. 577.
230. Gowen J. W. a. Zelle M. R. J. Inf. Dis., 1945, v. 77, p. 85.
231. Graham J., Graham R. M. Proc. Natron. Acad. of Scien., 1949, v. 35, N. 2, p. 102.
232. Graham J., Graham R., Neri L., Wright K. J. Immunol., 1956, v. 76, N. 2, pp. 103.
233. Gustafson G. E., Koletzky S. Proc. Soc. Exp. Biol. Med., 1953, v. 84, N. 2, p. 266.
234. Hale W. M., Stoner R. D. Radiat. research., 1954, v. 1, N. 5, p. 459.
235. Hammond C. W., Vogel H., Clark I., Cooper D., Miller C. Radiation Research, 1955, v. 2, N. 4, p. 354.
236. Hammond C. W., Ruml D., Cooper D. B., Miller C. Ph. J. Exp. Med., 1955, v. 102, N. 4, p. 403.
237. Hammond C. W., Colling M., Cooper D., Miller C. Ph. J. Exp. Med., 1954, v. 99, N. 5, p. 411.
238. Harris T. N., Harris S., Beale H., Smith I. J. Exp. Med., 1954, v. 100, N. 3, p. 289.
239. Harris T., Harris S., Farber M. J. Immunol., 1955, v. 75, N. 2, p. 112.
240. Hatch M. N., Chase H. B., Fenton P. E., Montagna W. a. Wilson J. W. Proc. Soc. Exp. Biol. Med., 1952, v. 80, N. 4, p. 632.
241. Heinrich H. Zbl. f. Bakt., 1913, Bd. 70, S. 421.
242. Hollinsworth J. W. Proc. Soc. Exp. Biol. Med., 1950, v. 75, N. 2, p. 477.
243. Hsu D. a. Geiman Q. Am. J. Trop. Med. a. Hyg., 1952, v. 1, p. 747.
244. Jaroslow B. a. Taliaferro W. J. Inf. Dis., 1956, v. 98, N. 1, p. 75.
245. Jacobson L. O., Robson M. J., Marks E. K. Proc. Soc. Exp. Biol. Med., 1950, v. 75, N. 1, p. 145.
246. Kalter S. S., Stuart D. C. a. Tepperman I. Proc. Soc. Exp. Biol. Med., 1950, v. 74, p. 605.
247. Kaplan H. S., Speck R. S. a. Jawetz E. J. Lab. Clin. Med., 1952, v. 40, p. 682.
248. Karady S. J. Immunol., 1939, v. 37, N. 5, p. 457.
249. Kipton M. a. Freund J. Proc. Soc. Exp. Biol. Med., 1952, v. 81, N. 1, p. 260.

