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PROBLEMS OF RADIATION SAFETY IN SPACE FLIGHTS

PHYSICAL AND BIOLOGICAL STUDIES WITH HIGH-ENERGY PROTONS

Yu. G. Nefedov, Editor-in-Chief

Atomizdat, Moscow, 1964

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION - WASHINGTON, D. C. DECEMBER 1965

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Translation of "Problemy radiatsionnoy bezopasnosti kosmicheskikh poletov; fizicheskiye i biologicheskiye issledovaniya s protonami bol'shikh energiy."
Atomizdat, Moscow, 1964.

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

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ANNOTATION

The monograph presents results of physical and biological experiments performed with high-energy protons in the synchrocyclotron of the United Institute of Atomic Investigations.

Characteristics of radiation effects under conditions of space flight and the development of permissible levels of radiation are examined. Materials on dosimetry and proton radiation protection are presented. Results of experiments on the biological action of high-energy protons and coefficients of their relative biological effectiveness are presented. Investigation results on prevention and cure of radiation sickness produced by the action of high-energy protons are published.

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FOREWORD

/3*

The conquest of space, initiated by the Soviet Union, requires a thorough solution of a number of complex problems. Among these the problem of medical and biological safety in space flight occupies an extremely important position.

In the decree of the Central Committee of the Communist Party of the Soviet Union and the Council of Ministers of the USSR entitled "Measures for Further Development of Biological Science and Its Link to Everyday Life,"[1] particular attention is devoted to the development of a new branch of natural science--space biology. One of the principal problems of space biology is the protection of the cosmonaut against ionizing radiation.

This monograph presents the principal results of physical and biological investigations made in 1960-1962 by the authors, using the synchrocyclotron of the Joint Institute for Nuclear Research (OIYaI) at Dubna.

The greater part of the radiation of solar flares and the Earth's inner radiation belt consists of high-energy protons (of the order of 100-700 Mev); the characteristics of the biological effect of this radiation remain virtually unstudied. For this reason it seemed necessary to investigate the biological effect of high-energy protons, using the proton beam of the OIYaI synchrocyclotron.

The collection of articles "Materialy po biologicheskomu deystviyu protonov vysokikh energiy" (Materials on the Biological Effect of High-Energy Protons), edited by A. A. Letavet and E. B. Kurlyandskaya (Izd-vo AMN SSSR, Moscow, 1962), gives the first results of investigations of protons with energies of 660 Mev. It should be noted that in the studies published in that collection of articles, as well as in the few investigations by foreign scientists with small laboratory animals, the problem considered was not that of ensuring radiation safety in space flight. In addition to the relative biological effect of protons, it appears necessary to evaluate the characteristics of their influence on vitally important organs and systems of man, under space flight conditions, and to develop highly effective methods for treatment of possible radiation injuries. Also of definite interest is a study of the biological effect of ionizing radiation on individual systems of the body, especially the vestibular apparatus.

Investigations of the biological effect of high-energy protons are necessary, in particular, for the solution of the many practical problems of planning radiation protection and dosimetry on spacecraft.

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*Numbers given in the margin indicate the pagination in the original foreign text.

[1] "Pravda," Jan. 24, 1963.

Weight of the spacecraft is the most decisive factor when developing radiation protection under space flight conditions. For this reason physicists must have sufficiently complete information on the intensity of radiation in space, the admissible limits of the radiation effect, absorption of ionizing radiation by different materials, etc. In order to ensure radiation safety of cosmonauts it is of particular importance to have a system of dosimetric control to estimate radiation conditions on a spacecraft clearly and opportunely. The development of reliable systems of dosimetric control requires radiation detectors which can determine the dose of cosmic radiation in biological equivalents of the rad (berad), and also the dose of secondary radiations arising in spacecraft components. Evaluation of radiation resistance of protective and construction materials under the intense bombardment of high-energy protons is an extremely timely problem. For this reason we felt it desirable to include the results of physical investigations in this monograph.

We wish to express deep appreciation to the directors of the State Committee on the Use of Atomic Energy of the USSR and the Joint Institute for Nuclear Research for kindly affording us the possibility of carrying out these investigations. We express sincere appreciation to Professor V. P. Dzhelepov, Director of the Laboratory of Nuclear Problems, for cooperation and assistance in carrying out the work on the synchrocyclotron.

CHAPTER 1. CHARACTERISTICS OF THE RADIATION EFFECT UNDER SPACE FLIGHT CONDITIONS AND WAYS TO DETERMINE ADMISSIBLE RADIATION LEVELS /5

V. Ye. Dudkin, Yu. G. Grigor'yev, Ye. Ye. Kovalev, A. V. Lebedinskiy, S. V. Levinskiy, Yu. G. Nefedov, N. I. Ryzhov, L. N. Smirennyy and M. A. Sychkov

Advancing successful implementation of the program for conquest of space requires the solution of a number of extremely important problems of a technical and medicobiological character.

Modern biological science must solve many problems of great practical importance in order to ensure the safety of space flights. How long can man survive weightlessness? How long can he endure presence in a sealed cabin of limited size in complete isolation? What danger is involved in the effect of ionizing radiations on the cosmonaut? In other words, on the path into space man must overcome a number of "barriers," and the success of space conquest depends on how successfully the problems involved in overcoming these barriers are solved.

With respect to overcoming accelerations (g-forces), the situation is already quite clear: these accelerations are surmountable. The flights of Soviet and American cosmonauts and numerous investigations, using special apparatus, have determined the limits to which man can withstand them.

There are considerably fewer data on the influence of weightlessness on the human body. However, the results of space flights of the Soviet cosmonauts Yu. A. Gagarin, G. S. Titov, A. G. Nikolayev, P. R. Popovich, V. F. Bykovskiy and V. V. Nikolayeva-Tereshkova give basis for considering the effect of weightlessness over a period of several days to be a factor not exerting a serious influence on the health and general condition of man.

The prolonged isolation of man in a sealed space of limited size is a major problem of independent interest, receiving the attention of scientists of many countries. Definite advances have already been made toward the solution of this problem.

The radiation barrier is a serious obstacle in the path of man into /6 space. Modern science has at its disposal data showing that beyond the limits of Earth's atmosphere there can be intense fields of many kinds of ionizing radiation (X- and γ-rays and heavy high-energy charged particles).

In an evaluation of radiation danger under space flight conditions we must take into account the possibility of the effect of primary cosmic radiation, radiation of solar flares, the Earth's outer and inner radiation belts and other possible regions with high radiation in interplanetary space.

Primary Cosmic Radiation

The intensity and composition of primary cosmic radiation have been investigated in experiments on space rockets and artificial Earth satellites and also in the launchings of geophysical rockets and pilot balloons. Primary cosmic radiation includes protons, α-particles and heavy nuclei with energies of the order of 10^9 ev or more.

At distances greater than 66,000 km from Earth the intensity of cosmic radiation is constant--1.8-4.0 particles/cm^2·sec (refs. 1-4). The most reliable value is 2.3 particles/cm^2·sec.

The differential spectrum for all nuclei of primary cosmic radiation with the total energy related to one nucleon has the form: $n(E)dE = cE^{-\gamma}dE$. For $E \geq 1.0$ Gev/nucleon, the value of the coefficient γ is 2.0-2.5.

In a period of normal solar activity the ionization created in the atmosphere by primary cosmic radiation remains almost constant at geomagnetic latitudes greater than 58°. This means that particles with energies below 1 Gev/nucleon (rigidity cutoff at a geomagnetic latitude of about 60°) are very few in number.

The absence of low-energy particles in high latitudes (even at the poles, where theoretically particles of any energy could be present) is called the high-latitude cutoff.

As a result of the high-latitude cutoff, the differential spectrum has a maximum at an energy below 1 Gev/nucleon. Computations of the dose created by primary cosmic radiation reveal that more than half the dose is created by heavy and very heavy nuclei (refs. 5-7). Table 1 gives the contribution to the dose from each component of primary cosmic radiation on the basis of data in reference 8.

The depth distribution of the absorbed dose created by primary cosmic radiation was computed in reference 8. The decrease of the dose in the body with depth is insignificant, due to the great hardness of primary cosmic radiation. When the thickness of the protection is up to 10 g/cm^2, the dose absorbed in the body increases from the surface to the center as a result of particle deceleration, and therefore as a result of an increase of their ionization losses. With further increase of the thickness of the protection the deceleration of /7 particles in the protection exerts an influence, and particle absorption in the body sets in. In this case the increase of the dose in the direction of the center ceases, and beginning with some thickness of the protection the dose absorbed in the body decreases from the surface to the center.

In prolonged space flights, primary cosmic radiation is a chronic irradiation factor. The maximum dose absorbed in the body, therefore, must be used as a criterion of radiation safety. However, computations have shown that the

decrease of the dose in the body does not exceed approximately 10 percent, and radiation danger created by primary cosmic radiation can therefore be estimated from the values of the mean tissue dose: 22.4 mrad/day, or 127.6 mrem/day.

TABLE 1. CONTRIBUTION OF DIFFERENT COMPONENTS OF PRIMARY COSMIC RADIATION TO THE MEAN TISSUE DOSE (WITHOUT PROTECTION)

Characteristics of dose		Component					
		Protons	α-particles	Group of intermediate nuclei ($Z = 6-9$)	Group of heavy nuclei ($Z \geq 10$)	Group of very heavy nuclei $Z \geq 20$	Primary cosmic radiation
Absorbed dose	mrad/day	6.2	3.7	3.4	5.0	4.1	22.4
	mrem/day	6.2	3.7	11.1	34.0	72.6	127.6
Contribution to dose, %	physical	27.7	16.5	15.2	22.3	18.3	100
	biological	4.9	2.9	8.7	26.6	56.9	100

With an increase of the thickness of the protection the dose of primary cosmic radiation remains virtually constant to a thickness of 10-15 g/cm^2. In the case of very large thicknesses (about 70-100 g/cm^2), the dose decreases by a factor of 1.5-2. The values of the dose do not contradict the data in the literature, such as 17.5 mrad/day in reference 6 (which can be recalculated to a flux of 2.3 particles/day); 19-27 mrad/day or 96-137 mrem/day in reference 7.

These values of the absorbed dose of primary cosmic radiation were obtained without taking into account the dose of secondary radiation created in the body by primary nuclei. Rigorous calculation of the dose created by secondary radiation arising in the protection and in the body of the cosmonaut is virtually impossible, due to the absence of data on the interaction of high-energy and multiple-charged particles with matter. The contribution of secondary radiation to the dose absorbed in the cosmonaut's body has been estimated in reference 8, and with respect to the height, dependence of total ionization created by primary cosmic radiation does not exceed 50-100 percent. Thus, the maximum absorbed dose created in interplanetary space in the absence of protection is 34-45 mrad/day or 190-250 mrem/day.

With approach to the Earth's surface ionization decreases by a factor of approximately 2 as a result of the increase of the screening effect of the /8
Earth (there is an increase of the solid angle at which the Earth is visible), and by several times more (depending on geomagnetic latitude) due to the increase of strength of the geomagnetic field deflecting cosmic particles.

The maximum dose when there is protection of 2 g/cm^2, computed for a spacecraft trajectory with an angle of inclination to the orbital plane of $i = 65^\circ$ and a height h = 200-600 km, is 8-11 mrad/day. The doses of primary cosmic radiation were measured directly during the flight of the second spacecraft in an orbit with the same parameters; these doses were 6-10 mrad/day, which agrees with the computed data.

It should be noted that at the present time almost all estimates of the dose of circumterrestrial radiation and galactic radiation in the literature were based on computing the absorbed energy of particles in an isolated gram of tissue. This value, which we will refer to hereafter as the local absorbed dose, cannot serve as a criterion of radiation danger during space flights.[1]

The estimates of radiation danger in space (ref. 8) are based on computation of the mean tissue dose, i.e., total energy absorbed in the body (integral dose) related to a unit mass. This method of computation is more correct, since it takes into account the increase of specific ionization losses of the particles during their deceleration, which is especially important if the paths of the particles lie entirely within the body. In addition, this method takes into account the self-screening effect, which is of considerable importance for particles with high specific ionization (for example, the low-energy protons of the inner radiation belt and solar flares). At the same time, radiation danger in space flight cannot be characterized solely by the value of the mean tissue dose. The most realistic evaluation of radiation danger caused by penetrating radiation during space flights can be made on the basis of the value of the mean tissue dose and the distribution of the absorbed dose in the body with depth.

Penetrating Radiation of Solar Flares. In addition to the constantly present primary cosmic radiation there are regions with a high intensity of penetrating radiation propagating from the Sun, which constitute a radiation danger for living beings. According to modern concepts, they consist of fluxes of protons generated or accelerated on the Sun at the time of chromospheric flares and escape from the Sun together with a frozen-in part of the magnetic field at a velocity of 10^5 km/sec (ref. 9). The penetrating radiation of /9 solar flares can be detected from an increase of intensity of cosmic rays in the atmosphere, and in particular from an increase of the neutron flux. In the case of large flares cosmic ray intensity at the boundary of the atmosphere increases in the middle latitudes by $1\text{-}3 \cdot 10^3$ times, i.e., it attains $2\text{-}6 \cdot 10^3$ particles/$cm^2 \cdot$sec (refs. 9-12).

The frequency of development of solar flares is related to solar activity and sunspot number. During a period of maximum solar activity there are 5-13 small flares annually. During a period of minimum solar activity the frequency of such flares decreases appreciably. Large flares occur considerably less frequently: 1 or 2 flares in 5 years.

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[1]Only in the case of primary cosmic radiation, which includes very high-energy particles, do the values of local and mean tissue doses differ little from one another.

It is customary to classify solar flares by importance in accordance with the brightness and size of the visible flare observed on the solar disk. However, in evaluating radiation danger it is more convenient to classify solar flares on the basis of the character of the proton spectrum.

Type A includes solar flares (such as May 10, 1959 and Nov. 12, 1960) in which the energy of the protons ranges from 30-400 Mev. The integral spectrum of such flares can be written in the form $\sim E^{-4}$, and the proton flux can be about 10^8 protons/cm^2.

Type B includes solar flares (such as Feb. 23, 1956) in which the proton energy is up to 50 Gev. The integral spectrum of type B flares has the form $(1 + E)^{-6}$, where E is expressed in Gev.

The values of the mean tissue dose created by protons after passing through an aluminum shield of 0.1, 1 and 10 g/cm are 230, 85 and 20 rad, respectively, for a type A flare and 110, 80 and 35 rad for a type B flare (ref. 8).

Table 2 gives the values of the absorbed dose created by protons of solar flares behind a spherical shield of aluminum with a mean thickness of 10 g/cm^2.

The represented values of the local dose agree with the data of other authors (refs. 13-14).

The biological dose was computed on the basis of the maximum values of the relative biological effect, determined for the case of chronic irradiation. /10 With acute irradiation, such as occurs at the time of a flare, the relative biological effect will be considerably less. In the case of solar flares it is, therefore, more correct to evaluate radiation danger on the basis of physical dose (in rad).

The values of this absorbed dose were obtained without taking into account secondary charged particles and neutrons. According to the estimates made in references 13-16, the dose caused by secondary charged particles attains 10 percent of the dose created by primary protons. The dose caused by neutrons in the case of type B flares apparently will be small, and according to the estimate made in reference 13 is 5-10 percent. For type A flares the dose created by neutrons behind a protective thickness of about 10 g/cm^2, according

TABLE 2. ABSORBED DOSES CREATED BY SOLAR FLARE PROTONS

Absorbed dose after flare		Type A	Type B
Mean tissue dose	rad.	20	35
	rem.	24	40
Local dose	rad.	78	80
	rem.	-	-

to the estimates cited in references 13 and 16, is comparable with the dose from primary protons.

Comparison of the mean tissue and local doses reveals that there is always an appreciable difference between them: when the protective thickness is about 1 g/cm^2, the local dose is approximately 10 times greater than the mean tissue dose, and when 20 g/cm^2, 2 or 3 times greater.

Therefore, a reconsideration of the principal criterion of radiation danger, leading to discarding of the concept of local doses, considerably changes the ideas prevailing up to now concerning radiation danger of solar flare protons.

The Earth's Outer Radiation Belt. Information on the outer radiation belt has been obtained by artificial Earth satellites and space rockets (refs. 1-3 and 17).

The lower boundary of the outer belt in the region of geomagnetic latitudes 55-70° is situated at a distance of about 300 km from the Earth's surface (ref. 2) and descends to 200 km in the South Atlantic (refs. 18 and 19). In the equatorial plane the outer belt extends to 55,000 km from the Earth's surface (refs. 1-3 and 17).

The intensity maximum in the outer belt is situated at a distance of approximately 10,000-20,000 km from the Earth's surface (ref. 2).

During recent years information on the total fluxes and the spectrum of electrons in the outer belt has changed appreciably. Former estimates of the electron fluxes in the belt, based on measurements with radiation counters, were found to be exaggerated by a factor of approximately 1,000 due to incorrect interpretation of the readings of the radiation counters. Existing estimates of the radiation danger in the outer belt were also found to be considerably exaggerated.

At the intensity maximum of the outer belt the flux of electrons with energies greater than 40 kev is $1 \cdot 10^8$ particles/cm^2·sec (ref. 20), and the flux of electrons with energies greater than 200 ev is $4 \cdot 10^7$ particles/cm^2·sec. In evaluations of the radiation danger it apparently is reasonable to use the flux value $1 \cdot 10^8$ particles/cm^2·sec. /11

With respect to the electron spectrum in the outer belt, the most reliable data are those of Van Allen et al. (ref. 20). By using these data, an expression of the form $N(E)dE = 1.8 \cdot 10^6 E^{-3} dE$ (where E is the electron energy in Mev) can be obtained for the differential electron spectrum. An electron spectrum of this form applies to the central part of the outer belt. There are no reliable data on the electron spectrum in other parts of the belt.

It should be noted that despite the new, lower value for the electron flux in the outer belt (10^8 particles/cm^2·sec in comparison with 10^{11} particles/cm^2·sec), the problems involved in the radiation safety of cosmonauts and accordingly the problem of protection of the manned compartments of spacecraft against electrons and bremsstrahlung of the outer belt have not lost their importance. If there is no protection, or only very thin protection, the doses at the maximum of the outer belt can range from several tens to several thousands rad/hour, i.e., a dose known to be dangerous. Therefore, if a cosmonaut is to emerge from the ship, he must have a space suit affording adequate protection, ensuring a decrease of the radiation dose to an admissible level.

In a general case, depending on the thickness of the protective covering, in computing the radiation effect in the Earth's outer radiation belt we must take into account both the effect of electrons and the contribution of the bremsstrahlung generated in the covering and the cosmonaut's body. If the protective covering has a thickness greater than the path of electrons with maximum energy, the radiation dose is caused entirely by bremsstrahlung. As a rule, this case applies when computing the protection of the manned compartment of a spacecraft. The design of a space suit is more complex, since apparently it is necessary to consider the radiation effect of the electrons penetrating the relatively thin protective covering of the space suit, in addition to bremsstrahlung.

The total intensity of the dose from electrons and bremsstrahlung varies from tens of rad/hours for small thicknesses to fractions of a rad/hour for a thickness greater than 1 g/cm^2. When the thickness of the protection is less than 1 g/cm^2, the overwhelming part of the total dose is created by electrons penetrating the protection. However, when the thickness of the protective covering is greater than 1-2 g/cm^2, the dose is determined entirely by bremsstrahlung. Depending on the thickness of the protection, there also is a change in the character of the depth distribution of the absorbed dose in the body. When the protection is thin and when electrons play a significant role, it is necessary to expect a sharp variation of the distribution of the absorbed dose in the body with depth. With a thicker protective layer the variation of the absorbed dose in the body with depth should be less sharp. These considerations must be taken into account in calculations of a protective covering, when selecting a radiation danger criterion.

It is obvious that the existence of a sharp decrease of the absorbed dose in the body with depth makes it necessary to have more detailed radiobiological information on the admissible levels of penetrating radiation for the /12 different organs and tissues of the body, when making estimates of the radiation danger. Without this information calculations of protection required against the electrons of the Earth's outer belt cannot be regarded sound.

Earth's Inner Radiation Belt. The inner zone of corpuscular radiation is bounded by the magnetic line of force intersecting the Earth's surface at geomagnetic latitude 45° (ref. 2). It begins at a height of 500-600 km in the western hemisphere and at 1,500 km in the eastern hemisphere and extends to a height of 5,000-10,000 km. The maximum radiation intensity is observed at a height of about 3,400-3,600 km (refs. 2 and 17).

Direct measurements with emulsion stacks (refs. 21 and 22) and analysis of the results obtained with artificial Earth satellites with particle counters (ref. 2) lead to the conclusion that the radiation of the Earth's inner radiation belt consists of fluxes of protons with energies of the order of 100 Mev or more.

At a height of about 1,000-1,200 km above the Earth the flux of protons passing through a protective layer of 5 g/cm^2 is 800 ± 200 particles/$cm^2 \cdot sec$ (ref. 22). In the central part of the inner radiation belt there is a flux of about 20,000 protons/$cm^2 \cdot sec$ with energies greater than 40 Mev. The radiation intensity decreases from the equator to the high latitudes, but for the time being there are no precise data.

In addition to protons the inner belt contains fluxes of electrons with an intensity of about $2 \cdot 10^{10}$ particles/$cm^2 \cdot sec$ for electrons with energies $E > 20$ kev and $\sim 10^8$ particles/$cm^2 \cdot sec$ for $E > 600$ kev (ref. 3).

Measurements made with an emulsion stack have shown that fluxes of π-mesons and other nuclear particles are negligibly small (ref. 22). The differential spectrum obtained in these measurements has the form $N(E)dE = N_0 E^{-n} dE$, where $75 < E < 700$ Mev; $n = 1.84 \pm 0.08$; $N_0 = (2.1^{+1.0}_{-0.7}) \cdot 10^3$ protons/$cm^2 \cdot sec \cdot Mev^{n-1}$ sterad.

In the low-energy region ($E_p < 30$ Mev) the form of the spectrum is strongly dependent on latitude: in the high latitudes the proton spectrum in the low-energy region becomes steeper ($\sim E^{-4.5}$), and there is also an intensity increase.

When data are available on the proton spectrum it is possible to compute the dose received through spherical protection. Some results of computations (ref. 8) of the intensity of the dose created by protons at the intensity maximum of the Earth's inner radiation belt despite protection by aluminum of different thicknesses are given in table 3.

The variation of the distribution of the dose absorbed in the body with depth reveals that the maximum dose is at the surface of the body. An increase in the thickness of the protection leads to a decrease of the drop between the maximum and minimum values of the dose.

TABLE 3. INTENSITY OF DOSE CREATED BY PROTONS OF THE EARTH'S INNER RADIATION BELT BEHIND ALUMINUM SHIELDS OF DIFFERENT THICKNESSES

Absorbed dose	1 g/cm^2		7.5 g/cm^2		20 g/cm^2	
	mrad/sec	mrem/sec	mrad/sec	mrem/sec	mrad/sec	mrem/sec
Mean tissue dose	0.88	1.11	0.56	0.68	0.31	0.38
Surface dose	2.81	3.26	1.09	1.33	0.50	0.57
Local dose	4.42	5.6	1.71	2.12	0.72	0.84

The intensity of the tissue dose was determined in these computations /13 (ref. 8) only for ionization losses of protons.

According to data in the literature (refs. 5, 13, 15, 23 and 24), the absorbed dose from secondary particles after protection by light material with a thickness of 20 g/cm^2 is 5-10 percent of the total dose and increases with an increase of the thickness of the protection. The dose of secondary radiation arising in heavy protection can attain 30-40 percent of the total dose.

The literature contains no estimate of the magnitude of the dose of secondary radiation, formed by protons in the human body. On the basis of experimental data on the passage of high-energy protons through tissue-equivalent matter, which we obtained using the synchrocyclotron of the Joint Institute for Nuclear Research, it can be concluded that the contribution of secondary radiation to the total dose is about 10 percent. Thus, the dose absorbed in the cosmonaut's body due to secondary particles formed in the body and the protection can be increased by 10 percent.

These are the presently known principal sources of radiation in space and the quantitative characteristics of ionizing radiation, which must be taken into account in evaluating possible radiobiological effects. However, in evaluating the biological effect of ionizing radiation in a plan for ensuring the safety of space flights, it is necessary to take into account not only the radiation doses, but also the relative biological effects of different kinds of radiation, the radio sensitivity of tissues and systems, and finally the possibility that the entire body can compensate and restore disrupted functions. The relative biological effect coefficient for a particular radiation is equal to the quotient from the division of the tissue dose of "standard" radiation by the tissue dose of the particular radiation giving the same effect as the standard radiation. The standard radiation usually used for this purpose is X-radiation (130-200 kv) or the γ-radiation of Co^{60}. Doses of different radiations causing the same biological effect usually characterize the effectiveness. Doses causing 50- or 100-percent fatalities to animals in a fixed period often are used for this purpose for acute forms of irradiation.

Under conditions of a sustained small dose, the coefficient of relative /14 biological effect is determined by an estimate of the time and degree of manifestation of postponed effects (malignant growths, genetic changes, etc.) and

the decrease of the mean lifetime for a particular population. The rate of restoration of impaired functions is also used in a comparative evaluation. The character of the studied effect can influence the value of the coefficient considerably. It was demonstrated in the collection of articles edited by A. A. Letavet and E. B. Kurlyandskaya (refs. 25-30) that the coefficient of biological effect of protons with respect to blastomogenic effect was larger than in a comparative evaluation of the lethal effect.

The coefficient of relative biological effect of one type of ionizing radiation can also be different for different biological objects (refs. 31-45). It is also important to take into account that the relative biological effect is influenced by the magnitude and intensity of the dose and the dose distribution with time (refs. 6, 11, 46 and 47). The determination of the value of the tissue dose in rems (i.e., the dose in rads, multiplied by the coefficient of relative biological effect) facilitates the quantitative description of the biological effect of radiation in the living organism.

It is assumed that the coefficient of relative biological effect is determined to a considerable degree by the linear density of losses of the energy of ionizing particles. It becomes necessary, therefore, to make a detailed study of the interaction between a particular type of radiation and living tissue and determine the spectrum of linear energy losses (LPE).

A change of the LPE in the range of 0.2-3.5 kev per micron of a particle path exerts little influence on the relative biological effect, whose coefficient in this case is equal to approximately 1. An increase of LPE from 3.5 to 50-200 kev per micron accordingly changes the coefficient of relative biological effect by 10-20. However, when the LPE is greater than 175 kev per micron there is no further significant change of relative biological effect (refs. 4, 38, 39, 41, 42 and 48).

In the determination of the admissible radiation levels for space flight it is necessary to take into account the wealth of experience accumulated by radiobiologists, hygienists and physicists in the normalization of the admissible limits of effect of ionizing radiations with respect to the conditions for use of various sources of ionizing radiation in different branches of the national economy.

We can use the following values as points of departure for determination of the admissible dose of radiation in space flight:

the daily dose of irradiation of a man on Earth, which is 0.4-2 mrem and consists of the effect of cosmic radiation, the radiation of radioactive elements in the Earth's crust and radioactive isotopes present in the human body;

the admissible dose of professional irradiation, which now is 150 rem /15
during a 30-year period, i.e., about 14 mrem per day. This value regulates the irradiation of a limited part of the population by nature of activity associated with the effect of radiations;

a dose of irradiation of about 50 rem, which can be regarded as the single admissible dose not causing severe radiation damage in the organism, even in the case of general irradiation;

doses of brief general irradiation of the organism of 100-400 rem, which can lead to the impairment of the ability to work, development of acute radiation sickness and, in individual cases, to death.

Taking into account the limits of the admissible radiation effect, on the one hand, and the exceptional character of the forthcoming manned flights in space on the other hand, we can adopt 50 rem as the admissible dose when the duration of space flight is from several days to one year, and 25-30 rem per year when the space flight duration is several years. These values are approximate and are in need of further refinement.

In estimating the biological effect of ionizing radiation under space flight conditions we take into account that the developed norms apply to a limited number of individuals, and genetic effects are not of decisive importance in this case. In addition, the great capacity of the human body for compensation and restoration of impaired functions is taken into account.

Modern radiobiological concepts indicate that a definite percentage of radiation somatic injuries are repaired. The restoration process, according to Davison, can be expressed by the formula $D_{eff} = D_0 [f + (1 - f)e^{-\beta t}]$, where D_{eff} is the effective dose in rads; D_0 is the total dose, in r; f is irreversible injuries, in percent; β is the constant rate of restoration, in percent per day; t is the number of days, and e is the base of natural logarithms.

Most authors assume that f for the human body is 10 percent. However, in evaluating the restorability of different tissues and systems, there can be deviations in either direction. For example, Baum (ref. 40) assumes that for an erythropoietic system f will be equal to 20 percent.

The character of restoration after single irradiation of man can be represented by the curve shown in figure 1. Repeated irradiation with a dose of 100 r can be permitted only after a definite time following the first radiation effect in order to avoid clinical manifestations of radiation sickness.

The reactions of the body transpiring with the participation of the nervous system have great compensatory possibilities. Our investigations (ref. 49) have shown that insignificant functional changes on the part of the vestibular apparatus, so important for the activity of the cosmonaut, appear when the total irradiation dose is greater than 50-100 r.

Radiation is not the only factor affecting man under space flight conditions. The cosmonaut is affected by radiation, overloads, changes of the gas and chemical composition of the air medium of the manned cabin and other factors characteristic of a sealed enclosed space. It must be emphasized that the radiation effect can lower the resistance of man to these factors. /16

It is also important that the combined effect of these factors can aggravate radiation injury, or, on the other hand, cause the effect of "combined" increase of the general reactivity of the body.

Despite the great practical importance of the problem of the combined effect of ionizing radiation and other space flight factors, only individual studies have so far been published on this problem (refs. 32, 33 and 50-56).

The influence of most of the operative factors, not complicated under space flight conditions, is not so significant as to lead to the development of any specific changes, such as the development of radiation sickness or the phenomenon of intoxication by harmful substances present in the air. However, each of the operative factors, even individually, causes a definite nonspecific reaction, directed primarily to the adjustment of the organisms to the modified, new conditions in the external medium. In the event that two or more factors operate simultaneously, this nonspecific effect can be accumulative, which leads to a more clearly expressed response. /17

As a result of the summing of the effect of the varied irritants there can be intensification of the specific reaction to the influence of any one of the irritants. For example, with an increase of air temperature due to intensification of perspiration there can also be an increase of the elimination by man of organic compounds and ammonia. This can be considered a specific reaction to the temperature increase. However, if ionizing radiation is added to the number of operative factors, the elimination of harmful impurities increases

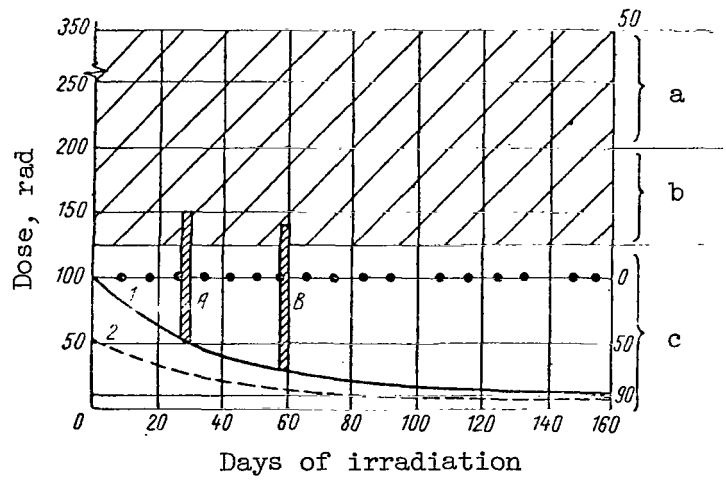

Figure 1. Character of restoration and irreversible injuries to man during a single exposure to ionizing radiation (doses of 50 and 100 rad) and repeated irradiation (dose of 100 rad. Absorbed dose of 100 rad (1) and 50 rad (2); A, repeated dose of 100 rad after 30 day period; B, after 60 day period; a, death; b, acute radiation effects; c, restoration and irreversible injuries.

still further. In this case the nonspecific influence of small doses of ionizing radiation intensifies the specific reaction to the effect of a high temperature.

Similarly, it is also possible to expect an intensification of specific reactions to the effect of ionizing radiation when its effect is combined with other irritants. An example can be cited to illustrate this possibility. It was impossible to detect any substantial changes in the blood-generating organs of a rabbit, which had been irradiated in a total dose of 300 rad for 2 months. However, if irradiation in this same dose is combined with the effect of vibration and decreased barometric pressure, one month after termination of the irradiation it was possible to detect clearly expressed changes in the bone marrow of the animal; these changes are characteristic of the effect of ionizing radiation. If there is a different combination of joint action of radiation and some other space flight factors, and the values of these effects are different, there can be a net effect moderating the radiation effect. As an illustration of this possibility we cite the results of investigations reported by A. A. Sveshnikov and A. V. Sevan'kayeva in reference 51 of the function of the vestibular apparatus. The experiments were made on eight dogs, four of which were subjected to fractional total irradiation of 9 roentgens a day to a total dose of 200 roentgens, while four others were subjected not only to irradiation in the same dose, but also to the additional effect of vibration, noise and decreased atmospheric pressure. In this case clearly expressed functional changes of the vestibular apparatus were noted only in the first group of animals (fig. 2), that is, in the dogs subjected only to irradiation.

When determining the admissible radiation levels, it also is necessary to take into account the individual radiosensitivity of man. Data from numerous radiobiological investigations indicate that identical radiation doses can cause a dissimilar degree of damage to individuals of the same species. Although until now sufficiently reliable tests have not been found for determination of individual radiosensitivity without preliminary irradiation, this phenomenon nevertheless should be one of the decisive factors when selecting cosmonauts. For this reason it is necessary to continue the search for reliable tests for evaluation of individual radiosensitivity.

The problem of preventing overirradiation of a cosmonaut or a biocomplex aboard a spacecraft by physical methods is very complex, because protection /19 against high-energy radiations is involved.

Dosimetric control, ensuring the precise determination and clear notification of radiation conditions, and also warning of possible overirradiation of a crew and biocomplex, occupies a particularly important place in a radiation safety system. A system of dosimetric control should ensure measurement of the dose of cosmic radiations, taking into account their biological effects. In this connection the problem arises of developing physical methods for measurement of the dose directly in biological equivalents of the rad (in rem).

Such are the characteristics of the radiation effect under space flight conditions and the means for determining admissible radiation levels.

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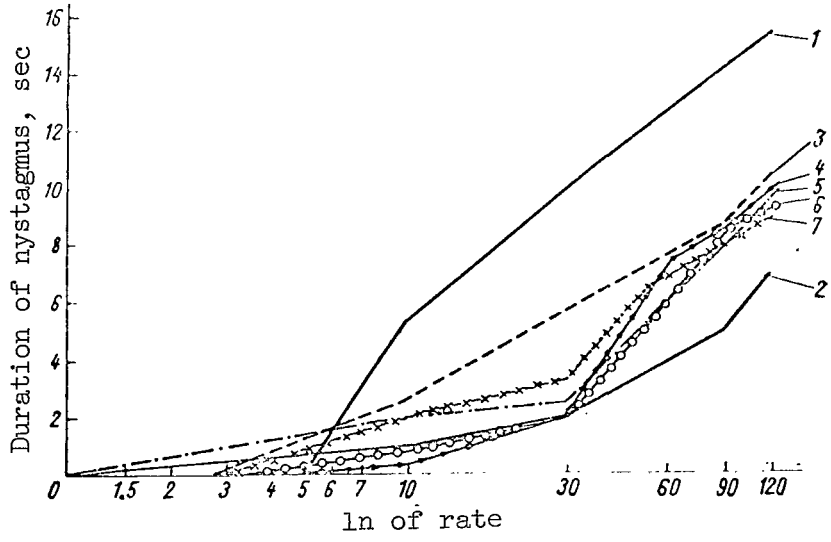

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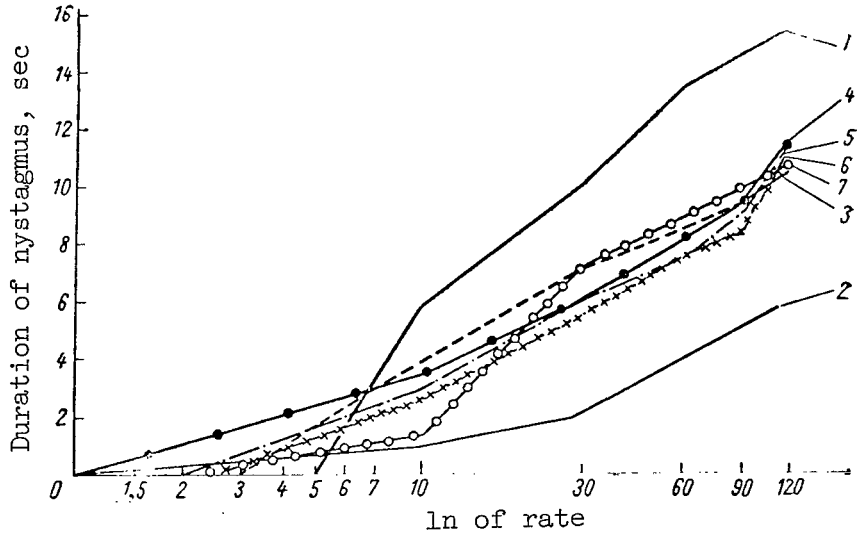

b

Figure 2. Diagrams of vestibular behavior of dogs after combined fractional irradiation (9 roentgens per day, total dose 200 roentgens), vibrations, noise and low atmospheric pressure (a) and isolated irradiation in the same dose (b): 1, 2, maximum and minimum indices of vestibular behavior of control animals; 3, curves of vestibular behavior constructed from mean values; 4-7, curves of vestibular behavior of experimental dogs.

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CHAPTER 2. DOSIMETRIC INVESTIGATIONS AND SHIELDING STUDIES USING THE SYNCHROCYCLOTRON OF THE JOINT INSTITUTE FOR NUCLEAR RESEARCH /22

V. P. Afanas'yev, Yu. S. Deyev, I. B. Keirim-Markus,
Ye. Ye. Kovalev, S. S. Kuznetsova, E. G. Litvinova,
V. A. Sakovich, L. N. Smirennyy, I. K. Sokolova and M. A. Sychkov

Requirements of Physical Conditions for Irradiation of Animals by High-Energy Protons

The synchrocyclotron of the Joint Institute for Nuclear Research, shown diagrammatically in figure 1, generates a beam of protons with energies of 667 ± 5 Mev in the form of pulses with a duration of 400 μsec at the rate of 100 pulses/sec. The divergence of the beam, investigated using photographic films, is ~ 40 min, and flux density is up to 10^{11} protons/cm^2·sec, which corresponds to an intensity of tissue dose up to $4 \cdot 10^3$ rad/sec. The total flux of protons in the beam is $1\text{-}4 \cdot 10^{11}$ protons/sec, which in the case of a uniform distribution of the proton flux in an area with a diameter of 40 cm makes it possible to obtain a tissue dose with an intensity of 10 rad/sec or less.

Figure 2 shows that the cross section of the proton beam has an irregular form: the flux density is greater at the center and decreases toward the edges.

In the experiments made earlier (ref. 1), the animals were placed in a collimator 7 (see fig. 1). A lead plate 1 cm thick was placed at the outlet of the beam to improve the uniformity of the dose field. Not more than three rats, placed side by side, were irradiated at the same time. The absorbed dose was computed from the flux measured by graphite indicators (ref. 2). This method of irradiation is not suitable for experiments with large groups of small animals, especially with dogs.

Irradiation of experimental animals by protons requires creation of a dose field meeting definite requirements.

First, the experimental conditions should ensure uniform irradiation of the bodies of the experimental animals. When dogs are irradiated, this requires a dose field which is uniform in the range ± 10 percent in an area with a diameter of 40 cm.

Second, admixture of accompanying radiations in the flux incident on the animals should not exceed ~ 10 percent of the absorbed dose (in rad). Only

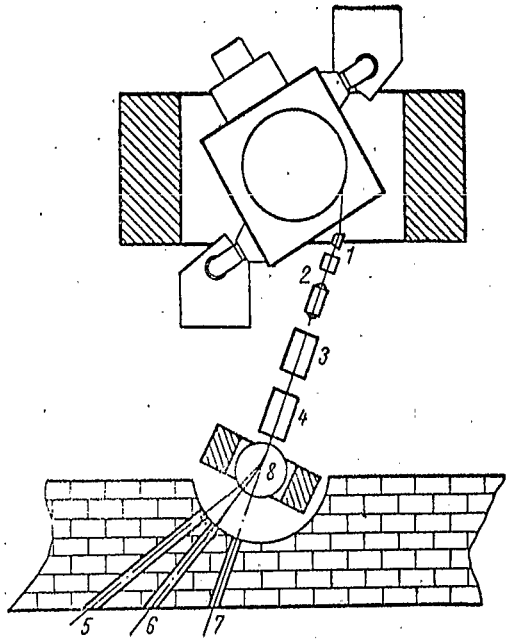

Figure 1. Diagram of layout of synchrocyclotron. 1, emergence of beam; 2-4, supports for experimental apparatus; 5-7, collimators in protective wall; 8, SP-37 magnet.

Figure 2. Photograph of cross section of proton beam.

in this case can the derived data be used later for computation of the results of irradiation by a flux of protons with an arbitrary energy spectrum. /24

Third, the intensity of the absorbed dose should fall in the range 0.1-1 rad/sec, since it is in this range that the fullest study has been made of the biological effect of X- and γ-rays. In addition, such a dose intensity makes it possible to irradiate large groups of animals by radiation in massive doses and to do so quite rapidly.

Fourth, it is desirable to be able to irradiate the animals with protons of different energies in the ranges 100-300 and 500-700 Mev without changing the operating regime of the accelerator.

Methods Used in Measurement of Fluxes and Doses of High-Energy Protons

In order to create a dose field meeting these requirements we used different methods for carrying out a number of dosimetric studies. Methods for measurement of an absorbed dose in a broad working range were the principal methods used.

Individual Luminescent Control Method (ILC). The individual luminescent control (ILC) method is based on the use of luminescent phosphors SrS (Eu, Sm) (refs. 3-5). Under the influence of radiations the luminescent phosphors accumulate absorbed energy over a long time. If an excited sample of the phosphor SrS (Eu, Sm) is subjected to infrared light with a wavelength of $\sim$ 100 mμ, it emits a burst of luminescence of an orange color. The brightness of this luminescence is the basis for judging the absorbed dose of radiation in the range $2 \cdot 10^{-3}$-$2 \cdot 10^{3}$ rad.

Tablets of SrS (Eu, Sm) weighing about 1 g in airtight and opaque holders are used in the ILC dosimeters. By means of filter casings the "hardness variation" of the γ-radiation of the ILC dosimeters is compensated with an accuracy to $\pm$ 12 percent in the range from 120 kev and above.

Measurement is accomplished from a special control panel from which the dosimeter holder is opened mechanically; the tablet is irradiated by infrared rays and the photocurrent of a FEU-29 photomultiplier, created by the burst of luminescence, is measured at this time. The instrument scale is calibrated in roentgens.

The readings of the ILC dosimeters have little dependence on temperature in the range 0-40° and on a dose intensity up to 15 r/sec. In the first three days of retention at room temperature after irradiation the accumulated dose decreases by 18 percent and thereafter (over a period of one month) changes little.

In the investigations the ILC dosimeters were calibrated using a Co^{60} standard source. In order to convert from the dosimeter readings in roentgens to "tissue" rads of protons we must multiply the readings (a) by the coefficient

0.878 of "air" rad/r and (b) by the ratio of the coefficients of electron transformation of the working medium of the ILC dosimeter (SrS) and air for the γ-rays of Co^{60}, equal to 0.90 (ref. 6) and, finally, the readings should be /25 divided by the ratio of the stopping powers of SrS and the biological tissue to protons of different energies.

In order to determine the relative stopping power by use of the Bethe formula we computed the LPE values S_{ion} of protons of some substances. No polarization correction was introduced, because polarization does not play a role in the investigated range of energies. We assumed 10 percent hydrogen and 90 percent air as the composition of biological tissue by weight. Special computations have confirmed that the results for such a composition agree in the range ± 1 percent with the results of computations for more precise compositions: 10.1 percent H; 12.1 percent C; 4.2 percent N and 73.6 percent O_2 (ref. 7) or 10 percent H; 18 percent C; 3 percent N; 65 percent O_2; 4 percent Al (ref. 8). For SrS we assumed a composition of 70 percent Sr and 30 percent S, since the luminophor contains a flux of 15 percent $SrCl_2$. Results of the computations are given in figure 3.

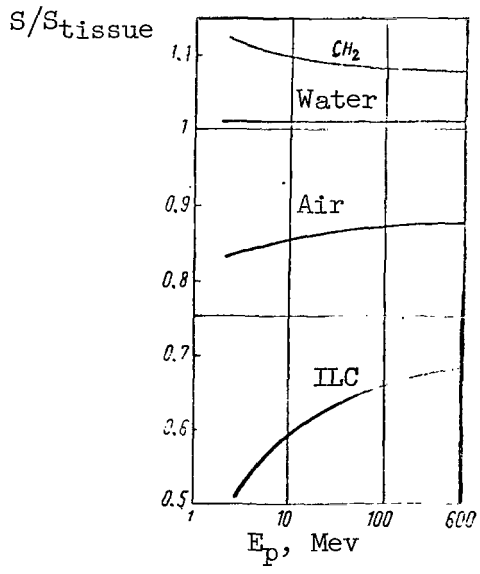

Figure 3. Relative stopping power of certain types of matter in comparison with tissue for protons of different energies (computed from ionization losses).

The additional transfer of energy to matter as a result of nuclear interactions ΔS_{nuc} gives a small correction to the ionization losses. The value ΔS_{nuc} was computed using the formula

$$\Delta S_{nuc} = \frac{1}{2} \sum_i n_i \sigma_i \int_{E_i} N(E) S_{ion}(E) dE, \qquad (1)$$

where n_i is the concentration of the ith element; σ_i is the cross section of inelastic scattering of protons; N(E) is the number of secondary particles with energies E emitted as a result of the interaction, and S_{ion} is their LPE.

Integration is carried out for cascade particles; in the case of evaporating particles the integral is replaced by the excitation energy of a residual nucleus after deduction of the sum of the binding energies of all the escaping particles. The energy transferred to protons and heavier recoil nuclei was neglected due to its smallness.

Table 1 gives some results of the computations.

The computations revealed that in the energy range from 100 to 660 Mev the relative stopping power of SrS falls in the range from 0.66 to 0.72.

Thus, the absorbed dose in "tissue" rads was determined from the ILC readings (in roentgens) multiplied by 1.15 ± 0.05, that is, increased by 15 ± 5 percent.

TABLE 1. SPECIFIC ABSORBED DOSE $S_{ion} + \Delta S_{nuc} = S \times 10^{-8}$ rad·cm^2/proton /26

Proton energy, Mev	Tissue	Water	Air	SrS	Pb
660	3.90 + 0.45 = = 4.35	3.99 + 0.42 = = 4.41	3.45 + 0.51 = = 3.99	2.75 + 0.39 = = 3.13	2.03 + 0.54 = = 2.57
500	4.35 + 0.35 = = 4.70	4.40 + 0.33 = = 4.73	3.80 + 0.42 = = 4.22	3.00 + 0.31 = = 3.31	2.22 + 0.42 = = 2.64
350	5.25 + 0.26 = = 5.51	5.30 + 0.24 = = 5.54	4.58 + 0.30 = = 4.88	3.54 + 0.21 = = 3.75	2.64 + 0.32 = = 2.96
200	7.20 + 0.22 = = 7.42	7.25 + 0.21 = = 7.46	6.25 + 0.27 = = 6.52	4.80 + 0.20 = = 5.00	3.50 + 0.22 = = 3.72
100	11.7 + 0.2 = = 11.9	11.8 + 0.2 = = 12.0	10.2 + 0.3 = = 10.5	7.60 + 0.20 = = 7.80	5.48 + 0.21 = = 5.69

Note: The numbers in the table should be multiplied by 1.6 for conversion to LPE in Mev·cm^2/g.

The dependence of the dosimeter readings on radiation energy or "hardness variation" is determined not only by the relative stopping power, but also by the change of the energy yield η with a change of the LPE of radiation. However, the dependence $\eta(S)$ has not been investigated for ILC dosimeters. Indirect data on the dependence $\eta(S)$ can be obtained by a comparison of the readings of ILC dosimeters and the readings of dosimeters of other types in different radiation fields.

Individual Dosimetric Control Method Using Glass Dosimeters (IKS). The IKS method is based on the appearance of thermoluminescence after irradiation of special aluminosulfate glass activated with manganese (refs. 9 and 10). When samples of such glass are heated to 350-400° there is thermoluminescence of an orange color; the quantity of luminescence recorded with a FEU-29 photomultiplier is the basis for judging the value of the absorbed dose. IKS glass dosimeters measuring 4 x 15 x 15 mm make it possible to record doses in an extremely broad range--from 0.02 to $2 \cdot 10^6$ rad--and retain information for an unlimited period of time after irradiation. The "hardness variation" of γ-radiation is not great, since with respect to effective atomic number the glass is close to Mg, and can be compensated further by a filter of 0.6 mm Sn + 0.5 mm Al with an accuracy of ± 20 percent in the range of γ-quanta energies from 40 kev and greater.

The stopping power of the glass to the γ-radiation of Co^{60} is 98 percent of the stopping power of air (ref. 6). The ratio of the stopping powers of glass and biological tissue relative to protons can be considered equal to the relative stopping power of aluminum, which changes from 0.745 to 0.815 with a change of proton energy from 10 to 1,000 Mev. At low energies nuclear interactions do not influence the value of the relative stopping power, but energies of 500-1,000 Mev increase it by 2-3 percent. This correction was taken into account in the cited values of the relative stopping power of aluminum.

Thus, for determination of the tissue dose (in rad) the readings of the IKS dosimeters (in roentgens) were increased by 1.10 ± 0.04 times, that is, by 10 ± 4 percent. /27

Available data on the dependence of the energy yield η of thermoluminescence of the IKS dosimeters on LPE show that in the range above 1 Mev η is not dependent on proton energy. For α-particles with energies of 5 Mev, η is an order of magnitude lower than for protons. Thus, the dependence $\eta(S)$ should not exert an appreciable influence on the measurement of the absorbed dose, using IKS dosimeters, as long as the contribution of heavy charged particles is not great.

Ferrosulfate Method of Chemical Dosimetry with a Ferrosulfate Dosimeter. This method is based on the reaction of oxidation of bivalent iron into trivalent iron ($Fe^{2+} \rightarrow Fe^{3+}$). The concentration of forming ions of trivalent iron is proportional to the absorbed dose of radiation in the range $4 \cdot 10^3$-$4 \cdot 10^4$ rad.

In chemical dosimetry it is customary to use $1 \cdot 10^{-3}$ M solutions of ammonium sulfate of bivalent iron (Mohr's salt) $(NH_4)_2 [Fe (SO_4)_2] \cdot 6H_2O$ in 0.8 normal sulfuric acid.

Due to the high radiation stability of sulfuric acid and the extremely small concentration of bivalent iron, it is assumed that the direct effect of radiation on dissolved matter is negligibly small, and the primary radiation-chemical process is the decomposition of water. The products of radiolysis of water interact with bivalent iron and the dissolved molecular oxygen as follows

$$Fe^{2+} + OH = Fe^{3+} + OH^-; \quad (2)$$

$$H + O_2 = HO_2; \quad (3)$$

$$Fe^{2+} + H_2O_2 = Fe^{3+} + OH + OH^-; \quad (4)$$

$$Fe^{2+} + H^+ + HO_2 = H_2O_2 + Fe^{3+}. \quad (5)$$

The yield of trivalent iron is 15.68 ± 0.07 ions per 100 ev for the γ-radiation of Co^{60} (ref. 11).