250. Knepper R. *Virch. Arch.*, 1935, Bd. 296, H. 2, S. 364.
251. Kolmer J. A., Pule A. M. a. Werner M. J. *Inf. Dis.*, 1937, v. 61, p. 53.
252. Lennox B., Depster W., Boag G. *Brit. J. Exp. Path.*, 1952, v. 33, p. 380.
253. Liebow A., Warren S. a. de Coursey E. *Am. J. Path.*, 1947, v. 25, N. 5, p. 853.
254. Lipton M. M., Fround J. J. *Immunol.*, 1953, v. 70, N. 3, p. 326.
255. Lowen A. *Mitt. a. d. Grenzgeb., d. Med. u. Chir.*, 1909. Bd. 19, S. 141.
256. Liu P. Y., Snyder J. C., Enders J. F. J. *Exp. Med.*, 1941, v. 73, N. 5, p. 669.
257. Luclany G., Vajda Gy., Toth E. *Arch. internat. pharmacol.*, 1955, Bd. 100, N. 3--4, S. 339.
258. Di Lusio T. R. *Am. J. Physiol.*, 1955, v. 181, N. 3, p. 595.
259. Marcus H., Donaldson D. M. *Proc. Soc. Exp. Biol. Med.*, 1953, v. 83, N. 1, p. 184.
260. Marcus S., Donaldson D., Esplin D. J. *Immunol.*, 1955, v. 74, N. 6, p. 494.
261. Marston R. Q., Ruth H. J., Smith W. W. *Proc. Soc. Exp. Biol. Med.*, 1953, N. 83, p. 289.
262. Marston R., Gonschery L., Alderman I., Smith W. W. *Am. J. Physiol.*, 1953, v. 172, N. 2, p. 365.
263. Menkin V., *Dynamics of Inflammation. Analysis of the Mechanism of Infectious Processes*, Medgiz, 1948.
264. Miller C. P., Hammond C. W., Tompkins M. *Science*, 1950, N. 2896, p. 719.
265. Miller C. P., Hammond C. W. a. Tomplins M. J. *Lab. Clin. Med.* 1951, v. 38, p. 331.
266. Miller C. P., Hammond C. W., Tompkins M. a. Shorter G. J. *Lab. Clin. Med.*, 1952, v. 39, p. 462.
267. Mogabgab W. J., Thomas L. J. *Lab. Clin. Med.*, 1952, v. 39, N. 2, p. 271.
268. Morgan J. J. *Exp. Med.*, 1947, v. 85, N. 1, p. 131.
269. Morton J. J. *Exp. Med.*, 1916, v. 24, N. 4, p. 419.
270. Muth S. *Frankfurter Zeitschrift fur Pathologie*, 1953, Bd. 64, H. 2, S. 235.
271. Naiman D. N. J. *Parasitol.*, 1944, v. 30, N. 4, p. 209.
272. Nelson C. T., Fox C., Freeman E. *Proc. Soc. Exp. Biol. Med.*, 1950, v. 75, N. 1, p. 181.
273. Olitsky P. K., Casals Y., Tal C. *Proc. Soc. Exp. Biol. Med.*, 1950, v. 75, N. 1, p. 276.
274. Osborne G. W., Bryan H. S., Quastler H., Rhoades H. E. *Am. J. Pathol.*, 1952, v. 170, N. 2, p. 444.
275. Peterson E. J. *Fac. Radiologists*, 1954, v. 5, N. 3, p. 189.
276. Pillemer L. *Trans. N. Y. Acad. Sci.*, 1955, v. 17, N. 7, p. 256.
277. Pillemer L., Blum L., Lepow I. H., Ross O. A., Todd E. W., Wardlow A. C. *Science*, 1954, v. 120, N. 3112, p. 279.
278. Pincerton H., Swank R. *Proc. Soc. Exp. Biol. Med.*, 1940, v. 45, p. 704.
279. Pollack A. D., Victor J. *Proc. Soc. Exp. Biol. Med.*, 1955, v. 89, N. 4, p. 561.
280. Portella A. *Boll. Soc. ital. biol. Sper.*, 1953, v. 29, N. 9--11, p. 1805.
281. Rigdon R. H. a. Rudisell H. *Proc. Soc. Exp. Biol. Med.*, 1945, v. 59, p. 167.
282. Rogers B., Converse S. *Plastic a. Reconstr. Surg.*, 1954, v. 14, N. 4, p. 261.
283. Root S. W., Andrews G. A., Kniseley R. M. *Tyor Malcolm Cancer*, 1954, v. 7, N. 5, p. 856.
284. Rossezlet a. Sarian J. *Schweiz. med. Wschr.*, 1944, Bd. 10, 260.
285. Salazar Mallen M. *Gas. med. Mexico*, 1953, v. 83, N. 1, p. 49.
286. Savitsky I. P. *Am. J. Physiol.*, 1955, v. 181, N. 1, p. 215.
287. Schlang H. *Proc. Soc. Exp. Biol. Med.*, 1950, v. 74, N. 4, p. 749.
288. Schmitt H. G., Thierfelder F. *Strahlentherapie*, 1954, Bd. 93, N. 3, S. 417.
289. Schönig A. *Strahlentherapie*, 1929, Bd. 33, S. 55.
290. Schwartzman G. *Proc. Soc. Exp. Biol. Med.*, 1951, v. 75, p. 835.
291. Schwartzman G., Schneierson S., Soffer L. *Proc. Soc. Exp. Biol. Med.*, 1950, v. 75, N. 1, p. 175.