Within the limits $1 \cdot 10^{-3}$-$1 \cdot 10^{-4}$ M G (Fe^{3+}) is not dependent on the concentration of bivalent iron. The most reproducible yield is attained when using a solution of salt of bivalent iron in sulfuric acid with concentrations of 0.2-1.5 normal solution; G (Fe^{2+}) is dependent on the oxygen content in the solution. A decrease of the concentration of O_2 in the solution below critical levels causes a bi- or trivalent decrease of yield. In the course of irradiation the dissolved oxygen "is burned out." If the system is irradiated in a closed container and is in a state of equilibrium with the air, G (Fe^{3+}) is linearly dependent on the dose up to $5 \cdot 10^4$ roentgens; in the region $5 \cdot 10^4$ roentgens there is an inflection of the curve; beginning with $5 \cdot 10^4$ and to $2 \cdot 10^5$ roentgens the dependence between the quantity of oxidized ions and the dose is maintained, but the output of the reaction is lower. G (Fe^{3+}) is not dependent on the intensity of the dose to $1 \cdot 10^6$ rad/sec. At higher intensities the dosimeter operates unreliably, since in this case there must be very high rates of mixing (virtually impossible) to fill the shortage of oxygen whose concentration in the reaction zone decreases. No temperature dependence is observed in the range from 0 to 50°. The value G (Fe^{3+}) increases in the presence of /28 organic admixtures. NaCl ($1 \cdot 10^{-3}$ M) is introduced into the solution for stabilization of G (Fe^{3+}).

As shown in references 12 and 13, in the LPE range from 2 to 50 Mev·cm^2/g, corresponding to proton energies from 1,000 to 10 Mev, the yield remains virtually constant, but with denser ionization G (Fe^{3+}) decreases (fig. 4).

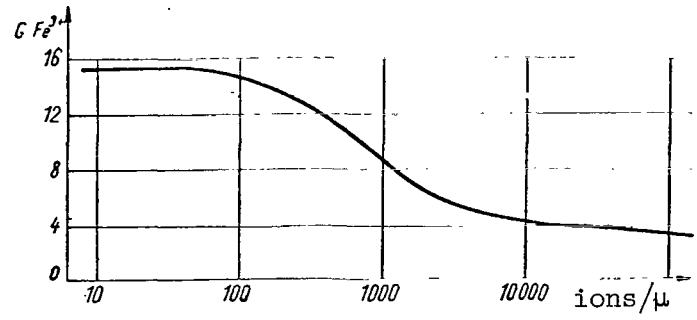

Figure 4. Values of radiation-chemical yield of Fe^{3+} as function of ionization density.

In composition ferrosulfate dosimeters differ little from water, and it follows, therefore, from figure 4 that they do not have a "hardness variation," at least to a proton energy of 10 Mev, below which S becomes greater than 50 Mev·cm^2/g and the yield of the reaction of oxidation of iron decreases (table 2).

When using carefully purified reagents and spectrophotometric analysis of iron ions the ferrosulfate method ensures an accuracy of ± 2 percent.

Such positive qualities of the ferrosulfate method as the high accuracy (± 2-5 percent), the broad range of absorbed doses ($4\cdot10^3$-$4\cdot10^4$ rad) and intensity of dose (to $1\cdot10^6$ rad/sec), constancy of G (Fe^{3+}) with a change of LPE /29

TABLE 2. COMPARATIVE CHARACTERISTICS OF A FERROSULFATE SYSTEM, WATER, AIR AND BIOLOGICAL TISSUE

Medium	Specific weight, g/cm^3	Effective atomic number	Electron density, electrons/ g·10^{-23}
0.001 M solution of $Fe(SO_4)_2$	1.027	7.68	3.33
Water	1.000	7.42	3.34
Biological tissue	1.000	7.42	3.36
Air	0.001293	7.64	3.01

from 2 to 50 Mev·cm^2/g and the absence of "hardness variation" at proton energies from ~ 10 Mev indicate the feasibility of the use of this method.

The initial solution used was a $1 \cdot 10^{-3}$ M solution of Mohr's salt and $1 \cdot 10^{-3}$ M NaCl in a 0.8 normal solution of sulfuric acid.

The concentration of forming ions of trivalent iron was determined by direct spectrophotometric measurement in the Fe^{3+} absorption band at a wavelength of 302 mμ. All measurements were made with a SF-4 instrument. The dosimetric solutions were poured into spherical vials made from chemically neutral glass with a minimum volume of 3 cm^3 or a maximum of 25 cm^2. In those cases when it was necessary to measure the dose volume (see below), flat containers measuring up to 400 x 400 mm, filled with a solution of Mohr's salt, were also used.

The value of the yield of oxidation used in measurements of the absorbed dose with a ferrosulfate dosimeter was $Fe^{2+} \rightarrow Fe^{3+}$ G (Fe^{3+}) = 15.68 ± 0.07 ions per 100 ev (ref. 11), correct for radiations with minimum stopping power.

The value G (Fe^{3+}) was checked experimentally for protons with energies of 120, 240 and 660 Mev. For these purposes the absorbed dose (in rad) was computed from the flux, measured using graphite indicators. Table 3 gives the derived values.

TABLE 3. VALUES G (Fe^{3+}) FOR HIGH-ENERGY PROTONS

Proton energy E_p, Mev	G (Fe^{3+}), ion/100 ev
660	16.4 ± 0.6
240	15.9 ± 1.1
120	15.4 ± 1.8

As follows from these data, the derived values G (Fe^{3+}) within the limits of measurement error do not differ from those given in reference 11.

An effort was made to determine the influence of the density flux of protons on the readings of the ferrosulfate dosimeter in the range $3 \cdot 10^7$-$9 \cdot 10^9$ protons/cm^2·sec, which corresponds to a mean dose intensity of protons of 1.3-$2.5 \cdot 10^2$ rad/sec and a pulse dose intensity of $0.3 \cdot 10^2$-$6.25 \cdot 10^3$ rad/sec. Figure 5 shows that, within these limits, the dosimeter readings are not dependent on the proton flux density, which agrees with the data in the literature (ref. 14) on γ-radiation.

Method for Measurement of a Proton Flux Using Graphite and Aluminum Indicators. Absolute measurements of proton fluxes were made by measurement of the

β-activity induced in carbon and aluminum samples. The following reactions were taken into account for this purpose

$$Al^{27}(p, 3pn)Na^{24} \quad \text{and} \quad C^{12}(p, pn)C^{11},$$

characterized by the fact that their cross sections have little dependence on /30 proton energies greater than 100 Mev. This makes it possible to measure proton fluxes distributed in conformity to some energy spectrum. Due to sufficiently precise information (ref. 15) (fig. 6) on the cross section of these reactions, it is convenient to use aluminum and carbon as monitors in working with monoenergetic protons of any energy above the threshold of these reactions.

The half-life of C^{11} is 20.5 min. As a result, the measurement of the total flux of protons is possible when the duration of the irradiation is not greater than 30 min.

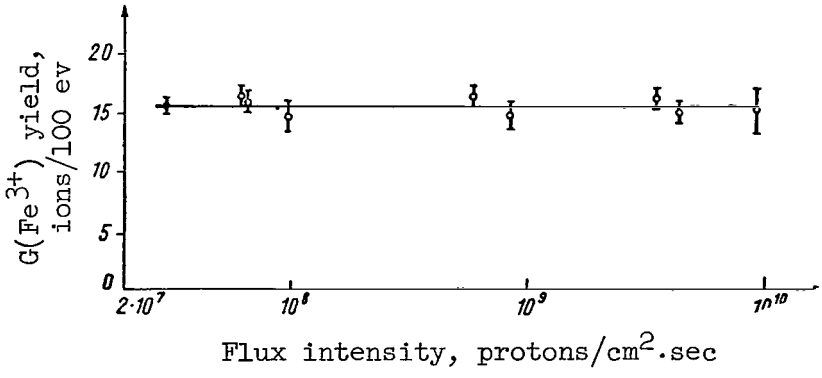

Figure 5. Readings of ferrosulfate dosimeter as function of density of proton flux.

Investigation of β-activity is made 1 hour after irradiation, or later, since β-active isotopes forming simultaneously with C^{11} in a carbon detector have half-lives not greater than 10 min.

The half-life of Na^{24}, which is 15.1 hours, is the longest of the half-lives of the isotopes formed during the irradiation of aluminum by protons.

The closest half-lives are 20 min (C^{11}) and 112 min (F^{18}), so that the measurements of β-activity of aluminum samples must be made one day after irradiation.

It is practical to accomplish the total count of protons with aluminum detectors by an irradiation exposure up to 20 hours. However, in the case of a proton flux of low intensity and short exposures, carbon gives a higher efficiency in recording due to a lesser half-life than aluminum at comparable activation cross sections.

The indicator samples were prepared in the form of aluminum plates measuring 20 x 20 x 1 mm and graphite plates measuring 20 x 20 x 5 mm. The

radioactive radiations of the samples were measured by an end-window counter in a lead housing. The activity of carbon was computed, taking into account corrections necessary in absolute measurements of the activity of thick-layered samples (ref. 16). The aluminum detectors were calibrated by irradiation in a homogeneous beam of protons with energies of 660 Mev, simultaneously with small samples of aluminum whose absolute activity was measured with a 4π-counter, and an apparatus for absolute measurements in a small solid angle using an end- /31 window counter. The measurements made using these instruments were in agreement.

Calibrations of graphite and calibration of aluminum were made by joint irradiation.

The isotopes Na^{24} and C^{11} can also be formed in the reactions Al^{27} $(n\alpha)$ Na^{24} and C^{12} (n, 2n) C^{11}. Therefore, in measurement of the proton flux it was necessary to be sure of the absence of high-energy neutrons. On the other hand, the variation of the cross sections of these reactions with the energies of neutrons (fig. 6) makes it possible by a comparison of the ratios of activity of aluminum and carbon samples to judge the change of the contribution of neutrons to the makeup of radiation under different conditions, such as behind protection or in a phantom.

An evaluation of the composition of radiation can also be made by comparison of the readings of other detectors having different efficiencies of recording of protons and neutrons at different energies.

In order to evaluate the compositon of the radiation we also made a comparison of the computed specific absorbed dose (per unit flux of protons) and the specific absorbed dose of protons, obtained experimentally from measurements of the flux of protons and the absorbed dose created by them. /32

Ionization Method. In the study of the passage of a proton beam through layers of plastic, when the dimensions of the dose field were small, a DIGD instrument was used in making relative measurements. This instrument has a flat ionization chamber with a diameter of 100 x 100 mm, filled with pure He to a pressure of 0.5 atm. The windows of the chamber and the three inner electrodes were made of copper foil 0.1 mm thick. The same chamber was used in a number of experiments as a monitor. The voltage fed to the chamber ensured a saturation current.

The selected measurement methods are characterized by a broad range, both with respect to value (from $2 \cdot 10^{-2}$ to $2 \cdot 10^{6}$ rad) and dose intensity (up to $\sim 10^{6}$ rad/sec), and the used dosimeters retain information a long time, making it possible to obtain cartograms of the dose fields by simultaneous measurements by a large number of detectors. The mean error of the used dosimetric methods is approximately ± 10 percent.

A comparison of the different methods was made in proton beams with energies of 660, 380, 240 and 120 Mev. In these ranges of measurement it was found convenient to irradiate with ILC in combination with carbon detectors, and when chemical dosimeters are used, to employ them in combination with aluminum detectors. The IKS dosimeters have such a broad measurement range that irradiation was possible using either carbon or aluminum detectors, thus combining all methods into one.

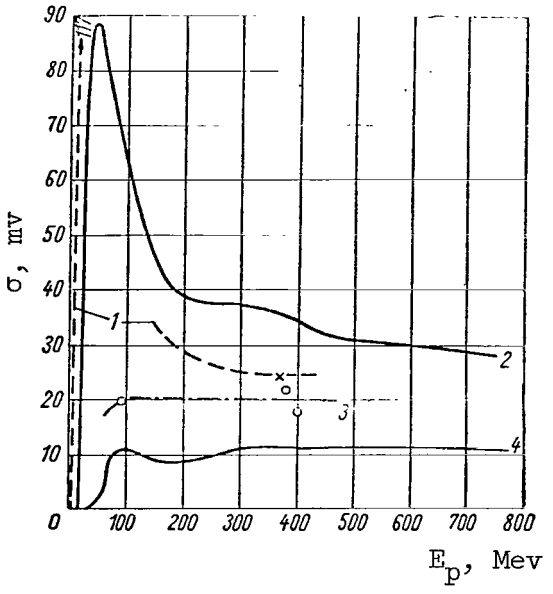

Figure 6. Cross sections of nuclear reactions: 1, Al^{27} (n, α) Na^{24}; 2, C^{12} (p, pn) C^{11}; 3, C^{12} (n, 2n) C^{11}; 4, Al^{27} (p, 3pn) Na^{24}.

Absorbers of polyethylene or paraffin were placed at the point of emergence of the beam from the accelerator (see fig. 1) in order to obtain protons with energies less than 660 Mev. The thickness of the absorbers was computed using the data from references 17 and 19. An SP-37 magnet (fig. 1) was used to deflect the proton beam into the collimator 6, thereby purifying it from accompanying radiations. The detectors were irradiated simultaneously beyond the collimator. Table 4 gives the measurement results.

The experimental values of the dose per proton agree well with the computed values. It follows, therefore, that the absolute measurements of activity do not contain a large systematic error. The agreement of the results obtained by the ILC and chemical methods is evidence that at least to S = 7 Mev·cm^2/g the energy yield (S) of SrS phosphorus remains constant. At the same time, the close values of the specific absorbed dose per proton obtained by measurements with the ILC and the ferrosulfate dosimeters and the good agreement in the intensity of the absorbed dose obtained in simultaneous measurements with chemical and thermoluminescent dosimeters is evidence of the reliability of dosimetric measurements.

Table 4 shows that the readings of different dosimeters agree satisfactorily with one another and correspond to the computed values. Consequently, the change of the specific absorbed dose can be evidence of a change in the /34 composition of the radiation. An increase (during measurement of the flux by

TABLE 4. RESULTS OF EXPERIMENTAL COMPARISON OF READINGS OF VARIOUS DOSIMETERS FOR PROTONS WITH ENERGIES OF 660, 380, 240 AND 120 MEV

Proton energy, Mev	Intensity of absorbed dose, rad/sec			Flux density, protons/ $cm^2 \cdot sec \cdot 10^6$			Specific absorbed dose, $rad \cdot cm^2$/ $proton \cdot 10^{-8}$			
	ILC	Chemical	IKS*	Chemical	IKS	C	ILC C	Chemical Al	IKS Al	Computed
660	-	-	-	820		840	-	-	-	4.35
	-	-	-	820		820	-	-	-	
	-	-	-	150		167	-	-	-	
	9.9	-	-	-		240	4.1	-	-	
	35.4	-	-	-		720	4.9	-	-	
	37.1	-	-	-		790	4.7	-	-	
	-	26.5	13.9**	600	460**	-	-	4.5	3.1**	
	-	24.5	-	580	-	-	-	4.4	-	
380	0.52	-	-	-	-	9.5	5.5	-	-	5.27
240	0.23	-	-	-	-	3.1	7.4	-	-	7.42
	0.17	-	-	-	-	1.9	8.9	-	-	
	-	0.27	0.24	3.4	3.7	-	-	7.8	6.7	
	-	0.22	0.21	-	-	-	-	-	-	
120	0.083	-	-	-	-	0.80	10.4	-	-	10.7
	-	0.141	0.127	1.61	1.19	-	-	8.8	10.6	

*Thermoluminescent dosimeters of IKS glass were used in a number of experiments (refs. 9 and 10) (the measurements were made by I. A. Bochvar). The glass was calibrated in the same sample source as the ILC.

**Due to nonhomogeneity of the beam, the detectors were not irradiated identically and this increased the measurement error.

Al) will mean that lower-energy protons with large S give an appreciable contribution to the dose. On the other hand, a decrease of the specific absorbed dose in comparison with the computed value will indicate that there are many neutrons in the radiation.

The use of dosimetry methods is therefore suitable for measurements in fields of protons with energies of 100-660 Mev. At the same time, they possibly distort the contribution in the absorbed dose from low-energy secondary particles. Since such particles are responsible for approximately half the value ΔS_{nuc} (in light matter), the error in dose determination due to "hardness variation" of the detectors does not exceed several percent.

Study of Dose Fields

One of the problems in this investigation was the need for widening the proton beam without exciting appreciable accompanying radiation. For this reason it was decided to perform the irradiation in the room housing the synchrocyclotron, not in the collimators. The beam was widened by proton scattering in absorbers. Since the intensity of the secondary radiations forming in the absorber increases with its thickness, it was necessary to select those materials which cause the necessary scattering of the beam with use of a minimum thickness. The mean angle of multiple scattering of protons increases with the atomic number of the element; for lead it is 3 times greater than for carbon. In addition, the number of secondary cascade protons forming per 1 g/cm^2 is several times less in lead than in carbon. For this reason lead blocks 10 cm thick were used as absorbers. When such a filter is used, the angle of multiple scattering, at which the proton flux decreases by 15 percent, is $\sim 2^{\circ}$ (ref. 18), which at a distance of 5-7 m from the absorber (curves 3 and 4, fig. 6) made it possible to obtain a radiation field of the required size with a diameter of $\sim$ 35 cm. In this case the energy of the protons was decreased to 510 Mev (ref. 19).

Using the data in reference 20, it can be calculated that the proton /35
flux is attenuated by 43 percent by inelastic scattering and the admixture of soft cascade protons, neutrons and π-mesons appearing during nuclear interactions in the lead at the place where the animals are irradiated is less than 0.8, 1.4 and 0.07 percent, respectively. The contribution of secondary evaporating particles can be neglected due to their isotropic angular distribution and short paths. Comparison of data obtained using different measurement methods has confirmed the absence of appreciable admixtures of accompanying radiations.

For an experimental checking of the contribution of neutrons and γ-radiation we also measured the absorbed dose of radiation beyond the collimator 7 (fig. 1) with the magnet cut in and cut out. In the second case the readings of the ILC dosimeters decreased to 0.04 percent and those of the graphite flux detectors to 0.5 percent of the initial value. The contribution of neutrons and γ-quanta in the radiation field, therefore, apparently was unimportant. However, since the angular distribution of secondary particles was less directed than the primary particles, such an experiment gives only the lower estimate of the admixture.

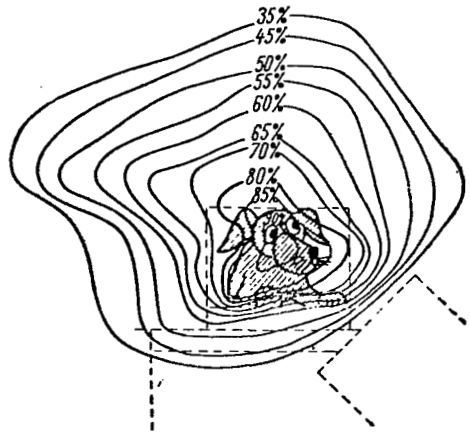

Figure 7. Dose field of irradiation of animals by protons with energies of 510 Mev. Filter of 10 cm Pb. Dashed lines indicate support and box for irradiation of dog. At bottom right--projection of quadrupole lens situated in front of table with animals.

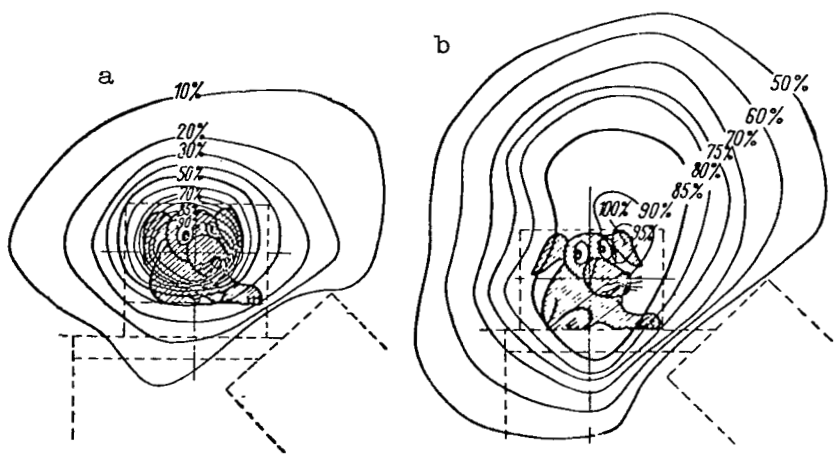

Figure 8. Dose field of irradiation of animals by protons with energies of 100 Mev: a, filter of 180 cm paraffin; b, 150 cm of paraffin and 6 cm of Pb.

Using dosimeters placed in aluminum frames (fig. 1, 3), we obtained a cartogram of the dose field in the plane perpendicular to the proton beam. The determined dose field at the place where the animals were irradiated (fig. 1, 8) for an energy of 510 Mev is shown in figure 7.

Figure 7 shows that the size of the field is approximately 20 percent, and the intensity of the absorbed dose at the center of the field is 1-2 rad/sec; it can be decreased to 0.03-0.1 rad/sec by a change of intensity of the synchrocyclotron beam. The created field therefore meets all necessary requirements. /36

Figure 8 shows cartograms of the dose fields resulting from irradiation by protons with energies of about 100 Mev. In this case decrease of proton energy was accomplished by a paraffin absorber. Due to the great thickness of the paraffin filter, the distance from it to the animals is decreased to such an extent that in the case of paraffin alone it is not possible to create a field of irradiation of the required size (fig. 8b). After the part of the paraffin at the end of the absorber turned toward the animals was replaced by lead, it was possible to broaden the field of irradiation adequately.

Determination of Tissue Doses

Phantoms were prepared for study of the distribution of radiation in the bodies of animals. The phantom for a rat (fig. 9a) was a plastic container with a base 50 mm in diameter, a height of 143 mm and a wall thickness of 1.8 mm, filled with a solution of Mohr's salt. The solution had a volume of 180 cm^3 and a weight of 185 g, which corresponds to the weight of a pubescent rat. The upper conical part of the container, 43 mm in height, was removable and filled with solution separately. On the basis of samples taken from the conical and cylindrical parts we can determine the mean tissue absorbed dose in the body and head of the rat. The phantom was irradiated by protons with energies of 510 Mev, together with animals in a box for irradiation of rats. The results of measurements of the absorbed dose in the head and body and the readings of the ILC dosimeters, irradiated together with the animals, coincided. During irradiation, the ILC dosimeters were replaced several times, and the readings then were summed up for comparison with chemical dosimeters irradiated during the entire time. The small animals therefore were subjected to uniform irradiation. /37

The study of radiation distribution in the bodies of dogs is of the greatest interest.

The phantom for a dog (fig. 9b) is a cylinder 56 cm long and 20.6 cm in diameter, assembled from paraffin disks. The detectors were placed within the cylinder. The phantom was irradiated by protons with an energy of 510 Mev in position 3 (fig. 1) in a special box in which dogs were irradiated later.

The results, shown in figure 10, reveal that when the dogs are irradiated in a longitudinal direction, the tissue dose at the emergence of the beam (curve 1) is 80 percent of the dose at the entry and 67 percent of the maximum tissue dose at a depth of 11 cm. A greater uniformity of the tissue doses is obtained when there is two-directional irradiation (curve 2): one half of the dose from the direction of the head and the other half from the direction of the tail. When this method is used, nonuniformity of the tissue dose does not exceed ± 10 percent. The mean tissue dose coincides with the surface absorbed dose. Curves 1, 3 and 4 show that there is an accumulation of secondary cascade

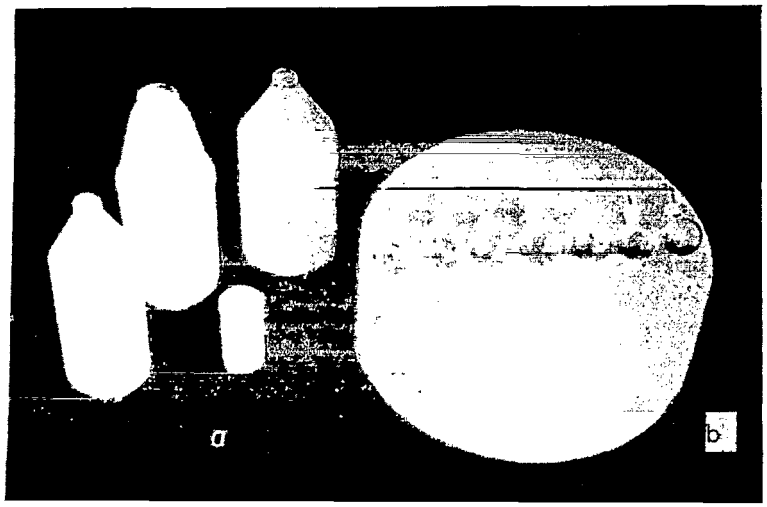

Figure 9. Phantoms of rat (a) and dog (b).

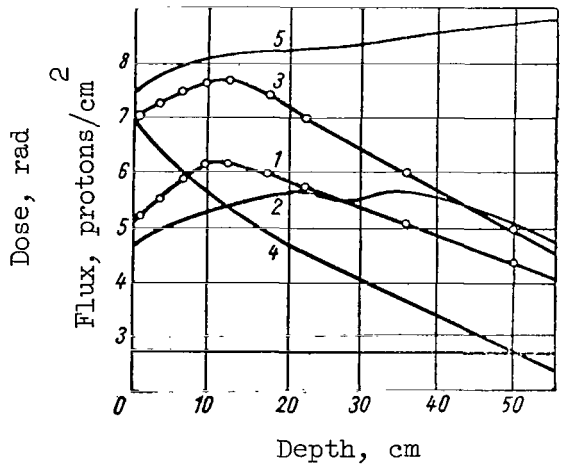

Figure 10. Tissue doses in phantom of dog during irradiation by protons with energies of 510 Mev: 1, absorbed dose, x 10 rad; 2, absorbed dose, x 10 rad in case of two-directional irradiation; 3 proton flux indicated by readings of graphite detectors, x 10^8 protons/cm^2; 4, proton flux according to computations, taking into account beam deflection and loss of protons due to inelastic interactions in paraffin; 5, absorbed dose per proton, x 10^8 rad·cm^2/proton.

protons in the body of the animal, with a mean path in the paraffin of about 12 cm (~ 150 Mev). If the change of the reaction cross section (p, pn) C^{11} with proton energy (fig. 6) is taken into account, by a rough estimate the quasi-equilibrium flux of cascade protons at a depth greater than 15 cm of paraffin is approximately 30 percent of the flux of primary protons. If we use as the specific absorbed dose per proton the values $S = 4.7 \cdot 10^{-8}$ rad·cm^2/proton for 510 Mev and $S = 12 \cdot 10^{-8}$ rad·cm^2/proton for 100 Mev, the value S for a quasi- /38 equilibrium radiation spectrum near the maximum of the absorbed dose should be $4.7 \cdot 10^{-8} \cdot 10 + 12 \cdot 10^{-8} \cdot 0.3 = 8.3 \cdot 10^{-8}$ rad·cm^2/proton and cascade protons are $12 \cdot 10^{-8} \cdot 0.3 = 3.6 \cdot 10^{-8}$ rad·cm^2/proton, or 43 percent of the absorbed dose. This value is not much greater than the actual contribution of cascade protons to the tissue dose, which, to judge from curve 1, can be estimated at 25-40 percent of the total dose at a depth greater than 12 cm.

The same reasoning can be applied to curve 5. The slow increase of curve 5 at great depths has a quantitative explanation in the increase of S, because the energy of the primary protons decreases with their passage through the paraffin.

By use of ILC dosimeters, in which there is a high sensitivity to γ-radiation with quanta energies less than 0.2-0.3 Mev when used without filter cases, it was established that the contribution of secondary γ-radiation to the absorbed dose was insignificant. By comparing the readings of ILC dosimeters (with and without filter cases), placed in identical positions within the phantom, it was possible to detect the differences.

Thus, in the case of two-directional irradiation by a pure flux of protons with an energy of 510 Mev, the dogs were subjected to irradiation which was uniform throughout the body; this irradiation was by protons with energies of about 500 Mev (~ 70 percent of the absorbed dose), by protons with energies in the 100-Mev range (~ 20 percent of the absorbed dose) and apparently by slower particles of nuclear stars (~ percent of the absorbed dose).

The contribution of the products of star formation to the absorbed dose is of particular interest, due to their high biological effect. It is necessary, therefore, to have further, more detailed information concerning the composition of the radiation within the phantom.

Method of Experimental Study of Protective Properties of Materials in the Proton Beam of the Synchrocyclotron of the Joint Institute for Nuclear Research

In connection with the possibility of radiation injuries during space flights, the problem of protection of the cosmonaut from penetrating radiations now has assumed great practical importance. In order to decrease the levels of irradiation of the cosmonaut to admissible values, it apparently is necessary to

have a considerable thickness of the protection of the occupied cabin of the ship. For example, for the absorption of high-energy protons appearing at the time of solar flares it is necessary to have about 400 g/cm^2 of carbon or 700 g/cm^2 of tungsten. Due to the very obvious rigid requirements imposed on the weight of spacecraft, in the planning of the protection of the latter it is necessary to have quite complete information on the doses of radiation in space and clear recommendations on the admissible limits of the radiation effect /39 under space flight conditions and to know thoroughly the attenuation of the radiation dose in different protective materials.

With complete absorption of protons (such as is achieved, for example, in the protection of accelerators) the dose beyond the protection is determined by secondary neutrons.

At present there are no reliable methods for computing protection from high-energy protons and little initial data for computations; it is necessary, therefore, to formulate model experiments.

Since in space it is necessary to deal with protons having different angle and energy distributions, the model experiments should ensure the study of protection from a broad parallel beam of monoenergetic protons for different energies of the latter and different angles of incidence on the protection.

For this study we selected construction materials and materials having specific protective properties. The protective properties of the materials should be evaluated from the attenuation of the tissue dose of radiation (in rems or rads). In the latter case it is necessary to know the relative biological effect of the radiation beyond the protection, that is, have information on the dose composition of the radiation.

The characteristics of the external proton beam of the synchrocyclotron of the Joint Institute for Nuclear Research make it possible to model the most important part of the energy spectrum of the cosmic radiation protons.

The principal methodological problems involved in the use of the proton beam of the synchrocyclotron involve the modeling of a broad beam of protons, study of the protective properties in the case of slant incidence of the beam, evaluation of the contribution of secondary radiations formed in the protection and obtaining monochromatic proton beams with different energies in the range 100-700 Mev.

For modeling a broad monodirectional proton beam it is possible to use the concepts developed in reference 21 for the problem of the passage of γ-rays through matter. Figure 11 shows that there is an unambiguous correspondence of the protons from the narrow beam, arriving at the detectors situated at points A_0, A_1, A_2, ... to each proton in the broad beam incident on detector A. The angles of deflection and the paths traveled in the protection before and after interaction are identical for these protons. It also is obvious that the

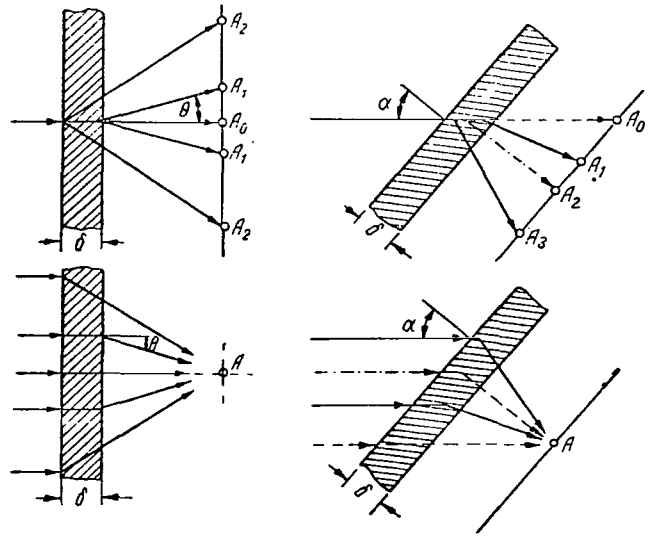

Figure 11. Diagram of transition from narrow to broad beam.

conditions for passage through the protection for protons which do not experience scattering are identical in both cases.

Thus, if in the case of a narrow beam we measure the absorbed radiation dose not only at the point A_0, but also at a sufficient number of other points situated in a plane parallel to the layer of protection, on the basis of these data we can obtain full information on attenuation in the protection of a broad parallel proton beam. /40

During passage through the protection, the protons lose their energy in collisions of atoms with electrons (ionization losses) and experience elastic (Coulomb and diffraction) scattering and inelastic interactions with nuclei of matter. As a result there is a gradual decrease of proton energy, deflection from the direction of initial movement, partial absorption of the proton beam by nuclei of matter and formation of secondary particles in the protection. With this taken into account, total absorption of the dose in a thin layer of the thickness d_0 (g/cm^2), parallel to the plane of the protection on which the narrow proton beam is normally incident, is determined by the expression

$$Q = 2\pi k \Pi N d_0 \int_0^{\delta} dy \int_0^{\pi/2} e^{-\sigma_a y} \frac{d\sigma(\theta, y, E)}{d\Omega} S(\theta, y, E) \times \times \left(-\frac{\overline{dE}}{dx}\right)_{\theta y} tg\theta d\theta, \quad (6)$$

where

Q is the total absorbed dose (g·rad);
Π is the total flux of protons in the beam (protons/sec);
k is a conversion factor;
N is the number of atoms per 1 g of matter in the protection;
y is distance in the protection, read from the point of emergence of the beam;
δ is the thickness of the protection (g/cm^2);
σ_a is the cross section of inelastic interaction of protons with the matter of the protection;
$\frac{d\sigma\ (\theta,y)\ E}{d\Omega}$ is the effective differential cross section, which takes into account all the processes of elastic scattering of protons in the matter of the protection; /41
S (θ, y, E) is a function taking into account the dose contribution of secondary particles formed in the protection as a result of nuclear interactions of protons;
$\left(-\frac{\overline{dE}}{dx}\right)_{\theta y}$ are the mass losses of proton energy in the d_0, averaged for protons scattered at the angle θ (Mev·cm^2/g).

We will now consider a case when a broad beam of protons with the flux density Φ (protons/cm^2·sec) is incident on the protection. In this case the absorbed dose D (in rad) beyond the protection is

$$D = 2\pi k\Phi N \int_0^{\delta} dy \int_0^{\pi/2} e^{-\sigma_a y} \frac{d\sigma\ (\theta, y, E)}{d\Omega} \times$$

$$\times S(\theta, y, E) \times \left(-\frac{\overline{dE}}{dx}\right)_{\theta y} tg\ \theta d\theta. \qquad (7)$$

In order to find the total absorbed dose Q for a narrow beam and the absorbed dose D for a broad beam, the principal difficulties involved are the finding of the functions $\frac{d\sigma\ (\theta,y,E)}{d\Omega}$ and S (θ, y, E).

However, from the point of view of the experimental study of the protective properties of materials in the proton beam of the synchrocyclotron, it is sufficient that in both cases these functions be identical. Thus, from expressions (6) and (7) we obtain

$$\frac{D}{\Phi} = \frac{Q}{P d_0} = q, \qquad (8)$$

that is, the absorbed dose per unit flux of protons in the broad beam is equal to the integral absorbed dose per unit flux of protons in the narrow beam imparted to a layer with matter of the thickness 1 g/cm^2.

It follows from expression (8) that $D = q\Phi$.

However, the absorbed dose D from a broad proton beam with the flux density Φ, created beyond a protective layer of the thickness δ, can be written in the form

$$D = q_0 f(\delta, E_0)\Phi, \tag{9}$$

where $f(0, E_0)$ is the dose factor of attenuation and accumulation; the subscript "0" applies to the proton beam before reaching the protection. It is obvious that

$$q = q_0 f(\delta, E_0). \tag{10}$$

In measurements with a narrow beam f can be determined as /42

$$f = \frac{Q(\delta)}{Q(0)},$$

where $Q(0)$ and $Q(\delta)$ are the total absorbed doses in the absence of protection and beyond a protection of the thickness δ, respectively.

Thus, the study of protection from a broad beam of protons essentially involves the experimental determination of q or Q. It follows from formula (6) that determination of Q requires integration for the volume of the detector, parallel to its protection. Experimentally such integration can be accomplished by use of an adequate number of sensors, such as ILC dosimeters or ampules containing a solution of Mohr's salt, and by summing up their readings.

This method is convenient because the ratio of the readings of the outermost detectors to the readings of the detectors at the center of the dose field can be used to judge the error arising due to the finite size of the area on which the detectors are placed. The distribution of the absorbed dose beyond the protection in the plane perpendicular to the beam was determined experimentally to great distances from the axis of the beam. The error in measurement by the detectors situated at selected distances from the axis did not exceed 5 percent.

Another method for the determination of Q is based on the use of dosimeters with a quite large surface, for example, flat containers with a solution of Mohr's salt. The advantage of these dosimeters is in the simplicity of the measurements. At the same time, the chemical dosimeter differs little from water in composition, and the absorption of radiations of different types and energies therefore is by means of processes of interaction, close to the processes occurring in the tissues or fluids of an organism; for this reason a dosimeter of this type has a small "hardness variation."

In the study of the slant incidence of a broad proton beam on a protection it also is possible to use the dose factor of attenuation and accumulation, which by analogy with a case of normal incidence can be described in the form

$$f_\alpha(\delta, E_0) = \frac{Q_\alpha(\delta)}{Q_\alpha(0)}.$$

Here Q_α (0) and Q_α (δ) are the total absorbed doses from a narrow beam in the case of no protection and beyond a protection of thickness δ, situated at the angle α to the direction of the proton flux (fig. 11); Q_α (δ) is measured in the plane parallel to the protection.

As mentioned previously, in order to obtain a proton beam with lower proton energies, the protons from the synchrocyclotron were passed through a polyethylene absorber, in which they lost part of their energy. In order to improve the monoenergetic character of the beam and purify it from secondary radiations emerging from the absorber, the beam was deflected by a magnet /43 and passed through a collimator in a 3-meter protective wall.

An estimate of the value of the neutron flux was made by a comparison of the readings of detectors with appreciably different sensitivity to neutrons and protons. The detectors used for this purpose were those measuring absorbed energy, such as chemical dosimeters, a luminescent dosimeter (ILC) and a glass thermoluminescent dosimeter (IKS) (refs. 9 and 10), as well as indicators. Preliminary experiments revealed that the efficiency of ILC and IKS dosimeters for neutrons is more than an order of magnitude less than their efficiency for protons.

On the basis of comparisons of the readings of the dose detectors (chemical, ILC, IKS) and flux indicators (C and Al) it was found that the admixture of the neutron flux in the beam emerging from the collimator does not exceed 5-6 percent in the range of proton energies 100-600 Mev. Thus, the method used for disposing of proton energy made it possible to obtain quite pure radiation.

The value of proton energy beyond the absorber was determined by the magnetic analysis method and by use of the path-energy relation in lead and aluminum. The discrepancy in determination of energy by these two methods did not exceed 6 percent.

TABLE 5. CHARACTERISTICS OF SOME PROTECTIVE MATERIALS

Material	Chemical formula	Density, g/cm^3	Content of elements by weight, in %			
Polyethylene	$(CH_2)_n$	0.94		C 85.6		H 14.4
Polycarbonate	$(C_{16}O_3H_{14})_n$	1.2		C 75.53	O 18.96	H 5.51
Polyvinylchloride	$(C_2H_3Cl)_n$	1.4		C 38.4	H 4.8	Cl 56.8
Glass-base textolite, with EDF-6 resin	-	1.8	O 37.5	Si 17.0	Al 1.78	Ba 15.9
			Zn 2.72	B 3.22	C 20.1	H 1.78
Glass-fiber reinforced material (SVAM)	-	2.0	O 39.8	Si 19.62	Al 2.03	Ba 17.3
			Zn 2.95	B 3.5	C 13.6	H 1.2
Aluminum	Al	2.7			Al 100	
Lead	Pb	11.3			Pb 100	

In the protection investigations we studied a number of typical materials, whose characteristics are represented in table 5. Among them was polyethylene--a material with an optimal content of hydrogen, thus a good material for modeling water. Due to the great concentration of hydrogen, these materials /44 should be the best with respect to the weight of the protection, since they possess the maximum stopping power.

The remaining plastic and aluminum represent materials which, together with good construction properties, are characterized by low density. Finally, lead is a representative of the heavy materials necessary for protection from bremsstrahlung.

The protective properties of the materials were studied behind the protective wall of the room housing the synchrocyclotron (ref. 22), under the geometric conditions shown schematically in figure 12. The proton beam from the collimator C, 2 cm in diameter, passed through the ionization chamber-monitor M and was directed normally onto a layer of protective material P. The detector D was placed in contact with the material to be investigated; the thickness of the latter increased from the direction of incidence of the beam.

The total absorbed dose Q (δ) beyond the protection was measured with an ionization chamber or a plastic container filled with Mohr's salt, measuring 40 x 30 x 1 cm. The dosimeter readings gave the mean absorbed dose; therefore, in order to obtain Q, they were multiplied by the area of the dosimeter cross section. In measurements of Q the ILC dosimeters were arranged directly next to one another in a plane to the beam in a panel measuring 40 x 30 cm. Since

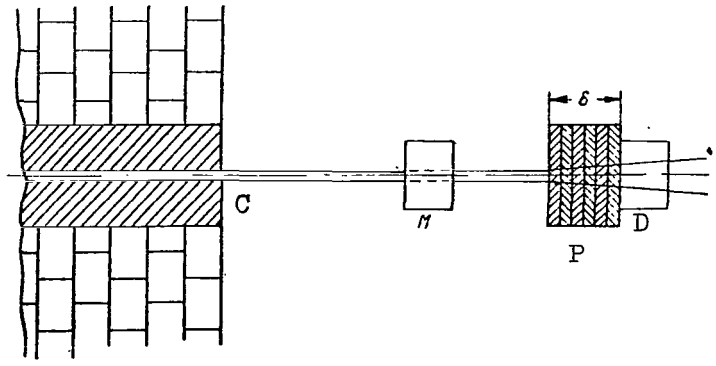

Figure 12. Diagram of experiment for investigation of protective properties of materials: C, collimator; M, monitor; P, absorber of studied material; D, detector.

the measurements confirmed that the dose field possesses circular symmetry, in some of the subsequent experiments the ILC dosimeters were placed only along one of the radii of the field, in contact with one another, with the ends toward the beam, and Q was obtained by numerical integration. The value Q was normalized from the monitor readings.

Protection from protons with energies of 667 Mev was studied beyond /45
the collimator 7 (fig. 1). Experiments for study of protection from protons of lesser energies were made in the proton beam, deflected by magnet 8 into collimator 6. The proton energies and the flux intensity of the protons incident on the protection are given in table 6.

The results of the experiments were represented in the form of the dependence of the dose factor of attentuation and accumulation of the broad proton beam f (δ) on the thickness δ of the protection (figs. 13a, b and c).

Figure 13 shows that for protons with energies in the range 100-200 Mev there is a characteristic change of the absorbed dose, similar to the classical

TABLE 6. PROTON ENERGY AND FLUX INTENSITY USED DURING STUDY OF PROTECTIVE PROPERTIES OF MATERIALS

Proton energy, Mev	Proton flux intensity, protons/cm^{2}sec Mev·10^6
667 ± 5	2000
384 ± 23	10
240 ± 15	3
126 ± 80	1

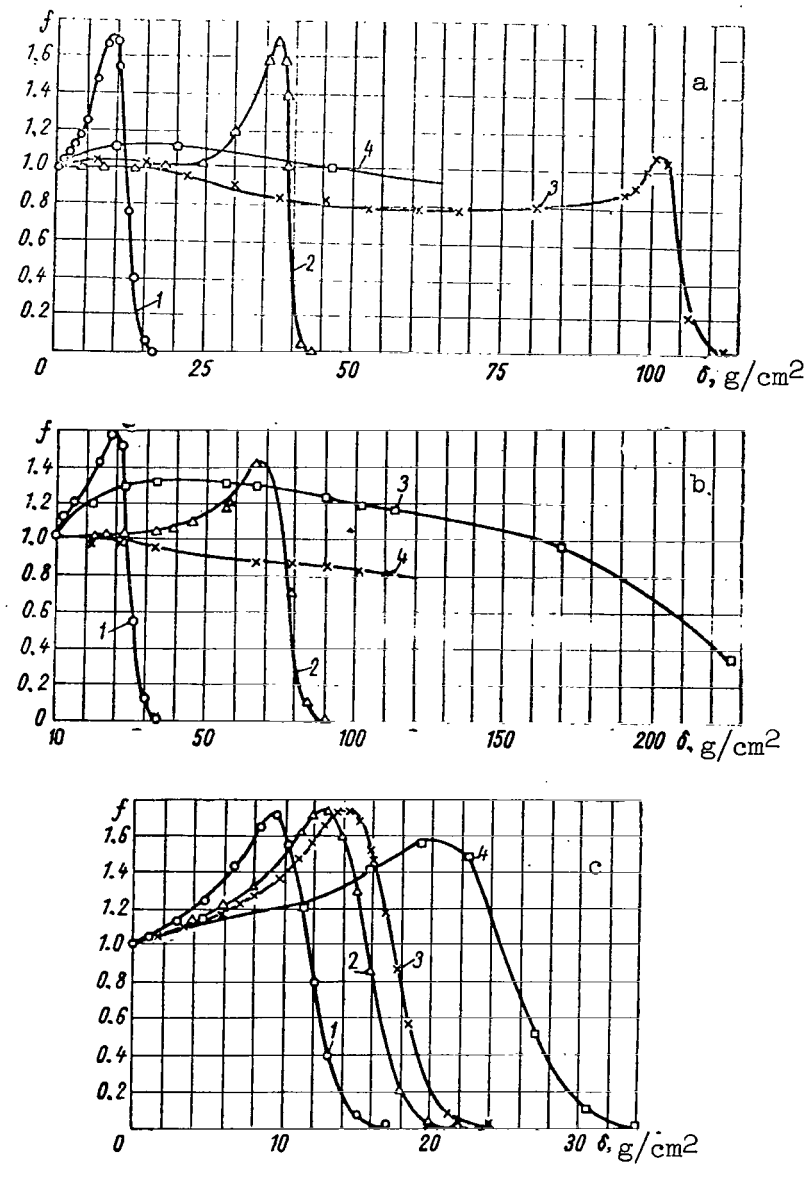

Figure 13. Change of absorbed dose: a, behind absorber of polyethylene from broad beam of protons with energies (in Mev): 1, 126 ± 8; 2, 240 ± 15; 3, 384 ± 23; 4, 667 ± 5; b, behind absorber of lead from broad beam of protons with energies (in Mev): 1, 126 ± 8; 2, 240 ± 15; 3, 384 ± 23; 4, 667 ± 5; c, behind protection of various materials from broad beam of protons with energies of 126 ± 8 Mev: (1) polyethylene; (2) SVAM glass-base textolite; (3) aluminum; (4) lead.

Bragg curve. The gradual ascent of the curve with an increase of the thickness of the penetrated layer usually is attributed to the increase of ionization losses of energy with slowing of protons, and the steep drop following the maximum usually is attributed to stopping of protons when there is a total loss of kinetic energy. The differences of the Bragg curve and the dependence of $\left(-\frac{dE}{dx}\right)$ on the thickness of the absorber can be attributed satisfactorily to the influence of fluctuations of energy losses and multiple scattering.

In the case of high-energy protons there is a characteristic increase of the absorbed dose, leading to a maximum on the f (δ) curve when the thickness of the protection is about 5-10 g/cm^2. Thus, the accumulation of secondary radiations first overlaps the attenuation of the primary beam due to inelastic interaction proportional to $\left(-\frac{dE}{dx}\right)_\delta . e^{-\sigma_{inelastic}\delta} \Big/ \left(-\frac{dE}{dx}\right)_0$ and is appreciable at these energies, since (-dE/dx) changes little. As a quasiequilibrium state sets in between accumulation and attenuation, there is an onset of a decrease of f (δ), which at the end of the path competes with a rapid increase of ionization losses of energy. With an increase of the energy of the protons the accumulation of secondary radiations becomes more significant, and the Bragg maximum is less clearly expressed.

An experiment was made for evaluation of the secondary radiations. A lead absorber was placed in position 2 (fig. 1) and the radiation detectors beyond collimator 7. Measurements were made with the magnet 8 cut out and then with the magnet cut in, driving the primary protons and all the charged secondary particles from the collimator. In all measurements the monitor was an aluminum detector situated at the exit window of the accelerator (fig. 1, 1). These measurements determined the contribution to the absorbed dose from neutron and γ-radiation moving in the direction of the primary beam. The results are shown in figure 14. /47

Figure 14 shows that the absorbed dose from neutron and γ-radiation attains a maximum at 5 g/cm^2 Pb, that is, at approximately the same thickness as on curve 4 (fig. 13b). When there is a great thickness of the protection, the absorbed dose of neutron and γ-radiation remains virtually unchanged, and when the thickness of the protection is of the order of the path of the primary protons, it attains 10 percent of the absorbed dose of the charged particles.

Since the neutron and γ-radiation, being secondary radiation, is more isotropic than the primary and scattered protons, its contribution to the absorbed dose in the broad beam will be greater than the measured value. The latter can be used as a lower estimate. The total contribution of the neutron and γ-radiation in a broad beam can be obtained by making similar investigations at different angles to the layer of the absorber.

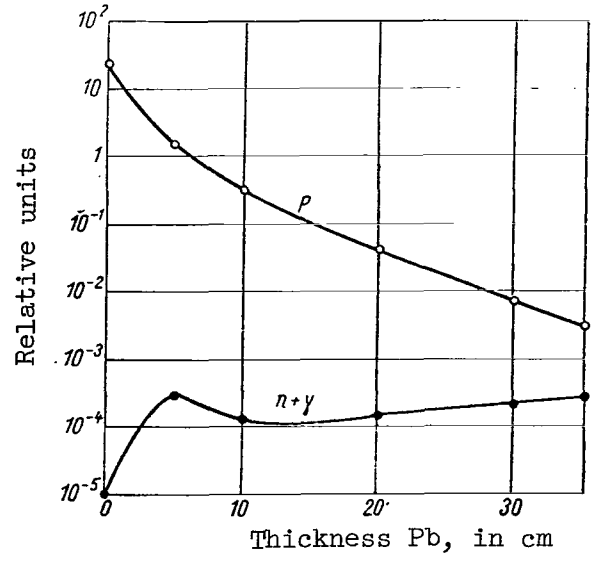

Figure 14. Relation between primary and secondary radiations behind protection in direction of beam.

The shape of the f (δ) curves of a broad proton beam is slightly dependent on the material of the protection. However, as is well known, due to the difference in stopping power the protective properties of the materials are not equivalent. This is shown clearly in figure 13. For example, for protons with energies of 126 Mev the absorbed dose beyond a protection of polyethylene decreases to zero already beyond a layer δ = 16.5 g/cm^2, whereas this requires a thickness of lead of δ = 36 g/cm^2. Thus, for total protection each square centimeter of the lead shield is 19.5 g heavier than a polyethylene shield. At higher proton energies the difference in protective properties of materials is related to the difference in the characteristics of the products of nuclear interactions. /48

For practical use in computations of protection from protons with a complex spectrum, the derived results are represented more conveniently in the form of a family of curves showing the dependence of the dose factor of attenuation and accumulation f on proton energy, selecting as a parameter of the family the thickness δ of the protection (fig. 15).

The absorbed dose beyond a particular protection can be determined using similar curves by numerical integration of the expression

$$D\delta = \int_E q_0(E)\, f\,\delta(E)\, \Phi(E),$$

where q_0 (E) are determined by computation or from tables, fδ (E) are obtained from the curves and Φ (E) is given by the initial proton spectrum.

Figure 15 should be considered as illustrative, since for precise construction of the curves at the present time there are insufficient data. It is necessary to obtain additional information for other proton energy values. However, even from this illustration it follows that to a thickness of protection equal to the proton path there is no attenuation of the absorbed dose of protons; on the contrary, there is an accumulation up to 70 percent of the initial value.

The results of these investigations can be used for computation of the /49 protection from a broad beam of protons incident normal on the protection. For some other angular distribution of protons the change of the absorbed dose with thickness of the protection will differ from the values cited above. Therefore, in order to obtain full data for computation of the protection from protons, it is necessary to continue the experimental investigation of attenuation of broad proton beams for the case of slant incidence on the protection.

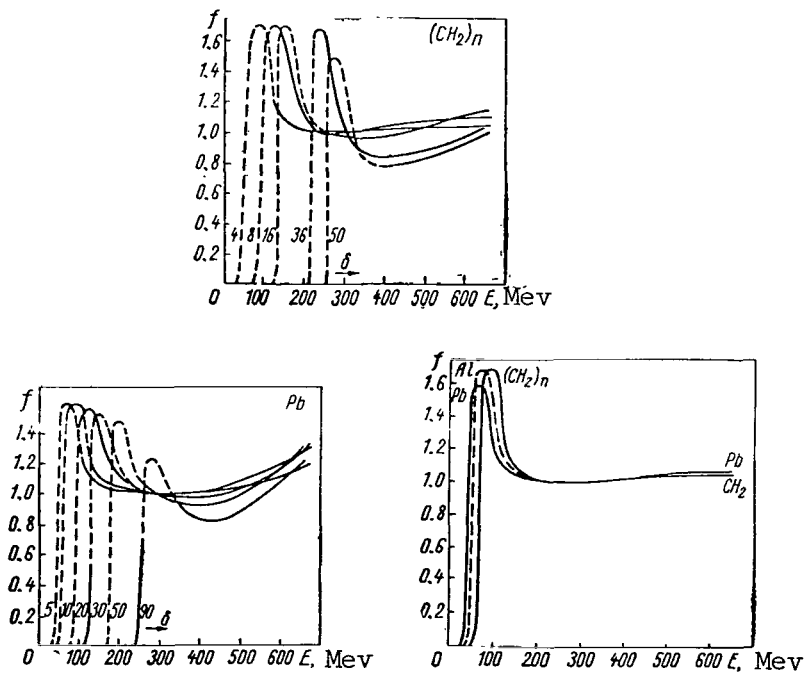

Figure 15. Dependence of dose factor of attenuation and accumulation on proton energy for different thicknesses of protection (in g/cm^2) of polyethylene lead, and polyethylene, aluminum and lead.

Study of the Radiation Resistance of Materials

In connection with the possibility of intense irradiation of the parts of a spacecraft, the problem of the study of radiation resistance of materials under the influence of proton radiation arises. Although there is a sufficient number of reports in the literature on problems of radiation resistance of materials (refs. 23-25), the overwhelming majority of experimental investigations have been made on the sources of electromagnetic and neutron radiations. However, data on the change of properties of the materials under the influence of proton bombardment cannot be found in the literature.

Our institute, in collaboration with the Scientific Research Institute of Plastics, carried out investigations of the radiation resistance of polymeric materials, using the synchrocyclotron of the Joint Institute for Nuclear Research. The radiation resistance of the materials used in these experiments had not been investigated in general at an earlier date, even using ordinary sources of nuclear radiations.

The radiation resistance of the plastics, therefore, was studied simultaneously using high-energy protons at the Joint Institute for Nuclear Research and the K-60,000 apparatus of the L. Ya. Karpov Physicochemical Institute, in which the radiation source was a Co^{60} preparation with a total activity of 18,200 g·equiv of radium. The objects investigated were various plastics which can be used in the parts of spacecraft and in protective equipment, including a number of glass plastics, film and thermoplastic materials. In addition to the radiation investigations, we used specially created apparatus for modeling other effect factors present in space (ultraviolet radiation and thermal stresses under the conditions prevailing in deep vacuum).

An evaluation of the radiation resistance of materials was made on the basis of investigations of a number of physicomechanical characteristics (tensile strength, relative elongation and statistical bending strength).

Five or ten samples of each material were subjected to irradiation simultaneously. All subsequent measurements of the physicomechanical characteristics were made with the methods described in GOST (State Standard) 4648-56 and GOST 4649-49.

Table 7 gives the preliminary results of measurement of the strength characteristics of plastics under the influence of proton and γ-radiation.

Analysis of the data reveals that most materials satisfactorily maintain /51 strength in the case of absorbed doses up to 30-40 Mrad. The radiation resistance of glass plastics is higher than for thermoplastics, whose properties must be modified by the introduction of matter protecting the polymer from the effect of penetrating radiations. Some materials (pentone and polyformaldehyde) do not withstand a prolonged radiation effect.

With respect to the difference of the sensitivity of plastics to proton and γ-radiation, it must be said that whereas with an equal absorbed dose there are identical changes in the glass plastics EN-5 and EDP-2, such a reaction

TABLE 7. CHANGE OF STRENGTH CHARACTERISTICS OF PLASTICS UNDER INFLUENCE OF RADIATION

Material	Absorbed dose (Mrad), protons with E = 660 Mev	Physical-mechanical properties			Absorbed dose (Mrad), rays E = 1.3 Mev	Bending strength, kg/cm^2
		Tensile strength, kg/cm^2	Relative linear expansion, %	Bending strength, kg/cm^2		
EDF-6 glass-base textolite	0			4500		
	49.0			4594		
	120.1			4762		
	180.0			4294		
EDP-2 glass-base textolite	0			5227	0	5227
	63.1			6390	30	5288
	124.3			4866	60	5233
	181.0			4380	100	5217
SVAM glass-base textolite	0			4736	0	4736
	63.1			4553	30	5180
	124.3			4300	60	5312
	180			4060	100	4284
54/6 glass-base textolite	0			3480	0	3494
	63.1			3100	30	3686
	124.3			2693	60	3384
	181.0			2650	100	2438
EN-5 glass-base textolite	0			5494	0	5480
	63.1			5380	30	5623
	124.3			5034	60	5358
	181.0			4300	100	5230
Polyethylene terephthalate film	0	1156	29			
	63	1167	20			
	124	890	13			
	181.0	Destroyed				
Polycarbonate film	0	419	66			
	63	683	39			
	124	352	10			
	181	Destroyed				
Polypropylene	0	300	48			
	26.2	126	0			
	57.7	90	0			
	181.0	Destroyed				

TABLE 7 (Continued)

Material	Absorbed dose (Mrad), protons with E = 660 Mev	Physical-mechanical properties			Absorbed dose (Mrad), rays E = 1.3 Mev	Bending strength, kg/cm^2
		Tensile strength, kg/cm^2	Relative linear expansion, %	Bending strength, kg/cm^2		
Pentone	0	460	28			
	26.2	98	0			
	57.7	8.7	0			
High-strength polystyrene (with filler)	0			570		
	26.2			608		
	57.7			236		
High-strength polystyrene (without filler)	0	Destroyed before tests				
	26.1					
Polyformaldehyde	0			1100		
	26.2			400		
	57.7			366		

is not found for other materials, and the final solution of this problem requires additional investigations.

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CHAPTER 3. BIOLOGICAL EFFECT OF PROTONS WITH ENERGIES OF 510 MEV IN CASES OF MULTIPLE IRRADIATION /53

A. F. Bibikova, N. G. Darenskaya, M. P. Domshlak, A. N. Ganshina, G. K. Gerasimova, V. I. Lebedev, Yu. G. Nefedov, N. I. Ryzhov, L. T. Tutochkina, M. S. Uspenskaya and V. V. Yakovlev

The biological effect of high-energy protons has not yet been investigated adequately. Available studies are devoted for the most part to the local influence of this type of radiation. The injuries caused to the brain and other organs of laboratory animals (primarily small animals) under the influence of a narrow beam of protons with energies of 200 and 700 Mev have been described in considerable detail by American and Swedish investigators (refs. 1-11). With the exception of the description of individual investigations with mice (ref. 12) we find no information in the foreign literature on the biological effect of high-energy protons during general irradiation of animals. The investigations of Soviet authors (refs. 13-19) to a certain degree fill this substantial gap. Unfortunately, there are few such studies. Until now we have not succeeded in finding descriptions of investigations made on large experimental animals.

It should be remembered that in irradiation of animals of a size commensurable with the length of the proton path there can be a more clearly expressed biological effect than occurs with particles of this same energy on small laboratory animals. These considerations were one of the factors determining the conduct of these investigations--study of the biological effect of high-energy protons.

As experimental animals we used mongrel dogs, rats of the Wistar species and mongrel white rats. The dogs were subjected to fractional and single irradiation by protons with energies of 510 Mev in doses of 250-650 rad and rats in doses of 100-1,200 rad.

A special proton beam apparatus was developed for irradiation of animals, as stated previously; this made it possible to create a dose field of protons with energies of 510 Mev in an area with a cross section of 40 cm with a dose intensity of about 1-1.5 rad/sec without an admixture of accompanying radiations.

The dogs were irradiated from two directions. This ensured greater /54 uniformity of the distribution of radiation in the body of the animal. A study of tissue doses in the phantoms of the experimental animals revealed that low-energy protons contribute substantially to the total dose in the body of the dogs.

Evaluation of the harmful effect of protons was made using the following indices:

-- general state of the animals (external appearance, activity, state of hair, fur and mucous membranes, body temperature, weight dynamics, food irritability, etc.);

-- condition of the peripheral blood and bone marrow;

-- data from a biochemical investigation of the blood serum for the content of total protein, its individual fractions, serum mucoid, cholinesterase;

-- state of the cardiovascular system (maximum arterial pressure, arterial tone, rate of propagation of the pulse wave, electrocardiography, plethysmography);

-- state of the oxidation processes in the organism;

-- state of the nonspecific resistance of the organism to infection;

-- pathologic-morphological and histological characteristics of the central and peripheral nervous systems, cardiovascular system, respiration, gastrointestinal tract, blood-generating organs, etc.

Evaluation of the gravity of radiation injuries in rats was accomplished on the basis of analysis of clinical, hematological and pathologic-morphological investigations.

The study of the biological effect of protons in the case of a fractional effect was made using 17 mongrel dogs of both sexes, weighing 8-17 kg, and 58 white male rats weighing 180-200 g.

The dogs, depending on irradiation conditions, were divided into three groups: group I (6 dogs)--nineteen-fold irradiation in the course of five weeks to a total dose of 650 rad; group II (6 dogs)--eight-fold irradiation /55 in the course of two weeks to a total dose of 690 rad; group III (5 dogs)--seventeen fold irradiation in the course of four weeks to a total dose of 550 rad (table 1).

TABLE 1. REPEATED IRRADIATION OF A DOG WITH 510 MEV PROTONS

Group	No. of animals	Total dose, rad	No. of irradiations and period of time	Result	Average life span, days
I	6	650	19 in 5 weeks	All died	43.3
II	6	690	8 in 2 weeks	All died	22.8
III	5	550	17 in 4 weeks	All survived	-

Experiments on Dogs

Multiple irradiation of dogs with protons having energies of 510 Mev led to the development of radiation sickness in the animals. Since the total doses of irradiation and also the multiple character and total time of exposure for the animals in the different groups were not identical, the manifestations of radiation sickness were also different.

In order to determine the characteristics of the biological effect of proton irradiation during multiple effect in a number of cases, we formulated control experiments with exposure of the animals to X-rays in equal doses and under identical irradiation conditions.

Clinical State. Multiple irradiation of the dogs of group I during a long period did not affect behavior and activity; the general state of these animals was good (table 2). On the 28th-30th day, when the total dose reached 400-450 rad, the dogs remained active, had good appetite and outwardly in almost no way differed from healthy, nonirradiated animals. After a total dose of about 500 rad, on the 35th-36th day, despite a satisfactory state of the dogs, there was blanching of the mucous membranes, decrease in food irritability, and in some animals there were petechial subcutaneous hemorrhages. A sharp deterioration of the general state of all animals set in on the 37th-38th days, when total radiation dose was 600 rad. Beginning at this time, the animals revealed progressive weakness to the point of adynamia, increase of body temperature to 39.5° or above, deterioration of appetite, appearance of multiple subcutaneous and subconjunctival hemorrhages, gangrenous changes in the mouth and diarrhea, frequently with blood. All animals died between the 41st and the 49th day from the beginning of exposure with extremely clear manifestation of these phenomena.

The clinical course of illness of the dogs in groups II and I was similar in many respects. However, since the irradiation of these animals was twice as rapid, there was a more acute development of the illness with a relatively brief latent period (10-12 days from the onset of irradiation) and earlier manifestation of the principal symptoms. All animals died between the 3d and 19th days after irradiation ceased.

The external manifestations of radiation sickness in the dogs of group III during the time of radiation effects, and also in the period which fol- /57
lowed, were extremely limited. During the first 25-30 days the animals did not differ in external appearance and behavior from healthy nonirradiated dogs. Only after completion of the entire cycle of irradiation (on the 30th-40th days), when the total dose reached 550 rad, did some of the dogs reveal decreased activity, deterioration of appetite, dishevelled appearance, dullness and patchiness of the fur, rather clearly expressed blanching of the mucous membranes and the appearance of small subcutaneous hemorrhages on the paws. These phenomena were observed for 1.5-2 weeks, after which they disappeared. During the entire period of observation the body temperature remained within the limits of normal variations.

In generalizing the data on the clinical course of the illness, it can be noted that there is a relatively prolonged latent period during which external

TABLE 2. RHYTHM OF IRRADIATION AND SINGLE DOSES (RAD) RECEIVED BY DOGS IN PERIOD FROM JULY 10-AUG. 18, 1961

Name of dog	July									
	10 (1)	12 (2)	15 (3)	17 (4)	19 (5)	21 (6)	24 (7)	26 (8)	28 (9)	31 (10)
Kashtanka	13,0	23,0/36,0	23,0/59,0	21,0/80,0	27,0/107,0	8,3/115,3	28/143,3	29/172,3	36/208,3	18/226,3
Tsygan	12,0	22,0/34,0	23,0/57,0	38,0/95,0	40,0/135,0	22,0/157,0	28,0/185,0	11,0/196,0	40,0/236,0	15,0/251,0
Chernushka	12,5	20,0/32,5	24,0/56,5	26,0/82,5	29,0/111,5	18,9/130,4	33,0/163,4	21,0/184,4	40,0/224,4	14,0/238,4
Kabul	11,0	20,0/31,0	20,0/51,0	36,0/87,0	18,2/105,2	23,8/129,0	35,0/164,0	24,0/188,0	34,0/222,0	13,0/235,0
Krivoy	10,0	22,0/32,0	20,0/52,0	30,0/82,0	22,2/104,2	21,0/125,2	21,0/146,2	10,0/156,2	78,0/234,2	32,0/266,2
Tigr	13,0	19,0/32,0	22,0/54,0	30,0/84,0	47,7/131,7	21,3/153,0	24,0/177,0	23,0/200,0	34,0/234,0	35,0/269,0

Name of dog	August								Total dose
	2 (11)	4 (12)	7 (13)	9 (14)	11 (15)	14 (16)	16 (17)	18 (18)	
Kashtanka	62/288,3	78/366,3	26/392,3	46/438,3	42/480,3	75/555,3	52/607,3	28/635,3	635,3
Tsygan	62,0/313,0	55,0/368,0	18,0/386,0	49,0/435,0	46,0/481,0	79,0/560,0	46,0/606,0	33,0/639,0	639,0
Chernushka	64,0/302,4	57,0/359,4	15,0/374,4	47,0/421,4	53,0/474,4	68,0/542,4	55,0/597,4	40,0/637,4	637,4
Kabul	62,0/297,0	71,0/368,0	21,0/389,0	54,0/443,0	59,0/502,0	80,0/582,0	34,0/616,0	36,0/652,0	652,0
Krivoy	62,0/328,2	62,0/390,2	31,0/421,2	46,0/467,2	53,0/520,2	68 0/588,2	44,0/632,2	28,0/660,2	660,2
Tigr	60,0/329,0	51,0/380,0	55,0/435,0	45,0/480,0	51,0/531,0	34,0/565,0	44,0/609,0	36,9/645,9	645,9

Note.-In numerator--single doses of irradiation; in denominator--total doses. Sequence number of irradiation given in parentheses.

manifestations of illness are absent. On completion of this period, phenomena develop (without precursors), corresponding clinically to the period of the height of radiation sickness caused by a single general irradiation by X- or γ-rays.

Composition of Peripheral Blood. The changes in the peripheral blood in general were also characteristic of acute and subacute forms of radiation sickness. These changes were noted in animals of all groups long before the appearance of the first external indications of illness.

TABLE 3. *MEAN DATA ON CHANGE OF INDICES OF PERIPHERAL BLOOD IN DOGS OF GROUP I*

Time of examination of dogs, days	Number of erythrocytes, millions per 1 mm^3	Hemoglobin content, g %	Number of thrombocytes, thousands per 1 mm^3	Number of reticulocytes, ‰	Number of leukocytes, thousands per 1 mm^3	RBE mm/hour
Before irradiation	6.40 ± 0.22	14.2 ± 0.3	452 ± 23	9.6 ± 1.7	12.3 ± 0.4	2
" "	6.90 ± 0.16	14.1 ± 0.2	320 ± 56	6.0 ± 2.0	9.7 ± 1.3	3
" "	6.38 ± 0.36	13.8 ± 0.2	311 ± 29	6.8 ± 1.6	12.3 ± 1.5	3
Mean before irradiation	6.56 ± 0.24	14.0 ± 0.4	361 ± 30	7.4 ± 1.1	11.4 ± 0.8	3
At time of irradiation						
8th	6.38 ± 0.20	12.9 ± 0.3	336 ± 12	4.0 ± 0.9	9.8 ± 1.0	4
15th	6.10 ± 0.13	16.6 ± 0.4	277 ± 25	3.6 ± 0.9	9.9 ± 0.9	6
22d	6.40 ± 0.26	15.1 ± 0.5	287 ± 14	1.3 ± 0.3	10.7 ± 1.7	9
29th	5.65 ± 0.15	12.9 ± 0.5	162 ± 13	0.1 ± 0.1	5.4 ± 0.6	10
38th	5.14 ± 0.08	13.3 ± 0.5	170 ± 34	0.0	1.9 ± 0.4	15
43d	4.10 ± 0.17	10.9 ± 0.0	-	-	1.00 ± 0.6	22

In the first 20 days of irradiation, the dogs of group I revealed on insignificant (4-7 percent of the initial value) decrease in the quantity of erythrocytes and thereafter a sharp decrease, particularly in the last period of life when in individual animals it dropped to 53 percent in comparison with the background data, on the average from 6.56 to 4.1 million cells (table 3).

After the first irradiations the animals of group II revealed a decrease in the quantity of erythrocytes, expressed most clearly on the 12th day (at the height of radiation sickness). By this period the quantity of erythrocytes /58 attained 55-60 percent of the initial value (table 4).

TABLE 4. MEAN DATA ON CHANGE OF INDICES OF PERIPHERAL BLOOD IN DOGS OF GROUP II

Time of examination	Number of erythrocytes, millions per 1 mm^3	Hemoglobin content, %, Sahli method	Number of thrombocytes, thousands per 1 mm^3	Number of leukocytes, thousands per 1 mm^3
Before irradiation	6.23	92	244	14.1
" "	6.22	88	254	9.6
" "	6.39	90	168	11.3
Mean before irradiation	6.28	90	222	11.6
Days from beginning of irradiation, 4th, 12th, 19th	5.75	84	132	6.8
	5.56	82	21	3.2
	3.60	54	11	1.1

Appreciable changes in the quantity of erythrocytes in the animals of group III were also observed with the onset of irradiation. However, in the first 20 days the decrease in the quantity of erythrocytes was small and did not exceed 10 percent of the initial value. With an increase of the dose, erythropenia progressed. By the end of exposure the quantity of erythrocytes had decreased by 17-20 percent of the initial value (on the average from 6.12 to 5.09 million cells per 1 mm^3). The decrease of the content of erythrocytes continued for 3-4 weeks after ending of irradiation. By this period the mean level of erythrocytes had decreased to 4.74-4.78 million cells per 1 mm^3, which was 77-78 percent of the initial quantity. Thereafter, during the entire period of observation (2 months after cessation of irradiation), there was restoration of erythropoiesis, but it was not complete. Sixty-seven days after cessation of irradiation the blood contained only 88 percent of the initial quantity of cells (table 5).

The changes in the content of reticulocytes in the animals of all groups were considerable. A substantial decrease of their quantity already was noted by the 10th-15th days. By this time, that is, long before cessation of irradiation, the reticulocytes in the peripheral blood of the animals of all groups had almost disappeared. In the surviving animals (group III), restoration was observed 1.5-2 months after cessation of irradiation.

TABLE 5. MEAN DATA ON CHANGE OF INDICES OF PERIPHERAL BLOOD IN DOGS OF GROUP III /59

Time of examination		Number of erythrocytes, millions per 1 mm^3	Hemoglobin content, %, Sahli method	Number of thrombocytes, thousands per 1 mm^3	Number of leukocytes, thousands per 1 mm^3
Before irradiation		6.51 ± 0.22	89 ± 5.6	242 ± 52	13.60 ± 0.90
" "		6.14 ± 0.19	90 ± 3.9	237 ± 29	9.20 ± 0.70
" "		6.12 ± 0.08	82 ± 4.8	189 ± 31	10.65 ± 0.81
Days from beginning of irradiation	4th	5.60 ± 0.21	81 ± 5.4	168 ± 57	7.50 ± 0.80
	12th	5.90 ± 0.20	88 ± 3.5	89 ± 13	8.90 ± 0.11
	19th	5.60 ± 0.14	80 ± 2.3	64 ± 12	6.50 ± 0.11
	26th	5.38 ± 0.19	88 ± 2.5	47 ± 14	4.00 ± 0.88
Days after end of irradiation	5th	5.09 ± 0.31	74 ± 3.5	57 ± 16	3.40 ± 0.48
	13th	4.93 ± 0.13	67 ± 0.8	54 ± 10	3.31 ± 0.51
	20th	4.74 ± 0.25	66 ± 3.6	125 ± 40	5.72 ± 0.45
	28th	4.78 ± 0.43	74 ± 3.2	139 ± 45	8.52 ± 0.99
	45th	5.17 ± 0.25	77 ± 3.5	141 ± 36	8.28 ± 0.67
	70th	5.40 ± 0.30	66 ± 2.7	-	8.13 ± 0.60

Extremely profound changes were discovered in the content of blood platelets. An appreciable decrease of their quantity was observed after the first irradiations. At the end of exposure the level of the content of platelets in animals of groups I and II did not exceed 5 percent of the initial values. Even in the dogs of group III, in which radiation sickness was less well expressed, the quantity of platelets had decreased by 75 percent. Later (after irradiation), even though there was a clearly expressed and intensive restoration of the quantity of platelets, it was far from complete and by the 45th day after irradiation had not attained 75 percent of the initial content.

The dynamics of change of the quantity of leukocytes in the animals of the different groups was not identical and was dependent on the repetition, total and single irradiation dose. In the first days after onset of irradiation, all animals revealed decrease in the quantity of leukocytes. However, in the dogs of groups I and III this decrease in quantity was relatively small by the 20th day and did not exceed 15-17 percent of the initial value. Moreover, by the end of this period, there was a clear tendency to restoration of the quantity of leukocytes to the initial level. In particular, this applies to the neutrophils, whose quantity for the animals of groups I and III increased on the average to 108 and 90 percent, respectively. Thereafter (after the total dose exceeded 230 rad), the quantity of leukocytes decreased extremely rapidly. By the 38th-40th days the blood of dogs in group I contained not more than 1,500 cells per 1 mm^3. There was restoration of the quantity of leukocytes in the dogs of group III, after irradiation, but there was characteristic relatively low rate of the restoration processes. For example, by the 70th day after irradiation the peripheral blood contained only 76 percent of the initial level of leukocytes. Changes of the quantity of leukocytes in the dogs of group II were characteristic of acute radiation sickness. /60

An analysis of the leukocytic formula revealed that in the first period of irradiation, with the attainment of total doses of about 230-250 rad, leukopenia was caused to a considerable degree by decrease in the number of lymphocytes. At the same time, the changes in the quantity of neutrophils were less significant. Moreover, in animals of groups I and III in this period (to the 20th-22d day from the onset of irradiation), there was an absolute increase in neutrophilic cells. Thereafter (during irradiation of animals with large doses), there was an intense absolute and relative decrease of the number of neutrophils, so that by the end of the period of irradiation there was relative and absolute neutropenia and relative lymphocytosis (tables 6 and 7). In addition, cytological investigation showed that the blood contained degenerative forms of cells, chromotinolysis, fragmentation of nuclei, increase of cytolysis, appearance of toxic granularity in the protoplasm of the neutrophils, etc.

TABLE 6. MEAN DATA ON CONTENT OF DIFFERENT FORMS OF LEUKOCYTES IN PERIPHERAL BLOOD OF DOGS OF GROUP I

Time of examination (days from beginning of irradiation)	Number of leukocytes, thousands per 1 mm3	Number of neutrophils, thousands per 1 mm3	Number of lymphocytes, thousands per 1 mm3	Leukocytic formula, %						
				Eosinophils	Young	Rod-shaped-nuclear	Segmented-nuclear	Lymphocytes	Monocytes	Plasmocytes
Before irradiation	11.4	8.35	1.63	7.2	-	2.6	70.7	14.3	6.2	1.2
8th	9.8	7.21	1.21	5.9	-	2.7	70.9	12.4	9.8	0.7
15th	9.9	7.60	1.08	4.4	3.0	3.3	73.5	11.0	6.3	1.1
22d	10.7	9.03	0.93	2.6	5.5	4.8	79.6	8.7	6.4	3.5
29th	5.4	4.24	0.42	6.5	-	3.7	75.0	7.8	4.7	1.3
38th	1.9	1.48	0.32	7.0	-	7.2	70.9	17.0	4.0	1.0
43d	0.5	0.27	0.15	11.0	-	10.0	45.0	32.0	2.0	-

No appreciable changes were observed in the relative content of eosinophils and other cells. The decrease of their absolute number was proportional to the decrease of the total number of leukocytes.

Analysis of specimens of marrow obtained by puncturing, taken before and after the entire cycle of irradiations, revealed decrease of the absolute number of cells in the marrow, accompanied by decrease in the number of cells of the eythroblastic and myeloid series. At the same time there was a more clearly expressed decrease (to the point of total disappearance) of the cells of the early generations of erythroblastic and granulocytic parts of the marrow, and also an appreciable decrease of erythroblasts in the state of mitosis and monocytes. At the same time, as might be expected, there was considerable increase

TABLE 7. CONTENT OF DIFFERENT FORMS OF LEUKOCYTES IN DOG DEKAEDR /61
(TOTAL DOSE 550 RAD, GROUP III)

Time of examination		Number of leukocytes per 1 mm³	Number of neutrophils, thousands per 1 mm³	Number of lymphocytes, thousands per 1 mm³	Leukocytic formula, %						
					Young	Rod-shaped-nuclear	Segmented-nuclear	Eosinophils	Monocytes	Lymphocytes	Plasmocytes
Before irradiation		10.25	5.54	3.590	-	12	42	2	8	35	1
" "		13.20	5.28	5.810	2	13	27	7	6	44	1
Days from beginning of irradiation	4th	9.50	-	-	-	-	-	-	-	-	-
	12th	6.25	4.50	0.750	-	17	55	7	8	12	1
	19th	6.60	4.89	1.120	-	12	62	2	7	17	-
	26th	6.10	4.27	1.220	-	7	63	2	5	20	3
Days after end of irradiation	5th	4.60	3.94	0.530	-	11	70	2	5	11	1
	13th	5.25	3.15	1.440	4	16	44	4	3	28	1
	20th	5.50	3.19	1.320	6	14	44	8	1	24	3
	28th	10.00	5.80	1.200	2	8	50	17	8	12	3
	45th	9.15	4.19	2.640	4	7	39	15	4	29	2

in the number of poorly differentiated reticuloendothelial cells and a sharp relative increase of the number of lymphocytes and plasmocytes and the appearance of fibroblasts.

In the cytological investigation of the marrow cells many degenerative forms of cells were found, with pyknosis, chromatinolysis, fragmentosis, etc.

On the whole, analysis of the state of the peripheral blood, and the specimens obtained by puncturing, indicated serious damage of the blood-generating organs. However, there was no total devastation of the marrow and disappearance of leukocytes from the peripheral blood. This fact indicates that there is some specific effect of protons on the blood-generating function of marrow. At the same time, it can by no means serve as a basis for the conclusion that there is a relatively lesser effect of the protons on the marrow than is the case for X-rays; rather, it indicates that the death of the animals was not related to disruption of blood generation, but was probably caused by the sharp development of a hemorrhagic syndrome (in particular, formation of hemorrhages in the vitally important organs and tissues).

In concluding the discussion of the state of the peripheral blood and marrow, it should be noted that fractional irradiation by protons causes sharp disruptions of the blood-generating processes. The marrow of injured dogs reveals: considerable decrease of cell elements of the erythroblastic and myeloid series; decrease of cells of the early generations; decrease of the /62 number of erythroblasts and myeloblasts in a state of mitosis; relative increase of poorly differentiated reticuloendothelial cells; presence of a large number of degenerative forms of cells, etc. The impairment of marrow blood-generation caused decrease of the content of formed elements in the peripheral blood.

In the development of these changes, as in fractional irradiation by X-rays (ref. 20), there are phase variations. There are four extremely clear periods (phases): initial changes, relative stabilization, intensive or terminal changes, and restorative. This classification of the phases of hematological changes corresponds for the most part to the classification for fractional irradiation by X-rays (ref. 21). However, there also are some differences which apparently reflect the specific character of proton injuries caused by multiple irradiation. In particular, we note the suppression of erythropoiesis, more clearly expressed than in X-ray injury, and the relatively low rates of the processes of repair in the marrow in the restorative period, as a result of which the normalization of the content of erythrocytes and other formed elements in the peripheral blood sets in relatively late.

Biochemical Investigations of Blood Serum. The clinical importance of electrophoretic determination of the protein fractions of blood serum as a characteristic of the course of many illnesses is well known. It has been shown in a number of investigations that the content of total protein and its individual fractions reflects the dynamics and gravity of the illness, and in certain cases indicates the specific character of the manifestation of the pathological process in individual tissues (refs. 15 and 22-26). In our investigations we used this method.

The paper electrophoretic method was used to determine the total content of protein and the relative content of individual protein fractions; we supplemented this complex with a determination of low-molecular acidic blood serum mucoids (SM) and the activity of cholinesterases (AKhE).

Interest in SM developed because they are the most low-molecular and labile fractions of blood proteins. For this reason and also due to the high content of carbohydrates it is easier for SM to penetrate into the tissues and emerge from them than is true of other blood proteins. Although SM are apparently synthesized in the liver, there is a close relationship between the content of SM and the exchange of the basic matter of the connective tissue. The impregnation of the tissues, the deposition of fibrinoid, amyloidosis and the hyalinization of the vessels are also apparently closely related to the level of exchange of SM. However, the high content of carbohydrates (especially sialic acid) gives a high capability to mucoproteins (and especially SM) for the formation of complexes. The effects of an increase of SM in the blood have not yet been established. There can be favorable effects for the organisms /63 (binding of toxins) or unfavorable effects (inactivation of enzymes and hormones). With different illnesses the SM content changes considerably and

can frequently serve as an indicator of the degree of damage and the degree of development of the pathological process (refs. 27-30). In acute and subacute forms of radiation sickness caused by external and internal radiation there are qualitative and quantitative changes of the SM fraction (refs. 24 and 31-35).

The total content of protein (OB) was determined refractrometrically.

An electrophoretic study of the proteins of the blood serum was made for an 18-hour period in horizontal apparatus in a barbital buffer with pH = 8.6-8.9, using a voltage of 220 v. The electrophoretic records were stained a bromophenol blue. The protein fractions were eluated and subjected to colorimetric study, using a green filter with a 550 mμ maximum.

The SM were determined by the Wintzler method as modified by Veymer and Moshin.

Tables 8-10 and figure 1 give mean data on the content of OB, SM and protein fractions in all three groups of dogs subjected to fractional irradiation by protons.

In the dogs of group III (table 8), which received the minimum irradiation dose and survived, there were insignificant changes in the total protein content and relative content of protein fractions, not exceeding the limits of physiological variation. At the same time the SM level clearly decreased during the period from the 20th to the 60th day after commencement of irradiation.

In the dogs of group I (table 9), beginning on the 29th day, there were noticeable changes of the total content of the protein fractions, which progressed to the death of the animals. The total concentration of protein and the relative content of albumins decreased and the relative content of α_1-, α_2- and γ-globulins increased. From the ninth to the 15th days the SM level decreased and then increased, exceeding the initial level. Thus, in this group, in comparison with the preceding group, the decrease of the concentration of SM was less clearly expressed and less persistent.

Figure 1. Level of serum mucoids during fractional irradiation by protons: (1) 690 rad; (2) 650 rad; (3) 550 rad.

TABLE 8. CHANGE OF PROTEIN COMPOSITION OF SERUM IN DOGS OF GROUP III /64

Time of examination, days		Total protein, g %	SM, mg % tyrosine	Relative content of protein fractions, %					
				Albumins	Globulins				
					α_1	α_2	β_1	β_2	γ
Before irradiation		7.4	2.5	57.7	6.6	8.4	17.8		9.2
" "		8.1	2.5	56.8	6.0	8.1	7.8	10.1	10.6
Period of irradiation	5th	6.5	2.2	53.2	6.5	9.9	9.2	9.8	11.1
	12th	7.2	3.1	55.5	6.8	8.0	8.2	9.5	11.0
	20th	7.0	2.0	53.8	6.3	8.4	9.6	10.1	11.7
	27th	7.2	1.5	52.3	7.3	9.8	9.8	9.1	10.3
After irradiation	36th	6.9	1.3	57.6	6.1	10.2	8.4	8.3	9.8
	44th	7.2	1.2	53.8	7.1	11.2	9.3	8.6	9.6
	51st	6.6	2.1	55.5	7.4	9.0	8.2	9.9	11.2
	62d	6.9	1.9	50.7	6.8	9.2	10.0	11.6	11.7
	74th	7.6	2.3	55.4	5.8	8.3	9.7	9.7	10.8

TABLE 9. PROTEIN COMPOSITION OF BLOOD SERUM OF DOGS IN GROUP I

Time of examination, days	OB, g %	SM, mg % tyrosine	Relative content of protein fractions, %					
			Albumins	Globulins				
				α_1	α_2	β_1	β_2	γ
Before irradiation	7.3	2.6	59.5	5.4	6.5	9.1	12.2	8.7
Days from beginning of irradiation:								
9th	7.1	2.2	54.0	5.4	7.1	13.1	9.5	9.3
15th	7.8	1.8	55.6	5.1	7.1	9.3	9.8	12.4
29th	6.5	2.3	49.4	6.9	8.8	11.0	10.2	13.5
38th	6.7	3.0	49.7	8.4	10.0	9.6	10.2	11.5
43d	5.5	3.0	43.5	10.6	10.4	11.4	11.0	13.3

In the dogs of group II (table 10), in which the sickness transpired /65 most acutely, the changes of the total concentration of protein and individual fractions were clearly noticeable on the 20th day. At this time we found a decrease of total protein and albumins, a considerable increase of α_1- and α_2-globulins and a less clearly expressed increase of β_1-, β_2- and γ-globulins. The SM level increased, beginning on the 12th day. The phase of decrease of concentration of SM was virtually absent (there was only a tendency to a decrease on the 5th day).

In the dogs of groups I and III (in which radiation sickness developed over a relatively extended period of time) there was a phase of decrease in the concentration of SM. Such changes were observed in rats during subacute radiation injury caused by Sr^{90}. Clinically this phase corresponds to the latent period of the illness and, to use Selye's terminology, is the stage of autodefense.

Changes of AKhE. Cholinesterase is one of the enzymes playing a decisive role in the process of transmission of nervous irritability. Produced in the nerve formations and particularly in the synapses, acetylcholine, constituting an acetylcholine chloride, is rapidly hydrolyzed by cholinesterase, which ensures coordinated functioning of the nervous system. It has now been demonstrated (ref. 36) that there are two different cholinesterases--specific, discovered in the erythrocytes and nervous system, which is called true cholinesterase, and nonspecific cholinesterase, discovered in the blood serum, which hydrolizes not only the acetylcholine, but also other complex ethers (benzoylcholine). In particular, the state of AKhE in the liver, where it is formed (ref. 37), should be determined. For this reason a study of the activity of nonspecific cholinesterase in the blood serum can serve as a valuable diagnostic indicator of the functional state of the liver (refs. 38 and 39).

Many investigations have been devoted to the study of the activity of /66 specific cholinesterase in the tissues and organs under the influence of external radiation on the organism (refs. 34, 35 and 40-44), but there are few data on the influence of ionizing radiation on the activity of nonspecific cholinesterase. The experimental work of a number of investigators (refs. 34 and 45-47) has revealed different deviations from the norm in the AKhE of the blood serum at the time of radiation damage to the organism. For example, it has been shown that the irradiation of mice by X-rays in doses of 250, 500 and 1,000 roentgens causes an inhibition of AKhE in the blood serum (ref. 48). After 5 and 30 minutes, 2 and 6 hours and 1, 3 and 7 days from onset of general X-ray irradiation of dogs in a dose of 500 roentgens there was found to be a decrease of AKhE in the serum (ref. 49); by the beginning of the 14th day after onset of irradiation the activity of the enzyme returned to normal. Five minutes and 3 days after exposure to X-rays in a dose of 1,000 roentgens, there was considerable decrease of AKhE in the serum of the dogs, but 30 minutes and 2 hours later, there was an increase of its activity. It was found that the AKhE was below normal (ref. 50) 14 months later in the blood serum of dogs which had experienced acute radiation sickness caused by the γ-radiation of Co^{60} in the case of general irradiation in a dose of 400 roentgens. Indications of fat infiltration were noted in histological examination of the liver of

TABLE 10. PROTEIN COMPOSITION OF BLOOD SERUM OF DOGS IN GROUP II

Time of examination (days from beginning of irradiation)	OB, g %	SM, mg % tyrosine	Relative content of protein fractions, %					
			Albumins	Globulins				
				α_1	α_2	β_1	β_2	γ
Before irradiation	7.3	2.2	54.6	5.7	3.5	20.4		10.6
" "	8.2	2.3	55.2	5.5	8.8	9.2	10.7	9.3
5th	7.2	2.0	55.5	6.2	10.4	9.7	8.9	8.9
12th	7.1	3.5	57.5	6.0	8.3	10.3	9.1	8.3
20th	6.8	4.1	42.2	7.8	13.9	11.2	13.0	11.7

killed dogs. A rather clearly defined dependence was established between decrease of AKhE in the blood serum and fat infiltration.

In our investigations for determination of cholinesterase we used the G. V. Pravdich-Neminskaya method (ref. 31); the basis for this is a quantitative calculation of the acetic acid liberated from the acetylcholine chloride in the course of its hydrolysis. The quantity of acetic acid being set free was determined by titration in the presence of a bromothymol blue indicator of the quantity of alkali expended on its neutralization. Thereafter the quantity of acetylcholine chloride (in mg) destroyed in a unit time by 1 milliliter of serum at 37° and pH = 7.6 was computed.

The determination of AKhE in the blood serum of dogs was made 2-3 times before irradiation and in different periods of the development of radiation sickness.

Figure 2 shows the results of a study of the activity of cholinesterase of the blood serum of individual dogs. Figure 2 shows that the fractional irradiation of the dogs of group I caused different changes of AKhE in the serum. On the ninth day there was a reliably determined increase in the activity of the enzyme (by 36 percent), in three dogs, whereas in two there was a decrease. On the 15th day we noted a not too clearly expressed tendency to a decrease of AKhE. After 29 days the activity of the enzyme was within the limits of normal and only one week before death (on the 38th and 43d days) a statistically reliable decrease of AKhE by 18 ± 3 and 58 ± 9 percent, respectively was noted.

In the dogs of group II, in the case of fractional irradiation, there was a statistically reliable increase of AKhE by 27 ± 4 and 15 ± 5 percent, /67 when the dose received by the dogs was 360 and 600 rad respectively. By the 19th day the activity of the enzyme in the two dogs had returned to normal.

The changes of AKhE in the blood serum of the dogs of group III (fig. 2) had a phase character. On the fifth day after onset of irradiations the activity of the enzyme had increased appreciably (by 28 ± 8 percent) from the initial

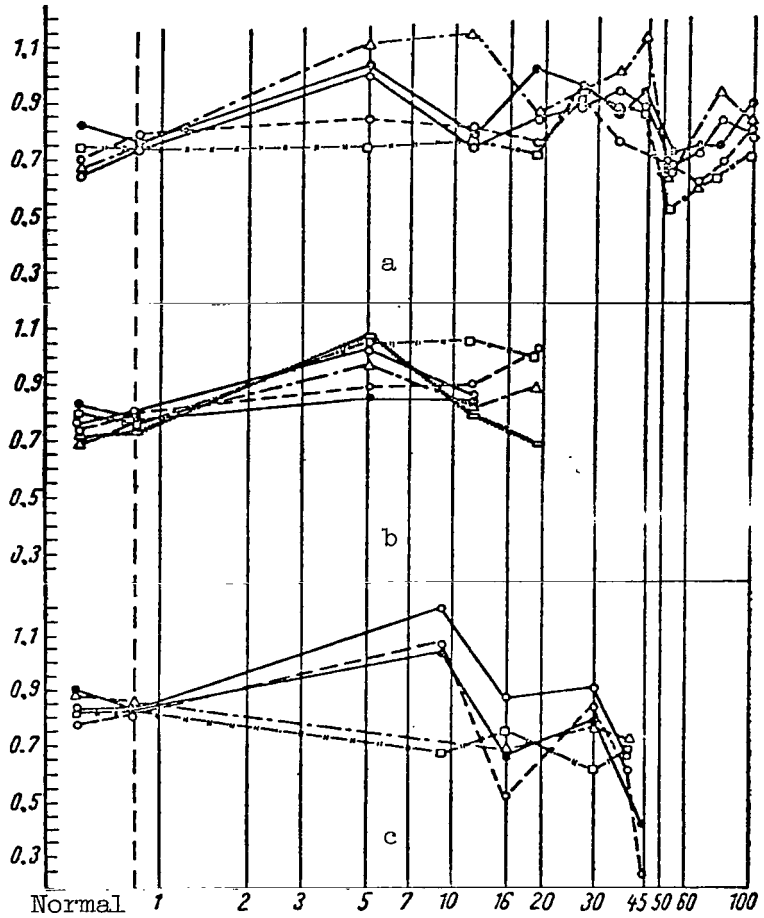


Figure 2. Change of activity of cholinesterase in blood serum of individual dogs. Along y-axis--quantity of acetylcholine (mg) hydrolized by 1 ml of serum in 1 hour; along x-axis--days after beginning of irradiation: (a) group III (550 rad); (b) group II (690 rad); (c) group I (650 rad).

level. On the 12th and 19th days the AKhE of all dogs, with the exception of one (on the 12th day), was within the limits of variation of the normal. At the later times of examination (on the 27th, 36th and 44th days), the AKhE in the blood serum had increased again (by 24 ± 3, 21 ± 5 and 30 ± 8 percent). By the 51st day we noted some tendency to a decrease in the activity of /68 the enzyme (by 11 ± 4 percent) from the initial level. Later (on the 62d and 74th days), the AKhE in the serum (according to mean data) had returned to the initial level, but by the 100th day there again was an insignificant increase of its activity (by 12 ± 4 percent). Statistical analysis of the collected numerical data revealed that under the specific experimental conditions a reliable increase of AKhE in the blood serum of the irradiated dogs occurred on the 5th, 27th, 36th, 44th and 100th days of the radiation sickness.

Condition of the Cardiovascular System. The pulse rate in the animals of all groups in the course of the first half of the period of irradiations did not change appreciably and ranged from 85-110/min. Thereafter, in the dogs of group I and especially in those of group II, it increased appreciably; the most clearly expressed changes were noted at the end of the irradiations, at the height of the sickness and in the terminal period, when the pulse rate was as great as 125-130/min or more. In the animals of group III the increase of pulse rate was less significant and was observed only in the course of a relatively brief period, at the end of the irradiations when the total dose was about 475 rad. It should be noted that immediately after cessation of irradiation there was a return of the pulse rate to its initial level in these animals. Moreover, at later times there was a stable and exceedingly well-defined decrease of pulse rate to 70-72 (in comparison with 96/min one minute before irradiation.

Maximum arterial pressure was manifested differently in the animals of the different groups. In group I during the initial period of irradiation (to the 15th day) there was a tendency to an insignificant increase (by 4-6 mm Hg). At later times there was a stable decrease of pressure by 15-20 mm Hg. A decrease of arterial pressure occurred also in the animals of group II, but it was observed immediately after the first irradiations and was considerably more clearly expressed; at the end of the period of irradiations it had dropped to 80-90 mm Hg (table 11). The maximum arterial pressure in the animals of group III, both during the time of and after termination of the period of irradiation was above the initial level by 6-20 mm Hg, and only in individual cases did it drop below normal.

Thus, it can be noted that in the case of fractional irradiation in relatively small single doses there first was a phase of increase of maximum arterial pressure which with an increase of a single and the total dose was replaced by the opposite reaction--by a decrease of pressure; the degree of this decrease was proportional to the intensity with which the total irradiation dose increased, i.e., the intensity of the dose. In those cases when the single dose of irradiation was quite large, as it was in the case of the dogs of group II, there was no initial increase of pressure: it decreased immediately.

Tone of Vessels. One of the most important indices of the functional /70 state of the vascular system, as is well known, is the tone of the vessels. At the present time, due to the difficulty in direct measurement of vascular tone, conclusions concerning its magnitude are based primarily on indirect data.

In our investigations the determination of vascular tone was based on an analysis of data on the change in the rate of propagation of the pulse wave by recording the time in which the pulse wave traveled the path from the semilunar valves to the dorsal artery of the foot of one of the rear extremities. It corresponded to the segment on the electrocardiogram falling between the S wave and the point determining the beginning of the ascending part of the pulse rise on the simultaneously recorded sphygmogram.

The determination of the rate of propagation of the pulse wave was made for the dogs in groups II and III. The results of these investigations revealed

TABLE 11. MEAN DATA ON CHANGE OF ARTERIAL PRESSURE AND OTHER HEMODYNAMIC INDICES IN DOGS

Group I		Time of examination of dogs of groups II and III, days after first irradiation	Group II			Group III		
Time of examination, days	Maximum arterial pressure, mm Hg		Maximum arterial pressure, mm Hg	Rate of propagation of pulse wave, m/sec	Pulse beats/min	Maximum arterial pressure, mm Hg	Rate of propagation of pulse wave, m/sec	Pulse beats/min
Before irradiation	-	Before irradiation	117	5.12	103	121	-	101
" "	110	" "	112	4.60	102	116	4.55	111
" "	112	" "	115	4.80	103	119	4.90	92
Mean before irradiation	111	Mean before irradiation	115	4.83	103	119	4.72	96
Period of irradiation		Period of irradiation						
8th	115	4th	115	5.66	97	136	5.33	95
15th	117	12th	97	5.66	102	140	5.05	100
22d	91	19th	95	4.80	124	128	5.45	100
29th	100	26th	-	-	-	-	5.00	107
		After irradiation						
38th	104	34th	-	-	-	126	4.90	99
43d	87	42d	-	-	-	120	5.17	80
		49th	-	-	-	122	5.17	86
		57th	-	-	-	128	4.77	90
		64th	-	-	-	110	4.77	83
		100th	-	-	-	129	5.00	72

that fractional irradiation of the dogs of group II caused some improvement of tone, primarily in the latent period of the illness. As shown by the data in table 11, in this period there is an approximately 15 percent increase in the rate of propagation of the pulse wave. With onset of the clinical symptoms of the illness the tone of the main vessels decreased. However, the decrease was significant only in some animals, and then only on the eve of their death.

Decrease of tone was not observed clearly in the dogs of group III. In the course of the entire period of irradiations and also a considerable time after its termination (up to 4 weeks) there was an insignificant but stable increase of vascular tone, which indicates a corresponding increase in the rate of propagation of the pulse wave.

It is known that the effect of ionizing radiation on the organism of animals leads to considerable structural changes and functional impairments of the vascular system. In the studies devoted to the study of this problem during radiation illness, great attention was devoted to the cutaneous vessels. Considerable restriction of the cutaneous vessels was found during irradiation of the dogs by X-rays in lethal doses. The maximum change was noted in the period of the height of the illness. This was confirmed by a number of investigators who used the thermometry method (refs. 50-54). The constriction of the cutaneous vessels was noted in various laboratory animals (monkeys, dogs, rabbits) after exposure to X- and γ-rays. Investigations of monkeys, made a considerable time after acute radiation sickness, revealed that even after three, and sometimes even four years from the time of irradiation there was constriction of the vessels and considerable decrease of their reaction to a thermal irritant (ref. 55).

The peripheral vascular system possesses great lability. Therefore, for correct evaluation of the observed changes it is necessary to record con- /71
tinuously several indices of its condition. This problem can be solved using the universal angiocardiograph proposed by V. V. Yakovlev. This records continuously, on any part of the surface of the body, such indices of the state of the peripheral vessels as maximum arterial pressure, vein pressure, arterial and vein tone and the rate of blood flow (ref. 56). The principle of the method is the recording of a plethysmogram from the distal part of the auricle of dogs with simultaneous compression of the blood vessels passing through it by a cuff.

These indices are evaluated from the plethysmogram in the following way. Maximum arterial pressure corresponds to the pressure in the cuff (detector) at the time of appearance of pulse variations during the decompression of the auricle. The pressure in the cuff, at which there is a rapid increase of the volume of the organ (onset of rise of the plethysmographic curve), corresponds to the pressure in the veins. A quantitative evaluation of arterial tone was made from the inverse value of the pulse amplitude on the plethysmogram, because there is a proportional dependence between the elasticity of the vascular wall and pulse amplitude (ref. 57). In the absence of pulse variations, the degree of vascular tone was arbitrarily denoted 2. Vein tone was expressed by a value inverse to the increase of the volume of the organ in which the state of the peripheral vascular system was investigated. The index used for

the volumetric rate of blood flow was the product of the tangent of the angle formed by the rising plethysmographic curve and the zero reference line and the rate of paper movement. Skin temperature was recorded by an ETM-3B point electric thermometer.

In order for the results of the investigation, obtained over a long period, to be comparable, we rigorously adhered to a set research method. In particular, the air temperature in the experimental room was maintained at the 18-20° level. The animal was acclimated to the room temperature for 20-30 min before each measurement. The study usually was made in the morning hours. The positions of the sensors and the tightened cuff on the auricle were constant. The rate of forcing air into the cuff, as well as the pressure of the plethysmographic sensor on the skin, were identical in all cases.

The state of the peripheral vascular system was studied for the dogs of groups II and III. It was established that fractional irradiation by protons leads to a substantial change of a number of indices characterizing the functional state of the peripheral vessels. These changes were observed after completion of the period of the irradiations--at the height of the illness and on the eve of the death of the animals. In these periods all the dogs of group II revealed a characteristic increase of the tone of the arteries and veins and a decrease of skin temperature (fig. 3). In the case of one dog, on the 20th-21st day from the onset of the irradiations, there was a singular dystonia of the arteries and veins: an increase of arterial tone, but low vein tone. On the 22d day the indices of vascular tone were appraised as /72 an angiospasm (fig. 4). The rate of blood flow had a tendency to decrease on the eve of the death of the animals (fig. 5).