292. Schwienhorst M. Beitr. zur path. Anat., 1928, Bd. 81, S. 375.
293. Shechmeister I. a. Bond V. Proc. Soc. Exp. Biol. Med., 1951, v. 77, N. 1, p. 77.
294. Shechmeister I. L., Bond V. P. a. Swift M. N. J. Immunol., 1952, v. 68, p. 87.
295. Shechmeister I. L., Paulissen L. J., Fishman M. Feder. Proc. 1952, v. 11, p. 146.
296. Shechmeister I. L., Adler F. L. J. Inf. Dis., 1953, v. 92, N. 3, p. 228.
297. Shechmeister I. L., Paulissen L. J., Fishman M. Proc. Soc. Exp. Biol. Med., 1953, v. 83, N. 2, p. 205.
298. Shechmeister I. L., Fishman M. J. Exp. Med., 1955, v. 101, N. 3, p. 259.
299. Shechmeister I. L., Paris W. H., Krause F. T., Paulissen L. J. a. Yunker R. Proc. Soc. Exp. Biol. Med., 1955, v. 89, N. 2, p. 228.
300. Singer T. J. Inf. Dis., 1953, v. 92, p. 97.
301. Smith F., Smith W. W., Gonshery L., Grenan M. Proc. Soc. Exp. Biol. Med., 1954, v. 87, N. 1, p. 23.
302. Smith W. W., Smith F., Ruth H., Cauter H., Grenan M. Am. J. Physiol., 1953, v. 172, N. 2, p. 351.
303. Smith W. W., Marston R. Q., Ruth H. J., Cornfield J. Am. J. Physiol., 1954, v. 178, N. 2, p. 228.
304. Smith W. W., Smith F., Alderman J. Am. J. Physiol., 1955, v. 182, N. 2, p. 400.
305. Smith W. W., Wood W. B. J. Exp. Med., 1956, v. 103, N. 4, p. 499.
306. Solarino G. Acta vitaminoli, 1954, v. 8, N. 4, p. 177.
307. Spar Y., Bale W., Wolff D., Goodland R. J. Immunol., 1956, v. 76, N. 2, p. 119.
308. Stoner R., Hale W. J. Immunol., 1954, v. 72, N. 5, p. 419.
309. Stroud A. N., Brues A. M., Summers M. M. J. Nat. Cancer Inst., 1955, v. 15, N. 4, p. 1109.
310. Swift H. J. Exp. Med., 1932, v. 36, p. 7.
311. Taliaferro W. H., Taliaferro L. G. a. Simmons E. L. J. Inf. Dis., 1945, v. 77, p. 158.
312. Taliaferro W. H., Taliaferro L. G. J. Immunol., 1951, v. 66, N. 2, p. 181.
313. Taliaferro W. H., Taliaferro L. G. J. Inf. Dis. 1954, v. 95, N. 2, p. 117.
314. Talmage D. Ann. Rev. Microbiol., 1955, v. 9, p. 335.
315. Taplin G. V., Grevior J. V., Finnegan C., 1 a Nier M. L., Dunn A. Feder Proc., 1952, v. 7, p. 396.
316. Thomas L., Paterson P. Y., Smith B. J. Exp. Med., 1950, v. 92, N. 2, p. 133.
317. Tullis J. L. Am. J. Pathol., 1949, v. 25, N. 5, p. 829.
318. Urbach E. Arch. of Dermat. and Syph., 1942, S. 697.
319. Zinsser H. a. Cataneda M. R. Proc. Soc. Exp. Biol. Med., 1932, v. 29, p. 840.
320. Vincent J. G., Vincent H. W., Dowdy A. W. Radiology, 1947, v. 48, N. 6, p. 610.
321. Vorlaender K. O. Acta allergolog., 1954, Bd. 7, S. 224.
322. Waksman B. H., Morrison L. R. J. Immunol., 1951, v. 6, N. 4, p. 421.
323. Waksman B., Bullington S. J. Immunol., 1956, v. 76, N. 6, p. 441.
324. Warren S. a. Draeger R. H. U. S. Nav. M. Bull. 1946, v. 46, N. 9, p. 1349.
325. Warren S. a. others. J. Am. Med. Ass., 1946, v. 131, N. 7, p. 598.
326. Wilkinson M. Blood, 1954, v. 9, N. 8, p. 810.