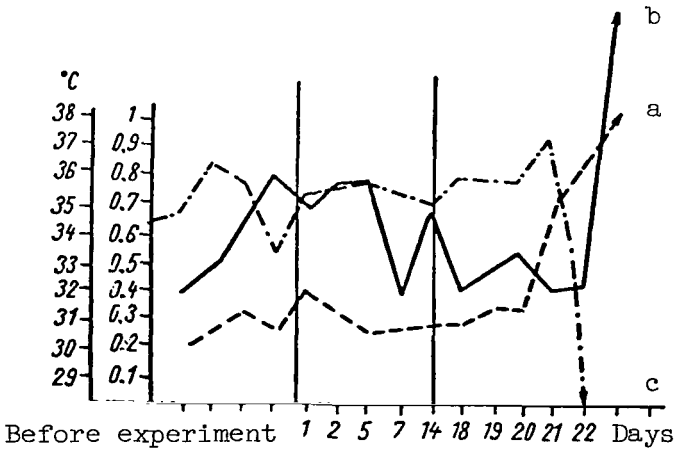


Figure 3. Change of arterial (a) in mm^{-1} and venous (b) in cm^{-1} tone of vessels of auricle and skin temperature (c) of dogs after fractional irradiation by protons. Vertical lines indicate period of irradiation.

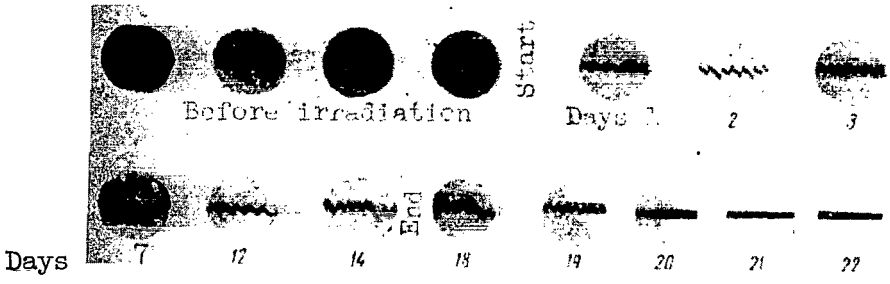


Figure 4. Plethysmogram recorded from distal part of auricle of dog before irradiation and at different times after it.

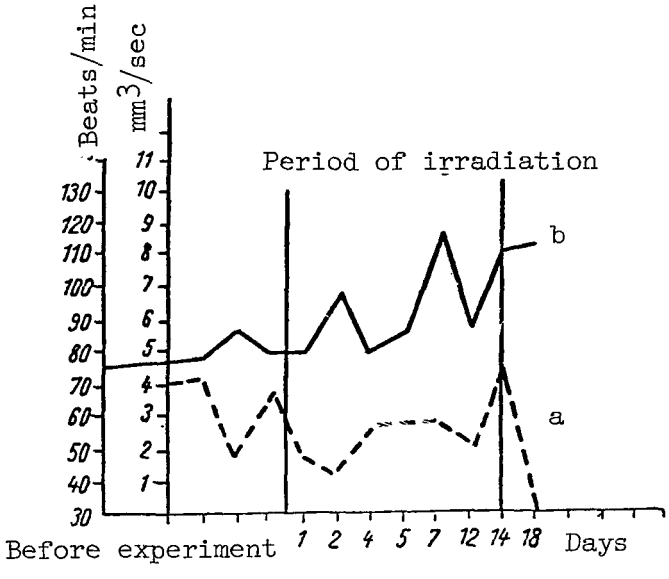


Figure 5. Rate of blood flow (a) and pulse rate (b) at different times after irradiation of one of the dogs of group II.

In the dogs of group III, which withstood the radiation sickness relatively well, there were brief changes of arterial and vein tone and skin temperature. In one of these animals in the period of irradiation from the 4th to the 12th days there was arterial-vein dystonia, expressed in a considerable increase of arterial tone (to 1) with low vein tone (0.4). On the graph this is rep- /73 resented in the form of the intersection of curves corresponding to arterial and vein tone (fig. 6). In another dog no appreciable changes in the state of the vessels were observed in the period of irradiation. On the 34th-36th days after the onset of irradiation, a considerable increase of arterial and vein tone was recorded for both animals. The skin temperature of the ear was minimal (21.8°) at this time. This state was appraised as an angiospasm of the

auricle. These changes apparently were of a functional character, since by the end of the experiment there was a constant increase of pulse variations accompanied by an increase of the skin temperature of the auricle. A normalization of the arterial and vein tone and skin temperature set in on the 36th-44th days. A decrease of the rate of blood flow set in at the same time as the increase of vascular tone and was restored by the 62d day.

Electrocardiographic data indicate that there were no substantial changes of the heart during the first two weeks of irradiation. In the observation intervals which followed, it was noted that there were changes of the electrocardiograms of individual animals of groups I and II, reflecting functional and organic injury of the myocardium and the circulatory system. At the height of the illness, and especially in the preterminal stage, in most of the animals there was splitting of the R wave, decrease of voltage and in individual cases inversion of the T wave in different abductions and displacement of the ST segment from the isoelectric line. The interval PQ for the dogs was not constant, but more frequently than before irradiation it was increased by 0.02-0.03 sec. No clearly defined changes were observed in the duration of the /74 complex QRS or the segments ST and QT, with the exception of some tendency to an increase of the systolic index in the late stages of the damage. At this same time there was "smoothing" of the arrhythmia of cardiac contractions.

In an analysis of the electrocardiograms of the animals of group III, in most cases the same changes were noted as in animals of groups I and II. However, they appeared for the most part in the late stages of the period of irradiation and after its completion. Clearly expressed wavelike changes of the duration of the interval PQ (from 0.14 to 0.22 sec) were noted. The clearest increase in the duration of PQ was observed 2 or 3 weeks after the onset of the irradiations. More frequently than normal there were also changes in the voltage and the shapes of the Q, R and S waves (appearance of jags and splittings). In individual cases there was inversion of the T wave, considerable decrease of its voltage and displacement of the segment ST below the isoline and its increase.

In conclusion we note that a number of substantial changes appear in the different components of the cardiovascular system during fractional irradiation by protons. In animals irradiated in lethal doses there was an increase of the frequency of the pulse, a decrease of the maximum and minimum arterial pressure in the main vessels and phase variations of the tone of the ar- /75 teries and veins. The heart underwent changes indicating the development of diffuse dystrophic processes in the myocardium and impairments of the antrioventricular and intraventricular conduction.

On the whole, the detected changes indicate that in addition to the other physiological systems the blood circulation apparatus also is extremely sensitive to protons. At the same time, the cardiovascular system also possesses relative resistance to the effect of this factor. The fact that most of the observed changes were noted at the height of the illness, when the total dose reached about 550-600 rad, indicates the conservation of compensatory mechanisms which maintain the blood supply of the organism at the required rate over a long period of time.

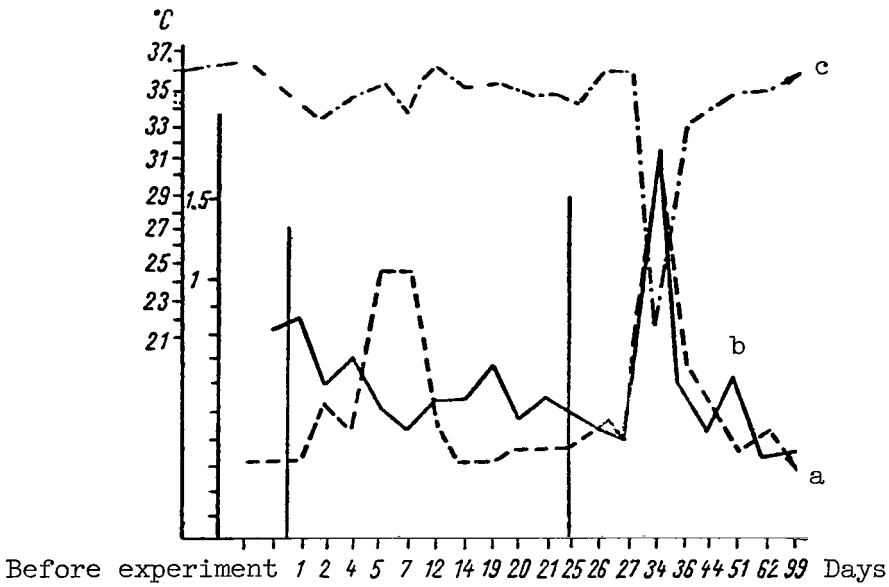


Figure 6. Changes of arterial (a) in mm^{-1} and venous (b) in cm^{-1} tone of vessels of auricle and skin temperature (c) of one of the dogs of group III. Vertical lines indicate period of irradiation.

Pathologic-morphological Investigations. There were autopsies on the dogs immediately after their death. Some animals were killed in the moribund state, and autopsies were made immediately after their killing.

In a macroscopic investigation of all animals we found changes characteristic of acute radiation sickness: a large number of hemorrhages in different organs and tissues of the body, atrophy of the lymphoidal apparatus, aplasia of the bone marrow, distrophic changes of the parenchymatous organs and the appearance of infectious complications. The largest number of hemorrhages was discovered in the lungs, heart and along the gastrointestinal tract. Hemorrhages appeared in the subcutaneous cellular tissue and also in the mucous membrane of the urinary tract. Punctate hemorrhages were seen in the kidneys, primarily in the cortical layer and capsule. In the heart multiple focal hemorrhages were found in the muscles of the atrium cordis dextrum and interventricular septum. Hemorrhagic diathesis was manifested very sharply in dogs which died after eight-fold irradiation (group II). The lymph nodes, especially retroperitoneal and mesenteric, increased in size, became filled with blood and edematous. The lymph follicles were indistinguishable; the bone marrow of the long tubular bones was plethoric, of a fluid consistency. The spleen decreased in size and thickened, and follicles could not be detected. The liver was swollen, flabby and of a yellowish-gray color. The configuration of the hepatic lobes was ill-defined. The kidneys had a similar appearance. In cross section there was no clearly defined boundary between the cortical and

medullary layers. The testicles were decreased in size and flabby; the vessels of the capsule were enlarged, and there were hemorrhages beneath the capsule.

Changes of the same type were observed in the histological investigation of the dogs of groups I and II. Exceptions were the spleen and the lymph nodes, in which, in addition to a general similarity (enlargement of the lymph spaces, hemorrhages, erythrophagia and a large quantity of hemosiderine), there /76 were some differences in their structure. Whereas the changes in the animals of group II were manifested on a background of almost complete disappearance of lymphocytes, in group I there was a considerable quantity of lymphocytes of medium and small diameter in the follicles of the spleen and also in the secondary nodules and soft ligaments of the lymph nodes (fig. 7). In the case of irradiation over a longer term, as occurred in group I, manifestations of repair appeared, accompanied by multiplication of lymphocytes.

The microscopic picture of damage to other organs was identical in both groups. Multiple massive parenchymatous and perivascular hemorrhages were seen in the lungs (fig. 8), but the parenchymatous hemorrhages in the dogs of group II had a more diffuse character. Edema of the lung was discovered in one of them. In all animals, in the adventitia of the large and small vessels there were many cells whose cytoplasm contained granules of hemosiderine (fig. 9). Abundant desquamation of the endothelium and epithelium of the bronchi was also noted.

Figure 7. Considerable quantity of lymphocytes in secondary nodes and pulpy ligaments of spleen of dog after 19-fold irradiation by protons. Magnification 7 x 20.

/77

Figure 8. Massive parenchymatous hemorrhage in lung of dog after 19-fold irradiation by protons (a) and perivascular hemorrhage around large vessel after 8-fold irradiation by protons (b). Magnification x 200.

Focal hemorrhages, edema and plasmatic impregnation of the walls of the vessels were observed in all investigated organs. This was supplemented in the heart by dystrophic changes of the myocardium. Manifestations of /78 dystrophy of the parenchyma were also discovered in the liver and kidneys. However, no clearly expressed changes of parenchymatous cells were noted in these organs. In the kidneys, in addition to a clearly expressed plethora of the glomerules, there was an albuminous effusion in the expanded cavity of the Bowman-Shumlyanskiy capsule.

Figure 9. Large quantity of cells filled with granules of hemosiderin in adventitia of vessel. Magnification x 200.

Necrobiotic changes of the epithelium (and locally its total exfoliation) were noted in the urinary bladder and urinary tract. In the mucous membrane and tunica submucosa of the urinary bladder massive hemorrhages were noted, leading to diffuse impregnation of all membranes of the organ by blood. Equally clearly expressed manifestations of hemorrhagic diathesis were observed in the gastrointestinal tract as well. Extensive hemorrhages were discovered in the mucous membrane and tunica submucosa of all parts of the stomach and the small and large intestine. In the lower and pyloric parts of the stomach there was autolysis of the tegmental epithelium and necrobiotic changes of the main and parietal acid cells. No clearly expressed changes were observed in the tegmental epithelium of the small intestines. A considerable number of mitoses was noted in its crypts. On the other hand, in the large intestine there were clearly expressed necrobiotic changes of the tegmental epithelium, primarily of the goblet cells. As mentioned earlier, the same differences were manifested in the spleen as in the lymph nodes.

There was almost complete disappearance of lymphoidal tissue in the dogs of group II. The remaining follicles were very small and contained a small number of mature lymphocytes (fig. 10a); the pulp was edematous and plethoric. /79

On the other hand, the spleens of the dogs in group I revealed a considerable quantity of young and mature lymphocytes, scattered diffusely in the pulp and around the small vessels (fig. 10b), which indicated a tendency to regeneration. At the time of death of the animals of both groups there was no complete devastation of the sperm ductules. In individual ductules morphologically undamaged spermatogenic cells were found in a state of mitosis and

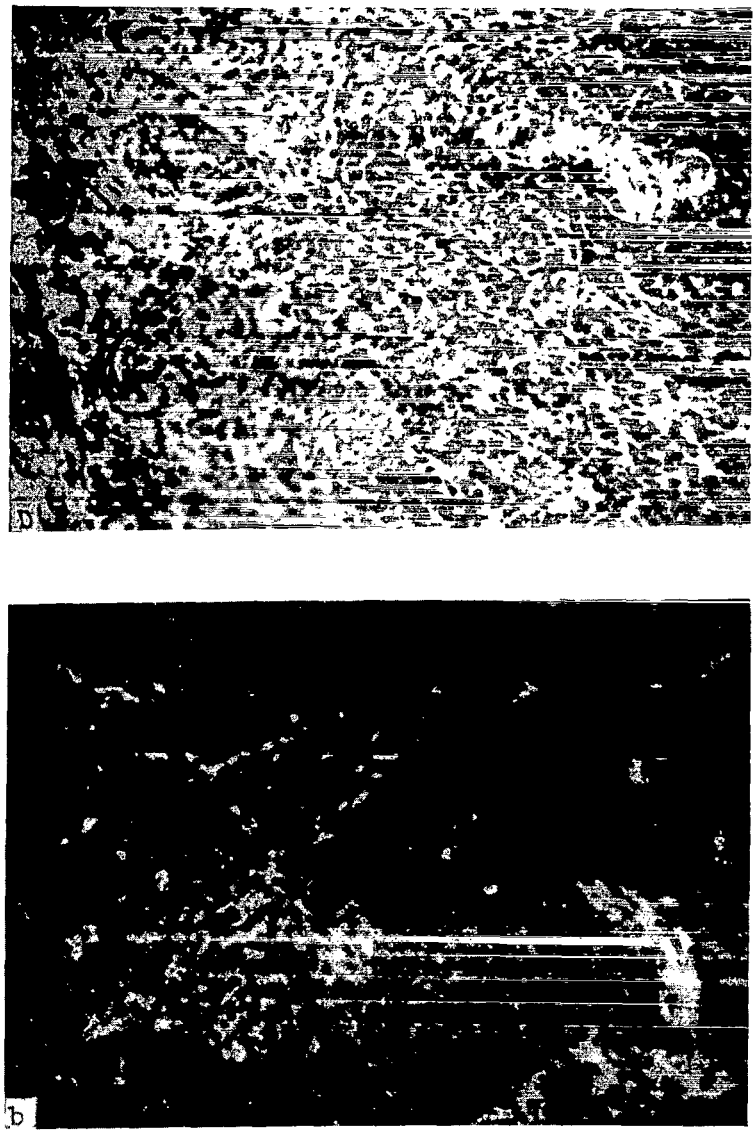


Figure 10. Devastation of spleen of dog after 8-fold irradiation by protons (a) and considerable quantity of young and mature lymphocytes diffusely scattered in spleen of dogs after 19-fold irradiation by protons (b). Magnification x 200.

at different stages of development (fig. 11). Hemorrhages were seen under the capsule and in the interstitial tissue, and granules of hemosiderin were seen in the cytoplasm of the cells of the adventitia of the vessels, indicating an increase of vascular permeability.

Figure 11. Considerable quantity of spermatogenic cells at different stages of division and development after 8-fold irradiation by protons. Magnification x 400.

In the pathologic-morphological investigation of the central and peripheral nervous system we noted minor opacification and anemia of the meninx and medulla. A focus of hemorrhaging, the size of a 3 kopeck coin, was discovered in one of the dogs after 8-fold irradiation in the sagittal cross section of the cerebellum. Small hemorrhagic foci were discovered in all dogs and in all parts of the brain.

A microscopic investigation of the brain and spinal cord made it possible to detect edema of the soft meninges locally with their separation into fibers and an insignificant increase of collagenic fibers of surface meninges in the region of the cortex of the hemispheres, somewhat greater in the meninges of the base of the brain. A more conspicuous increase of the number of collagenic fibers was noted in the soft meninges of the spinal cord, which was not characteristic for the age of these dogs (1.5-2 years).

The changes of the vessels of the brain essentially involved changes of vascular tone and the distension of the perivascular spaces. The structure of the walls of the collapsing vessels in most cases was indistinguishable. It was noted that there was a gross destruction of veins with perivenous hemorrhaging, sometimes an extraordinary distension of the lumen of the veins and liquefaction of perivascular cerebral matter.

The presence of a large number of hemorrhages was extremely characteristic of the histological picture of the brain and spinal cord. Small hemorrhagic foci were encountered in all parts of the brain. In one dog a major focus of hemorrhaging with destruction of the brain tissue over a great area was noted in the cortex of the vermis, which agrees with the macroscopic description

(fig. 12). Foci of hemorrhaging were observed in the reticular matter at the level of the midbrain. Multiple microfoci of necrosis of the cerebral /82 tissue were also discovered in the cortex of the hemispheres (frontal, temporal, occipital lobes and hippocampal gyrus), in subcortical-stem formations, in the cerebellum and in the white matter of the spinal cord. Massive necrosis with disintegration of tissue was detected in the cortex of the temporal region (fig. 13). The massive hemorrhages and necroses of the cerebral tissue apparently were preceded by small hemorrhagic and necrotic foci, since in some places there was a clearly expressed merging of individual micronecroses. The changes in the formation of the circulatory (lymph-blood) system are worth noting. For example, there was edema of the tissue at the base of all cerebral ventricles; there was focal disintegration of the ependymal lining in the third and fourth ventricles and also in the cerebral aqueduct (fig. 14). Changes of a degenerative character were detected in the cells of the ependyma and in the epithelial cells of the villi of the vascular plexi. Edematous disintegration, porosity and, in places, softening of the tissue were observed in the conducting systems of the stem and spinal cord.

No gross pathology of the cortical cells was observed, although foci of cellular obliteration were present in all parts (fig. 15); considerable cellular devastation was observed in the hippocampal gyrus. The grossest pathological deviations were noted in the nerve cells of the subcortical-stem formations, especially in the hypothalamic region. In some cells of the tuber cinereum there was degeneration of nuclei in the form of their segmentation and /84 hyperchromatosis. In the cells of the central gray matter, in addition to very slight staining of the nuclei, there was karyolysis and presence of shriveled

Figure 12. Massive focus of hemorrhaging in cerebellum with disintegration of cerebral tissue. Staining by the van Gieson method. Magnification by hand lens.

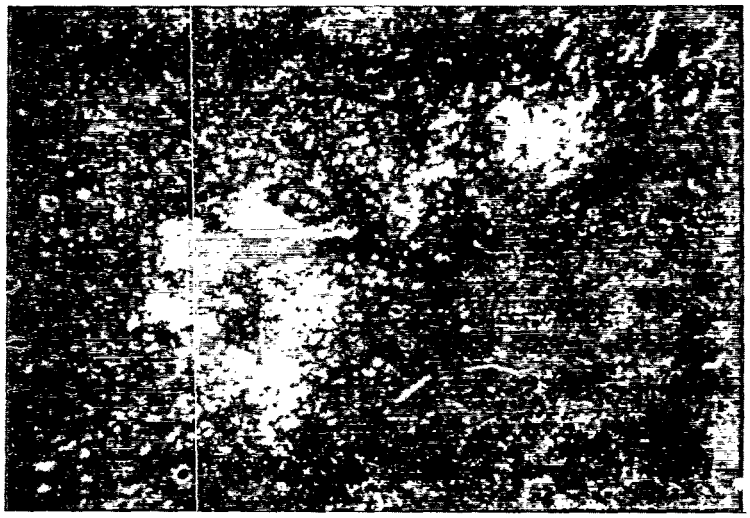


Figure 13. Necrotic disintegration of cerebral tissue in frontal lobe. Staining with hematoxylin-eosin. Magnification x 50.

Figure 14. Necrobiosis and necrosis of cells of ependyma of third cerebral ventricle. Staining by van Gieson method. Magnification x 200.

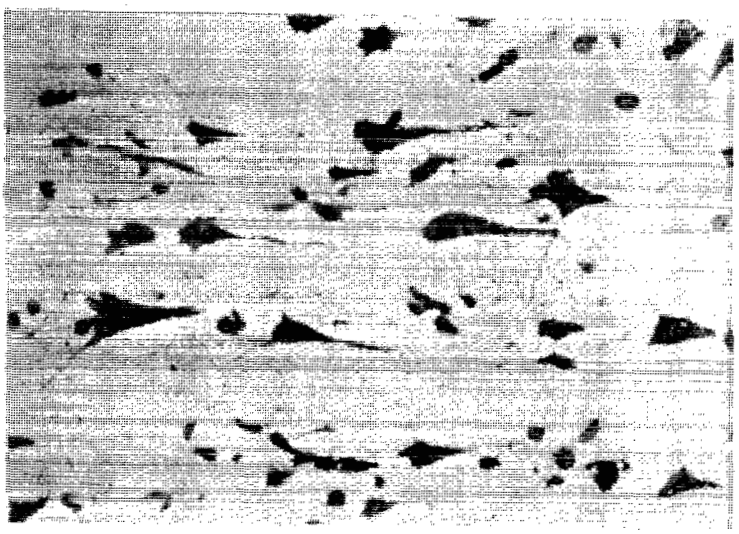


Figure 15. Shriveling and atrophy of corticospinal cells of second and third layers of motor apparatus. Staining by Nissl method. Magnification x 630.

cells. The pathology of the nucleoles of many cells was characterized by slight reception of stain and deformations. The changes in the underlying formations of the stem had a clearly expressed selective character. Among the cells of the motor nuclei of the stem there were exceedingly distended motor neurons and shriveled cells. Great spatial variation of injury of the nerve cells was observed in the nuclei of the pons Variolii and in the reticular matter of the stem. In the medulla oblongata the most widespread pathological changes were in the cells of the inferior olives (chromatolysis and homogenization of many cells), the cells of the dorsal nucleus of the vagus nerve and the cells of the vestibular nuclei.

In the cerebellum we observed various stages and forms of pathological changes of the Purkinje cells (chromatolysis, hyperchromatosis of the nuclei, shadow cells, cytolysis and foci of cellular shedding over a great area). Among the granular cells there were only individual cells with pyknosis of the nucleus. The changes of some cells of the molecular layer were expressed in hyperchromatosis of the nucleus. Changes of the nerve cells were combined with a pathological change in the myelinous nerve fibers. Manifestations of slight demyelinization were noted in the white matter of all parts, and in places there also were major foci of disintegration of the myelinous covering (fig. 16). We observed manifestations of the degeneration of myelinous nerve fibers both in the subcortical white matter and in the conducting systems of the stem and spinal cord without preceding disintegration of the myelinous covering of the nerve fiber.

Investigation of the astroglia and oligodendrology in the cerebral cortex indicated an inhibition of its reaction. Small accumulations of degeneratively

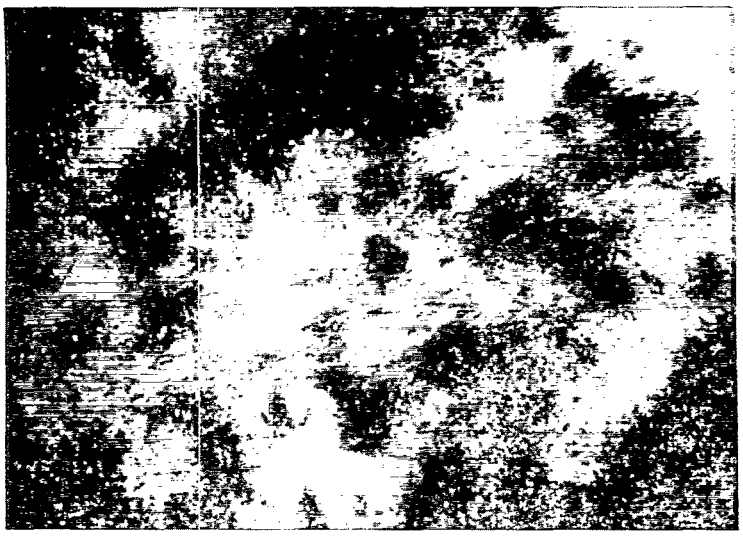


Figure 16. Foci of dimyelinization in white subcortical matter of cerebellum. Staining by Sokolyanskiy method. Magnification x 500.

modified astrocytes were observed in the white subcortical matter at the boundary with the gray matter. A proliferative-degenerative character of the astroglia was observed in sections of the medulla oblongata, in the region of the dorsal nucleus of the vagus nerve and the nuclei of the fasciculus cuneatus and fasciculus gracilis. The disintegration of astroglial appendices was observed around some destructively modified vessels of the subcortical-stem formations. There was disintegration of astrocytes in the tissue surrounding massive foci of necroses and hemorrhages. Only at some distance from the focus of a hemorrhage, such as in the cerebellum, was there a focal reaction of astrocytes of a progressive character (fig. 17).

In conclusion we point out: (a) uniformity of the character of the pathological process developing in the nervous system of dogs of different groups; (b) similarity to the pathological process in the central nervous system of dogs suffering from acute radiation sickness caused by general X-ray irradiation; (c) a distinguishing feature of the pathological process in the central nervous system of dogs injured by proton radiation is a predominance of /86 the destructive process in the intracerebral vessels, and as a result, more frequent focal hemorrhages and necroses than in dogs subjected to X-radiation in adequate doses.

In the peripheral part of the nervous system a study was made of the cervical, thoracic and lumbar spinal ganglia, the nodose and Gasserian ganglia and also the sympathetic ganglia: superior cervical and stellate ganglia and nodes of the solar plexus. Ordinary neurohistological methods of investigation were used.

In a microscopic study all nerve ganglia of the peripheral part of the nervous system were found to have characteristic changes of the nucleolar apparatus of the neurons. The nucleoles of the nerve cells were stained poorly by thionine, became faded, increased in size and assumed an irregular form. Many nucleoles in this case appeared to be clusters of small vacuoles and sometimes contained 1 or 2 large vacuoles (figs. 18 and 19). Similar changes of the nucleoles were seen in nerve cells, both with distended, enlarged and bubble-like nuclei and with nuclei decreased in size, which stained vividly. In many cases it was possible to observe an ejection of the nucleole from the nucleus into the cytoplasm of a neuron or the appearance of additional nucleoles /87 in the nucleus.

In the sensory ganglia, in addition to changes of the nucleoles of the nerve cells, in individual parts there is a decrease in the size of nuclei and their vivid, frequently metachromatic staining. Similar changes are observed very frequently in the Gasserian ganglion, where the nuclei sometimes assume the form of small clusters of an irregular form with a dark stain having uneven outlines (fig. 20). Individual shriveled nerve cells are encountered. In the nodose ganglion, in contrast to the other nodes, there are no dark-stained and especially no shriveled nerve cells. However, chromatolysis is noted rather frequently, usually of a peripheral character. In such cases chromatophilic matter is retained only in the form of clusters of tiny granules of such matter around the distended and eccentrically situated nucleus. In many cases it is possible to see neurons containing one large vacuole (cricoid cells) or a great many small ones.

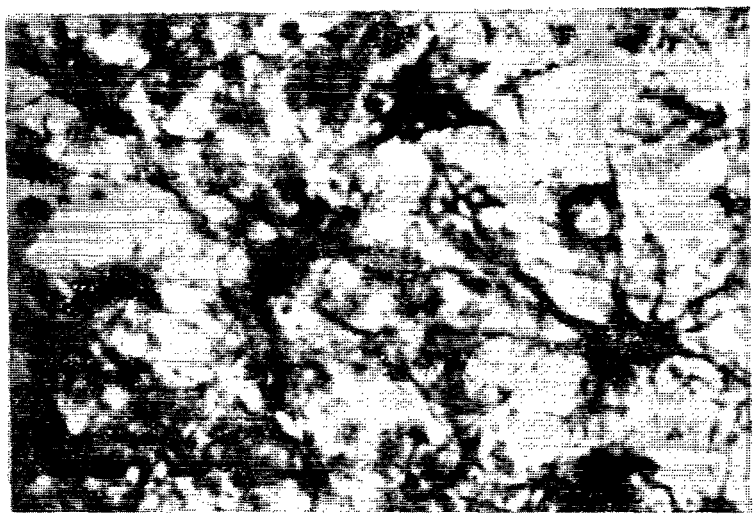


Figure 17. Degeneration and disintegration of astrocytes in granular layer of cerebellum. Staining by Kakhal method. Magnification x 630.

Figure 18. Stellate ganglion (well-expressed chromatolysis of nerve cells, distension of nuclei of neurons and fading of nucleoles). Staining by Nissl method with thionine Magnification x 400.

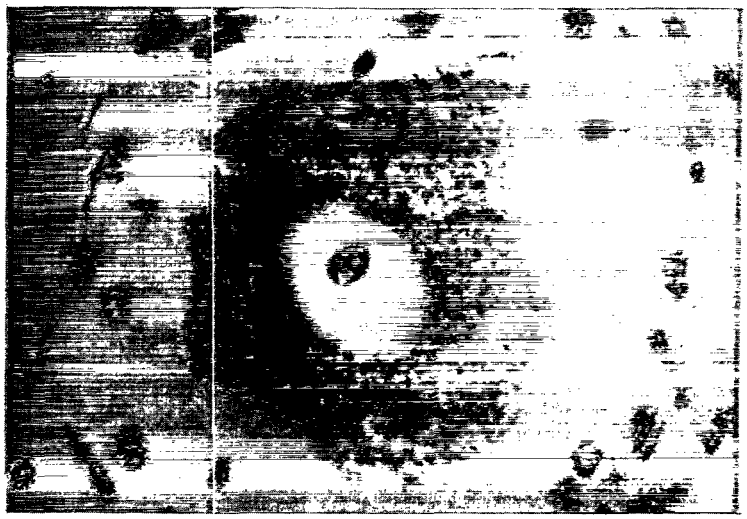


Figure 19. Cervical spinal ganglion (vacuoles in nucleole of nerve cell). Staining by Nissl method with thionine. Magnification x 1350.

Liquefaction of the chromatophilic matter of the neurons is also observed in the other ganglia, but it is of a different character. For example, in the neurons of the Gasserian ganglion there is for the most part focal liquefaction of the chromatophilic matter. In cases of well-expressed chromatolysis such cells have the form of neurons in a state of vacuolar degeneration. It should be noted that in all sensory ganglia the gross morphological changes of /88 the nerve elements had a strictly focal character. In those places where modified nerve cells predominated, there was a polymorphism of the nuclei of the peripheral glia.

In contrast to the sensory ganglia, in the sympathetic ganglia the injury of the nerve structures did not have a well-expressed focal character. Almost all nerve cells had changes of the acute dilatation type. The neurons increase in size, their nuclei are distended sharply and seemingly are ejected from the bodies of the neurons; they increased sharply in size and occupied almost half the area of the entire nerve cell. The cytoplasm of the neurons was almost without chromatophilic matter. Granules of this matter are retained in the form of individual small lumps only along the periphery of the body of the neuron. In many cases there is basophilic staining of the hyaloplasm of the neuron, thus making it possible to see the open reticular pattern of its structure. Individual shadow cells and vacuolized neurons are encountered.

In evaluating the state of the structures of the peripheral nervous system it is important to note the considerable injury of the neurons of the sympathetic ganglia. In actuality, their changes frequently are gross, and what is very important, widespread. The ganglia include no nerve cells having an ordinary structure; all appear changed. A similar character of injury /89

Figure 20. Gasserian node (gross modification of nucleus of nerve cell). Staining by hematoxylin-eosin. Magnification x 1350.

of the sympathetic structures also was noted in the case of acute radiation sickness caused by external X-ray irradiation. In the sensory ganglia, in contrast to the sympathetic ganglia, it was quite common to see unmodified nerve cells. However, this fact cannot serve as an indication of unimportant injury of these structures. It indicates only the more clearly expressed properties of plasticity of the afferent nerve formations, produced in phylogenesis, properties directed toward long preservation during various pathological processes of functional activity (ref. 58). As confirmation we cite the peculiarities of the injury of the sensory structures, specifically, a sharply expressed focal character. The preserved nerve elements to a certain degree are probably capable of functionally compensating existing morphological impairments. In actuality, impairments of the afferent structures are more serious. In these ganglia it is common to observe neurons with gross changes in their nuclei, indicating severe damage of the nerve cells, since the injury of the nuclear apparatus, the trophic center of the entire cell, often is the initial indication of death of the neuron (ref. 59). In addition, in many cases it is possible to see neurons in a state of necrobiosis. Considerable changes in the sensory nerve ganglia are not characteristic of acute radiation sickness caused by external X-ray or γ-irradiation.

After a comparative analysis of available data it is difficult to say which of the two systems, afferent or efferent, suffers to the greater degree.

The most characteristic manifestation of morphological impairments of all investigated nerve formations in this particular case was the change of the nucleoles of the nerve cells. These changes are evidence of considerable functional stress of the peripheral part of the nervous system as a whole, because the characteristic reaction of the nucleoles is observed in almost all the neurons of the afferent and efferent ganglia (refs. 59 and 60).

Experiments on Rats

We used 58 white male rats weighing 180-200 g in the experiment.

Depending on the total dose, the rats were divided into two numerically equal groups, in each of which the animals were subjected to 20-fold irradiation by protons with energies of 510 Mev over a 5-week period (table 12) with the total doses being 750 rad (group I) and 1,015 rad (group II). The single doses for the animals of groups I and II averaged 37 and 51 rad, respectively. The radiation dose intensity was 1-1.5 rad/sec. During the time of the ir-/ radiation the rats were in a cylindrical plexiglass box divided into 15 compartments. The box was rotated at a velocity of 20-25 rpm in a horizontal /90 plane, thus exposing all the animals to equal irradiation. During the time of irradiation the animals were accompanied by individual luminescent control (ILK) detectors.

On the basis of the readings of these detectors it was possible to draw conclusions concerning the single and total dose of irradiation of the animals. Before and after exposure, and also during the entire period of

TABLE 12. RESULT OF 20-FOLD IRRADIATION OF RATS IN 5-WEEK PERIOD BY PROTONS WITH ENERGIES OF 510 MEV DURING 280-DAY PERIOD OF OBSERVATION

Group	Number	Total dose, rad	Result
I	29	750	1 died
II	29	1015	4 died

observations, the animals were given food consisting of white bread, millet gruel, milk, meat, grain, etc.[1]

As tests characterizing the state of the animals we used: weight dynamics, content of erythrocytes, leukocytes and thrombocytes and the leukocytic formula. At different times during the sickness the rats were killed for morphological investigations.

Course of Radiation Sickness. After the first 3-4 irradiations the animals of both groups showed decreased activity, deterioration of appetite and dishevelled fur. Similar changes were also observed in the last ten days of irradiations. After the initial manifestations, the condition of the rats improved, and external clearly expressed signs of sickness were not discovered until the 25th-26th day from the beginning of irradiations. However, despite the relatively good general condition of the experimental animals, there were clearly expressed deviations in the weight dynamics of the body. Figure 21 shows that in the period of irradiations the increase of body weight stopped in the animals of both groups, and there even was some decrease. Only after termination of irradiations did the weight of the animals again begin to increase, but relatively slowly. As a result, in the course of all subsequent periods of observation there was progressive increase in the weight gap between the experimental and control animals. There were no clearly expressed differences in the dynamics of weight change of rats irradiated in doses of 750 and 1,015 rad.

Changes in Peripheral Blood. Tables 13 and 14 give the changes of the /91 peripheral blood of rats of groups I and II. It can be seen that during the entire period of irradiations there was progressive decrease of the number of erythrocytes from 8.15 and 7.8 million to 6.4 and 5.4 million in groups of rats I and II, respectively. Upon completion of the period of irradiations there was an insignificant tendency to restoration and then a further, but more /92 weakly expressed decrease in the content of erythrocytes. Two months after termination of the irradiations there still was a rather stable erythropenia. The content of erythrocytes by this time was 20-24 percent lower than the initial values.

[1] The daily norm for the rats was: bread--15 g, meat--5 g, groats--5 g, oats--15 g, vegetables--3 g, milk--8 g, salt--2 g, hay--2 g or grass--6 g.

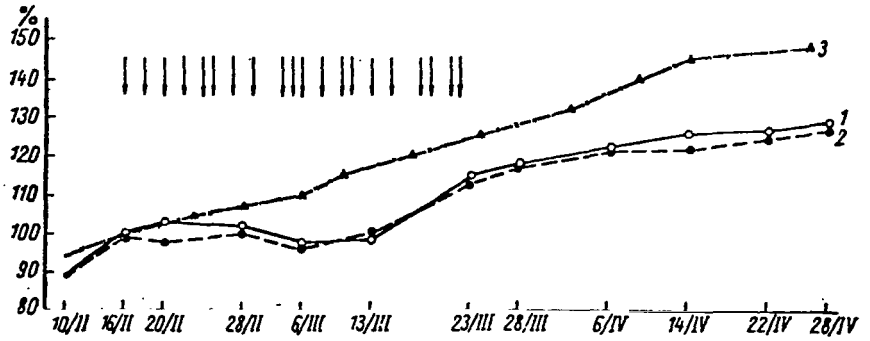


Figure 21. Change (in percent of initial level) of weight of rats in case of irradiation with dose of 750 rad (1) and 1,015 rad (2), also control data (3).

TABLE 13. CHANGES OF BODY WEIGHT AND INDICES OF PERIPHERAL BLOOD OF RATS OF GROUP I (TOTAL DOSE; 750 RAD)

Date and time of examination		Weight, g	Number of erythrocytes, millions per 1 mm^3	Number of leukocytes, thousands per 1 mm^3	Number of thrombocytes, thousands per 1 mm^3
Before irradiations	1 Feb.	192 ± 6.1	9.01 ● 0.23	17.75 ± 1.4	187 ± 18
	10 Feb.	202 ± 4.5	7.80 ± 0.12	15.68 ± 1.2	-
In period of irradiations*	16 Feb.	225 ± 5.5	8.15 ± 1.10	15.31 ± 0.7	-
	20 Feb.	232 ± 5.5	7.58 ± 0.15	8.14 ± 0.5	164 ± 34
	28 Feb.	229 ± 4.0	7.78 ± 0.22	8.52 ± 0.5	147 ± 8
	6 Mar.	221 ± 4.1	7.73 ± 0.20	6.92 ± 0.8	139 ± 56
	13 Mar.	224 ± 4.8	7.14 ± 0.19	6.96 ± 0.7	195 ± 24
After irradiations	23 Mar.	259 ± 5.6	6.44 ± 0.12	6.21 ± 0.5	156 ± 18
	28 Mar.	267 ± 4.7	6.94 ± 0.13	7.66 ± 0.4	184 ± 14
	6 Apr.	275 ± 6.3	7.05 ± 0.12	16.50 ± 1.4	264 ± 69
	14 Apr.	283 ± 6.1	6.46 ± 0.12	12.20 ● 0.7	313 ± 28
	22 Apr.	286 ± 8.3	6.47 ± 0.13	11.45 ± 0.9	124 ± 14
	28 Apr.	290 ± 8.6	6.30 ± 0.12	14.25 ± 1.0	159 ± 18
	5 May	302 ± 9.0	6.28 ± 0.28	15.57 ± 1.2	135 ± 16
	12 May	313 ± 11.0	6.83 ± 0.26	16.37 ± 0.8	135 ± 16
	22 May	314 ± 11.0	6.37 ± 0.15	17.88 ± 0.4	201 ± 53

*Irradiation of the animals took place in the period of Feb. 16-Mar. 22, 1961.

A sharp decrease in the number of leukocytes (by 50 percent or more) was noted immediately after the first exposures to irradiation. As a result of subsequent exposures there was further but more moderate decrease in the number of leukocytes. The maximum decrease was observed at the end of the period of irradiations, when the quantity of leukocytes was at the level of 39 and 29 percent of the initial values for groups I and II, respectively. Upon termination

TABLE 14. CHANGES OF BODY WEIGHT AND INDICES OF PERIPHERAL BLOOD OF RATS OF GROUP II (TOTAL DOSE; 1,015 RAD)

Date and time of examination		Weight, g	Number of erythrocytes, millions per 1 mm^3	Number of leukocytes, thousands per 1 mm^3	Number of thrombocytes, thousands per 1 mm^3
Before irradiations	1 Feb.	192 ± 3.0	8.2 ± 0.30	15.9 ± 0.7	483 ± 22
	10 Feb.	198 ± 2.5	7.4 ± 0.02	16.3 ± 0.6	342 ± 93
In period of irradiations	16 Feb.	221 ± 2.4	7.8 ± 0.36	16.1 ± 1.30	413 ± 51
	20 Feb.	218 ± 2.7	8.0 ± 0.30	7.5 ± 0.47	360 ± 76
	28 Feb.	225 ± 3.6	7.7 ± 0.21	7.4 ± 0.53	264 ± 33
	6 Mar.	214 ± 3.2	7.5 ± 0.10	5.8 ± 0.30	284 ± 39
	13 Mar.	222 ± 3.5	7.2 ± 0.27	4.9 ± 0.18	289 ± 90
After irradiations	23 Mar.	253 ± 3.4	6.7 ± 0.08	4.7 ± 0.26	220 ± 31
	28 Mar.	261 ± 3.8	6.5 ± 0.11	6.7 ± 0.40	427 ± 37
	6 Apr.	269 ± 4.5	6.2 ± 0.17	13.4 ± 1.0	758 ± 16
	14 Apr.	270 ± 5.0	6.0 ± 0.09	10.5 ± 0.85	702 ± 86
	22 Apr.	276 ± 9.0	5.4 ± 0.14	11.4 ± 1.10	740 ± 92
	28 Apr.	281 ± 7.8	6.4 ± 0.11	8.6 ± 0.92	583 ± 10
	5 May	286 ± 6.7	6.2 ± 0.10	15.4 ± 0.70	423 ± 86
	12 May	298 ± 7.1	6.3 ± 0.13	15.1 ± 0.68	486 ± 11
	22 May	306 ± 8.6	6.3 ± 0.30	15.8 ± 0.70	641 ± 91

of the irradiations there was a rapid, but temporary increase in the number of leukocytes. Fourteen days after cessation of the radiation exposure, the quantity of leukocytes attained 103 percent of the initial values in group I, but 84 percent in group II. Later, there was a rather prolonged decrease in the number of leukocytes. These changes were caused primarily by changes in the number of lymphocytes, which had decreased by the end of the irradiation period from 8,000-10,000 cells to 500-1,000/mm^3 (i.e., by a factor of 10-15), whereas the total content of leukocytes had decreased only by a factor of 3 by this time. The change of the other forms of leukocytes for the most part was proportional to the change of their total number.

In the irradiation period the content of thrombocytes changed, but, /93 unlike in the dogs, the changes were relatively small and variable (see cited samples). A difference in the total doses of irradiation (750 and 1,015 rad) exerted little influence on the change of quantity of thrombocytes. Immediately after termination of the irradiations there was an intense increase of the number of thrombocytes, after 2-3 weeks attaining 160-180 percent of the initial level. Later, there was a new wave of decrease of the quantity of thrombocytes to the initial values and even lower.

Pathologic-morphological Investigation. In the morphological analysis of the internal organs (heart, lungs, liver, kidneys, spleen and testes) of the irradiated rats an attempt was made to trace the dynamics of development of radiation damage with time. For this purpose the animals were killed after 2-3 weeks, 1, 1.5 and 2 months after termination of irradiation. In the autopsy

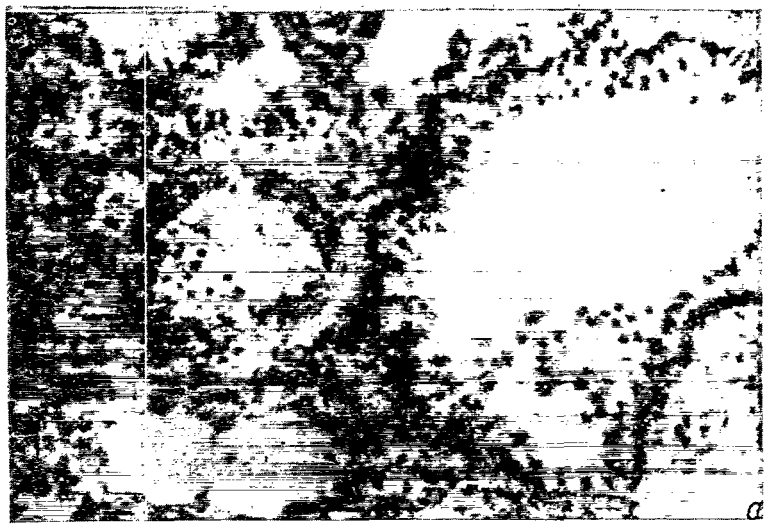
of the animals killed two weeks after termination of the radiations no sharply expressed pathological changes were discovered, with the exception of a decrease of size of the spleen and testes and old and new hemorrhages in the lungs, which were encountered rather frequently. At later times (1-1.5 months later), macroscopically visible hemorrhages in the lungs were encountered less frequently and the size of the spleen increased gradually; however, the decrease in size and flabbiness of the testes progressed.

In a microscopic examination both groups of rats were found to have characteristic indications of acute radiation sickness and as in the dogs the hemorrhagic syndrome was clearly expressed. Massive hemorrhages, perivascular edema and desquamation of the endothelium, as well as extensive foci of atelactasis in the lungs, were encountered most frequently in the first 2 weeks after the irradiations (fig. 22). Hemorrhages were encountered considerably less frequently after 1-1.5 months, but, as before, there was desquamation of the endothelium and epithelium of the bronchi, sectors of atelactasis, and destruction (fig. 22b) and obliteration of the small vessels. In the heart, as in the dogs, there were dystrophic changes in the myocardium, atonia of the vessels, small foci of parenchymatous hemorrhages and hemorrhages around the large vessels. Impairment of hemodynamics in the kidneys was expressed in plethora of the vascular glomerules, distension of the vessels and congestion in the cortical and medullary layers. A considerable time after irradiations it was found in some cases that there was dystrophic changes in the cells of the renal tubules and hemorrhages in the region of the renal pelvis. The impairment of hemodynamics and dystrophic changes of the parenchymatous cells of the liver were less significant.

In addition, no clearly expressed changes were noted in the small intestine. However, at different times after the end of the irradiation large numbers of cells containing granules of hemosiderin were found in the stroma of the villi, evidence of the impairment of vascular permeability in the intestine of the irradiated rats. /95

In the first 2 weeks after the end of the irradiations there was observed almost total destruction of the lymph follicles (fig. 23), plethora of the organ and distension of the reticular stroma. After 1 to 1-1/2 months there was partial restoration of the lymphoid apparatus. The follicles became more numerous and larger. The stroma was infiltrated diffusely by a considerable number of lymphocytes. Thus, at these times there was a great similarity to the structure of the spleen in the dogs of group I. In all observations there was noted a plethora of a congestive character, and there was a very large quantity of hemosiderin and brown pigment.

The total devastation of the sperm ductules in the testes appeared 2 or 3 weeks after the end of the experiment. At this time both spermatogenic and Sertoli cells had disappeared (fig. 24a). The capillaries surrounding the ductules were distended: a considerable quantity of interstitial tissue was filled with granules of hemosiderin. After 1 month the changes progressed. As before there was a total absence of the seminific epithelium and Sertoli cells. The interior of the ductules was filled with soft reticular tissue with a small quantity of homogeneous, slightly differentiated nuclei. In addition,



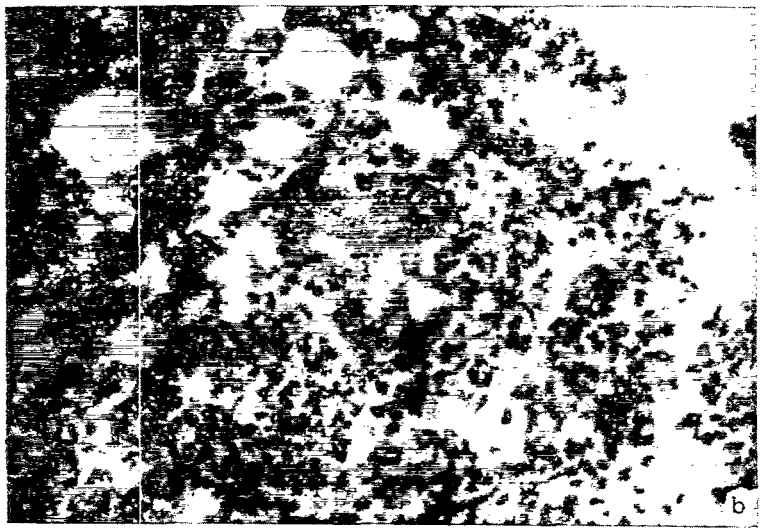


Figure 22. Abundant desquamation of endothelium and epithelium of bronchi in lung of rat (a) and destruction of wall of vessel (b). Magnification x 200.

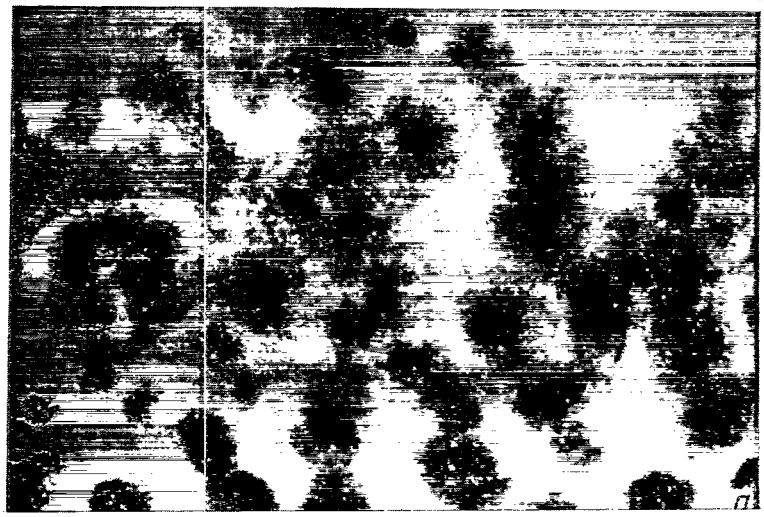
there was an abundant growth of interstitial tissue, not noted in the dogs. /98 The hemorrhagic syndrome became more sharply expressed, since in the place of the capillaries extensive lacunae filled with blood were formed in the interstitia (fig. 24b). Histocytes filled with granules of hemosiderin covered the walls of the capillaries. After 1.5-2 months these changes were accompanied in individual ductules by spermatogonia and larger cells (spermatocytes). Some of them were in a state of division (fig. 25a). However, in the anaphase stage

Figure 23. Destruction of lymph follicles in spleen of rat 2 weeks after end of irradiation. Magnification x 200.

there sometimes was observed a picture of divergence of the chromosomes, evidence of incompleteness of the occurring divisions. In the lumens of the ductules there were also atypical two- and multi-nuclei cells (fig. 25b). In some parts of the ductules the process of spermatogenesis reached the stage of formation of sperm cells (fig. 26).

In summarizing the data from the morphological investigations we noted that in the internal organs of rats, subjected to multiple irradiation by protons with energies of 510 Mev in total doses of 750 and 1,015 rad, there is development of changes characteristic of acute radiation sickness, and these are expressed most clearly in the first 2 or 3 weeks after termination of irradiations. Later repair sets in the lymphoidal organs, and indications of the hemorrhagic syndrome disappear in all the internal organs except the testes. In the latter the most frequent hemorrhages are observed 1-1.5 months after /99 termination of the experiment. Simultaneously, regeneration is observed in the ductules at this time, like in the spleen. A small number of cells of the seminific epithelium and Sertoli cells appears in individual ductules. The latter indicates the possibility of restoration, although incomplete, of spermatogenesis in later periods after irradiation.

Figure 24. Devastation of sperm ductules (a) and hemorrhages in interstitial tissue (b). Magnification x 200.



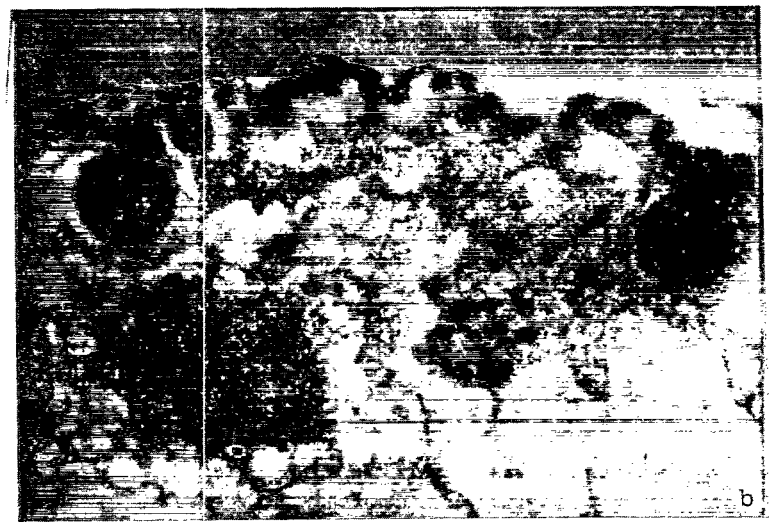


Figure 25. Division of spermatogenic cells 1.5 months after irradiation (a); gigantic multinuclear cells in lumen of ductule (b). Magnification x 200.

Figure 26. Restoration of spermatogenesis 2 months after irradiation. Magnification x 200.

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CHAPTER 4. BIOLOGICAL EFFECT OF PROTONS WITH ENERGIES OF 510 MEV DURING SINGLE IRRADIATION /101

A. F. Bibikova, N. G. Darenskaya, N. A. Derbeneva, M. P. Domshlak, A. N. Ganshina, G. K. Gerasimova, G. T. Ivanenko, G. M. L'vitsina, Yu. I. Moskalev, Yu. G. Nefedov, I. K. Petrovich, A. L. Pozdnyakov, N. I. Ryzhov, V. V. Shikhodyrov, L. T. Tutochkina, M. S. Uspenskaya and V. V. Yakovlev

Experiments on Dogs

The experiments were made on mongrel dogs of both sexes weighing 8-16 kg. Twenty-one dogs were used in the experiment. The animals were irradiated by protons with energies of 510 Mev with a single dose of 550 rad (group I--5 dogs), 400 rad (group II--5 dogs), 250 rad (group III--5 dogs) and 330-690 rad (group IV--6 dogs). The dose intensity was 1-1.5 rad/sec. The dogs were irradiated in a plastic box. In order to create a more uniform spatial distribution of radiation in the body of the dogs, radiation was directed from two sides (head-tail and tail-head). Table 1 shows the four series of experiments; table 2 gives data on the lifespan of the animals; table 3 gives the results of series IV of the experiments.[1]

Acute radiation sickness developed in all the animals after exposure /102 to the protons. The duration of the latent period depended on the magnitude of the dose and was 3-8 days for a dose of 550 rad (dogs of group I) and 9-15 days for a dose of 250 rad (dogs of group III). On completion of the latent period, on the 4th-9th or 10th-16th days, there were clinical symptoms of illness characterized by increasing listlessness of the animals, deterioration of alimentary excitability, diarrhea, increase of body temperature to 39.7-40°, hemorrhaging in mucous membranes, skin and subcutaneous cellular tissue and necrotic angina (table 3). Due to the extreme manifestation of these symptoms, 18 of the 21 dogs died on the 7th to the 14th day after irradiation. Three dogs of group III, irradiated with a dose of 250 rad, survived, but experienced a severe form of radiation sickness.

Peripheral Blood. Changes of the peripheral blood were characterized by a sharp decrease in the quantity of erythrocytes, leukocytes, thrombocytes and reticulocytes.

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[1] The dogs of group IV were irradiated with unidentical doses as a result of a gradual decrease in the intensity of the proton beam of the synchrocyclotron at the time of irradiation (see the results of irradiation of dogs of group IV). The irradiation of the dogs of groups I-III was done on June 30, 1962.

TABLE 1. PLAN FOR SINGLE IRRADIATION OF DOGS BY PROTONS WITH ENERGIES OF 510 MEV /102

Group	Dose, rad	Number of animals	Number which died	Average lifespan, days
I	550	5	5	7.6
II	400	5	5	11.4
III	250	5	2	14.2
IV	330-690	6	6	8.1

TABLE 2. RESULTS OF SINGLE IRRADIATION BY PROTONS WITH ENERGIES OF 510 MEV OF DOGS IN GROUP IV

Name of dog	Dose, in rad	Result	Lifespan, days
Strelka.	690	All dogs died	4.0
Belka.	550		9.0
Kortik	520		9.0
Peschinka.	495		9.0
Trusikha	440		9.0
Kameliya	330		9.0

The decrease in the quantity of erythrocytes was observed already on the 1st-3d day after irradiation. The maximum decrease occurred on the last days of life of the animals. A more clearly expressed decrease in the quantity of erythrocytes was observed in dogs irradiated with a dose of 250 rad (group III); this can be attributed to the greater lifetime of the animals, making possible fuller manifestation of the changes (fig. 1).

The change in the content of hemoglobin was parallel to the changes in the quantity of erythrocytes. As a result, the color index did not change significantly, with the exception of the 1st and 3d days when there was an insignificant, but statistically reliable increase in animals of groups I and II, which were irradiated with doses of 550 and 400 rad.

The change in the quantity of reticulocytes was the most pronounced. The intensity of its decrease during the first hours after exposure was so great that in the overwhelming majority of the animals of all groups there were no reticulocytes in the peripheral blood 24 hours after irradiation. The appearance of reticulocytes was noted only in the surviving animals of group III on the 19th day.

The changes in the content of leukocytes were expressed very sharply as well (fig. 1 and tables 4-6). There were no appreciable differences in the rate and depth of development of leukocytes in the dogs of groups I and II. A considerable decrease in the quantity of leukocytes (up to 200-300 cells per 1 mm^3 of blood) was already observed on the 7th-12th day. In the 3 dogs which

survived irradiation with a dose of 250 rad the decrease of leukocytes was not expressed clearly, and the quantity of leukocytes did not drop below 1,300 per 1 mm^3. Leukopenia was caused by decrease in the quantity of neutrophilic cells and lymphocytes. /105

TABLE 3. CLINICAL SYMPTOMS OF SICKNESS AND TIME WHEN OBSERVED (MEAN DATA FOR EACH GROUP OF ANIMALS)

Symptoms	Group I (550 rad)		Group II (400 rad)		Group III (250 rad)	
	Time of occurrence	Frequency of occurrence	Time of occurrence	Frequency of occurrence	Time of occurrence	Frequency of occurrence
Decrease of motor activity.....	4.6	All dogs	7.6	All dogs	11.4	All dogs
Decrease of alimentary excitability..	2.6	"	6.0	"	9.6	"
Blanching of mucous membranes.....	3.6	"	6.8	"	9.9	"
Increase of body temperature.....	5.0	"	7.6	"	13.4	"
Gingivitis.....	6.1	4 dogs	9.8	4 dogs	12.5	2 dogs
Necrotic angina....	7.2	"	11.0	3 dogs	14.0	3 dogs
Diarrhea.....	6.8	"	11.0	4 dogs	14.0	2 dogs
Hemorrhages in mucous membranes...	6.6	"	11.0	1 dog	15.0	"
Hemorrhages in skin and subcutaneous cellular structure.	7.6	2 dogs	7.0	2 dogs	15.0	"
Hemorrhages in gastrointestinal tract	6.3	All dogs	11.0	3 dogs	14.0	1 dog

The changes in the quantity of thrombocytes in the blood were extremely characteristic. On the first day there was an initial significant increase of the quantity of thrombocytes, especially in the animals of groups II and III, subjected to a dose of 400 and 250 rad (fig. 2). Maximum increase was observed on the first and third days, and thereafter there was a rapid decrease of the quantity of thrombocytes; by the seventh-tenth day their number was 8-30 percent of the initial value.

Cardiovascular System. During the latent period there was no appreciable change of the pulse rate; it remained in the range of 68-90 min. At the height of illness, especially in the preterminal period, there was sharp quickening of the pulse to 120/min or even more.

Changes of arterial pressure[1] had a phase character (table 7 and fig. 3). The reaction in the course of the first days after irradiation for the most part was identical for all groups of animals, and initially (1st-3rd day after irradiation) was characterized by a brief decrease of maximum and minimum pressure by 10-12 mm Hg; then there was normalization of arterial pressure. In the groups I and II of the animals, the maximum and minimum pressure by the beginning of the 5th day attained normal level. However, at the close of life there again was considerable decrease of arterial pressure. In the animals of group III there was relative stabilization and even some increase of maximum pressure from the 5th to the 10th day and then a considerable decrease (by 30-40 mm Hg) of arterial pressure. /106

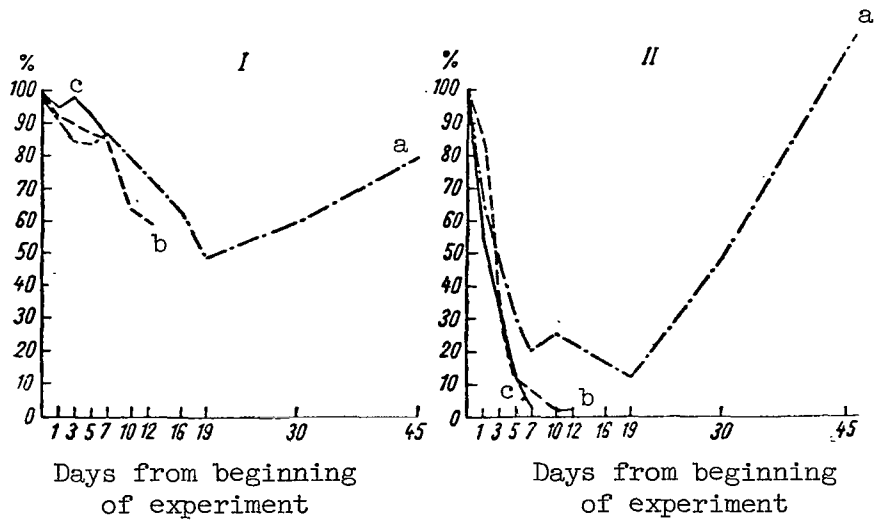


Figure 1. Changes of quantity of erythrocytes (I) and leukocytes (II) in dogs irradiated with 250 (a), 400 (b) and 500 rad (c).

The condition of the vascular system was studied only in the dogs of group IV, irradiated with doses of 330-690 rad. Maximum arterial and vein pressure, arterial and vein tone and the rate of blood flow in the vessels of the outer ears of the dogs were determined.

In 5 dogs (doses 550-330 rad) the changes in the peripheral vascular system developed uniformly. No appreciable impairments were observed immediately

[1]Arterial pressure was measured in the femoral artery of the rear leg. Multichannel amplifiers were used for simultaneous recording of pressure in a sensing element pressing the extremity near the hip, and a sphygmogram was obtained from the dorsal artery of the foot of this same extremity. The maximum arterial pressure corresponded to the pressure in the sensing element during appearance of arterial oscillations; minimum arterial pressure corresponded to the pressure at which all the waves of the sphygmogram became clearly noticeable.

after irradiation. Maximum changes of arterial and vein tone and skin /107
temperature were noted in later periods. Before irradiation the value of arterial tone in the different animals varied in the range 0.28-0.60, and the venous tone varied in the range 0.28-1.8. Before irradiation the skin temperature on the different days of the investigation varied in the range 33-37°. Change of these indices after irradiation occurred simultaneously. Decrease of skin temperature always was accompanied by an increase of the tone of both arteries and veins. These changes were recorded as early as the 1st-3rd day, followed by onset of a temporary normalization of the peripheral blood circulation. In the period of well-expressed manifestations of radiation sickness the arterial and venous tone increased again, and the temperature of the skin of the ear decreased sharply to 18-23° (fig. 4).

TABLE 4. CHANGES OF INDICES OF PERIPHERAL BLOOD IN DOGS OF GROUP I (550 RAD)

Date and time of examination, days	Number of erythrocytes, in 1 mm^3	Hemoglobin content, Sali method, %	Number of reticulocytes, %	Number of thrombocytes, thousands in 1 mm^3	Number of leukocytes, thousands in 1 mm^3
Before irradiation					
23/VI	7.13±0.40	101±6.3	9	296±45	9.54±1.26
26/VI	7.25±0.75	96±3.5	8	256±55	9.26±1.73
28/VI	6.71±0.69	93±3.8	3	---	9.78±1.67
Mean before irradiation	7.03±0.45	97±4.2	7	276±30	9.52±1.65
1st	6.70±0.77	96±3.8	0	274±51	5.46±0.71
3d	6.85±0.33	98±3.6	0	279±58	3.38±1.29
5th	6.47±0.37	91±4.5	0	206±30	1.28±0.32
7th	5.92±0.30	87±6.9	0	90±22	0.38±0.06

An increase of arterial pressure (to 120-140 mm Hg) was noted on the 2d day in two dogs. Low arterial pressure was recorded for 4 dogs on the last days of their life; the pressure dropped in individual cases to 20-40 mm Hg.

Venous pressure did not change significantly after irradiation. In individual cases it was not possible to determine blood flow due to its very slow rate.

The volumetric rate of blood flow, measured in the auricular artery of dogs, varied before irradiation in the range 4-10 mm^3/sec. In the case of 3 dogs it decreased on the 1st day after irradiation to 2-3.5 mm^3/sec. Before the death of the animals there was considerable slowing of the rate of blood flow to 1 mm^3/sec (fig. 5).

TABLE 5. CHANGES OF INDICES OF PERIPHERAL BLOOD IN DOGS OF GROUP II

Date and time of examination, days	Number of erythrocytes, in 1 mm^3	Hemoglobin content, Sali method, %	Number of reticulocytes, %	Number of thrombocytes, thousands in 1 mm^3	Number of leukocytes, thousands in 1 mm^3
Before irradiation					
23/VI	7.81±0.85	85±3.8	5	190±35	11.99±1.44
26/VI	7.45±0.44	87±2.3	11	153±40	11.95±1.58
28/VI	7.70±0.15	84±3.3	3	---	11.32±1.07
Mean before irradiation	7.65±0.35	85±2.5	.6	171±19	11.75±1.19
After irradiation					
1st	7.10±0.94	83±5.6	0	167±56	9.87±2.41
3d	6.86±0.10	83±1.36	0	257±45	3.78±0.52
5th	6.69±0.30	72±2.5	0	183±24	1.36±0.12
7th	6.49±0.40	68±6.6	0	84±19	0.93±0.24
10th.	4.91±1.25	58±3.4	0	14±8	0.26±0.09

TABLE 6. CHANGES OF INDICES OF PERIPHERAL BLOOD IN DOGS OF GROUP III

Date and time of examination, days	Number of erythrocytes, in 1 mm^3	Hemoglobin content, Sali method, %	Number of reticulocytes, %	Number of thrombocytes, thousands in 1 mm^3	Number of leukocytes, thousands in 1 mm^3
Before irradiation					
23/II	7.50±0.33	95±6.1	5	174±11	11.08±1.49
26/VI	7.62±0.93	86±4.5	6	217±25	11.45±1.01
28/VI	7.51±0.63	89±3.8	3	---	12.16±1.13
Mean before irradiation	7.54±0.48	90±5.5	5	189±15	11.15±1.23
After irradiation					
1st	7.08±0.42	87±4.1	0	263±13	7.36±0.67
3d	6.44±0.49	86±6.8	0	248±31	5.35±1.01
5th	6.34±0.35	78±4.7	0	206±32	3.51±0.34
7th	6.60±0.36	73±3.6	0	123±12	2.31±0.18
10th.	6.01±0.20	73±3.4	0	15±5	2.95±0.67
16th.	4.74±0.67	53±8.6	0	4±2	1.98±0.60
19th.	3.69±0.74	50±12.7	0	9±4	1.33±0.37
30th.	4.54±0.67	53±6.9	31	62±11	5.41±0.70
45th.	6.03±0.23	75±6.2	31	207±88	12.61±0.80

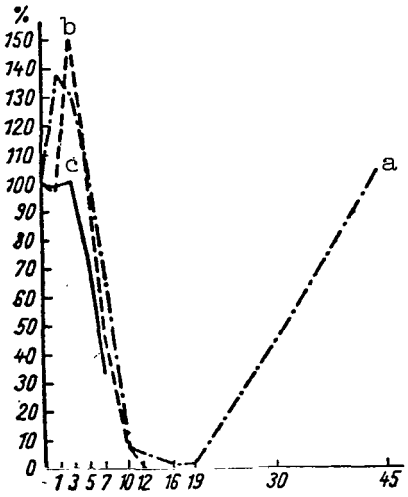


Figure 2. Change of quantity of thrombocytes in peripheral blood of dogs irradiated with 250 (a), 400 (b) and 550 rad (c).

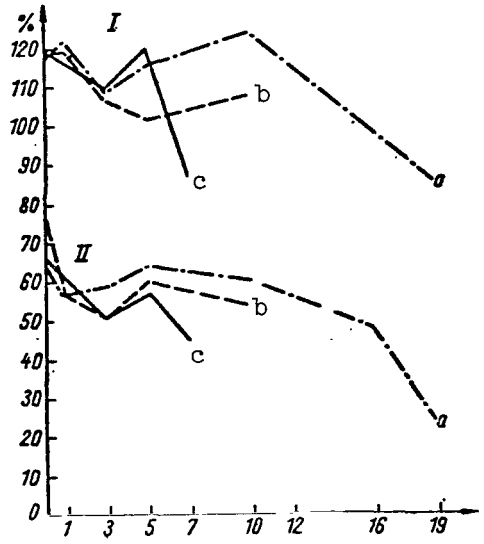


Figure 3. Change of maximum (I) and minimum (II) arterial pressure in dogs irradiated by protons with 250 (a), 400 (b) and 550 rad (c).

Data obtained during the investigation of one dog, irradiated with a dose of 690 rad, merit attention. Directly after irradiation this dog revealed an increase of arterial pressure to 100 mm Hg, in a comparison with 75 mm Hg before irradiation (table 8). On the 1st day arterial pressure decreased to 55 mm Hg and arterial and venous tone increased. At the same time there was /109 a drop in the rate of blood flow. On the 2d day there was some normalization of arterial and venous tone and the rate of blood flow, but skin temperature of the ear dropped to 32.3°. On the 3d day, despite the critical general condition of the dog, these indices differed little from the initial data. The value of venous tone was somewhat greater (0.8) than usual. However, at the end of this day there was onset of sharp impairment of blood circulation, the values of the arterial and venous tone increased to maximum values, there was decrease of the rate of blood flow, arterial pressure decreased to 58 mm Hg and skin temperature of the ear to 23°. After several hours (in the evening, on the 4th day after irradiation) the dog died.

Gaseous Interchange in a State of Relative Rest. Gaseous interchange was determined in the morning at a time of fasting. At this time the experimental animals were placed for 15 minutes in an airtight enclosed chamber with a volume of 136 liters. At the end of this period air samples were taken for study of the content of oxygen and carbon dioxide gas in a Holden apparatus. The results

TABLE 7. CHANGE OF MAXIMUM AND MINIMUM ARTERIAL PRESSURE IN DOGS OF GROUPS I, II AND III

	Group I (550 rad)		Group II (400 rad)		Group III (250 rad)	
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
Before irradiation						
26/VI. . . .	120 ± 1.9	69 ± 3.8	120 ± 4.8	70 ± 0	115 ± 4.7	68 ± 2.8
28/VI. . . .	120 ± 3.8	64 ± 3.8	116 ± 6.0	64 ± 3.6	119 ± 4.7	62 ± 0.9
Mean before irradiation. .	120 ± 2.8	67 ± 3.8	118 ± 5.4	77 ± 3.6	117 ± 4.7	65 ± 1.7
After irradiation						
1st.	117 ± 6.7	61 ± 3.8	120 ± 7.3	57 ± 3.6	122 ± 3.8	57 ± 2.8
3d	110 ± 6.7	51 ± 3.8	107 ± 4.9	51 ± 6.0	109 ± 7.6	59 ± 11.2
5th.	120 ± 3.8	57 ± 0.9	102 ± 1.2	60 ± 3.6	115 ± 5.7	64 ± 2.8
10th	-	-	108 ± 10.9	54 ± 2.4	124 ± 5.7	61 ± 2.8
16th	-	-	-	-	98 ± 12.0	48 ± 3.4
19th	-	-	-	-	85 ± 8.7	23 ± 8.6

of consumption of O_2 and release of CO_2 were reduced to normal conditions and converted to 1 kg of weight per min. Determination of gaseous exchange in a state of relative rest was made using animals (groups II and III) irradiated with a dose of 400 and 250 rad. The dogs were kept for a long time in an airtight chamber to obtain stable background data.

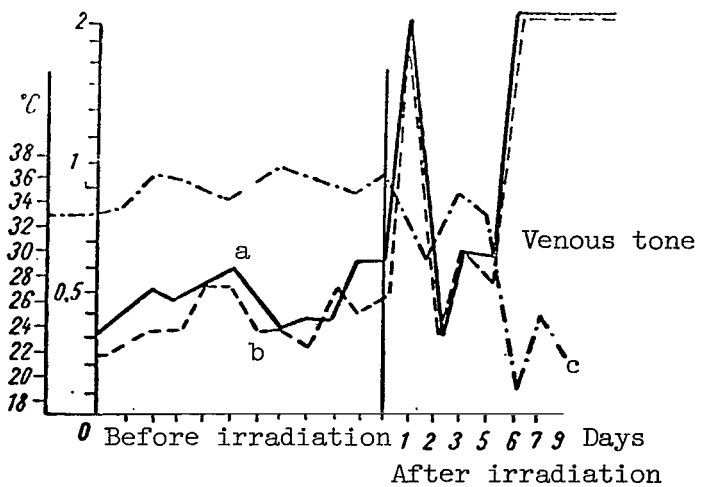


Figure 4. Change of arterial (a--in mm^{-1}) and vein (b--in cm^{-1}) tone in vessels of auricle and in skin temperature (c) in dog Peschinka, irradiated by protons with 495 rad.

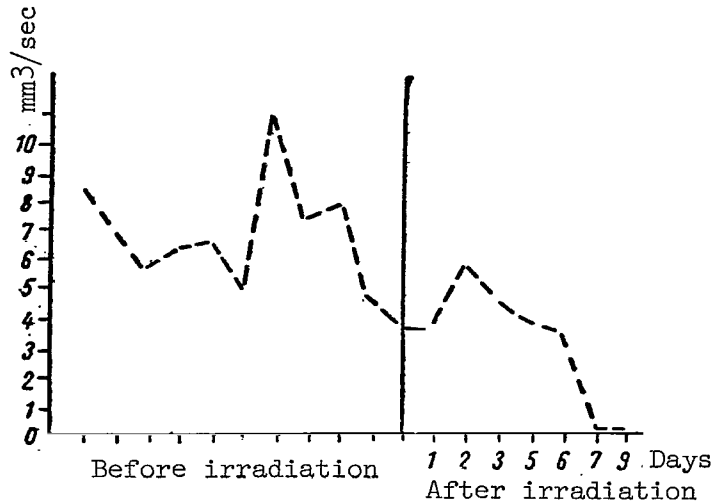


Figure 5. Change of circulation rate in vessels of auricle in dog Peschinka, irradiated by protons with 495 rad.

The most characteristic reaction to irradiation was a decrease (by 35-50 percent) of the liberation of carbon dioxide gas and the consumption of oxygen (fig. 6). These changes usually appeared immediately after irradiation and continued to the death of the animals. In the 3 dogs of group III, which survived after irradiation with a dose of 250 rad, this decrease was also observed during the period of the most clearly expressed manifestations of the illness. A characteristic aspect of these changes is the relatively clear manifestation

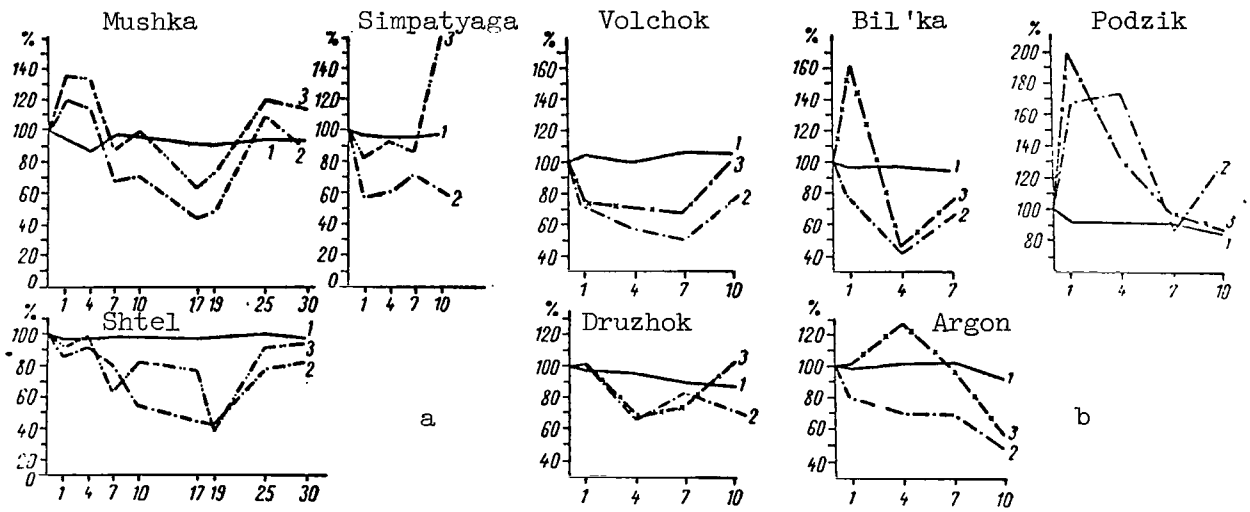


Figure 6. Oxygen consumption and release of carbon dioxide gas; dogs irradiated by protons with 250 rad (a) and 400 rad (b): (1) weight; (2) O_2 absorption; (3) CO_2 release. Along x-axis--days from beginning of experiment (a) and days after irradiation (b).

of changes in the consumption of oxygen: on a general background of decrease of exchange processes there was an increase of the respiratory coefficient. In some cases this decrease in the level of liberation of carbon dioxide gas was preceded by a 3-7 day period of extremely pronounced (30-60 percent) increase in liberation of carbon dioxide gas and a considerable increase of the consumption of oxygen. Only in one case there was a reaction characterized by a sharp increase of the consumption of oxygen and liberation of carbon dioxide (the dog Lobzik) (fig. 6b).

TABLE 8. CHANGE OF SOME INDICES OF CONDITION OF PERIPHERAL VASCULAR SYSTEM OF DOG STRELKA AFTER SINGLE IRRADIATION BY PROTONS WITH ENERGIES OF 510 MEV WITH DOSE OF 690 RAD (GROUP IV)

Days	Tone		Pressure, mm Hg		Blood flow rate, mm^3/sec	Pulse rate, beats/min	Ear skin temperature, °C
	Arterial mm^{-1}	Venous cm^{-1}	Arterial	Venous			
Before irradiation	0.40	0.66	--	--	5.3	100	35.5
	0.28	0.58	--	--	7.3	80	36.3
	0.50	0.50	--	--	8.2	78	37.0
	0.50	0.40	--	--	6.5	68	34.6
	0.33	0.50	--	--	7.1	68	35.0
	0.40	0.58	--	--	6.3	84	35.7
	0.33	0.66	--	--	6.5	80	36.4
	0.33	0.66	--	--	4.5	100	38.9
	0.33	0.62	80	4	4.3	96	35.0
	0.33	0.52	75	0—2	4.5	108	35.6
After irradiation							
0.....	0.50	0.66	100	20.0	3.8	124	35.0
1st.....	0.66	2.00	55	13.5	2.0	80	33.4
2d.....	0.40	0.38	--	5.0	4.3	72	32.3
3d morning	0.47	0.80	80	11.0	4.2	120	33.3
3d evening	2.00	5.00	58	0—2	0.8	124	23.0

Note. Recording of background indices of condition of peripheral vascular system was carried out for 2 weeks before irradiation.

Thus, the irradiation of dogs by protons with energies of 510 Mev with dose of 400 and 250 rad (groups II and III) caused a substantial change of exchange processes. A decrease of the general level of oxidation processes with a predominant decrease of the consumption of oxygen and an increase of the respiratory coefficient was most characteristic. These changes apparently are the result of the predominance of an anaerobic type of tissue respiration.

Change of Immunobiological Reaction. Changes of the immunobiological reaction, and in particular a nonspecific resistance to infection, are of great importance in the pathogenesis of infectious complications during radiation sickness.

The state of nonspecific resistance of the organism to infection was determined using the following indices: bactericidal properties of the cutaneous coverings, quantitative and qualitative composition of the flora of the oral cavity, bactericidal properties of the serum and phagocytic activity of the neutrophils of the blood.

As is well known, the cutaneous and mucous coverings possess a considerable bactericidal effect. The change of the bactericidal properties of the cutaneous coverings was studied for the first time in radiation sickness by N. N. Klemparskaya (ref. 1).

Investigation of the bactericidal properties of the cutaneous coverings of the experimental animals was made by the Klemparskaya method (ref. 1). Impressions were taken from shaved parts of the skin on the sides of the torsos of the dogs. The index of the bactericidal character of the skin was calculated (percentage of dead colonies 15 min after application of Bacterium coli to the skin). Data on the change of the index of bactericidal effect of the /112 skin of dogs of groups III, II and I, irradiated with doses of 250, 400 and 550 rad, are given in figure 7, which shows that dogs irradiated by protons manifested a considerable decrease of bactericidal effects of the skin. The character of the change of the bactericidal effect was almost identical in the dogs of all groups. A clear decrease of bactericidal properties of the skin was already noted on the 1st day after irradiation. On the 3d-5th day the bactericidal properties of the skin intensified somewhat. However, in all animals this function of the skin always remained below the initial level. In the dogs of groups I and II, irradiated with doses of 550-400 rad, a second sharp decrease of the bactericidal effect was observed beginning on the 7th day and up to the time of their death. For example, in the dogs of group II, irradiated with protons with a dose of 400 rad, the index of the bactericidal effect on the 10th day after irradiation dropped by 40 percent in comparison with the initial data. In 3 dogs of group III which survived after irradiation with a dose of 250 rad the bactericidal properties of the cutaneous coverings remained low during the entire time of observation (19 days).

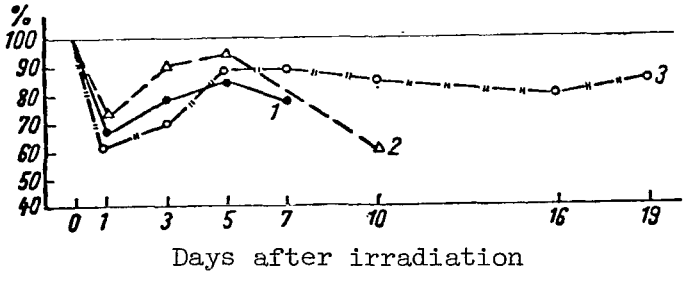


Figure 7. Change of bactericidal index (percent of loss of colonies). Horizontal line -- initial bactericidal level: (1) 550 rad; (2) 400 rad; (3) 250 rad.

TABLE 9. VALUES X (IN PERCENT) IN DOGS AT DIFFERENT TIMES AFTER IRRADIATION (FOR INDEX OF BACTERICIDAL EFFECT OF SKIN)

Irradiation dose, rad	Days after irradiation						
	1st	3d	5th	7th	10th	16th	19th
550	2.99/98	2.65/95	Not reliable	2.99/98	--	--	--
400	3.31/99	Not reliable	Not reliable	2.85/95	Not reliable	--	--
250	3.31/99	3.31/99	Not reliable	Not reliable	Not reliable	2.35/95	2.35/95

Note. Numerator gives value X, denominator value p, expressed in percent.

Statistical analysis of the values of the index of bactericidal effect of the skin (using criterion X) confirmed the reliability of the values of decrease of the bactericidal effect after irradiation in a comparison of these values with the initial data (table 9).

In the course of radiation sickness there is a change of bactericidal properties not only of the cutaneous coverings, but other tissues as well. There is also a considerable change of the bacteriolytic properties of the mucous membranes. The antibacterial properties of the mucous membranes are ensured by a considerable number of factors: adsorption of bacteria by the epithelial cells, phagocytosis by leukocytes and the effect of the lysozyme enzyme.

According to data presented by O. G. Alekseyeva (ref. 2), in the course of radiation sickness there is decrease both of the quantity of saliva and of

TABLE 10. DISTRIBUTION OF ANIMALS AS FUNCTION OF PRESENCE OF COCCAL FLORA IN ORAL CAVITY OF DOGS BEFORE AND AFTER IRRADIATION

Days after irradiation	Number of dogs corresponding to quantity of coccal flora					
	0	0-50	50-100	100-500	500-1000	1000
Initial level	15	0	0	0	0	0
1st	0	14	1	0	0	0
3d.	0	10	3	1	1	0
5th	0	0	0	0	0	15
7th	15	0	0	0	0	0
10th. . . .	8	0	0	0	0	0
16th. . . .	3	0	0	0	0	0
19th. . . .	0	1	0	2	0	0

lysozyme. The bacteriolytic properties of the mucous membranes can be judged indirectly on the basis of an analysis of microflora. It is known that in most cases particular species of microflora live in the oral cavity of /113 different animals. They enter the oral cavity from without and in part from the tonsils, upper respiratory tract, etc. The greatest part of the microorganisms entering the oral cavity of organisms is annihilated rapidly as a result of the effect of the antimicrobal properties of the saliva.

Illnesses of the upper respiratory tract are accompanied by changes of the qualitative and quantitative composition of the flora in the oral cavity.

The problem of the state of the microflora of the oral cavity during radiation illness has been investigated by G. A. Shal'nova (ref. 3). She found both an increase in the quantity of microbes and a change of their qualitative composition and the appearance in the oral cavity of Bacterium coli, normally absent. The study of microflora in the oral cavity was made by the method developed by Shal'nova.

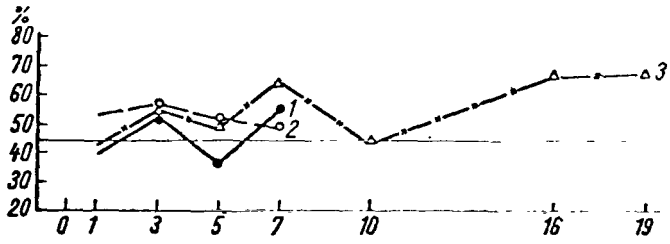
An investigation of the dogs before irradiation revealed that the content of microbes in the oral cavity was small. The growth of microbes usually was not noted when the emulsion was seeded from disk impressions onto an Endo medium. Irradiation of the dogs by protons with energies of 510 Mev led to an increase of the quantity of coccal flora in the oral cavity. The dynamics of change of the quantity of coccal flora in dogs in different periods after irradiation is indicated in table 10. Beginning on the 1st-5th day after irradiation there was an increase of microflora in the oral cavity. The maximum number of colonies was sown on the 1st-3d day in dogs of groups I and II, irradiated with doses of 550 and 400 rad. The maximum quantity of microbes (more than 1,000 colonies) was sown on the 5th day in all dogs, regardless of the dose to which they had been subjected. On the subsequent days after irradiation (7th-16th day) there was a considerable decrease of coccal flora in the oral cavity.

In the 3 dogs of group III which survived after irradiation with a /114 dose of 250 rad the increase of microflora in the oral cavity occurred on the 19th day. The increase of the quantity of cocci in the oral cavity of dogs undoubtedly was related to a considerable decrease of the bacteriolytic properties of the mucous membrane after irradiation.

The collected data indicate that in dogs irradiated by protons with energies of 510 Mev there is an appreciable decrease of the bactericidal function of the covering tissues.

However, it is not only the covering tissues which possess bactericidal properties. This property is also characteristic of the internal medium. The blood serum plays an important role in the purification of the blood from bacteria. Bacteria usually do not multiply in the blood due to the high bactericidal properties of the serum.

Determination of the bactericidal properties of the serum was made with respect to Bacterium coli strain 675 in experiments in vitro. Immediately after taking blood, the serum was prepared and 0.5 ml of whole undiluted serum was mixed with a 0.01 ml suspension of Bacterium coli (the dilution contained



Days after irradiation

Figure 8. Bactericidal change (percent of death of colonies) of serum in dogs. (Annotations same as in figure 7.)

50,000 microbe cells). Immediately after mixing serum and culture, 0.1 ml of this mixture was sown in an Endo medium. This mixture was then placed in a thermostat for 1 hour at 37°, after which 0.1 ml again was sown on an Endo medium. After its incubation with serum for a period of 1 hour the percentage of dead colonies of Bacterium coli was computed.

Data on the change of the index of bactericidal effect of the serum for dogs of groups I-III are given in figure 8. The data presented show that the dogs irradiated with protons with energies of 510 Mev did not show a decrease of bactericidal properties of the serum after irradiation. Some decrease of bactericidal properties of the serum on the 1st and 5th days after irradiation is not statistically reliable. A small activation of bactericidal properties of the serum on the 3d and 7th days after exposure was statistically reliable. In the dogs which died on the 7th-10th day after irradiation no decrease /115 of bactericidal properties was observed, even in the last days of life.

The bactericidal properties of the blood are dependent on a variety of factors: presence of specific antibodies in the serum, level of the complement, properdine, etc. The phagocytic activity of the blood cells is of great importance for purification of the blood from microbes. The phagocytic activity of the neutrophils of the blood was determined with respect to a live culture of Staphylococcus albus.

The percentage of actively phagocytic neutrophils (quantity of phagocytic neutrophils per 100 cells) and the phagocytic index (number of cocci subjected to phagocytic action by one neutrophil per 50 counted cells) were computed.

The phagocytic activity of the neutrophils of dogs irradiated by protons with energies of 510 Mev changes very rapidly.

The dynamics of the phagocytic activity of the neutrophils of the blood of dogs are shown in figure 9. The changes of the phagocytic activity were already noted on the 1st day after irradiation. In the dogs of group III, irradiated with a dose of 250 rad, there was considerable stimulation of the phagocytic activity on the 1st day after irradiation; beginning on the 5th day there was again some increase. However, it attained only 50 percent of the normal level. Moreover, in the dogs of groups I and II, irradiated with doses of 550 and 400

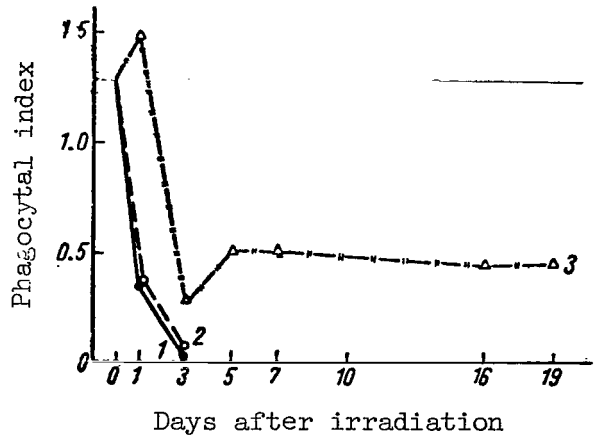


Figure 9. Change of phagocytal activity (index) of neutrophils in dogs. (Annotations same as in fig. 7.)

rad, there was a well-expressed inhibition of phagocytosis which thereafter progressed. Beginning on the 5th day after irradiation it was virtually impossible to count the necessary quantity of neutrophils (due to their absence in the smears).

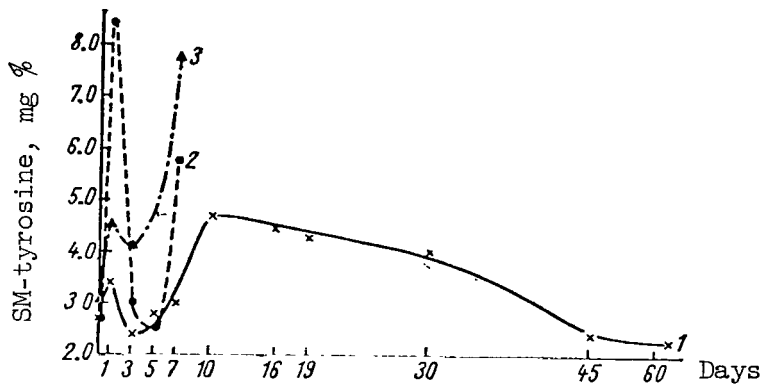


Figure 10. Concentration of serum mucoids in blood of dogs with single irradiation by protons with 250 (1), 400 (2) and (3) 550 rad.

Therefore, during irradiation of dogs by protons in absolutely lethal doses there was an appreciable decrease of phagocytic activity, beginning with the 1st day after irradiation. In the case of lesser doses there was a phase change of phagocytic activity: the activation of phagocytosis on the 1st day and its subsequent inhibition.

These data from the study of the state of natural immunity show that /116 in the dogs irradiated by protons there is an impairment of nonspecific resistance to infection. To one degree or another there was involvement of all

TABLE 11. PROTEIN COMPOSITION OF BLOOD SERUM IN DOGS OF GROUP III IRRADIATED IN DOSE OF 250 RAD (MEAN DATA FOR 5 ANIMALS)

Date & time of examination, days	Total protein, %	SM, mg % tyrosine	Relative content of protein fractions, %				
			A	α_1	α_2	β	γ
26/VI	7.3	2.4	57.00	5.90	7.30	20.9	8.50
28/VI	7.3	2.8	55.00	5.90	7.50	21.1	10.40
1st	6.6	3.4	53.55	5.87	8.51	21.8	10.77
3d	6.1	2.4	52.02	6.66	8.00	20.7	12.48
5th	6.1	2.8	51.25	6.47	8.06	20.5	12.57
7th	6.3	3.0	47.70	8.20	9.00	21.4	12.30
10th	6.5	4.7	48.82	8.49	9.13	23.4	10.65
16th	6.5	4.5	50.83	6.35	9.82	21.7	11.11
19th	7.0	4.3	46.33	7.30	14.14	23.1	8.83
30th	6.6	4.0	44.63	7.03	11.70	25.7	10.51
45th	7.2	2.4	45.13	8.77	9.62	23.8	12.35
60th	6.4	2.3	50.80	7.60	7.90	22.3	10.80

TABLE 12. PROTEIN COMPOSITION OF BLOOD SERUM IN DOGS OF GROUP II IRRADIATED IN DOSE OF 400 RAD (MEAN DATA FOR 4 ANIMALS)

Time of analysis, days	Total protein, g %	SM, mg % tyrosine	Relative content of protein fractions, %				
			A	α_1	α_2	β	γ
Before irradiation							
26/VI 1962	7.4	2.5	60.2	5.3	8.90	17.0	8.5
28/VI 1962	7.5	2.9	57.7	6.4	7.50	18.4	9.4
After irradiation							
1st	7.0	9.0	54.7	5.7	10.16	16.9	10.8
3d	6.1	3.2	50.9	5.9	12.70	19.1	11.5
5th	6.3	2.9	53.7	5.5	13.70	19.8	8.6
7th	6.1	6.7	43.5	7.0	14.40	23.6	11.6

Note. The dog Volchok has been excluded from group II because the initial indices for the protein composition of her blood differed sharply from the average.

studied links of nonspecific reaction. The earliest change was noted in the functions of the cutaneous coverings and the phagocytic activity of the neutrophils of the blood.

Change of the Protein Composition of the Blood Serum. Clear changes of the protein composition of the blood were detected beginning on the 1st day after irradiation. There was a persistent decrease of the total concentration of protein, a decrease of the relative content of albumins and an increase of the relative content of all globulin fractions. There was a particularly significant increase in the concentration of serum mucoids (SM), and α_1- and α_2-globulins (fig. 10 and tables 11-13). /117

The SM changes had a phase character. In the dogs of groups I, II and III there was an increase of the SM level a day after irradiation. On the 3d-5th day there was a decrease to the initial level. This was followed by a phase of increase of the SM level (moderate in the case of a dose of 250 rad and progressive up to the death of the animals with doses of 400 and 550 rad). In 3 dogs which survived after irradiation with a dose of 250 rad the SM level was normalized after 2 months.

The changes of the total concentration of proteins and the composition of the proteins of the serum increased gradually. This could be seen unusually clearly in the dogs of group I, irradiated with a dose of 550 rad (table /118 13). In the dogs of group II, irradiated with a dose of 400 rad, there was a partial normalization on the 5th day after irradiation (table 12). In the dogs of group III, irradiated with a dose of 250 rad, the maximum changes of the protein fractions developed in the period from the 20th to the 45th day. There was no complete normalization of the composition of the protein fractions by the end of the observations (60th day).

TABLE 13. PROTEIN COMPOSITION OF BLOOD SERUM OF DOGS OF GROUP I IRRADIATED IN DOSE OF 550 RAD (MEAN DATA FOR 5 ANIMALS)

Date and time of examination, days	Total protein, g %	SM, mg % tyrosine	Relative content of protein fractions, %				
			A	α_1	α_2	β	γ
Before irradiation							
26/VI	7.4	2.4	55.2	5.6	7.8	21.7	9.0
28 VI	7.5	3.6	55.2	6.0	7.9	20.5	10.1
After irradiation							
1st	6.1	4.5	51.2	5.8	9.7	19.0	13.9
2d	7.7	4.1	50.6	6.3	10.7	18.8	14.6
5th	7.1	4.8	46.5	6.7	13.2	20.2	12.9
7th	6.7	7.7	41.8	7.1	18.2	21.6	10.5

After analyzing these data it can be seen that the most sensitive index is the SM concentration; it should be emphasized that there is a clear dependence between the change of this index and the proton dose.

Activity of Cholinesterases of the Blood Serum. Changes of the activity of cholinesterases (AKhE) in the blood serum of individual dogs in a normal state and after irradiation by protons are shown in figure 11.

A sharp and rather persistent decrease of AKhE in the blood serum was observed in all dogs regardless of the dose. Before irradiation the AKhE was 0.79 ± 0.03 mg of acetylcholine chloride, broken down by 1 ml of serum in 1 hour at t = 37° and at pH = 7.6 (mean data for 15 dogs). In the dogs irradiated by protons with a dose of 550 rad (group I) the activity of the enzyme 1, 3, 5 and 7 days after irradiation decreased sharply and was 0.48 ± 0.04, 0.56 ± 0.03, 0.49 ± 0.03 and 0.46 ± 0.02 mg, respectively. In no dog of this group was there restoration of AkhE to the initial level during the entire lifespan.

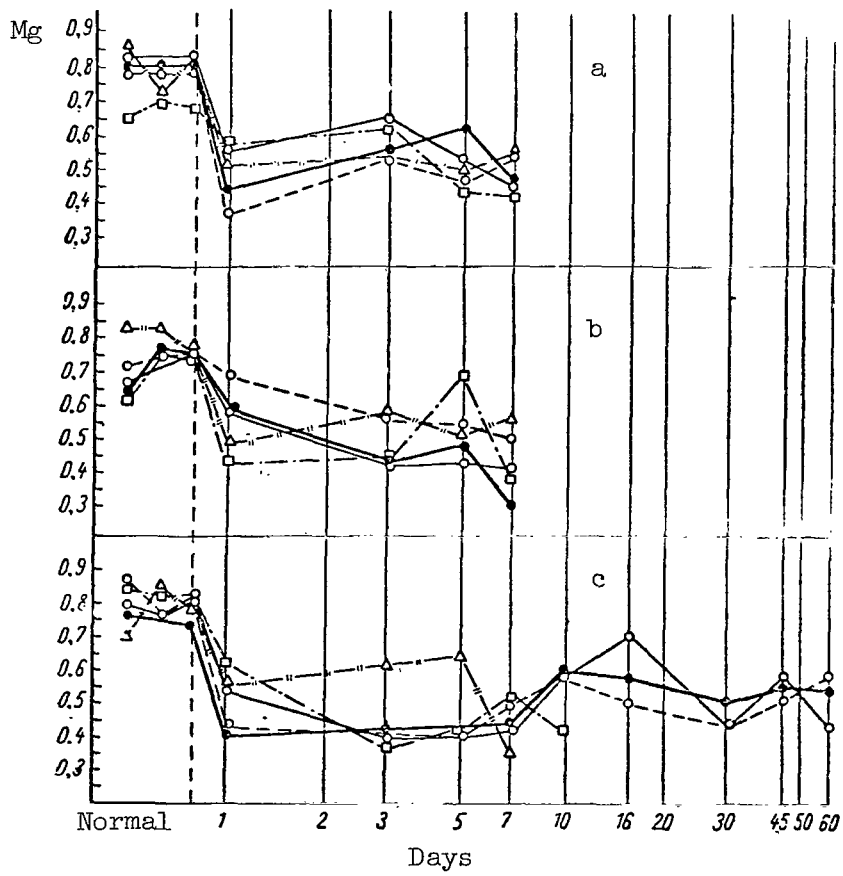


Figure 11. Individual changes of activity of cholinesterase of blood serum in individual dogs at different times following single irradiation with 550 (a), 400 (b) and (c) 250 rad. Along y-axis--quantity of acetylcholine (mg) destroyed by 1 ml of serum in 1 hour. Along x-axis--time of investigation, days.

In the animals irradiated by protons with a dose of 400 rad (group II) there was also decrease in the activity of the enzyme during the entire period of the investigation. After 1, 3, 5 and 7 days from the onset of irradiation the mean data for AKhE for the dogs of this group were 0.55 ± 0.04, 0.48 ± 0.03, 0.53 ± 0.04 and 0.42 ± 0.04 mg, respectively. The maximum decrease of the activity of the enzyme in the case of a dose of 400 rad, as in the case of a dose of 550 rad, therefore was on the 7th day.

In the dogs irradiated by protons with a dose of 250 rad (group III) /119 there was a similar decrease of AKhE. For example, 1, 3, 5 and 7 days after irradiation the AKhE level for the dogs of this group was in the range 0.52 ± 0.04, 0.46 ± 0.04, 0.48 ± 0.05 and 0.46 ± 0.03 mg, respectively. On the 10th, 16th, 30th, 45th and 60th days of the illness the activity of the cholinesterase remained depressed by 30-40 percent; the maximum decrease (by 40 percent) in the 3 surviving dogs was observed on the 30th day after irradiation. The statistical analysis of the derived numerical data revealed that the decrease of AKhE of the blood serum after irradiation by protons at different /120 times in the investigation was reliable.

As a comparison, figure 12 shows the mean data for change of AKhE in the blood serum of dogs after irradiation by protons with doses of 250, 400 and 550 rad. Figure 12 shows that with all doses of irradiation a day later there was a sharp decrease of AKhE in the blood serum by 34 ± 2, 30 ± 1 and 39 ± 5 percent, respectively, in relation to the initial level; on the 3d day a further decrease of the activity of the enzyme appeared: by 41 ± 5, 39 ± 4 and 29 ± 4 percent, respectively. On the 5th day after irradiation the activity of the nonspecific cholinesterase of the serum continued to remain low by 39 ± 3, 33 ± 5 and 38 ± 4 percent, respectively. On the 7th day there was a maximum decrease of AKhE in the dogs of group III by 41 ± 3 percent, in group II by 47 ± 5 percent and in group I by 42 ± 2 percent, relative to the initial data. These data indicate

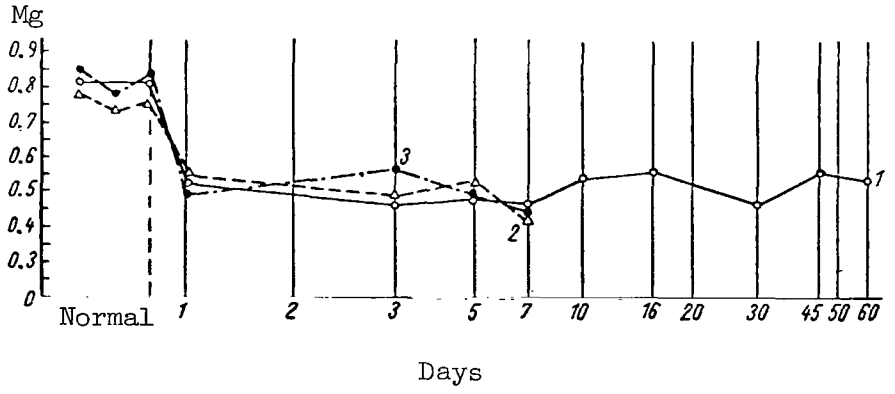


Figure 12. Change of activity of cholinesterase in blood serum at different times following single irradiation of dogs (mean data for group) in dose of 250 (1), 400 (2) and 500 rad (3). Along y-axis--quantity of acetylcholine decomposed by 1 ml of serum in 1 hour. Along x-axis--time of investigation, days.

considerable changes of the activity of the investigated enzyme. It should be noted that there is no dependence between the changes of AKhE and the magnitude of the proton dose.

Pathologic-morphological Investigations. The pathologic-morphological changes developing in dogs after a single irradiation by protons resemble the changes which are noted in acute radiation sickness caused by X-ray or γ-irradiation. The most characteristic manifestations were multiple hemorrhages in the subcutaneous cellular tissue of the torso and neck and also in the internal organs and tissues of the body. A maximum number of hemorrhages was noted in the lungs, heart and along the gastrointestinal tract. Hemorrhages were dis- /121 covered very frequently in the kidneys, primarily in the cortical layer and capsule; in many cases they were found in the urinary tract. The character of the hemorrhages in the different organs and tissues was not the same. In the cellular tissue and lungs the hemorrhages were in the form of extensive hematoma, whereas punctulate hemorrhages were noted in the gastrointestinal tract, kidneys and heart. Damage to the blood-generating organs was sharply expressed. There was aplasia of the spleen, atrophy of the lymphoid mechanism and an almost total devastation of the active bone marrow; the marrow yielded only a bloody fluid instead of a pulpy mass.

In addition to these impairments there usually were dystrophic changes of the internal organs. In most cases there was damage to the liver in the form of flabbiness of the organ and poor definition of its configuration. The kidneys and heart muscle showed less damage. Quite frequently the lungs showed signs of incipient edema, and less frequently a picture of well-expressed edema involving a large part or the entire lung. At autopsy infectious complications

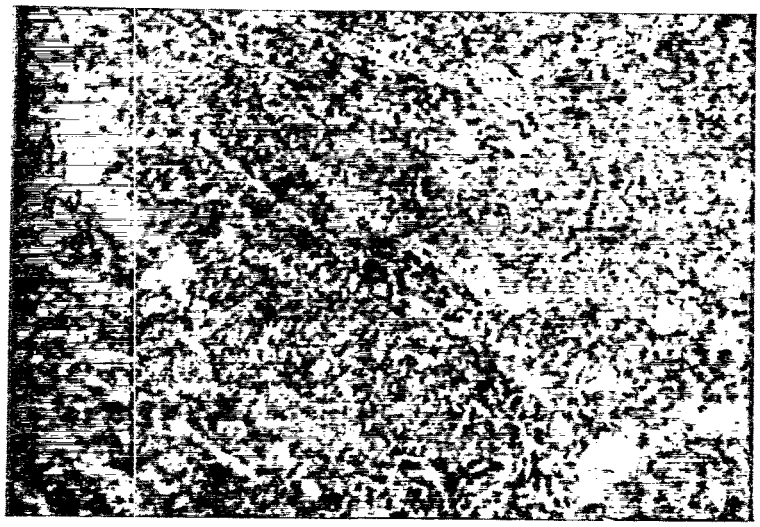


Figure 13. Spleen of dog (30th day after irradiation). Devastation of lymphoid tissue. Hemorrhage. Staining with hematoxylineosin. Magnification 20x10.

in the form of amygdalitis were frequently found. The testicles were usually decreased in size, had a flabby appearance and hemorrhages were frequently found under the capsule.

Microscopic investigation also showed changes in the organs and tissues characteristic of acute radiation sickness. The bone marrow appeared extremely devastated. For the most part cellular elements were preserved which are related to reticular syncytium. From time to time near the bone structures there were individual hemocytoblasts and dying cells of the myeloid series. In the lymph nodes and lymph follicles of the gastrointestinal tract there was considerable loss of lymphoidal tissue. The secondary nodes were poorly expressed and contained virtually no lymphocytes. The cells of the follicles consisted mostly of reticular and plasmatic elements. The lymph spaces were distended and erythrocytes and macrophagia were noted in their lumen. There was sharply expressed edema and plethora. A similar picture was observed in the spleen. The lymphoidal tissue was almost entirely reduced (fig. 13). The red pulp had a uniform appearance and was poor in cellular elements.

The predominant forms of cells were reticular and plasmatic, situated in small clusters around the central arteries and in the spaces near the trabeculae. Considerable erythrophagia was observed. In many cases there were hemorrhages, well-expressed edema and plethora of the vessels.

The lungs usually had a "mottled" appearance, and the fields of hemorrhages alternated with fields of emphysema and atelectasis. In most cases the hemorrhages were situated around the bronchial tubes. The walls of the vessels were distended, edematous and some were homogenized. In the adventitia along the course of the large and often the small vessels there was a large quantity /122 of cells whose cytoplasm was filled with an iron-containing pigment. There was abundant desquamation of the endothelium of the vessels and epithelium of the bronchi. In the alveolar part of the lung there were sectors of accumulation of an albuminous exudate with an admixture of erythrocytes. At the same time there were accumulations of an exudate not containing cellular elements. In many cases it was possible to see foci of necrosis of pulmonary tissue. In most instances they were situated at the center of hemorrhages and usually were characterized by lack of a cellular reaction.

The heart showed distension and opacity of the muscle fibers and small foci of adipose distrophy. In the coronary vessels there were shriveling and vacuolization of the endothelium and in some cases plasmatic impregnation of the walls.

In the gastrointestinal tract there were necrobiosis of the mucous layer and also hemorrhages and edema in the submucosa. These changes were manifested most clearly in the large intestine, where there were considerable foci of necrosis. There was no cellular reaction on the boundaries of the necrosis.

Clearly expressed impairment of the blood and lymph circulatory system and degenerative-necrobiotic changes of the parenchyma of the organ were detected in the liver (fig. 14). The central, collecting veins and the interlobular capillaries were distended and filled with blood. The walls of the vessels /123 were homogenized. In all cases there was disintegration of the hepatic

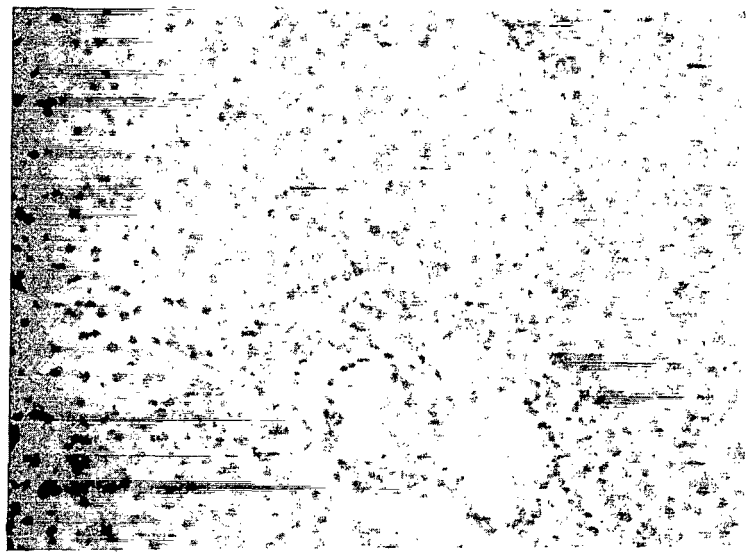


Figure 14. Liver of dog (15th day after irradiation). Disintegration of hepatic lobes. Dilatation and blood congestion of central vein and capillaries. Staining with hematoxylin-eosin. Magnification 20x10.

trabeculae and indications of adipose distrophy with fine drops of fat in the cytoplasm of the hepatic cells at the center of the lobes. The hepatic cells looked swollen and frequently contained 2 or 3 nuclei.

In the kidneys (fig. 15) there were well-expressed impairment of blood circulation and dystrophic changes of the epithelium of the tubules. In the vessels, for the most part in the arterioles of the glomerules, there were foci of homogenization of the wall. In many cases in the glomerules it was possible to see an accumulation of an albuminous liquid very clearly. In the lumen of the straight and convoluted tubules there frequently were erythrocytes and sometimes hyaline casts. The epithelium of the tubules was distended and in places had peeled.

Degenerative and atrophic changes had occurred in the endocrine glands, indicating depression of their function. In particular, in the thyroid gland we found decrease and thinning of colloids. In the thyroid gland there was destruction of insular tissue, which was rich in bright large α-cells. In the testes there was clearly expressed depression of spermatogenesis and /124 degeneration of the seminific epithelium. In the lumen of the sperm ductules there remained only abnormal gigantic cellular forms and remnants of destroyed cells.

The interstitial tissue, in contrast to such tissue subjected to X-ray irradiation (refs. 4-6), was damaged and the number of its cells decreased. In the ovaries there was atrophy and death of the maturing follicles. In the stroma of the gonads there were hemorrhages, distension of the capillaries and granules of hemosiderin.

Thus, the pathologic-morphological investigation of the internal organs revealed that in animals subjected to a single exposure by protons with energies of 510 Mev there is a typical picture of acute radiation sickness with a symptom complex of morphological changes characteristic of the particular illness. However, some difference can be noted. It is expressed in the extraordinarily large number of hemorrhages, accompanied by extensive edema, especially in the subcutaneous cellular tissue and in the lungs.

Macroscopic examination of the brain and spinal cord revealed no changes.

The histologically detected changes in the central nervous system for the most part were similar to the changes noted in dogs with acute radiation sickness, caused by general multiple irradiation by protons with energies /125 of 510 Mev in doses of 650 rad. However, there were some distinguishing characteristics. In particular, there was a clearly expressed fibrous thickening of the pia mater of the brain and spinal cord. Also, there was a sharply expressed fibrosis of the pia mater at the base of the brain in the region of the anterior (chiasma level) and posterior (infundibulum) hypothalamus. The pia mater covering the infundibulum appeared thickened. There was an ingrowth of sclerosed vessels from the pia mater of the base of the brain in the region of the supraoptical nuclei. The most important difference in the histopathological changes in the case of single irradiation must be regarded as the absence of massive hemorrhages in the brain, whereas in the case of multiple irradiation there were foci of extensive intracerebral hemorrhaging or multiple small hemorrhagic foci. However, in this particular case hemorrhagic microfoci were noted

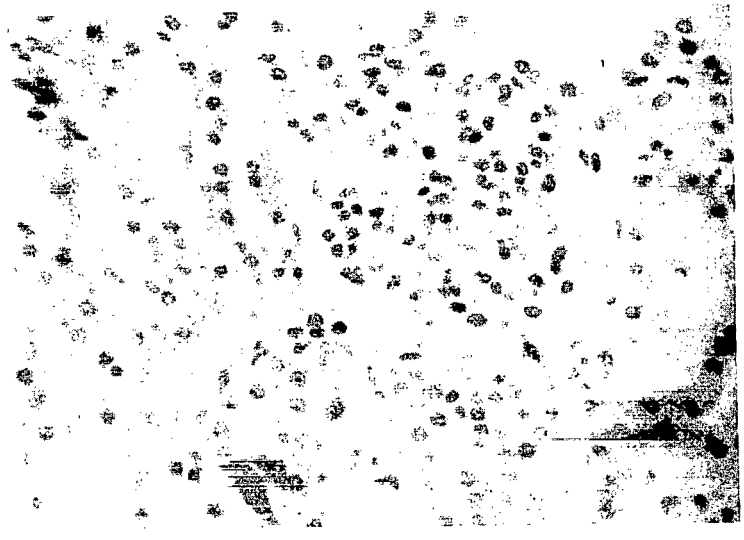


Figure 15. Kidney of dog (14th day after irradiation). Dystrophic changes of epithelium of convoluted tubules and accumulation of albuminous fluid in lumen of tubules and capsule. Staining with hematoxylin-eosin. Magnification 20x10.

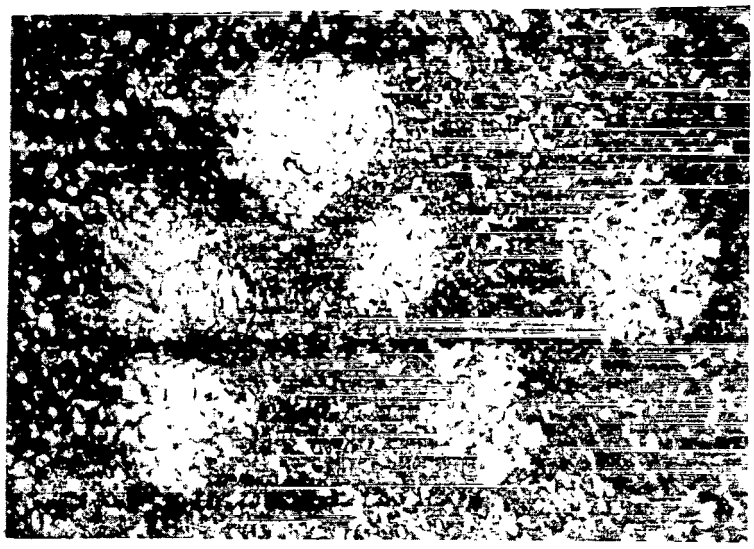


Figure 16. Lysis of nerve fibers in white matter of spinal cord. Magnification 20x10.

only in the hypothalamic region (supraoptical nuclei). In addition, a peculiarity of the histopathology of the central nervous system in the case of single irradiation by protons was the lesser propagation of necroses with disintegration of cerebral white matter, in comparison with multiple irradiation. At the same time, it was noted that there were many "spotty" necroses not associated with the vessels in the white matter of the spinal cord. Among the characteristic features of such necroses are: (a) the strict boundaries between them and the surrounding tissue, so that the necroses appeared in the form of bright spots caused by limited lysis of the nerve fibers, and (b) their localization--for the most part in the white matter (fig. 16).

In sections of the medulla oblongata and pons Variolii in some cases we observed lysis of the perivascular medulla, not only in the white matter, but in the gray matter as well (fig. 17). Vascular dystonia, although observed in the cerebral matter, was not so widespread, for example, as in dogs with a long lifetime (treated dogs). Regardless of the conditions of irradiation, a common characteristic of all animals was change in the circulatory system; these were characterized by edema under the ependyma, pyknosis and necrobiosis of the ependymal cells in many parts. In virtually all cases there was necrobiosis and necrosis of the ependyma of the third ventricle with transition into the infundibulum and in the infundibulum itself. In places on the base of the fourth ventricle there was disintegration of the myelinous nerve fibers. Edema in the region of the cerebral ventricles apparently plays more than a small role in autonomic impairments and, it must be supposed, also in creation of vascular tone.

The nerve cells were damaged selectively. As is well known, selective damage of the nerve cells of the central nervous system is a characteristic of radiation sickness caused by general X-ray and γ-irradiation.

Experiments with Rats /127

The investigation was made using 400 white rats of the Wistar breed, weighing 180-220 g. The animals were subjected to total single irradiation by protons with energies of 510 Mev with doses of 100, 275, 330, 400, 420, 470, 520, 620, 730, 850, 920 and 950 rad, respectively. The irradiation conditions, dosimetric control and maintenance of the animals for the most part were similar to those used in the investigation of the biological effect of multiple irradiations.

The following tests were used for characterizing the condition of the animals: weight, composition of the peripheral blood (quantity of erythrocytes and leukocytes, the leukocytic formula and qualitative changes of the blood cells), as well as data from pathologic-morphological investigations and the general clinical picture. The animals were investigated on the 1st, 3d, 5th, 7th, 10th, 15th, 20th and 30th day after irradiation and then monthly over a 1-1/2-year period.

Clinical Picture. The radiation illness developing in the rats after irradiation by protons with energies of 510 Mev with doses up to 400 rad was light and did not lead to a fatal outcome (table 14). In a period of 3-5 days after irradiation there was some decrease in the activity of the animals, listlessness, deterioration of appetite and a decrease in the intensity or a temporary cessation of weight increase. These phenomena later disappeared rapidly, and before the end of the observations the external symptoms of radiation injury had disappeared.

In the case of irradiation with doses of 420-620 rad passivity and /128
anorexia were expressed more clearly. On the 3d-6th day there was salivation,

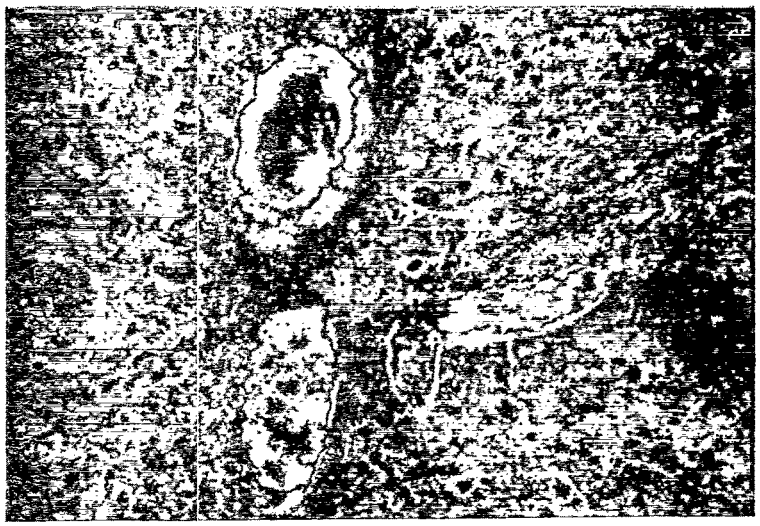


Figure 17. Lysis of perivascular medulla in gray matter of medulla oblongata. Magnification 20x10.

ichorous discharges from the mouth and subconjuctival hemorrhages. The fur was lusterless and dishevelled. In the first 5-7 days after irradiation the animals were slovenly and many had diarrhea. Later these symptoms gradually disappeared in most animals. In the other part of the group of rats the manifestations of radiation injury progressed, and death of the animals set in. Most animals irradiated with doses greater than 600 rad died from the increasing gravity of the symptoms of radiation sickness.

The reaction of the peripheral blood to single irradiation by protons was characteristic for acute radiation sickness, and was manifested by a sharp decrease of the quantity of formed elements of the blood. Clearly expressed changes of the quantity of erythrocytes were observed beginning on the 5th day (table 15). By the 10th day, if the animals still remained alive at that time, the changes attained maximum severity. By this time the quantity of erythrocytes in animals irradiated with doses of 470-520 rad had decreased to 3.6-4.9 million cells/1 mm^3 (fig. 18), which was 60-65 percent of the initial level. Gradual restoration of the quantity of erythrocytes was noted later in the surviving animals. By the beginning of the second month, the quantity of erythrocytes averaged 108, 97, 88 and 71 percent in animals irradiated in doses of 400, 420, 470 and 520 rad, respectively. In the periods which followed (up to 4 months), the content of erythrocytes was restored to the initial level. However, a decrease was then observed. After 12 months the quantity of erythrocytes did not exceed 55-75 percent of the initial level (fig. 19). With the development of erythropenia there were indications of irritation of /129 the red matter of the bone marrow: anisocytosis, increase of the number of polychromatophylic erythrocytes and appearance of erythroblasts in the peripheral blood.

TABLE 14. INJURY TO RATS AS FUNCTION OF DOSE OF RADIATION BY FLUX OF PROTONS WITH ENERGIES OF 510 MEV

Group	Dose, rad	Total number of animals	Number of dead animals	Dead animals, %	Mean life span of dead animals, days
I	330	21	0	0	All survived
II	400	49	0	0	All survived
III	420	21	1	4	16
IV	470	25	7	28	12
V	520	21	7	33	20
VI	620	22	20	91	6
VII	730	25	24	96	5
VIII	850	10	10	100	3.5
IX	920	15	15	100	3.5
X	950	15	15	100	3.5

Note. Total number of animals does not include control animals or those killed for pathologic-morphological investigations.

Still more clearly expressed changes were discovered in the content of leukocytes. Their quantity began to decrease with the 1st day after irradiation. By the 5th-7th day the intensity of the decrease was maximal. By this time the quantity of leukocytes in rats irradiated in doses of 470, 520 and 620 rad did not exceed 3-6 percent of the initial level. In the period from the 7th-10th day there was an insignificant increase in the quantity of leukocytes. Later the rate of restoration increased, but at the end of two months the mean level of the content of leukocytes in most animals had not reached the initial values (fig. 20 and table 16). Eight or nine months later the total quantity of /130 leukocytes with all irradiation doses was 30-40 percent lower than the initial values (fig. 21).

These changes in the total quantity of leukocytes depended on the changes of the absolute quantity of neutrophils and lymphocytes. In the first 7 days after irradiation the absolute quantity of neutrophils and lymphocytes decreased proportionally. Restoration of the quantity of neutrophils began 7 days later. One month later their quantity attained the initial values and later even exceeded them. The increased quantity of neutrophils was maintained during the entire course of the observations, including a long time thereafter (12-16 months). The absolute quantity of lymphocytes in the first 1-7 days decreased by 80-90 percent or more. In the succeeding periods there was restoration, but it was by no means complete. By the end of the first month after irradiation the absolute quantity of lymphocytes did not exceed 55-58 percent. It remained at this level during the entire remaining period of observation -- 2 to 18 months (see fig. 21).

On the whole, it should be noted that the observed changes in the /131 content of leukocytes depend on the irradiation dose. However, analysis of the changes of the number of cells in the leukocytic formula reveals that the shape of the dose-effect curve can change with time and can be different for different kinds of cells (neutrophils, lymphocytes, etc.). Figure 22 presents data

TABLE 15. CONTENT OF ERYTHROCYTES (MILLIONS PER 1 MM^3) IN PERIPHERAL BLOOD OF RATS SUBJECTED TO SINGLE IRRADIATION BY PROTONS WITH ENERGIES OF 510 MEV

Group	Dose, rad	Number of erythrocytes before irradiation	Number of erythrocytes after irradiation, days							
			1st	3d	5th	10th	15th	20th	30th	45th
I	330	7.30	7.20	6.32	6.29	6.57	5.89	6.85	5.35	6.68
II	400	6.61	7.33	6.98	5.18	5.21	5.53	5.56	5.78	6.49
III	420	6.87	8.00	7.54	6.20	6.10	6.84	6.32	6.28	6.10
IV	470	5.93	7.80	7.45	5.50	3.60	4.10	6.00	5.70	6.35
V	520	7.50	7.31	8.31	6.65	4.96	4.87	6.80	5.90	5.81
VI	620	7.20	7.54	7.35	6.15	-	-	-	-	-
VII	730	6.93	6.65	6.56	5.90	-	-	-	-	-
X	950	8.20	-	8.64	-	-	-	-	-	-

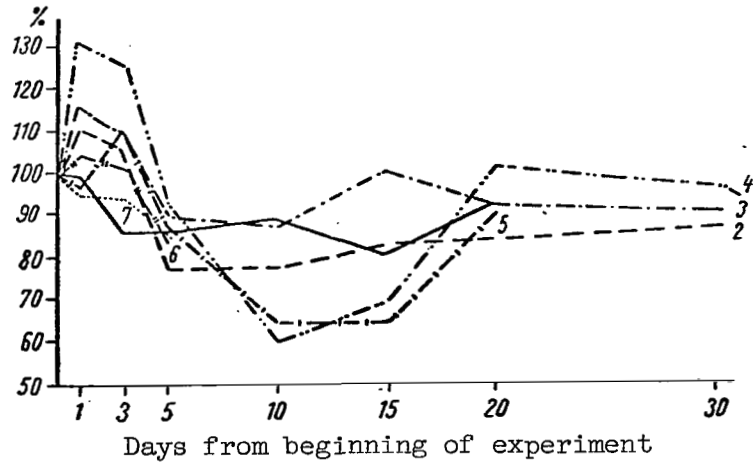


Figure 18. Change of quantity of erythrocytes in blood of rats during first 45 days after irradiation by protons in doses of 330 (1), 400 (2), 420 (3), 470 (4), 520 (5), 620 (6) and (7) 730 rad.

on the changes of the quantity of neutrophils as a function of the irradiation dose and the time of observation from the 1st day to the 14th-15th month. Figure 23 also shows data on the change in the number of lymphocytes. /132

The form of the dose-effect curve for lymphocytes in the period from the 1st-90th day has an S-shape, since the quantity of lymphocytes decreases with increase of the irradiation dose (fig. 23). In later periods the dose-effect curve has a linear character and almost is not dependent on the irradiation dose.

The change of the number of neutrophils has a different and more complex character. Initially (1st-14th day) the dose-effect curve for neutrophils has a linear character, but later (180th day) the quantity of neutrophils increases proportional to the increase of the irradiation dose, and the curve approaches the x-axis. The mechanism of this reaction is not clear, and it can be assumed that neutrophilic leukocytosis is caused by infectious processes complicating the radiation sickness.

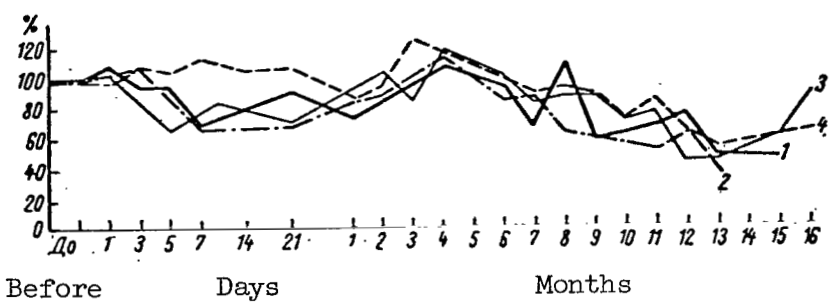


Figure 19. Change of quantity of erythrocytes (percent) in blood of rats in first month after irradiation and much later (months): (1) 850 r; (2) 750 r; (3) 275 r; (4) 100 r.

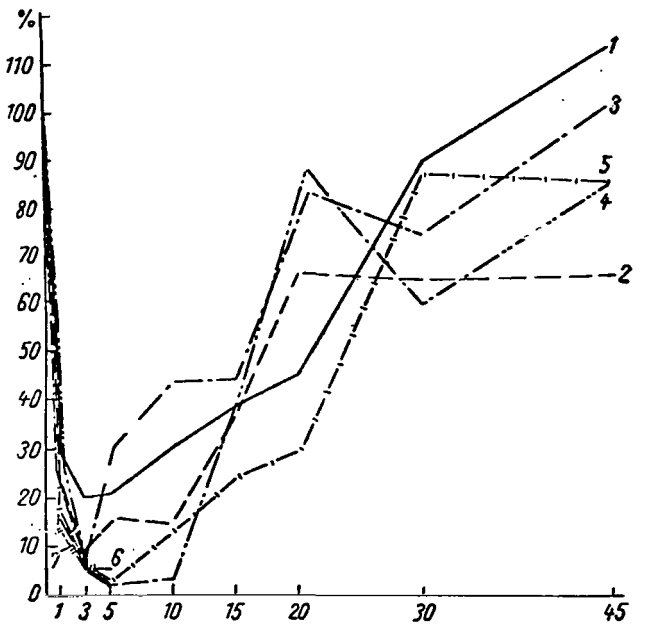
Thus, the reaction of lymphoidal and myelenoidal hemorrhaging is different, and these differences depend not only on the radiation dose, but also on the physiological characteristics of the cells and on the times when the study of the quantity of these cells in the blood of the animal is made.

Pathologic-morphological investigations of the internal organs were made in 50 rats irradiated with doses of 330, 420, 520 and 620 rad. The animals were killed on the 3d, 7th, 15th, 30th and 45th day after irradiation for study of the dynamics of morphological changes.

Perivascular edema, swelling of the walls of the vessels and foci of parenchymous hemorrhages were detected in the lungs on the third day after irradiation. The presence of a considerable quantity of grains of iron-bearing pigment in the cells situated along the capillaries indicated an increase of vascular permeability, obviously developing at an early stage after irradiation.

In individual cases on the seventh day there were not only massive /133
parenchymatous hemorrhages, but perivascular hemorrhages as well along the large veins, plasmatic impregnation of the walls of the vessels and destruction of some small arteries around which small "casings" formed, consisting primarily of lymphocytes.

Later (after 15, 30 and 45 days), the number of vessels, including large ones, experiencing destruction increased. There also was an increase of the



Days from beginning of experiment

Figure 20. Change of quantity of leukocytes (percent) in blood of rats during first 45 days after irradiation by protons in dose of 330 (1), 400 (2), 420 (3), 470 (4), 520 (5), 620 (6) and 730 rad (7).

size of the lymphoidal "casings" (fig. 24). A picture was observed similar to nodular periarteritis. In many cases there were foci of intermediate pneumonia. At all times in the investigation there was a large quantity of cells filled with grains of hemosiderin in the walls of the large and small vessels.

Investigation of the heart revealed edema of the myocardium, expansion and plethora of the vessels and swelling of their walls. In rats irradiated with doses of 520-620 rad there were hemorrhages near the aorta, atrium cordis dextrum and interventricular septum, most frequently in the first two weeks after irradiation. Dystrophic changes became detectable later, and small agglomerations of lymphocytes in the myocardium were discovered. /134

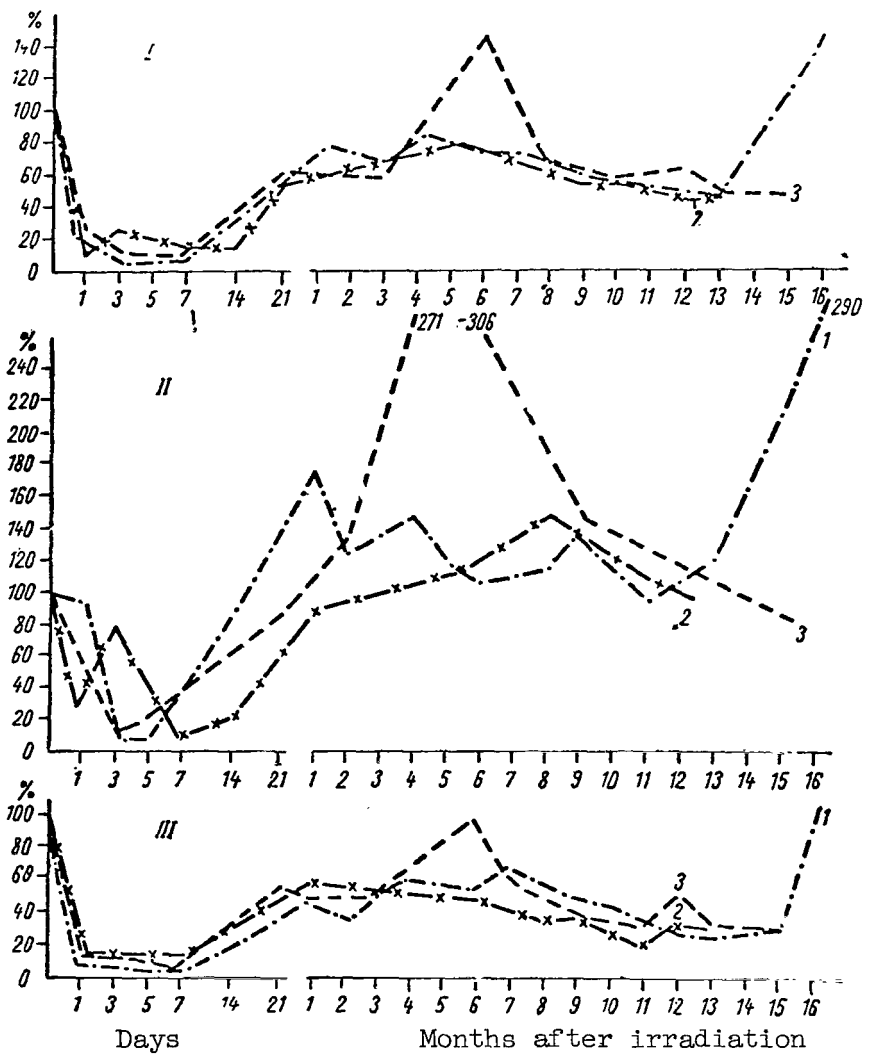


Figure 21. Change (percent) of total quantity of leukocytes (I), neutrophils (II) and lymphocytes (III) during first month after irradiation and much later: (1) 420 rad; (2) 730-760 rad; (3) 850 rad.

TABLE 16. CONTENT OF LEUKOCYTES (THOUSANDS PER 1 MM^3) IN PERIPHERAL BLOOD OF RATS SUBJECTED TO SINGLE IRRADIATION BY PROTONS WITH ENERGIES OF 510 MEV

Group	Dose, rad	Number of leukocytes before irradiation	After irradiation, days							
			1st	3d	5th	10th	15th	20th	30th	45th
I	330	13.80	3.96	2.74	2.98	4.37	5.40	6.40	12.40	15.80
II	400	15.00	3.60	1.36	2.40	2.30	5.80	10.10	9.96	10.10
III	420	16.00	4.20	1.24	4.90	7.00	7.30	13.30	12.00	16.30
IV	470	16.50	4.43	1.14	0.54	0.60	6.58	14.80	10.10	14.10
V	520	13.30	2.40	0.93	0.44	1.80	3.30	4.00	11.70	11.40
VI	620	14.30	2.0	1.04	0.90	-	-	-	-	-
VII	730	16.80	2.3	0.9	0.4	-	-	-	-	-
VIII	950	16.30	-	0.6	-	-	-	-	-	-

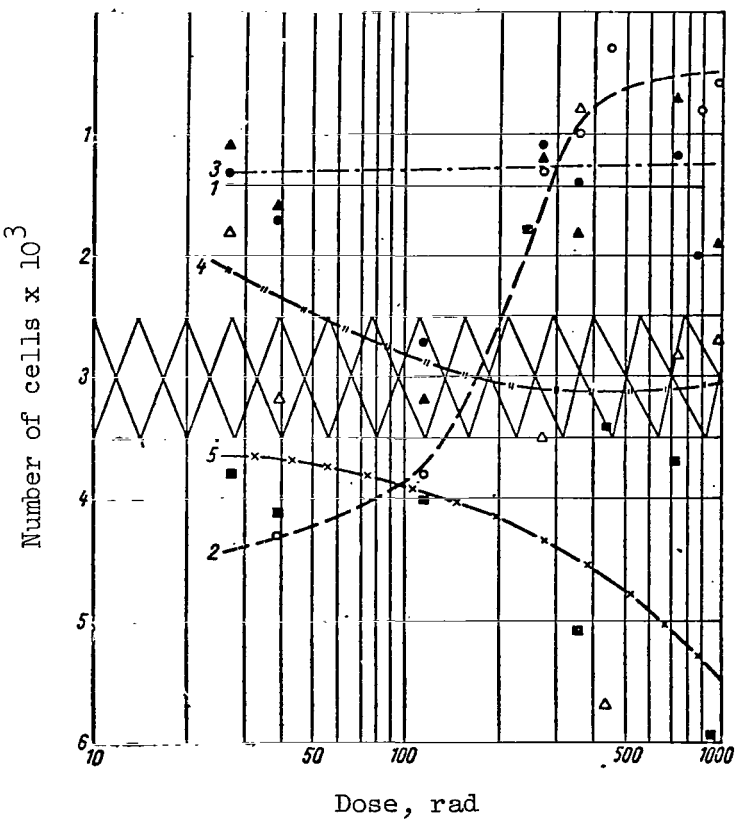


Figure 22. Change of quantity of neutrophils as function of irradiation dose (rad) and time of observation: (1) 1st day; (2) 5th day; (3) 14th day; (4) 28th day; (5) 180th day.

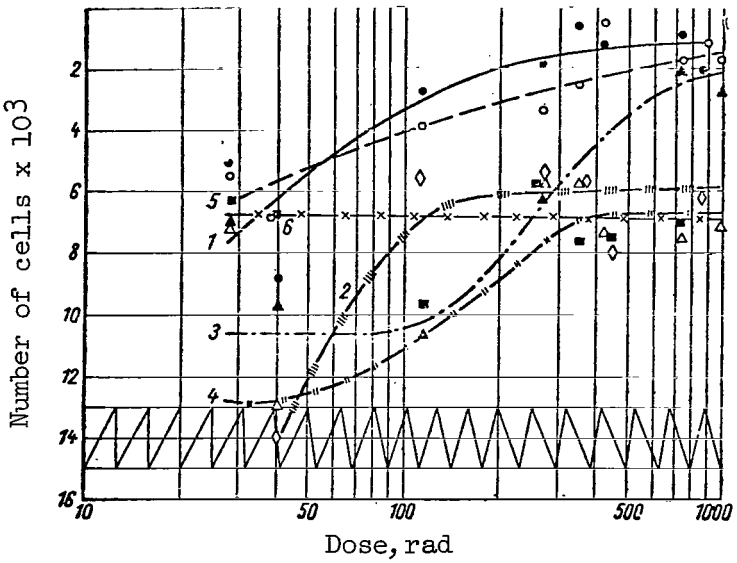


Figure 23. Change of quantity of lymphocytes as function of irradiation dose and time of observation: (1) 1st day; (2) 5th day; (3) 14th day; (4) 28th day; (5) 90th day; (6) 180th day.

Figure 24. Lymphoid "casings" around vessels in lungs. Magnification 10x20.

Clearly expressed changes in the liver were not detected earlier than the seventh day. In some animals there were individual liver cells with pyknotic nuclei and basophilously coloring cytoplasm, while in others the cytoplasm became "washed out" and contained large basophilic lumps. A large number of fatty inclusions were found in individual cells.

Small foci of necrosis were visible in the hepatic lobes in rats irradiated with a dose of 420 rad. All these changes in the animals of the different groups were detected in a nonuniform degree; they were manifested most clearly in rats irradiated with a dose of 620 rad.

A different pathological picture was discovered in all groups of rats 30-45 days after irradiation. Autopsies made during this time revealed flabbiness of the liver. Microscopic study showed clearly expressed phenomena of dys- /135 trophy of the parenchyma of the organ, expansion of the intraobular capillaries and interlobular veins and massive hemorrhages in the parenchyma and disintegration of the hepatic ligaments. The intact cells were large in size with vacuolated cytoplasm and gigantic nuclei in a state of necrobiosis. Adiposity of the liver was noted when staining for fat (fig. 25).

In the kidneys, as in the liver, clearly expressed changes were observed not earlier than 30-45 days after irradiation. At that time there were well-expressed dilatation of the blood vessels (fig. 26) and hemorrhages in the cortex and medulla, near the renal pelvis and under the capsule. There were clearly expressed dystrophic changes in the epithelium of the tubulus contortus. At the same time there was an expansion of the cavity of Bowman's capsule, which contained an albuminous effusion, and there was plasmatic impregnation of the walls of the vessels.

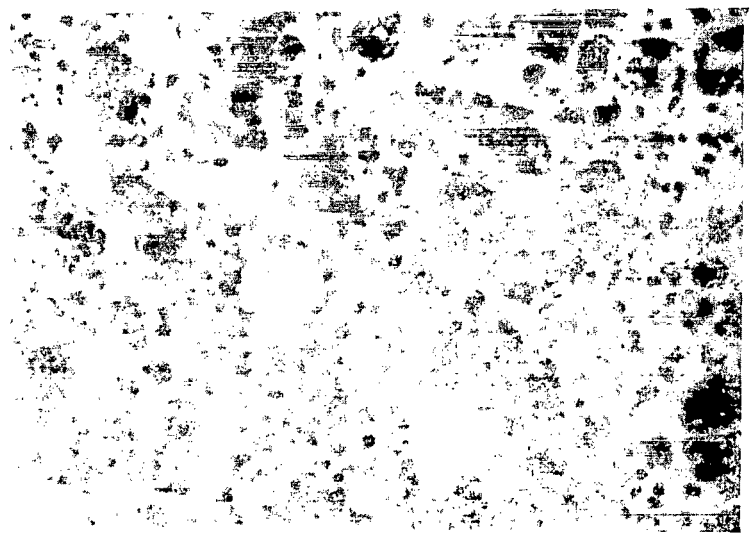


Figure 25. Fat encroachment in liver. Magnification 10x20.

Figure 26. Well-expressed plethora and dilation of vessels in cortical and medullary layers of kidneys. Magnification 8x10.

Plethora was observed in the spleen after the third day (fig. 27a). During this time the most complete devastation of the lymphatic follicles was noted in rats irradiated with a dose of 620 rad. Seven to 15 days (fig. 27b) after initial irradiation, a tendency to regeneration was noted in the spleen. This indicated the presence of mitoses in the follicles and a considerable quantity of lymphocytes, diffusely scattered in the pulp and forming agglomerations around the small vessels. The plethora of the organ continued during this period. /138

The spleen became anemic 30-45 days after the irradiation. Its size increased due to hyperplasia of the reticular stroma. As before, the follicles were small and the configuration of the organ had an eroded appearance (fig. 28). The pulp not only contained diffusely scattered lymphocytes, but also tiny foci of myeloidal hemorrhaging. In all animals, killed at different times after irradiation, the spleen contained a large quantity of grains of hemosiderin and brown pigment, evidence of a high rate of disintegration of erythrocytes.

Clearly expressed pathologic-morphological changes were observed in the stomach and in the small and large intestines only in a late period after the irradiation. After 30-45 days the tegmental epithelium and the epithelium of the glands of the inferior and pyloric parts showed necrobiotic changes and in many cases a detachment of the mucous membranes of the small and large intestine. In the connective tissue part of the mucous membrane and submucosa and in the muscles there were no clearly expressed dystrophic changes. The content of granules of hemosiderin in the cells of the stroma of the villi of the gastric mucosa and large intestine, noted at different times during the investigation, /139 indicated prolonged impairment of vascular permeability.

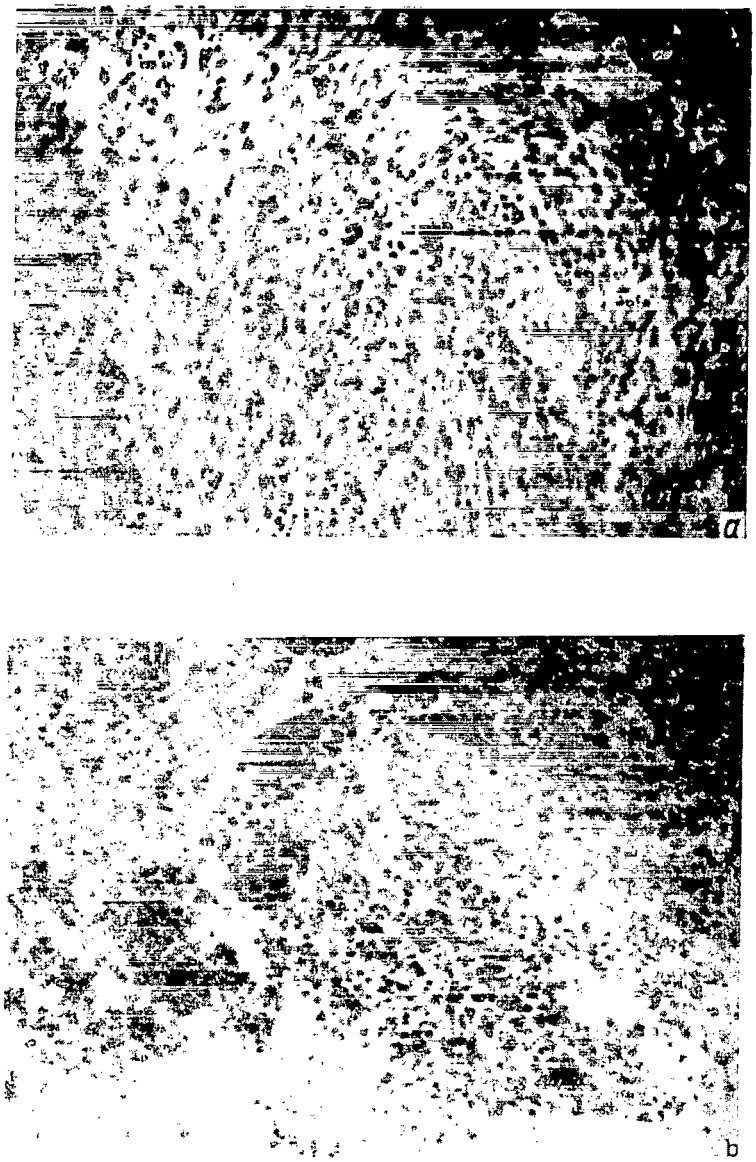


Figure 27. Devastation of spleen after irradiation with 620 rad after 3d day (a) and appearance of regeneration 7 days after irradiation (b). Magnification 10x20.

Morphological changes in the testes, as in the liver and kidneys, developed 1-1.5 months after irradiation. In the first two weeks after exposure no clearly expressed changes were observed in the seminific epithelium of the sperm ductules (fig. 29). In the lumen of the tubules there were cells in all stages of development as well as mature sperm cells. There was a large quantity of dividing cells. Some pathologic mitoses (agglutination of chromosomes, their irregular divergence toward the poles, gigantic multinuclear cells) were

observed. The syncytium of the Sertoli cells, as in the case of irradiation by X-rays (refs. 6-8), retained a normal structure. However, there were no cases of growth of interstitial tissue or a well-expressed dilatation of capillaries, such as observed in rats after multiple irradiation by protons. A moderate number of cells containing hemosiderin were discovered.

After 1-1.5 months there was total devastation of the sperm ductules (fig. 30). However, at this time some of the ductules revealed foci of Sertoli cells and a small quantity of cells with vividly staining nuclei, morphologically similar to the nuclei of spermatogonia. There was moderate growth of interstitial tissue with a small number of cells, whose cytoplasm included granules of iron-containing pigment.

In summarizing our observations it can be said that the single irradiation of white rats with protons with doses of 330-620 rad, like multiple irradiation with doses of 750 and 1,015 rad, causes acute radiation sickness with uniform morphological changes of the internal organs.

The height of the sickness in rats subjected to a single irradiation by protons is observed approximately from the 3d to the 10th day after irradiation, the same as after single irradiation by X-rays.

On the first days of sickness the most sharply expressed phenomena were hemorrhagic diathesis (primarily in the lungs) and shrinking of the lymphoidal apparatus of the spleen. Later, during the first two weeks after irradiation, there was regeneration of the lymphocytes and partial restoration of the follicles. The manifestations of hemorrhagic diathesis became less clearly

Figure 28. Hyperplasia of reticular stroma of spleen and eroded structure of organ 45 days after irradiation with 330 rad. Magnification 10x20.

expressed and hemorrhages occurred more rarely, although indications of impairment of hemodynamics and vascular impregnation remained evident in the later stage of the illness.

It should be noted that there was development of dystrophic changes in the epithelium and glands of the gastrointestinal tract and a sharply expressed

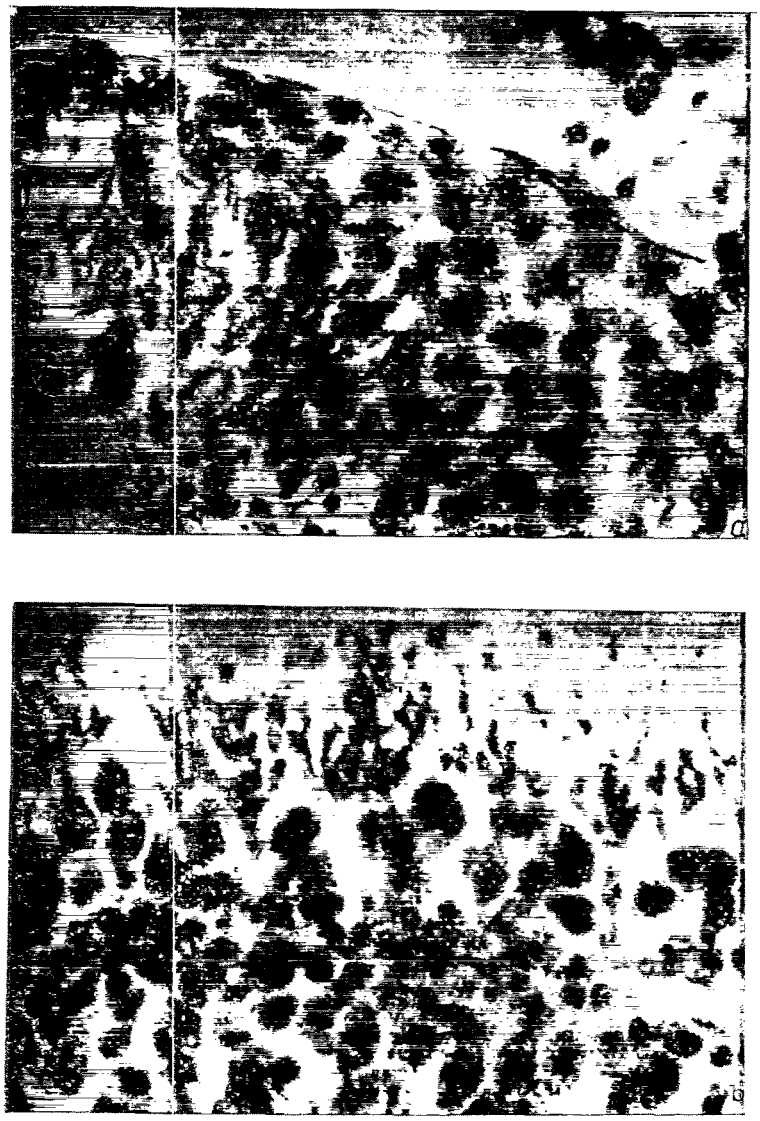


Figure 29. Changes in the testes on 3d day after irradiation with 620 rad (a) and 7 days after irradiation with 520 rad (b). Magnification 15x40.

circulatory disturbance and degenerative changes of the parenchyma of the liver and kidneys in late stages after irradiation. At these stages there were increases in the size of the spleen as a result of hyperplasia of the reticular stroma and an eroded character of the structure of the organ as a result of incomplete restoration of the lymphoidal apparatus. The manifestation of at least a modest regeneration of the spermatic epithelium and syncytium of Sertoli cells 1.5 months after irradiation is evidence of the possibility of restoration of spermatogenesis.

Figure 30. Changes in testes 15 days after irradiation with 620 rad (a) and devastation of sperm ductules 45 days after irradiation with 330 rad (b). Magnification 15x40.

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CHAPTER 5. BIOLOGICAL EFFECT OF PROTONS WITH ENERGIES OF 126 MEV ON CORNEAL EPITHELIA OF MICE /143

V. M. Mastryukova and A. D. Strzhizhovskiy

Interest has increased in the study of the biological effect of high-energy particles as a result of the successes achieved in space research.

In this chapter, when evaluating the biological effect of high-energy protons, we have used the process of physiological regeneration of tissues possessing high radiosensitivity. The corneal epithelia of mice, the selected object of our investigation, long ago became a standard in the study of radiation damage of the process of physiological regeneration, which facilitates considerably the determination of the relative biological effect (RBE) of radiation and ensures significant methodological advantages. The proposed method for mathematical analysis of experimental data made it possible to obtain rigorous quantitative conclusions.

Laboratory male mice weighing 15-20 g were given total radiation with protons with an energy of 126 Mev in doses of 200 and 500 rad with a dose intensity of 0.3 rad/sec. After 1, 1.5, 2.5, 3, 4.5, 5, 6.5, 7, 8.5 and 9 days from onset of irradiation they were killed by decapitation at the rate of 12 experimental and 5 control mice. A total of 170 mice was used in the experiments.

The material was fixed in Bowen fluid and total preparations were prepared which then were stained by the Weigert method. Then the number of cells in the field of view of the microscope, the number of mitoses per 10,000 cells and the content of chromosome aberrations in the anaphase stage (chromosome bridges, breakage and fragmentation) were counted in the two lower reproductive layers of the epithelium on the periphery and at the center of the cornea.

There was an average of 203.6 ± 1.4 cells in the epithelium of the corneas of the control animals as seen in the field of view of a MBI-6 microscope with a magnification of 7 x 2, 5 x 60. The mitotic index of the corneal epithelium (ratio of the number of mitoses to the number of cells) at 1000 hours was $(16.30 \pm 0.66)\cdot 10^{-3}$, and at 2200 hours--$(5.88 \pm 0.62)\cdot 10^{-3}$. The mean daily value of the mitotic index was $(11.09 \pm 0.45)\cdot 10^{-3}$. Virtually no pathological mitoses were encountered in the corneal epithelium of the control animals. /144

Irradiation by protons with energies of 126 Mev in doses of 200 and 500 rad causes a slowing of the mitotic activity of the corneal epithelium, a

decrease of the number of cells in the field of view of the microscope and the development of pathological forms of mitosis. Table 1 gives the corresponding data.

TABLE 1. INFLUENCE OF IRRADIATION BY PROTONS WITH ENERGIES OF 126 MEV ON MITOTIC ACTIVITY AND QUANTITY OF CORNEAL EPITHELIUM CELLS

Irradiation dose, rad	Time after irradiation, days	Mitotic index 10^{-3}	Relative quantity of cells in field of view of microscope	Relative content of pathological anaphases
200	1	5.81 ± 0.76	206.3 ± 3.1	0.164 ± 0.056
	1.5	13.61 ± 0.96	194.2 ± 3.0	0.091 ± 0.016
	2.5	9.05 ± 0.82	192.0 ± 3.0	0.063 ± 0.010
	3	9.59 ± 0.88	194.8 ± 2.5	0.031 ± 0.008
	4.5	8.36 ± 0.52	196.0 ± 2.8	0.028 ± 0.008
	5	10.63 ± 1.02	191.0 ± 2.6	0.047 ± 0.010
	6.5	11.40 ± 0.87	195.2 ± 2.7	0.028 ± 0.007
	7	3.41 ± 0.33	194.0 ± 2.9	0.020 ± 0.014
	8.5	12.28 ± 0.86	192.9 ± 2.8	0.022 ± 0.006
	9	5.25 ± 0.57	200.0 ± 2.9	0.020 ± 0.013
500	1	2.56 ± 0.29	188.0 ± 2.3	0.438 ± 0.056
	1.5	5.75 ± 0.50	188.1 ± 3.0	0.401 ± 0.033
	2.5	10.89 ± 0.81	185.0 ± 2.6	0.299 ± 0.026
	3	4.34 ± 0.49	192.5 ± 2.7	0.169 ± 0.045
	4.5	7.47 ± 0.58	198.7 ± 2.2	0.135 ± 0.020
	5	5.13 ± 0.26	200.1 ± 2.6	0.043 ± 0.013
	6.5	9.07 ± 0.69	199.7 ± 2.3	0.055 ± 0.010
	7	5.37 ± 0.48	202.2 ± 2.9	0.036 ± 0.014
	8.5	13.31 ± 0.43	203.0 ● 3.2	0.023 ± 0.006
	9	4.80 ± 0.53	201.8 ± 2.8	0.010 ● 0.005

It is easy to see that with an increase of the dose of irradiation the degree of suppression of mitotic activity increases and the intensity of restorative processes decreases. It is interesting to note a decrease of the amplitude of daily variations of mitotic activity, especially clearly expressed between the 3d and 5th days after irradiation in a dose of 200 rad. In the case of irradiation in a dose of 500 rad this effect is less clearly expressed, indicating the existence of an optimal dose of irradiation.

On the basis of a study of table 1 it can be established that irradiation in doses of 200 and 500 rad causes some decrease in the quantity of cells in the field of view of the microscope. However, its amplitude does not exceed /145
10 percent of the normal quantity of cells and over considerable periods of time the decrease in the quantity of cells in the field of view of the microscope in general is not statistically reliable.

The appearance of pathological mitoses in irradiated tissue is an indication of damage of the genetic structures of the cell. The dose dependence of the genetic effect of radiation, characterized by the maximum level of chromosome aberrations on the first day after irradiation, is linear within the limits of experimental error. Analyzing the corresponding data from table 1 by the least squares method, it can be represented analytically in the form $F(D) = 0.87 \cdot 10^{-3} \cdot D$, where F(D) is the relative content of pathological anaphases on the first day after irradiation in the dose D rad.

On the following days the content of chromosome aberrations in the corneal epithelium of the irradiated mice decreased monotonically. In the case of irradiation in a dose of 500 rad this decrease continues with an approximately constant intensity during the entire time of observation and by the end of the experiment (on the 9th day after irradiation) the relative content of pathological anaphases was 0.010 ± 0.005. In the case of irradiation in a dose of 200 rad the intensive decrease of the relative content of pathological anaphases continued only to approximately the third day after which its rate decreased sharply.

In actuality, as is established easily on the basis of an analysis of the data cited in table 1, in the case of irradiation in a dose of 500 rad the content of chromosome aberrations in the corneal epithelium of irradiated mice during the period from the 3d to the 9th day after irradiation decreases by a factor of almost 17, whereas in the case of irradiation in a dose of 200 rad it decreases only by a factor of approximately 1.5.

The basis for the analysis of the above-cited experimental data was a model of radiation damage of cell populations developed earlier by one of the authors and already published (ref. 1). This study presents in general form everything necessary for study of the mathematical approach and the formulas cited below are a corollary of the derived expressions, corresponding to the special case when the duration of the pathological mitosis is equal to approximately the normal duration.

By investigating the changes of mitotic activity and the content of chromosome aberrations in the corneal epithelium of irradiated animals simultaneously with changes of the number of cells in it we can compute the intensity of casting off of cells and draw conclusions concerning the causes of the latter. Taking into account that for each time t the change of the quantity of cells p (t) per unit time is determined by the relation between the mean probabilities of division k and casting off of cells $1/\tau$ per unit time, the following equation of cell balance can be written

$$\frac{dp(t)}{dt} = (k - 1/\tau)\, p(t). \qquad (1)$$

If the intensity of cell multiplication k and the relative change of the /146
quantity of cells per unit time $1/p(t) \cdot dp(t)/dt$ are known, by subtracting the

second from the first it is possible to compute the intensity of casting off of cells $1/\tau$. The value k (t) can be computed by knowing the mitotic index of the irradiated population I_{Σ} (t) and the mean duration of mitosis τ_M

$$k(t) = \frac{I_{\Sigma}(t)}{\tau_M}. \tag{2}$$

For computation of the value $1/p\ (t) \cdot dp\ (t)/dt$ it is possible to use the results of measurement of the quantity of cells in the field of view of the microscope. The investigation of the curve of change of the content of chromosome aberrations in irradiated tissue makes it possible to obtain some information concerning the fate of cells entering into pathological mitosis and the reasons for the change of intensity of casting off of cells. The expression for the content of pathological anaphases F(D, t) in irradiated tissue at the time t after irradiation in the dose D can be represented in the form

$$F(D, t) = \frac{1}{1 + \left(\frac{1}{F(D,0)} - 1\right) exp \int_0^t \frac{dt}{\tau_F}}, \tag{3}$$

where F(D, 0) is the initial value F(D, t) and $1/\tau_F$ is the intensity of disappearance of pathological anaphases from the irradiated tissue.

In a case when genetic impairments develop only at the time of irradiation and disappear only as a result of renewal of cells in the process of physiological regeneration, the value $1/\tau_F$ is equal to the intensity of the process of physiological regeneration k (t). Satisfaction of the condition $1/\tau_F > k$ indicates either partial reversibility of damage of the genetic structures of the cell or an increased intensity of dying away of cells with genetic impairments. Simultaneous determination of the intensity of casting off of cells from the tissue $1/\tau$ makes it easier to choose between these two possibilities. Satisfaction of the condition $1/\tau_F > 2k$ is proof of the partial reversibility of genetic impairments, but satisfaction of the condition $1/\tau_F < k$ is proof of the development of cells with genetic impairments in the period after radiation exposure.

We now will show how it is possible to determine the desired values k (t), $1/\tau$ and $1/\tau_F$, using the above-cited experimental data as a point of departure.

We will consider the time interval <il>, beginning i days after irradiation and ending after l days. Using formula (2) as a point of departure, the mean value k (t) in the time interval <il> $\{k\}_{il}$ can be written in the form /147

$$\{k\}_{il} = \frac{\{I_\Sigma\}_{il}}{\tau_M}, \tag{4}$$

where $\{I_\Sigma\}_{il}$ is the mean value of the mitotic index in the interval <il>.

Then expressing the value $1/\tau$ from formula (1) and replacing the derivative dp (t)/dt in the time interval <il> by the ratio of the increment of the function to the increment of the argument p (l)-p (i)/l-i, it is easy to obtain an expression for mean $1/\tau$ in the time interval <il> $\{1/\tau\}_{il}$

$$\{1/\tau\}_{il} = \{k\}_{il} - \frac{2}{p(l)+p(i)} \cdot \frac{p(l)-p(i)}{l-i}, \tag{5}$$

where p (l) and p (i) are the quantities of cells in the microscope field of view at the times l and i respectively.

Similarly, expressing $1/\tau_F$ from formula (3) and replacing the derivative $\frac{d \ln \left[\frac{1}{F(D,t)} - 1\right]}{dt}$ for the time interval <il> by the ratio of the increment of the function to the increment of the argument $\frac{\ln \left[\frac{1}{F(D,l)} - 1\right] - \ln \left[\frac{1}{F(D,i)} - 1\right]}{l-i}$, it is possible to obtain an expression for mean $1/\tau_F$ for the time interval <il> $\{1/\tau_F\}_{il}$

$$\{1/\tau_F\}_{il} = \frac{1}{l-i} \cdot \ln \frac{\left[\frac{1}{F(D,l)} - 1\right]}{\left[\frac{1}{F(D,i)} - 1\right]}. \tag{6}$$

According to data in the literature (refs. 2-4) $\tau_M \approx 35$ min. All other data necessary for computations using formulas (4)-(6) can be found in table 1. Table 2 gives the results of the computations.

In order to understand better the processes transpiring in irradiated tissue it is necessary to first investigate the process of cell renewal in the corneal epithelium of the control animals. According to the above-cited experimental data, there are daily variations of mitotic activity in the corneal epithelia of the control mice, whereas the quantity of cells in them does not change during the course of the day. By comparing the cell balance it is easy to establish that there also is a daily rhythm of casting off of cells associated with the daily rhythm of cell multiplication. As shown by precise

computations using formulas (4) and (5), the intensity of casting off of /148 cells $1/\tau$, at 1000 hours equal to 0.670 ± 0.027 per day, decreases by 2200 hours to 0.242 ± 0.026 per day, whereas in the morning hours it increases again. It therefore follows that in the corneal epithelium of control mice the principal factor responsible for the casting off of cells is their crowding out by those newly forming in the cell division process. This fact appears paradoxical since it means that the intensity of cell multiplication in the corneal epithelium of the control animals is far higher than that which is necessary for compensation of the dying off of cells in the process of natural aging, and as a result there is a casting off of viable cells from the tissue. However, this fact is explained easily if it is taken into account that a decrease of the energy efficiency in this case is compensated by an increase of the resistance of the tissue to external effects. In actuality, a slowing of cell multiplication can cause an appreciable decrease of the quantity of tissue cells only in a case if its duration is greater than the difference between the mean potential lifetime of the cell and the mean age of the tissue cells, decreasing with an increase of the intensity of the process of physiological regeneration.

TABLE 2. KINETIC PARAMETERS OF CORNEAL EPITHELIUM OF ANIMALS IRRADIATED BY PROTONS WITH ENERGIES OF 126 MEV

Irradiation dose, rad	Time interval, days	k (1/day)	$1/\tau$ (1/day)	$1/\tau_F$ (1/day)
Control		0.456 ± 0.019	0.456 ± 0.019	-
200	0-1.5	0.235 ± 0.010	0.269 ± 0.015	-
	1.5-2.5	0.393 ± 0.018	0.403 ± 0.021	0.399 ± 0.256
	2.5-4.5	0.382 ± 0.021	0.372 ± 0.024	0.423 ± 0.163
	4.5-6.5	0.385 ± 0.019	0.388 ± 0.021	0.000 ± 0.195
	6.5-8.5	0.323 ± 0.012	0.331 ± 0.016	0.135 ± 0.190
500	0-1.5	0.094 ± 0.005	0.149 ± 0.012	-
	1.5-2.5	0.242 ± 0.012	0.258 ± 0.016	0.451 ± 0.210
	2.5-4.5	0.282 ± 0.013	0.246 ± 0.016	0.498 ± 0.106
	4.5-6.5	0.277 ± 0.010	0.274 ± 0.013	0.495 ± 0.128
	6.5-8.5	0.338 ± 0.012	0.331 ± 0.016	0.452 ± 0.165

In conclusion, it is possible to make some predictions concerning the anticipated changes of the intensity of casting off of cells caused by changes of the mitotic activity of the corneal epithelium. In all cases when the value k is greater than the intensity of dying off of cells $1/T$ the value $1/\tau$ is not dependent on $1/T$ and is equal to k. However, if the condition $k \leq 1/T$ is satisfied, the casting off of viable cells from the tissue ceases and $1/\tau =$ /149 $1/T$.

We now turn to table 2. It is easy to see that only in the course of the first 1-1/2 days after irradiation in a dose of 500 rad, when the degree of

suppression of mitotic activity is maximal, is the condition $1/\tau > k$ satisfied. In all other cases the differences between these values are statistically unreliable. These facts correspond to the predictions made above. It therefore follows that in the course of the first 1-1/2 days after irradiation in a dose of 500 rad $1/T = 1/\tau = 0.149 \pm 0.012$ per day. This corresponds to the mean potential lifetime of the cell $T = 6.72 \pm 0.54$ days. Since irradiation can increase the ratio $1/T$, for the corneal epithelium of control mice $T \geq 6.72 \pm 0.54$ days. For comparison we note that the mean value of the maximum age of the cells of the corneal epithelia of the control animals, determined by the intensity of the process of physiological regeneration, is 2.20 ± 0.09 days.

The above-cited numerical value $1/T$ apparently somewhat exceeds the intensity of dying off of the cells of the corneal epithelium of a mouse in the process of natural aging (ref. 5).

In all the remaining cases, when the equation $k = 1/\tau$ is satisfied within the limits of experimental error, the principal cause of casting off of cells from the tissue was their crowding out by newly forming cells in the process of cell multiplication, which also masked the process of dying out of cells. As a result it is possible to determine the numerical value $1/T$ only for the first 1-1/2 days after irradiation in a dose of 500 rad; in the remaining cases it only is possible to state that

$$\frac{1}{T} < \frac{1}{\tau}$$

We now will consider the curves of change of the content of chromosome aberrations in the corneal epithelium of the irradiated animals. In the case of irradiation in a dose of 500 rad the rate of decrease of the content of pathological anaphases remained virtually constant during the course of the entire experiment. The mean value of the ratio $1/k\tau_F$ characterizing the result of pathological mitosis is 1.66 ± 0.28, which indicates either intensively transpiring processes of destruction of cells forming as a result of pathological mitosis or the partial reversibility of damage of genetic structures. On the basis of an investigation of the dose dependence of the effect it is easy to establish that the first hypothesis is the more probable. In actuality, if the pathological anaphases disappeared from the tissue as a result of restoration of the damage of the genetic structures of the cell, the intensity of this process would decrease with an increase of the dose of irradiation. How- /150 ever, the opposite effect is observed in the experiment, which indicates an increased intensity of the dying out of cells forming as a result of pathological mitosis.

On the curve corresponding to irradiation in a dose of 200 rad it is possible to define a region of rapid decrease of the content of pathological anaphases in the course of the first four days after irradiation, when $1/k\tau_F = 1.06 \pm 0.40$, and a region of slow decrease of the content of pathological anaphases, continuing from the 4th day after irradiation to the end of the experiment when $1/k\tau_F = 0.19 \pm 0.39$. The character of this curve is easy to

explain if it is assumed that irradiation by protons in the investigated range of doses causes the formation of mitotic anomalies of two types in the tissue. The cells forming as a result of pathological mitosis of the first type are nonviable and perish soon after developing. The reproductive capability of the cells forming as a result of pathological mitosis of the second kind is far closer to normal and for this reason the rate of disappearance of the corresponding cells from the tissue is not great. It is natural to assume that damage of the second type represents some intermediate stage between damage of the first kind and the norm.

The tissue content of cells with damage of the second type probably attains a maximum at some optimal dose of irradiation and then decreases with an increase of the dose, so that after irradiation in a dose of 500 rad the corneal epithelium contains virtually only damaged cells of the first kind. These cells also determine the intensity of the decrease of the curve of change of the content of pathological anaphases in the corneal epithelia of mice after irradiation by protons in a dose of 500 rad. In the case of irradiation in a dose of 200 rad, cells with damage of both the first and second type are formed in the corneal epithelium. However, the cells with damage of the first type perish rapidly and beginning approximately from the fourth day after irradiation the majority of the damaged cells are cells with damage of the second type, possessing a high reproductive viability.

In conclusion we will estimate the relative biological effect of protons with energies of 126 Mev. The most convenient index to use as a characteristic of the early effects of irradiation is the genetic effect of radiation, determined from the maximum level of chromosome aberrations on the first day after irradiation. For protons the dose dependence of the genetic effect of radiation can be represented in the form $F(D) = 0.87 \cdot 10^{-3} \cdot D$, and for X-ray radiation (ref. 5)--in the form $F(D) = 1.31\ 10^{-3} D$. With respect to the early genetic effect the relative biological effect of protons with energies of 126 Mev is 0.67, which confirms data in the literature (refs. 6 and 7).

Analysis of the curves of disappearance of chromosome aberrations /151
from the corneal epithelium of irradiated animals makes it possible to advance some assumptions concerning postponed effects of irradiation by high-energy protons. It can be postulated that cells with genetic damage of the second type, possessing high reproductive viability, are retained in the irradiated tissue for a long time and are responsible for tumors. The effectiveness of X-rays in the formation of this type of genetic damage is far less than the effectiveness of high-energy protons (ref. 5). It therefore follows that the relative biological effect of high-energy protons, determined on the basis of an analysis of postponed radiation effects, can be greater than the relative biological effect determined from early reactions. Some experiments (ref. 6) apparently confirm this point of view.

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CHAPTER 6. DETERMINATION OF THE BIOLOGICAL EFFECT OF PROTONS WITH ENERGIES OF 510 MEV and X-RAYS WITH ENERGIES OF 180 KV ON HEREDITARY STRUCTURES /152

Ye. D. Plotnikova and S. I. Strashnenko

In order to clarify the radiation danger involved in space flights, it is extremely important to investigate the effect of various components of cosmic radiation on hereditary structures, because the character of the effect of ionizing radiations on heredity differs from ordinary somatic effects. In the case of exposure of the organism as a whole to the effect of small doses, the restoration process leads rapidly to the elimination of effects from radiation. In contrast to the somatic effect, the genetic effect has a stable character. This indicates that in the solution of any problem associated with the effect of ionizing radiations on the organism of man or animals it is of basic importance to have data on heredity. We therefore carried out investigations for determination of the relative biological effect (RBE) of protons with energies of 500 Mev for hereditary structures.

As an index of hereditary changes caused by radiation we used dominant lethals. The nature of dominant mutations (ref. 1) is related to chromosome aberrations of different types and magnitudes. Some of these hereditary changes are possibly caused by other factors (ref. 2).

The dominant lethals developing in irradiated germ cells usually cause the death of the zygote, formed by such cells at early stages of development. A number of advantages of this method (less time-consuming than other methods of genetic analysis, considerably smaller number of investigated animals, etc.) permitted us to use it for determination of the relative biological effect (RBE) of protons.

Earlier (ref. 3) a study was made of the frequency of development of dominant lethals in rats under the influence of X-rays.

In our study we used nonlinear white rats weighing 250-300 g. The /153 males were subjected to total irradiation by protons with energies of 510 Mev with doses of 55, 200, 360, 520, 780 and 1,030 rad. After irradiation each male was kept for a period of 3 days with 2 or 3 females of the same age. The occurrence of pregnancy was judged from the appearance of blood in the vaginal membrane, observed on the 10th-12th day after exposure to the males. The females were killed on the 14th-16th day of pregnancy. After autopsy a count was made of the number of yellow bodies, places of implantation and embryos. We thus determined not only the number of dead embryos, but also the stage at which death occurred.

DEATH OF EMBRYOS TAKEN FROM UNIRRADIATED FEMALE RATS CROSSBRED WITH IRRADIATED MALES

Irradiation dose	Number of investigated females	Number of yellow bodies of pregnancy	Number of places of implantation	Number of live embryos	Number of dead embryos					
					Before implantation		After implantation		Total number	
					In fractions of unit[1]	In fractions of unit, with control taken into account[2]	In fractions of unit[1]	In fractions of unit, with control taken into account[3]	In fractions of unit[1] and [4]	In fractions of unit, with control taken into account[2] and [4]
					X-rays, roentgens					
Control	68	758	664	604	0.1240	-	0.0792	-	0.2032 ± 0.0237	-
134	48	568	484	399	0.1478	0.0272	0.1497	0.0908	0.2975 ± 0.0309	0.1183 ± 0.0290
268	35	398	345	227	0.1332	0.0105	0.2965	0.2736	0.4296 ± 0.0296	0.2841 ± 0.0338
402	36	419	323	150	0.2290	0.1200	0.4129	0.4307	0.6420 ± 0.0429	0.5507 ± 0.0306
670	37	403	255	90	0.3672	0.2776	0.4094	0.4421	0.7767 ± 0.0297	0.7197 ± 0.0266
804	23	258	148	30	0.4264	0.3452	0.4574	0.5089	0.8837 ± 0.0186	0.8541 ± 0.0252

(Footnotes appear at end of table)

TABLE (CONTINUED)

Irradiation dose	Number of investigated females	Number of yellow bodies of pregnancy	Number of places of implantation	Number of live embryos	Number of dead embryos: Before implantation: In fractions of unit[1]	Before implantation: In fractions of unit, with control taken into account[2]	After implantation: In fractions of unit[1]	After implantation: In fractions of unit, with control taken into account[3]	Total number: In fractions of unit[1] and 4	Total number: In fractions of unit, with control taken into account[2] and 4
					Protons, rad					
Control	21	271	243	220	0.1033	-	0.0749	-	0.1882 ± 0.0763	-
55 (44- 66)	29	361	327	290	0.0992	-	0.0975	0.0105	0.1967 ± 0.0488	0.0105 ± 0.0388
200 (180-227)	11	119	102	71	0.1429	0.0441	0.2605	0.2210	0.4043 ± 0.0822	0.2651 ± 0.0597
360 (346-382)	27	352	305	209	0.1335	0.0337	0.2728	0.2349	0.4063 ± 0.0330	0.2686 ± 0.0388
520	15	192	145	91	0.2448	0.1578	0.2813	0.2584	0.5261 ± 0.0552	0.4162 ± 0.0477
780 (744-822)	20	254	200	88	0.2126	0.1219	0.4016	0.4028	0.6142 ± 0.0550	0.5247 ± 0.0402
1030	17	201	75	13	0.6269	0.5839	0.3084	0.3464	0.9353 ± 0.0165	0.9303 ± 0.0216

[1]Number of dead embryos cited in relation to yellow bodies.
[2]Control based on use of Abbot formula (ref. 16).
[3]The data in this column were obtained by subtracting the values in the preceding column from the values in the last column.
[4]The cited estimates of errors were computed using the formula

$$\frac{\Sigma yi}{\Sigma xi}\sqrt{\frac{n}{n-1}\left[\frac{\Sigma\,(yi-\bar{y})^2}{(\Sigma yi)^2}+\frac{\Sigma\,(xi-\bar{x})^2}{(\Sigma xi)^2}+2\,\frac{\Sigma\,(xi-\bar{x})\,(yi-\bar{y})}{(\Sigma xi)\,(\Sigma yi)}\right]},$$

where n is the number of investigated females; xi is the number of yellow bodies; yi is the number of embryos in individual females; $\bar{x}$, $\bar{y}$ are the corresponding mean values computed for all females.

Since all embryos were the result of cross breeding, occurring only in the course of 3 days after irradiation of the males, the embryonal death was the result of dominant lethals arising under the influence of radiation in the sperm cells.

When carrying out a similar experiment with X-rays, the exposure of the gonads was local. However, as shown in a number of investigations (refs. 4 and 5), the method for irradiation of the gonads when studying the effect of radiation on them is without substantial importance. We therefore could use our earlier collected data for determination of the RBE of protons.

The derived data, characterizing the frequency of development of dominant lethals in rats under the influence of X-rays and protons, are given in the table.

In both cases the frequency of development of dominant lethals, established from the death of embryos of rats, increases with the irradiation dose. Two types of dominant lethals develop: the first, leading to death at the earliest stages of embryogeny, before implantation, and the second, causing death at later stages of development, after implantation of the embryo.

Earlier in the study of the dependence of the frequency of development of dominant lethals in rats on the dose of X-ray irradiation, we demonstrated (ref. 3) that this dependence is close to exponential. It was noted at the same time that the percentage of death observed in the case of the highest selected dose, 804 r, was considerably higher than expected for an exponential dependence. However, this difference was statistically unreliable.

In a study of the data obtained during irradiation by protons, it was found that even in this case the dependence will be exponential, if we do not take into account the percentage of development of dominant lethals for a dose of 1,030 rad. With respect to the embryonal death with a dose of 1,030 rad, it was much higher than anticipated for an exponential dependence, and this difference was statistically reliable. The exponential character of the dependence of the frequency of development of dominant lethals on the irradiation dose has been /155 noted repeatedly in mice (refs. 6 and 7) and in Drosophila (ref. 8). At the same time it was noted that in the case of relatively high doses the number of dominant lethals in Drosophila increases more rapidly (refs. 9 and 10), i.e., the deviations which we noted in rats occur. The reason for this is that the chromosomal rearrangements, regarded as dominant lethals, consist of two principal types: rearrangements arising on the basis of a single break in the chromosome (single-break rearrangements), and those developing on the basis of two or more breaks in different places in one chromosome or different chromosomes (two-break, three-break, etc.). In the range of relatively small doses it is most common for dominant lethals of the first kind to develop; this determines the linear dependence of the frequency of their development on dose. With high doses a considerably larger part of the dominant lethals is due to rearrangements of the second kind. This apparently is also reflected in the fact that at a dose of 1,030 rad there is a sharp increase of the percentage of embryos at the earliest stages of development (before implantation).

In the case of an exponential dependence there is a unique probability of the development of a dominant lethal in a single sperm cell per unit dose. This

probability is expressed by the coefficient (η) with the dose (D) in the formula for the exponential dependence of the number f(D) of embryos surviving from a dose of irradiation

$$f(D) = Ae^{-\eta D}$$

The existence of different mechanisms of development of dominant lethals makes it possible for us to compute from the dose curve obtained during irradiation with protons that segment of the curve (dose range 50-800 rad) where there is a predominance of single-break rearrangements (exponential dependence). The probability of the development of a dominant lethal in one sperm cell per 1 rad (mutation rate), computed by the least squares method, was equal for this range of doses to $1.0 \cdot 10^{-3}$. Using as a point of departure that the value of the mutation rate in experiments with X-radiation (neglecting the highest dose of 804 r) is $1.7 \cdot 10^{-3}$, we obtain an RBE value for protons equal to 0.6.

Earlier in the study of the dependence of the frequency of development of dominant lethals on the dose of X-radiation, it was noted (ref. 3) that there was some deviation of the experimental points from the exponential function in the direction of a sigmoid. The same is observed in the case of irradiation by protons, when the curve as a whole is considered. We therefore felt it desirable to analyze the data by the probit method. By using this method we determined the value LD_{50} (mean lethal dose) for the embryos of rats. In the case of irradiation by X-rays, LD_{50} was equal to 383 ± 15 r, and in the case of protons--565 ± 21 rad. The RBE of protons, determined from the frequency of development of dominant lethals, therefore was 0.7.

On the basis of the density of linear losses of energy of X-rays with /156 energies of 180 kv and protons with energies of 510 Mev, which are virtually identical, it can be postulated that the biological effect of these radiations also should be equal, i.e., the RBE of the protons should be 1.

One of the possible reasons why the RBE of protons was lower than 1 may be the method used in imparting the energy. Whereas the X-radiation is continuous, radiation of the synchrocyclotron is in pulses (100 pulses/sec), i.e., there is ultrafractionation. On the basis of various objects and using different rhythms of ultrafractionation, a number of authors have demonstrated that it can decrease the effect (by 20-30 percent) in comparison with continuous irradiation (refs. 11-15). This possibly occurred in our case as well.

It also is necessary to consider that, as a result of the interaction of protons with energies of 510 Mev with the tissues of the organism, nuclear reactions develop in the latter with the formation of "stars" having a high ionization density. It is, therefore, entirely possible that the derived value could be even smaller if this phenomenon did not occur.

It is interesting to note that the value of RBE of protons, which we determined, is close to the RBE value obtained on the basis of a number of somatic reactions (picture of blood, survival rate).

The coincidence of the RBE values detected in the study of genetic and somatic reactions apparently is not random. It is known that the postradiation changes in the nuclei of cells of the chromosomal rearrangement type appear in the tissues of the irradiated organism and are one of the principal causes of radiation injury of somatic tissues.

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CHAPTER 7. BIOLOGICAL EFFECT OF PROTONS WITH ENERGIES OF 130 MEV, X-RAYS WITH ENERGIES OF 180 KV AND CO^{60} GAMMA RADIATION ON THE VESTIBULAR APPARATUS /157

Yu. G. Grigor'yev, A. V. Sevan'kayev and A. A. Sveshnikov

The effect of ionizing radiation on the vestibular apparatus is of particular significance in the problem of ensuring radiation safety in space flight.

The results of successful implementation of prolonged manned space flights indicate the possibility of functional changes in the vestibular apparatus of a cosmonaut. First, the absence of a gravity field at the time of a space flight creates conditions under which there is a change of the otolithic apparatus, and there is impairment of the evolutionary functional relations between the otolithic apparatus and the semicircular canals. Second, during the time of a space flight the cosmonaut is effected by Coriolis acceleration, causing considerable irritation of the vestibular apparatus. Under such complex conditions of functioning of the nonauditory labyrinth there are real possibilities of the effect on it of a number of inadequate physical factors, especially ionizing radiation. It is, therefore, of primary importance to consider the problems of the character of functional changes of the vestibular apparatus under the influence of different doses of ionizing radiation and the possible subsequent change of the sensitivity to adequate irritants.

Whereas the influence of ionizing radiation on the functional and morphological state of a number of organs and systems has been studied in considerable detail and has been widely published, there is very little information on the state of the vestibular apparatus. For example, it has been noted that there are functional and morphological changes of the vestibular apparatus in patients who have been subjected to local irradiation for therapeutic purposes /158 (refs. 1-6). In 1954, describing the clinical manifestations of a chronic form of radiation sickness, N. A. Kurshakov (ref. 7) pointed out that there were accompanying optical-vestibular impairments. V. V. Blagoveshchenskaya (ref. 8) emphasized the possibility of dissociation of the caloric and rotary nystagmus and the intensification of vestibular-autonomic components during chronic radiation sickness, caused by the entry of radioactive substances into the human body.

In 1957 A. I. Titov (ref. 9) published some results of observations of the state of health of individuals working with sources of ionizing radiation, and who despite protective measures were subjected to the prolonged effect of penetrating radiation in small doses. In this group of individuals it was noted systematically that there were a variety of impairments of the functioning of the

otorhinolaryngological organs, including the inner ear. In an investigation of the vestibular function it was discovered that there was an appreciable change of the ordinary relationship between the sensory, somatic and autonomic reactions in the direction of a sympathetic or parasympathetic influence. In most cases slower vestibular-somatic reactions were found. These functional impairments were usually noted in an early, almost symptom-free period of development of chronic radiation sickness, or in that phase of the sickness when general phenomena were most prominent, such as weakness, fatigure, insomnia, headache, etc.

In addition to these clinical observations, the literature contains data on experimental investigations of the influence of ionizing radiation on the labyrinth (refs. 5 and 10-19).

The published data provide the basis for assuming that the character of the response of the vestibular apparatus to the influence of radiation depends primarily on the form of irradiation (general or local).

In the case of a local effect of radiation in small doses, it is impossible to detect appreciable changes in the functioning of the labyrinth (refs. 3 and 5) or they are brief (ref. 4). In the case of irradiation of the inner ear and in the case of radiation exposure in a dose of several thousand roentgens on the region of the ear and head, the symptoms are determined to a considerable degree by morphological changes in the labyrinth (pointed labyrinth) (refs. 1, 4, 5, 10-13, 15, 16 and 20-23).

In the case of general irradiation of the organism there is a phase change of the irritability of the vestibular apparatus; the central nervous system plays a definite role in the manifestation of these changes (refs. 17-19, 24 and 25). In the case of chronic radiation sickness, caused by the systematic effect of small doses of radiation, the irritability apparently is decreased in most cases (ref. 9).

At present it is impossible to stipulate with adequate assurance what extreme radiation dose can cause only reversible changes in the activity /159 of the vestibular apparatus and what dose, with sufficiently well-expressed compensatory mechanisms, will be a threshold dose for the functioning of the particular apparatus. Unfortunately, it must be stated that the literature still contains only a few clinical observations on this matter. It also must be noted that there has been an insufficiently rigorous approach on the part of certain investigators to evaluation of the physical parameters of ionizing radiation and the selection of radiation sources and an adequate irritant for the semicircular canals of the labyrinth. Practical needs (especially space medicine, in which the vestibular apparatus is of great importance) insistently require a detailed study of this problem.

Having at our disposal new methodological possibilities for a quantitative evaluation of the functional state of the vestibular apparatus, we decided to collect data characterizing: (a) the influence of high-energy protons on the vestibular apparatus; (b) the dose dependence of the studied effects, and (c) functional impairments of the activity of the semicircular canals during different periods of radiation sickness (experiments on rabbits and dogs).

The experiments were carried out with an apparatus for adequate irritation of the labyrinth; with this we obtained a wide range of angular accelerations and velocities: rotation at a velocity from 0.2 to 180°/sec, which can be attained with acceleration from 0.01 to 60°/sec^2 (ref. 26). In this work we were able to impart adequate irritation to the labyrinth in any desired intensity, and due to the presence of removable rings we could record objectively the vestibular-somatic and vestibular-autonomic reflexes.

Figure 1 shows the process of determination of the threshold and duration of nystagmus in a rabbit for stop-stimuli of different intensity, with simultaneous recording of breathing and pulse. At first the animal was rotated at small velocities of 0.5, 1 and 2°/sec before appearance of the first nystagmoidal movement; this was recorded in the form of a single "burst." We used this intensity of the irritant as the threshold value. Then, depending on the selected method, the animal was rotated at a uniform velocity of 10, 30, 60, 90, 120 and 180°/sec[1] for a period of 1 to 2 min; rotation was then suddenly stopped. Nystagmus of different duration, frequency and amplitude appeared, depending on the intensity of the irritant. By plotting the logarithm of angular velocity along the x-axis and the duration of postrotational nystagmus (in sec) along the y-axis and connecting the resulting points, we were able to draw a curve (vestibulogram), which to some degree characterizes the possibility of a reaction of the vestibular apparatus to an irritant of increasing intensity.

When there is adequate irritation of the labyrinth, it is impossible /161 to obtain isolated irritation of each semicircular canal (a combined reaction of both labyrinths is obtained); therefore, a caloric test was used in individual series of experiments. A dog was laid on its back with its head set perpendicular to a horizontal plane (the dog's nose was at a right angle to it); the semicircular canal then assumes a vertical position. In the external auditory canal (2/3 of its length) we placed a thin rubber tube 2 mm in diameter and then poured in water at a temperature of 20°, until the first nystagmoid movement was recorded objectively (respiration and pulse were recorded simultaneously). The second labyrinth was investigated 15-20 min later.

Many investigators assume that a quantitative character can be ascribed to the caloric sample. Thus, some authors determine the sensitivity of the labyrinth by the quantity of water necessary for causing nystagmus (refs. 27-33); others estimate the threshold of irritability from the time of onset of caloric nystagmus (refs. 34-38), and still others judge the state of the vestibular function from the duration of the vestibular-somatic reflex (refs. 39-41). In addition to an analysis of these indices we determined the intensity of the vestibular reaction, that is, the ratio of the duration of nystagmus to the quantity of water poured into the tube.

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[1] The indicated velocities were attained by subthreshold acceleration in order to exclude irritation of the vestibular apparatus at the beginning of rotation.

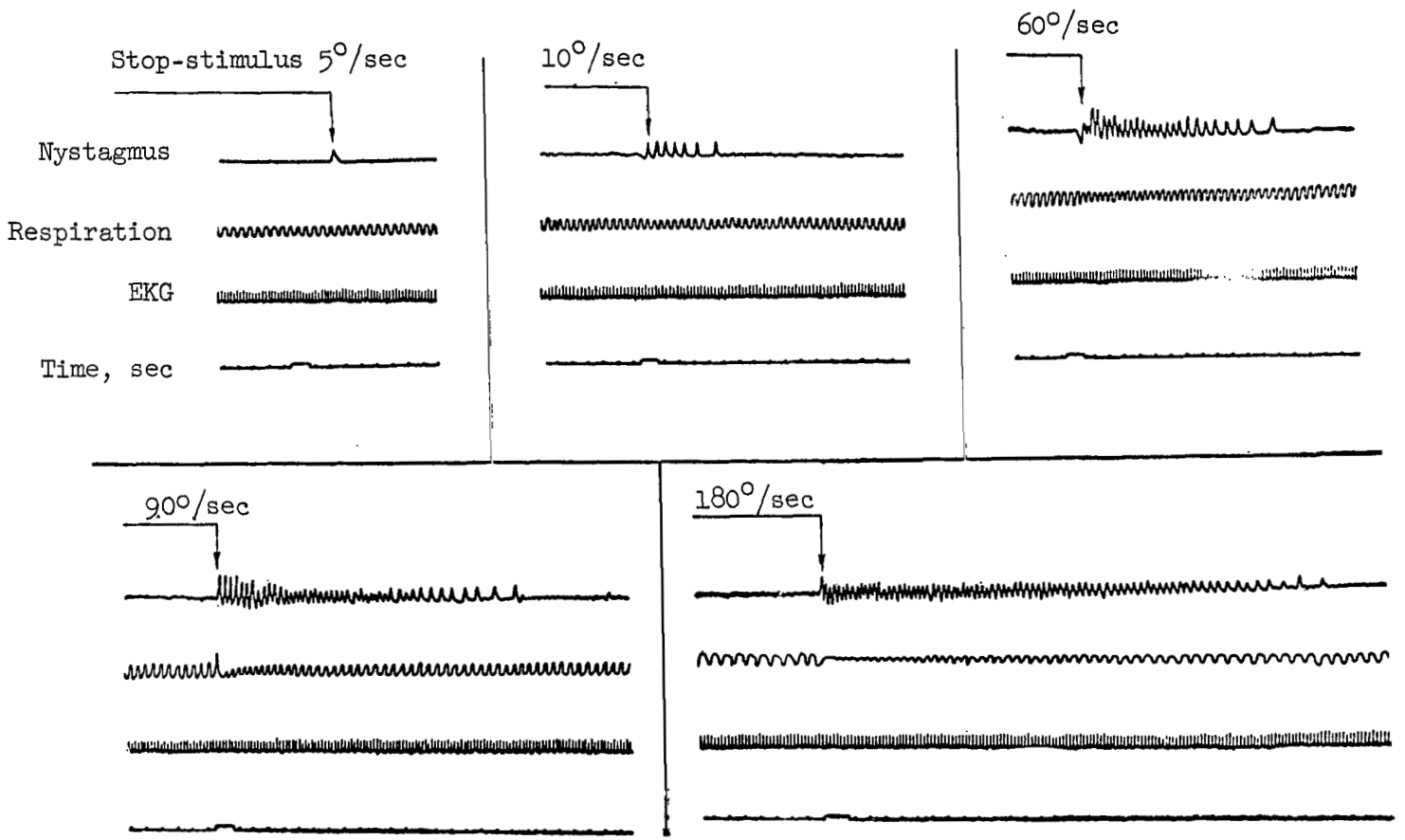

Figure 1. Determination of threshold and duration of nystagmus in rabbit. Stop-stimuli of different intensity and simultaneous recording of respiration and pulse.

Influence of Protons with Energies of 130 Mev on the Functioning of the Vestibular Apparatus

At present there are no published studies devoted to the study of the influence of high-energy protons on the functioning of the vestibular apparatus. Nevertheless, this problem is important for space radiobiology.

For this reason we investigated six dogs, three of which (Shustrik, Buyan and Lopushok) were irradiated by protons with energies of 130 Mev with a dose of 500 rad (group I) and three (Driada, Bezrodnaya and Pavlin) with a dose of 350 rad (group II).

The characteristics of the functional state of the vestibular apparatus of dogs after irradiation by protons show that the radiation illness transpired in a more acute form in group I.

The most acute radiation illness was experienced by the dog Shustrik, who died on the 12th day after irradiation. Despite such a severe course of radiation illness, a change of the threshold sensitivity of the vestibular apparatus in the direction of a decrease was observed only a day before the death of the dog (table 1).

As the radiation sickness developed, the reaction of the vestibular apparatus decreased and it was possible to note phase states of retardation in the apparatus. An adjustment phase was noted on the third day after irradiation /162 (fig. 2); with stimulation of increasing intensity, 30, 60, 90°/sec, nystagmus of an identical duration appeared. This was replaced by a paradoxical phase (fig. 2, curve 3): in the case of stimulation of greater intensity (120°/sec), the duration of the nystagmus was less than when the stimulation was less intense (90°/sec); a similar dependence is shown in curve 4. The general character of the change of the duration of the postrotational nystagmus, the number of beats and amplitudes are shown in figures 3-5 (curve 3).

A decrease of the threshold sensitivity also appeared in the dog Buyan a day before death (table 1); the decrease of reaction of the vestibular apparatus in this animal was sharper than in the dog Shustrik (curve 2, figs. 3-5). The development of the paradoxical phase of retardation was noted on the 6th and 11th day after irradiation.

TABLE 1. THRESHOLD SENSITIVITY OF VESTIBULAR APPARATUS (°/sec) AFTER IRRADIATION OF DOGS BY PROTONS WITH ENERGIES OF 130 MEV WITH DOSE OF 500 RAD

Name of dog	Initial background			Days after irradiation					
	9/V	12/V	15/V	3	6	9	11	14	16
Shustrik	4	5	4	4	4	5	7	x	
Buyan	3	2	3	3	3	3	3	5	x
Lopushok	4	3	4	3	4	4	3	4	5

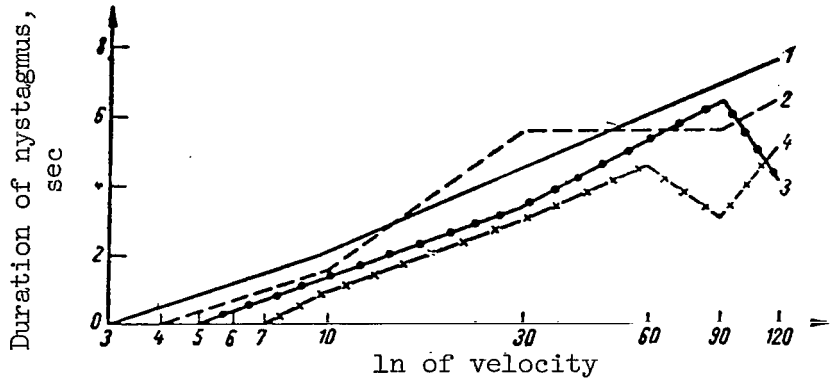

Figure 2. Curves of reaction of vestibular apparatus of dog Shustrik after irradiation with 500 rad: (1) curve for unirradiated animal; (2, 3 and 4) curves for 3d, 9th and 11th day, respectively, after irradiation.

The last of the dogs in group I to die was Lopushok (on the 17th day /164 after irradiation). In this dog also the decrease of threshold sensitivity was noted a day before death (table 1), and the reaction of the vestibular apparatus changed, as in the dogs Shustrik and Buyan, but the depression of the vestibular-somatic reflex in this animal was expressed to a greater degree (curve 1, figs. 3-5). The development of the paradoxical phase of retardation was noted on the 11th and 15th day after irradiation.

The course of the radiation sickness in the dogs of group II was less acute. Only one dog died in a time close to the time of the death of the dogs in group I (Driada, on the 20th day); two other dogs died on the 33d and 35th day after irradiation.

In the case of the dog Driada some decrease of threshold sensitivity was observed beginning with the 15th day after irradiation (table 2). The change in the duration, number of beats and amplitude of the postrotational nystagmus indicated a depression of the vestibular-somatic reflex (curve 1, figs. 6-8).

In the dog Bezrodnaya the decrease of threshold sensitivity was also observed, beginning on the 15th day after irradiation; thereafter it had a stable character.

However, due to the later time of death (33d day), this dog mani- /166 fested certain peculiarities of the change of the vestibular apparatus: a tendency to a wavelike change of reaction appeared on a background of low reaction of the vestibular apparatus (curve 3, figs. 6-8).

In the dog Pavlin the threshold sensitivity decreased, beginning on the 19th day after irradiation (table 2). In this dog, as in Bezrodnaya, there was a tendency to a wavelike change of reaction on a background of low reaction of the apparatus (curve 2, figs. 6-8).

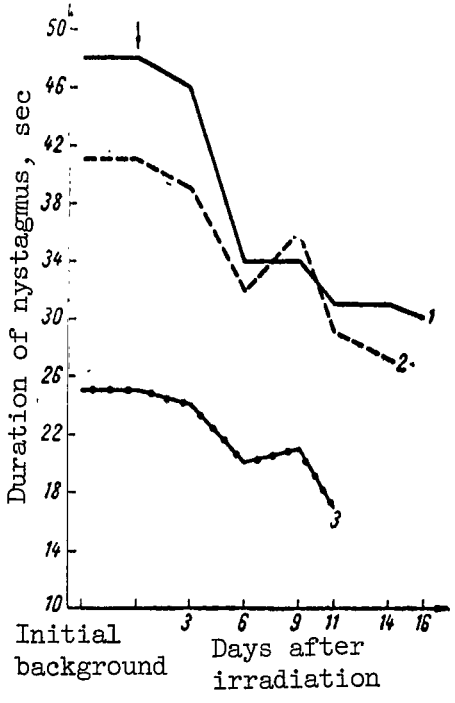

Figure 3. Change of duration of postrotational nystagmus after irradiation of dogs with dose of 500 rad: (1, 2 and 3) indices of dogs Lopushok, Buyan and Shustrik, respectively. Arrow indicates irradiation.

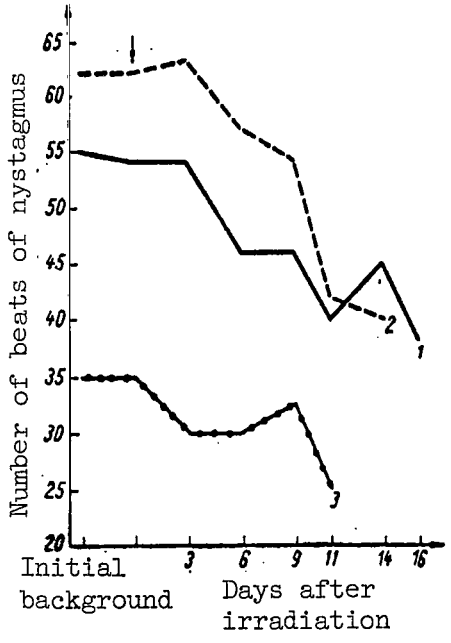

Figure 4. Change of number of beats of postrotational nystagmus after irradiation with dose of 500 rad. Notations same as in figure 3.

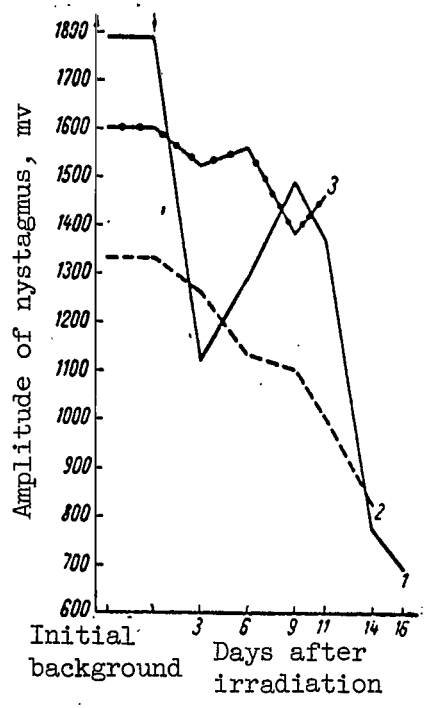

Figure 5. Change of the amplitude of postrotational nystagmus after irradiation of dogs with dose of 500 rad. Notations same as in figure 3.

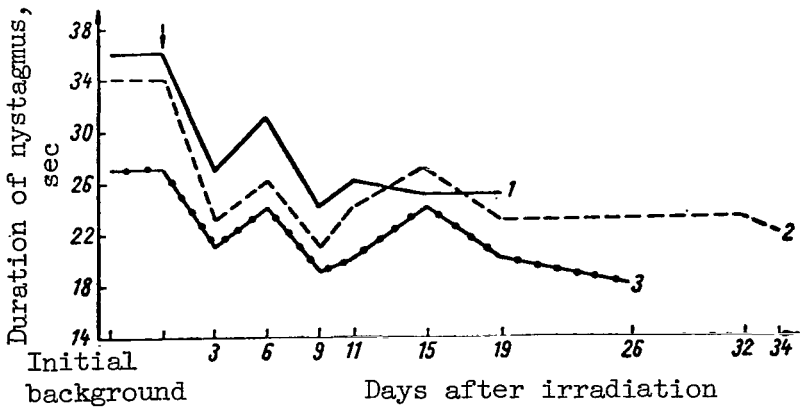

Figure 6. Change of duration of postrotational nystagmus after irradiation of dogs by protons with dose of 350 rad: (1, 2 and 3) data for dogs Driada, Pavlin and Bezrodnaya, respectively. Arrow indicates irradiation.

TABLE 2. THRESHOLD SENSITIVITY OF VESTIBULAR APPARATUS ($^{\circ}$/sec) AFTER IRRADIATION OF DOGS WITH PROTONS WITH ENERGIES OF 130 MEV WITH DOSE OF 350 RAD

Name of dog	Initial background			Days after irradiation								
	10/I	13/V	16/V	3	6	9	11	15	19	26	32	34
Driada	2	3	3	3	3	3	3	4	5	-	-	-
Bezrodnaya	5	4	4	4	4	5	4	6	7	7	7	-
Pavlin	4	4	3	3	3	4	4	4	5	7	7	7

In all dogs of group II we found development of a phase of retardation after irradiation; the adjustment phase, as in group I, was observed on the 3d day after irradiation (fig. 9), but the paradoxical phase was observed on the 6th-15th day. Beginning on the 26th day, curves 4 and 5 had a smooth slope although they were closer to the x-axis than before irradiation.

In both groups the changes of the vestibular-autonomic reflexes (respiration and pulse) were manifested only in stop-stimuli of 60-120°/sec; each dog had its individual peculiarities, which were expressed in a quickening or slowing of respiration and pulse in response to sudden stopping of rotation. Before irradiation respiration quickened or slowed by 6-8 acts per min; after irradiation these variations were expressed to a lesser degree, 3-5 acts per min; for the pulse--6-18 beats per min. It was not possible to establish such peculiarities for the animals which died earlier or later. /167

In the analysis of these indices we therefore detected a general pattern in the change of the functional state of the vestibular apparatus after irradiation by protons: a decrease of threshold sensitivity as a function of the

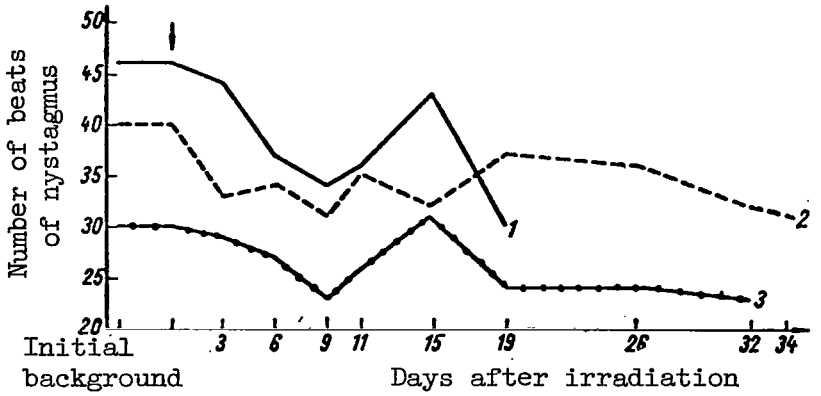

Figure 7. Change of number of beats of postrotational nystagmus after irradiation of dogs by protons with dose of 350 rad. Notations same as in figure 6.

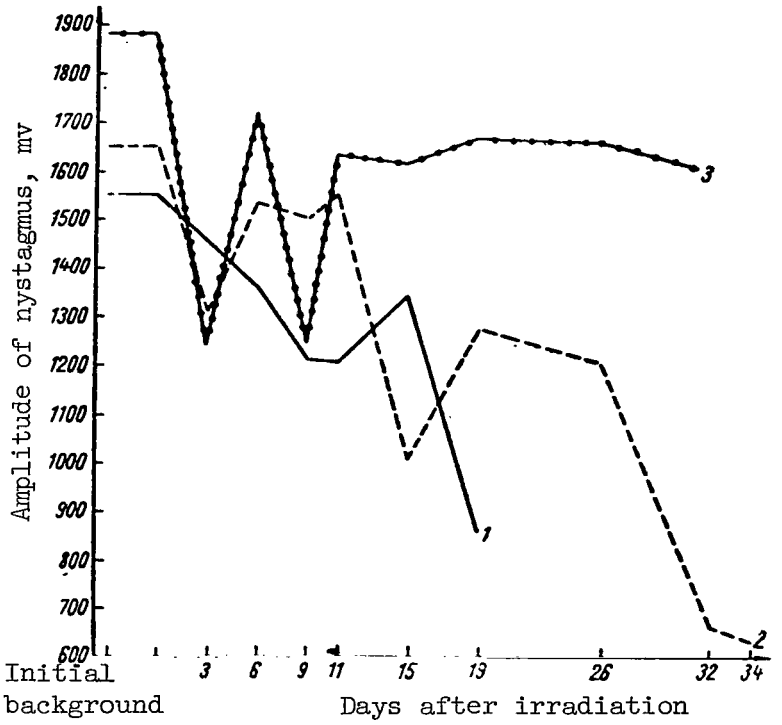

Figure 8. Change of amplitude of postrotational nystagmus after irradiation of dogs by protons with dose of 350 rad. Notations same as in figure 6.

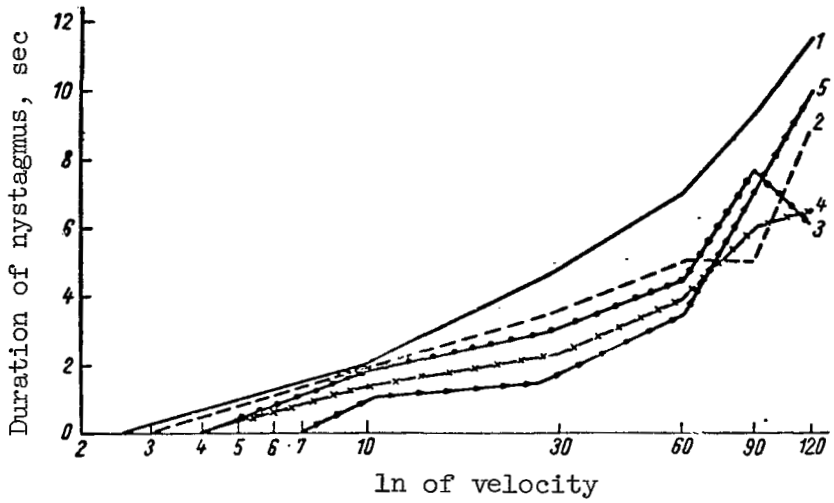

Figure 9. Curves of reaction of vestibular apparatus after irradiation of dog Pavlin by protons with dose of 350 rad: (1) curve before irradiation; (2, 3, 4 and 5) on the 3d, 9th, 11th and 35th day, respectively, after irradiation.

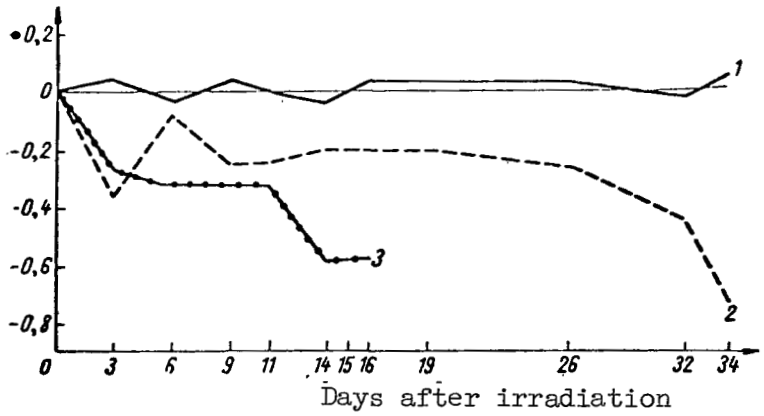

Figure 10. Change (in relative units) of intensity of postrotational nystagmus after irradiation of dogs by protons with doses of 500 and 350 rad (mean indices in group): (1) data for 6 control dogs; (2) after irradiation with dose of 350 rad; (3) after irradiation with dose of 500 rad.

lifetime of the irradiated animals and a decrease of the reaction of the vestibular apparatus (fig. 10).

Quantitative Relations between the Dose of Ionizing Radiation and the Functional State of the Vestibular Apparatus

The determination of norms with respect to the admissible levels of ionizing radiations applicable to space flight and the establishment of a sound basis for appropriate practical recommendations require that experimental investigations be carried out to determine the quantitative relations between the dose and the radiobiological effect.

Recently a large number of studies has been published in the field of quantitative radiobiology. Numerous investigations by Rayevskiy (ref. 42) and his school in the field of quantitative analysis of many radiation phenomena are well known.

The dose relationships have been established for a number of physicochemical reactions in elementary systems possessing low compensatory capability, and quantitative relations have been found between dose and many somatic effects. Along these lines it is of practical interest to consider data quantitatively, characterizing the radiosensitivity of the nervous system in general and /168 the vestibular apparatus in particular under the influence of different doses of radiation.

A definite relationship has been found between the intensity of the dose of ionizing radiation and the initial changes of the bioelectric activity of the cerebral cortex of a rabbit (ref. 43). However, for practical purposes it is necessary to establish similar quantitative relationships when evaluating the systemic reactions of the entire organism, that is, when there are the most favorable conditions for the compensation of impaired functions. For these purposes we used the vestibular apparatus, whose normal functioning under space flight conditions is mandatory.

For solution of the formulated problem we carried out five series of experiments on 100 rabbits weighing 2.5-3.5 kg (20 rabbits were subjected to each dose of irradiation): 1st series--50 r; 2d series--100 r; 3d series--500 r; 4th series--800 r, and 5th series--5,000 r. Twenty other rabbits of this same weight were used as control group.

These dosages interested us for two reasons. First, irradiation in different doses is possible during space flight at the time of solar chromospheric flares. In addition, a dose of 50 r now is recommended as the "justified risk" dose for a cosmonaut. Second, in order to establish a quantitative dependence between the dose of radiation and the initial functional changes of the vestibular apparatus, we used doses causing 50 percent (500 r) and 100 percent (800 r) death of animals in the course of 45 days, and also a dose (5,000 r) causing an acute form of radiation sickness.

The rabbits of the first series were subjected to general X-ray irradiation on a RUM-3 apparatus with a dose intensity of 16 r/min. All remaining animals (series 2-5) were subjected to general irradiation from a cobalt source on an EGO-2 apparatus with a dose intensity of 250 r/min.

A study was made of the initial reactions of the vestibular apparatus as a function of the radiation dose in the experimental animals. For this purpose we estimated the threshold sensitivity and the reaction of the apparatus to an adequate irritant 2 or 3 times before irradiation, immediately after irradiation and 2 and 5 hours later.

Investigation of the vestibular apparatus of control animals was made at the same time as for the experimental animals.

The vestibular-somatic reflex (nystagmus) and some vestibular-autonomic reactions (pulse, respiration) were recorded for all rabbits.

In the control group of animals the threshold sensitivity was normally 4-10°/sec. The threshold value for each rabbit was quite stable; the maximum variations of this value after "imaginary irradiation" in all 20 control /169 animals was not more than one degree in the direction of an increase or decrease of the threshold. An increase or decrease of the threshold after irradiation by more than 1° in comparison with the initial value was used in evaluating the change of the threshold sensitivity of the vestibular apparatus, and rabbits with such changes were considered as "reacting" to the radiation effect.

The initial reactions of any apparatus to an irritant are functional and usually are manifested in a change of its threshold sensitivity. It can be expected, therefore, that after exposure to radiation there will be an impairment of the functioning of the vestibular apparatus, and this impairment is manifested primarily in a change of threshold sensitivity. Table 3 gives the character of change of the threshold sensitivity of the vestibular apparatus after irradiation for each series of experiments.

Table 3 shows that in the first series of experiments (i.e., after irradiation with 50 r) the threshold sensitivity in 14 of 20 rabbits changed; in 7 of these animals there was a decrease of threshold sensitivity and in the others there was an increase.

TABLE 3. CHANGE OF THRESHOLD SENSITIVITY OF VESTIBULAR APPARATUS IN RABBITS IN FIRST 5 HOURS AFTER IRRADIATION WITH DIFFERENT DOSES

Series	Dose, r	Number of rabbits in series	Number of rabbits reacting to irradiation	Character of reaction		Number of rabbits not reacting to irradiation
				Decrease of threshold sensitivity	Increase of threshold sensitivity	
1st	50	20	14	7	7	6
2d	100	20	9	3	6	11
3d	500	20	18	18	-	2
4th	800	20	20	18	2	-
5th	5000	20	20	18	2	-

After irradiation with 100 r (2d series of experiments) there were changes noted in the activity of the vestibular apparatus in 9 of 20 rabbits: in 6 animals the threshold sensitivity decreased and in only 3 was there an increase.

Therefore, with an increase of the radiation dose there is a decrease of the number of cases of increase of irritability of the vestibular apparatus. However, in the case of irradiation with 50 r, impairment of the functioning of the vestibular apparatus was observed in general in a greater number of animals (14 animals) than in the case of irradiation with 100 r (9 rabbits). This can possibly be attributed to the fact that with an increase of the radiation dose from 50 to 100 r an increase of irritability (as a reaction of the apparatus to small doses of ionizing radiation) is manifested only in individual cases. /170 A qualitatively different character of the reaction in the form of depression of the functioning of the apparatus, characteristic of considerable radiation injuries, still did not develop to a proper degree. This in indicated by the data from our observations: a decrease of threshold sensitivity in the case of irradiation with 50 r was observed in 7 rabbits, and in the case of a dose of 100 r in 6 rabbits, that is, in both cases an almost identical number of animals; however, an increase of threshold sensitivity (and therefore an increase of the irritability of the vestibular apparatus) in the case of irradiation with 50 r was detected in 7 rabbits, but with irradiation with 100 r in only 3 rabbits.

The results from the use of a "reaction test," that is, after the use of an adequate irritant of increasing intensity (30, 60, 120°/sec), indicate some decrease of the reaction of the vestibular apparatus in some animals; this decrease was observed more clearly 5 hours after irradiation. In some of the animals there was increase of the reaction of the apparatus, together with an increase of threshold sensitivity.

In the 3d series of experiments (dose of 500 r), there were changes in the activity of the vestibular apparatus in 18 of 20 animals; in these animals there

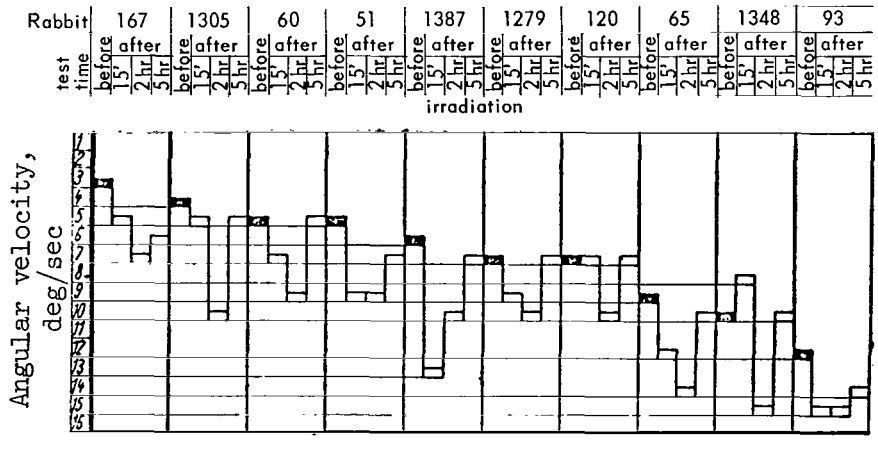

Figure 11. Change of threshold sensitivity of vestibular apparatus during irradiation of rabbits with dose of 500 r.

was an identical reaction of depression of the functioning of the vestibular apparatus. A decrease of the threshold sensitivity of these rabbits was expressed more clearly than in the rabbits of series 1 and 2, and its maximum decrease was observed 2 hours after irradiation. Five hours after exposure to radiation there was a tendency to restoration of the initial irritability (fig. 11).

In the fourth series of experiments (dose of 800 r) all animals showed /171 impairment of the functioning of the vestibular apparatus; in 18 of the animals there was also a decrease of the threshold sensitivity, whereas in the other two animals there was an increase. In some of the animals the decrease of the threshold sensitivity of the vestibular apparatus was expressed to a still higher degree, and its maximum decrease was also observed after 2 hours. Five hours after termination of exposure to the radiation, there was a tendency to restoration of the initial irritability.

The vestibulograms of the rabbits of the 3d and 4th series indicate a more clearly expressed decrease of the reaction of the apparatus. As an illustration we cite the vestibulogram of a rabbit after irradiation, 800 r (fig. 12). Figure 12 shows that the maximum decrease in the reaction is observed 2 hours after irradiation, that is, parallel with a decrease of the threshold sensitivity. Immediately after irradiation and during the first 5 hours, both the paradoxical and adjustment phases were observed. With a stop-stimulus with an angular velocity of 60°/sec the response sometimes was less than with a velocity of 30°/sec, but at a velocity of 120°/sec the reaction was the same as with a velocity of 30°/sec, but at a velocity of 120°/sec the reaction was the same as with a velocity of 30°/sec. Later there was some restoration of the initial reaction.

In the 5th series of experiments (dose of 5,000 r) there was an impairment of the functioning of the vestibular apparatus in all rabbits; as in the 4th series, 18 rabbits presented a decrease of the threshold sensitivity and 2 an increase. The decrease of threshold sensitivity in this series was expressed to a still greater degree than in the preceding series; in 10 of the rabbits the maximum of the decrease of the threshold was observed immediately after irradiation, and in the remaining 10 it was 2 hours after termination of exposure to radiation. Together with a decrease of the threshold sensitivity there was also noted a decreased reaction of the apparatus; the maximum decrease was observed immediately after irradiation, followed by some restoration of the initial reaction.

The character of the vestibular-autonomic reactions also changed. Before irradiation, irritation of the vestibular apparatus led to brief reflex /172 changes of respiration. In most cases there was a quickening of respiration and an increase or decrease of the amplitude of respiratory movements. No changes in the direction of quickening of the pulse were observed at this time. The intensity and duration of respiration reflex reactions usually changed proportional to the increase of negative angular acceleration. Following irradiation, it was found that the animals had impairments of the normal force relationships in the course of vestibular-autonomic reactions, manifested very

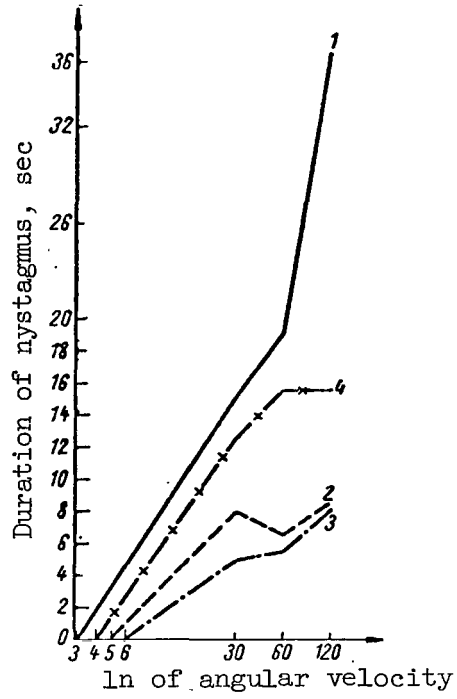

Figure 12. Vestibulogram after irradiation of rabbit with 800 r: (1) before irradiation; (2, 3 and 4) after 15 min, 2 and 5 hours after irradiation, respectively.

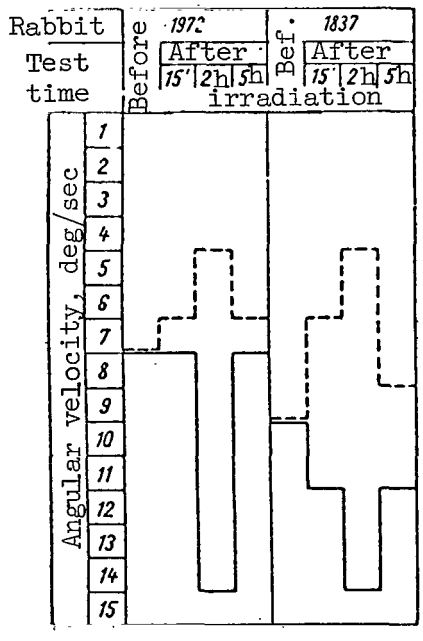

Figure 13. Change of threshold sensitivity of vestibular apparatus after irradiation of rabbit with 50 r (dashed lines) and 500 r (solid lines).

clearly after irradiation with 500, 800 and 5,000 r. Instead of the usual increase of intensity and duration of the reactions, with an increase of the intensity of the adequate irritation of the vestibular apparatus there was an almost identical picture of changes of respiration in response to weak and strong irritations (adjustment phase). In some of the animals the change in respiration appeared only in response to sufficiently strong stimulation of the labyrinth (120°/sec), but the magnitude of the effect was also less than before irradiation of the animal.

Thus, it was possible to establish a range of doses (500-5,000 r) which leads to a considerable impairment of the functional state of the vestibular apparatus in most of the animals, which is expressed in a decrease of irritability and reaction of the vestibular apparatus. In the range of doses 50-100 r some of the animals were observed to have a completely different character of functional changes--an increase of irritability. As an illustration we will cite the following observation (fig. 13). In rabbits No. 1972 and 1837, after irradiation with 50 r, there was an increase of the threshold sensitivity. A month later these same rabbits were irradiated with 500 r, and the character of the functional changes of the vestibular apparatus was then completely different: irritability had considerably decreased.

In developing practical measures, it is important that in the case of irradiation with a dose of 500 r and above, there be inhibition not only of the vestibular-somatic reflex, but the vestibular-autonomic reaction as well. /173 If this is done, the combined effect on the cosmonaut of radiation and adequate irritants for the nonauditory labyrinth (Coriolis force, weightlessness) will not cause development of autonomic impairments.

Functional Impairments of the Activity of the Semicircular Canals at Different Periods during Radiation Sickness

In determining the dose dependence in relation to initial changes of the functional state of the vestibular apparatus we noted that several hours after irradiation there was a quite clear tendency to a normalization of the impaired functions. It therefore was of great practical interest to obtain data on the character of possible impairments of the labyrinth in the later periods of development of radiation sickness.

Experiments on rabbits

Twenty rabbits, irradiated with a dose of 800 r, were observed during the entire development of the radiation sickness to the time of death of the animals. In all experimental animals acute radiation sickness developed to a severe degree. The life of the experimental animals for the most part terminated 7 to 13 days after exposure to radiation; one rabbit died on the fourth day and two rabbits survived. There was a vestibulometric investigation on the 2d, 3d, 5th, 7th, 10th and 12th days after irradiation. The 10 control rabbits were investigated at these same times.

Figure 14 shows the character of the variations of the threshold sensitivity of the control and experimental animals (those which died and those which survived)

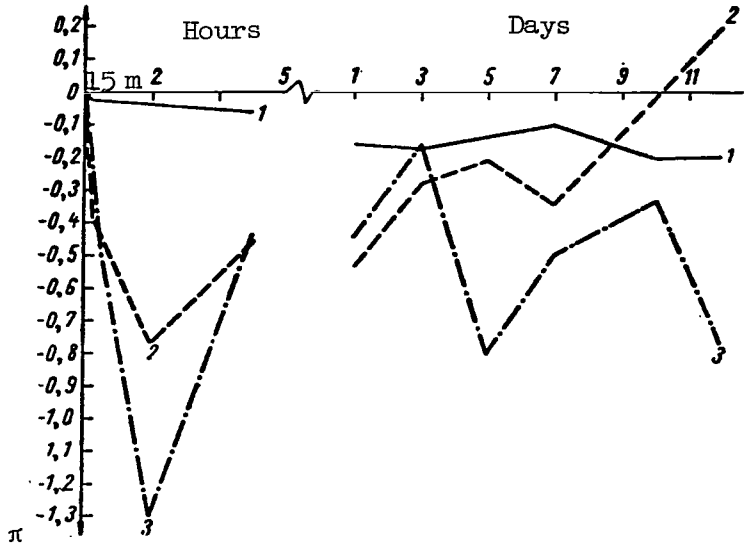

Figure 14. Change (relative units) of threshold sensitivity of vestibular apparatus of rabbits during acute radiation sickness: (1) control animals; (2) dead animals; (3) surviving animals.

at different times after exposure to radiation.[1] The left graph shows the character of the initial reactions of the vestibular apparatus, that is, 15 minutes, 2 hours and 5 hours after exposure to radiation; the right graph shows the character of variations of threshold sensitivity of these same rabbits during different periods of acute radiation sickness. The figure shows that immediately after irradiation of the experimental animals there is a decrease of threshold sensitivity, and 2 hours later this decrease was expressed to a considerable degree. It is interesting to note that in the surviving rabbits the threshold sensitivity decreased to a still greater degree than in those which died. Five hours later there was a tendency to restoration of the /174 initial sensitivity.

On the first day after irradiation the threshold sensitivity remained low. From the 3d through the 5th day, that is, in a period of relative stabilization in the course of the radiation sickness, there was a partial restoration of threshold sensitivity in the group of dying animals, but this restoration is

[1]The threshold value (II) for each rabbit was computed using the formula $Th = P-P_0/P_0$, where P_0 is the threshold value for each rabbit before irradiation; P is the threshold value after irradiation. Averaging was carried out for the 10 rabbits of the control group and for 20 rabbits of the experimental group, of which 18 died and 2 survived.

not complete. On the 7th day there again was a tendency to a decrease of irritability. In the terminal period of the radiation sickness, that is, by the 10th-12th days, there was a sharp increase of the threshold sensitivity. This effect was not observed in the surviving animals; in these rabbits the threshold sensitivity of the vestibular apparatus continued to remain low.

The results obtained using an adequate irritant of increasing intensity (stop-stimulus at velocities of 30, 60 and 120°/sec) indicate a considerable change of the reaction of the vestibular apparatus (fig. 15).[1] Immediately after irradiation the response to the stop-stimulus at all three velocities by the vestibular apparatus in the group of dying animals decreased sharply, and after 2 hours this decrease was expressed to a still greater degree. Five /176 hours after termination of exposure to radiation the excitability of the vestibular apparatus was restored and even had increased somewhat.

In the group of surviving animals the response of the vestibular apparatus to the effects of strong stimulation (30, 60 and 120°/sec) decreased sharply immediately after irradiation. Two hours later it remained at almost the same low level. Five hours later the response to a stop-stimulus at a velocity of 30°/sec increased somewhat, but remained lower than in the control group. At a velocity of 60°/sec this increase of response was expressed to a lesser degree, but at a velocity of 120°/sec there was generally no increase of the response.

A day after irradiation the response of the vestibular apparatus to the stop-stimulus at all three velocities remained low. On the 3d day the response increased to stop-stimuli of 30 and 60°/sec in the group of dying animals (to a greater extent than in the group of control animals). Then the shape of the curves for the dying animals at these velocities was uniform: after the 3d day the response decreased gradually and attained a maximum in the terminal period. With a stop-stimulus at a velocity of 120°/sec, a decrease of response on the first day was followed by a tendency to restoration of response and by the 7th day it attained the response of the control rabbits; in the terminal period (i.e., by the 10th-12th days) the excitability of the vestibular apparatus again decreased sharply.

On the 3d day after irradiation the reaction to the stop-stimulus at all three velocities decreased still further in the group of surviving animals than on the 1st day. Beginning with the 3d day, the curves for all three velocities fully duplicated the shape of the curves for the control animals, but the curves were at a lower level.

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[1] The reaction of the vestibular apparatus for each rabbit (R) was computed using the formula $R = r-r_0/r_0$, where r_0 is the duration of nystagmus after stop-stimulus for a certain velocity (30, 60 and 120°/sec) before irradiation, r-- after irradiation. Averaging in the control group was for 10 rabbits, in the experimental group for 20 animals, 18 of which died and 2 of which survived.

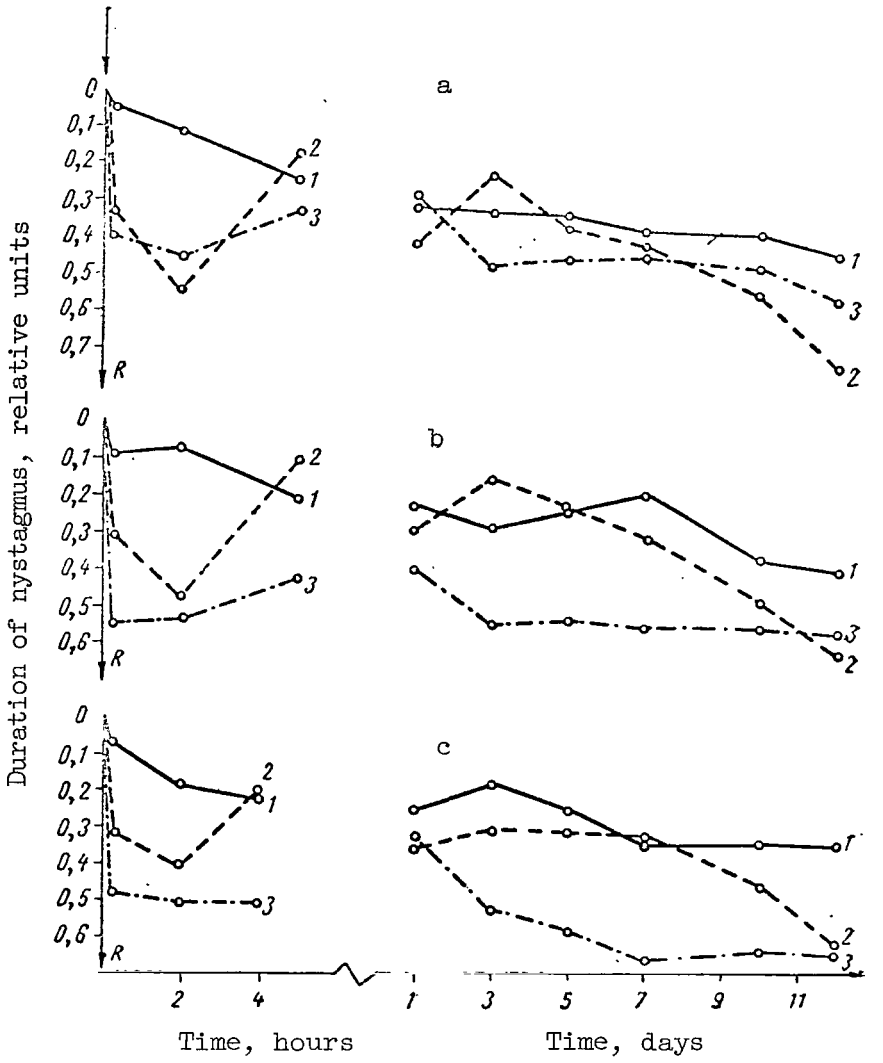

Figure 15. Change of reaction of vestibular apparatus of rabbits during acute radiation sickness. Angular velocity 30°/sec (a), 60°/sec (b), 120°/sec (c): (1) control animals; (2) dead animals; (3) surviving animals.

Thus, immediately after irradiation of rabbits with a dose of 800 r there was a sharp decrease of threshold sensitivity and reaction of the vestibular apparatus. Two hours later the depression of the functioning of the vestibular apparatus was expressed to a still greater degree. Five hours later the reaction of the vestibular apparatus was restored, and even the response to the adequate irritants of increasing intensity exceeded the response of the control animals. On the first day after irradiation the threshold sensitivity and the reaction of the vestibular apparatus remained low. In the period of "fictitious well-being" (from the 3d to the 5th day), the threshold sensitivity and reaction

of the vestibular apparatus was restored somewhat, but this restoration was not complete. In the terminal period (i.e., by the 10th-12th days) the threshold sensitivity increased sharply. Such an increase of threshold sensitivity was not observed in the group of surviving animals: it remained low.

Experiments on dogs /177

The experiments were made with single and fractional irradiation with a dose of 200 r. After a single irradiation 3 of the 4 dogs developed radiation sickness of average severity. The fourth dog of this group developed an acute form of radiation sickness with a lethal outcome on the 23d day after exposure to radiation (at autopsy it was found that there were massive hemorrhages in the lungs, in the atrioventricular septum of the heart and the mesenterium tenue).

In the three surviving dogs the changes of the vestibular apparatus were similar and were expressed in a decrease of excitability to caloric stimulation on the first two days after irradiation. Thereafter there was a phase change of irritability with a predominance of an increase (fig. 16). A normalization of irritability occurred toward the end of the second month. Investigation of the reaction of the vestibular apparatus revealed that it changed in the same direction as irritability.

In the fourth dog the functional state of the vestibular apparatus decreased as the radiation sickness developed (fig. 17). Investigation of the threshold sensitivity and the reaction of the vestibular apparatus revealed there was a decrease, beginning on the 15th day after irradiation. Reaction decreased as the radiation sickness developed. On the 15th day development of a paradoxical reaction to adequate irritants was noted (fig. 17, curve 3; as a response to stronger stimulation, 60°/sec, the duration of the reaction was less than for weaker stimulation, 30°/sec.

In the case of fractional irradiation with 200 r (at the rate of 9 r per day, with irradiations a day apart), there was an increase of the irritability of the vestibular apparatus on the first day after the dose; with an increase of the dose (27-63 r) decrease of the irritability followed. Thereafter there was a phase change of irritability with a predominance of increased irritability (fig. 18). Normalization of the functional state was established at the end of the second month, after termination of irradiation. The changes in the reaction of the vestibular apparatus to adequate stimuli were in the same direction as changes of excitability.

Changes of the vestibular-autonomic reflexes (respiration and pulse) were expressed in a perversion of the usual character of the changes of respiration and pulse during caloric stimulation of the labyrinth, in the appearance of salivation and less frequently in the appearance of a motor response. The data presented in figure 19 show that when caloric nystagmus appears in an unirradiated animal there is slowing of respiration and especially of the pulse rate (curves 3 and 1). After irradiation this pattern changed (curves 4 and 2): the frequency of respiration for the most part increased, but the pulse rate changed little during the caloric stimulation. The caloric response was

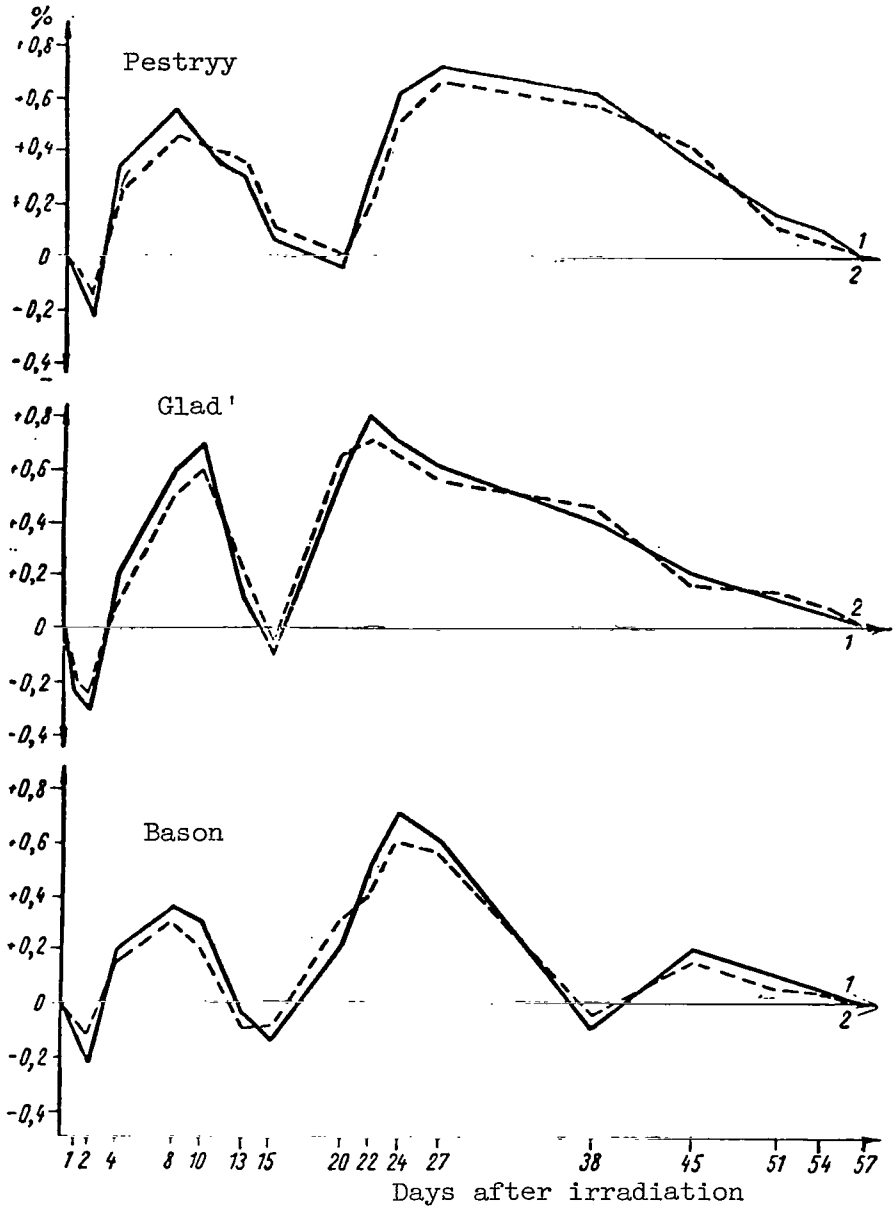

Figure 16. Change of intensity of caloric nystagmus in dogs during radiation sickness of medium severity (single general irradiation with 200 r): (1) right labyrinth; (2) left labyrinth.

accompanied by salivation. These data show that changes in the autonomic nervous system play a definite role.

Thus, the comparative study of the functional state of the vestibular apparatus with these doses provided the basis for assuming that there are

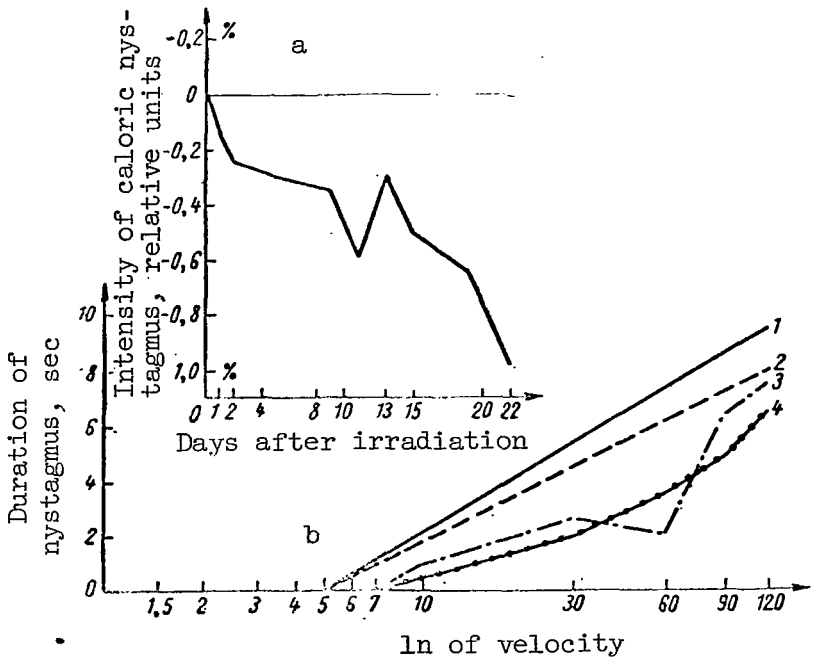

Figure 17. Change of intensity of caloric nystagmus (a) and reaction of vestibular apparatus (b) during severe stage of radiation sickness (dog Gulyaka, single general irradiation with 200 r): (1) before irradiation; (2, 3 and 4) on 2d, 15th and 22d day after irradiation.

definite differences in the effect of exposure to single and fractional irradiation.

It is known (refs. 6, 12, 17-19 and 45-48) that vestibular impairments under the influence of radiation represent one of the manifestations of damage to the central nervous system. For this reason we could postulate that the changes of irritability which we discovered were related to processes transpiring in the central nervous system; in particular, they are related to a different relationship of compensatory processes. For proof of this theory we formulated the following experiments on two dogs. First we carried out a unilateral labyrinthectomy, which was accompanied by impairment of the coordination of movements. Eight months later, when compensation of the impaired functions, having a cortical character, began, the dogs were exposed to a single irradiation with 200 r. Pertinent data are shown in figure 20. /182 These experiments revealed that in the period of maximum impairment of compensation there was decrease of irritability of the labyrinth. The process of increase of the compensatory function of the labyrinth was accompanied by an increase of irritability.

Thus, by using new methodological possibilities for investigation of the vestibular apparatus, we have been able to represent the dynamics of development of functional changes of the vestibular apparatus at different periods in acute radiation sickness, caused both by the effect of protons with energies of

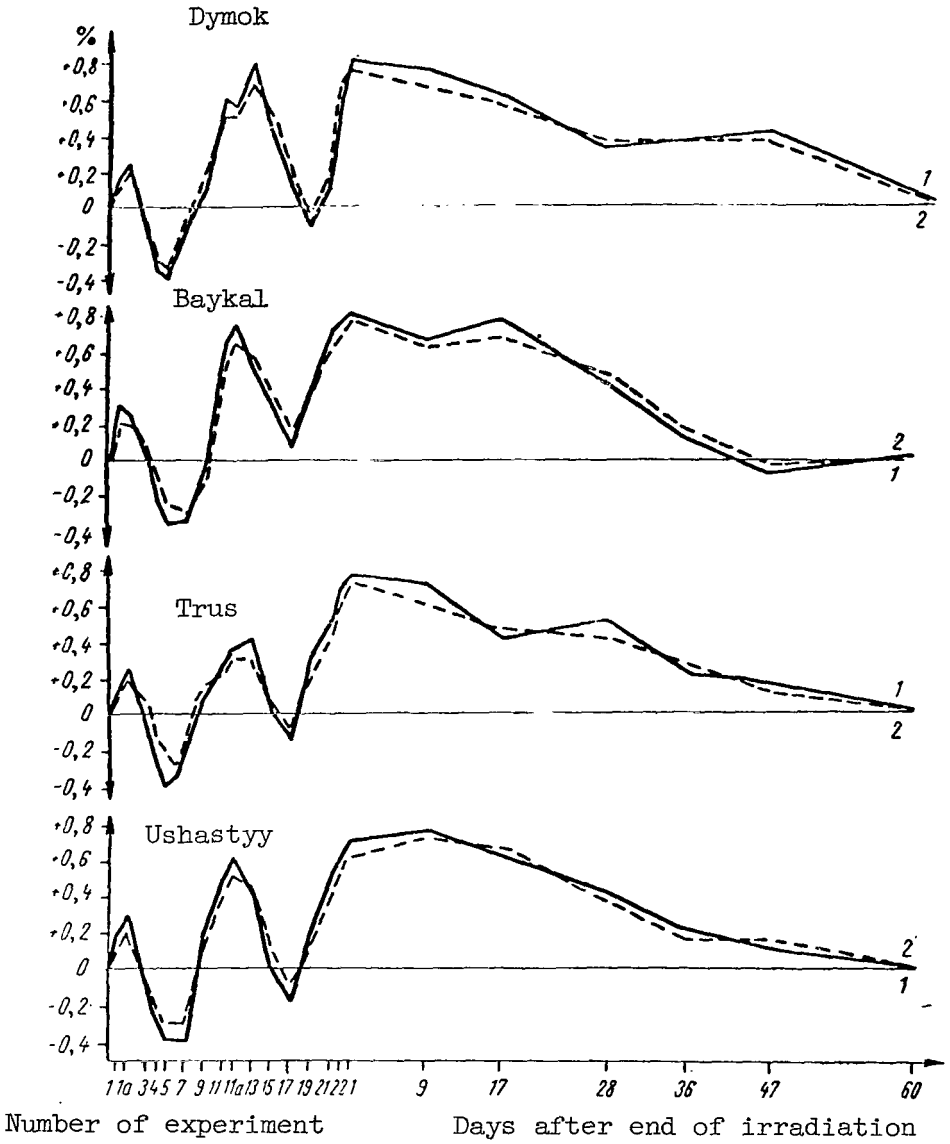

Figure 18. Change of intensity of caloric nystagmus during fractional irradiation of dogs (total dose 200 r): (1) right labyrinth; (2) left labyrinth.

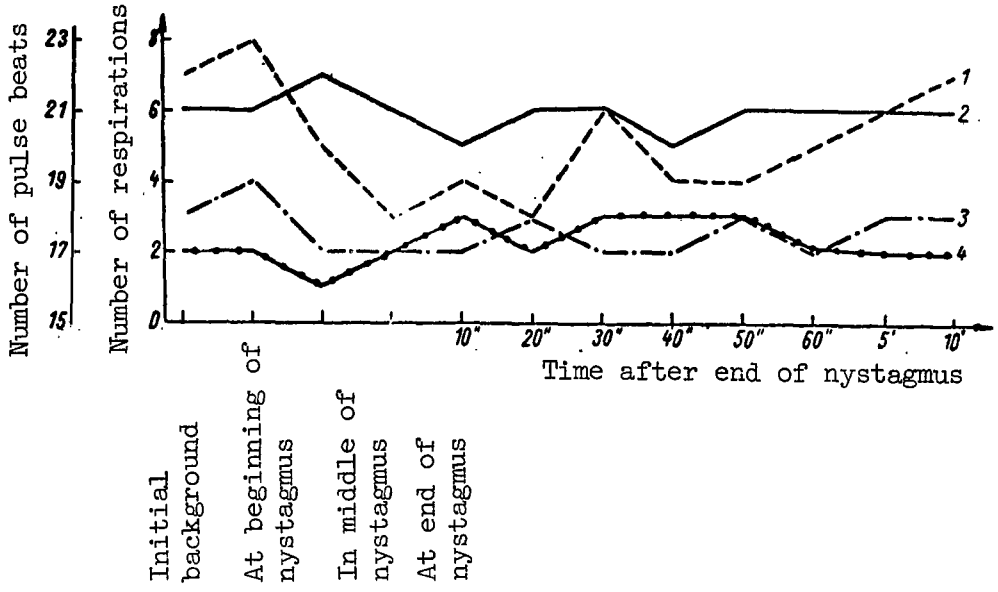

Figure 19. Character of change (after 10 sec) of respiration and pulse in caloric test before and after single irradiation with 200 r (dog Glad') (1 and 2) pulse rate; (3 and 4) frequency of respiration before and after irradiation, respectively.

Figure 20. Change of intensity of caloric nystagmus (1) and angle of inclination of head (2) of dog with unilateral labyrinthectomy after single irradiation with 200 r.

130 Mev and γ-radiation. We obtained data quantitatively characterizing the dependence between the dose of ionizing radiation and the effect. It was demonstrated that in the case of a single exposure with doses of 200 r and above there is a regular decrease of irritability of the vestibular apparatus and a decrease of its reaction in the first hours after irradiation. However, an initial increase of irritability is possible in the case of single exposure of the organism to lesser doses (50 and 100 r).

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CHAPTER 8. BIOLOGICAL EFFECT OF MULTIPLE X-RAY IRRADIATION WITH ENERGIES OF 180 KV COMPARED WITH PROTON IRRADIATION (DOSES AND TEMPORAL DISTRIBUTION) /184

N. G. Darenskaya, M. P. Domshlak, V. I. Grachev and N. I. Ryzhov

The relative biological effect of protons in fractional irradiation was determined by experiments with the corresponding irradiation of dogs and rats by X-rays with a production energy of 180 kv.

Table 1 gives the conditions for multiple irradiation of dogs and rats.

The condition of the animals (external appearance, motor and alimentary activity, state of the skin and fur, state of the mucous membranes, weight dynamics, body temperature, etc.), and the state of the peripheral blood and cardiovascular system (maximum arterial pressure, arterial tone, rate of propagation of the pulse wave, electrocardiography) were observed carefully and pathologic-morphological and histological investigations were made of the tissues and organs.

Experiments on dogs /185

Six mongrel dogs weighing 7-16 kg were subjected to multiple X-ray irradiation. The dogs were irradiated in a 12-tube X-ray apparatus with a voltage of 180 kv, a current of 24.8 ma, a focal length of 60 cm, filters of 0.5 mm Cu + 1 mm Al and a dose intensity of 60 r/min.

The character of the distribution of the X-rays in the phantom of a dog was close to the distribution of protons.

The rhythm of irradiation and magnitude of single and total doses are given in table 2.

TABLE 1. PLAN FOR FORMULATION OF EXPERIMENTS WITH MULTIPLE IRRADIATION OF DOGS AND RATS BY X-RAYS WITH ENERGIES OF 180 KV

Type and group of animals	Number of animals	Number of irradiations	Total dose, in r
Dogs.	6	17-19	613-667
Rats of group I	28	20	704-755
Rats of group II.	27	20	1011-1021

TABLE 2. RHYTHYM OF IRRADIATION IN SINGLE DOSES (AUG.-SEPT., 1961)

Name of dog / Irradiation dose, rad	5/VIII	7/VIII	10/VIII	12/VIII	14/VIII	16/VIII	19/VIII	21/VIII	24/VIII	26/VIII	28/VIII	30/VIII	2/IX	4/IX	6/IX	9/IX	11/IX	13/IX	16/IX	Total dose, r
Pyatnashka	13.0+	19.0/32.0	22.0/54.0	30.0/84.0	47.7/131.7	21.3/153.0	24.0/177.0	23.0/200.0	34.0/234.0	35.0/269.0	60.0/329.0	51.0/380.0	55/435.0	45.0/480.0	51.0/531.0	34.0/565.0	48.0/613.0	—		613
Grey	13.0+	23.0/36.0	19.0/55.0	21.0/76.0	27.9/103.9	8.3/112.2	28.0/140.2	29.0/169.2	36.0/205.2	18.0/223.2	62.0/285.2	78.0/363.2	26.0/389.2	46.0/435.2	42.0/477.2	75/552.2	52/604.2	28.0/632.2	—	632.2
Laskovyy	12.5+	20.0/32.5	24.0/56.5	26.0/82.5	29.1/111.6	18.9/130.5	33.0/163.5	21.0/184.5	40.0/224.5	14.0/238.5	64.0/302.5	57.0/359.5	15.0/374.5	47.0/421.5	53.0/474.5	68.0/542.5	55.0/597.0	40.0/637.0	—	637
Pamir	10.0+	22.0/32.0	20.0/52.0	30.0/82.0	19.2/101.2	61.0/162.2	21.0/183.2	10.0/193.2	38.0/231.2	32.0/263.2	62.0/325.2	62.0/387.2	31.0/418.2	46.0/464.2	53.0/517.2	68.0/585.2	44.0/629.2	28.0/657.2	—	657.2
Kazbek	12.0+	22.0/34.0	23.0/57.0	38.0/95.0	40.0/135.0	22.0/157.0	29.0/186.0	11.0/197.0	40.0/237.0	15.0/252.0	62.0/314.0	55.0/369.0	18.0/387.0	49.0/436.0	46.0/482.0	79.0/561.0	46.0/607.0	33.0/640.0	—	640
Mayka	11.0+	20.0/31.0	21.0/52.0	36.0/88.0	18.2/106.2	23.8/130.0	35.0/165.0	24.0/189.0	30.0/219.0	13.0/232.0	62.0/294.0	71.0/365.0	21.0/386.0	54.0/440.0	59.0/499.0	80.0/579.0	34.0/613.0	36.0/649.0	18.0/667.0	667

Note. Numerator shows single irradiation doses; denominator shows total doses.

TABLE 3. DURATION OF LIFE OF DOGS EXPOSED TO MULTIPLE X-RAY IRRADIATION (MEAN TOTAL DOSE FOR GROUP WAS 641 R DURING PERIOD OF IRRADIATION FROM AUG. TO SEPT. 16, 1961) /187

Name of dog	Number of irradiations	Total duration of period of irradiation, days	Total dose, r	Time of death after beginning of irradiation, days
Pyatnashka	17	37	613	37
Grey	18	40	632	56
Laskovyy	18	40	637	59
Pamir.	18	40	657	40
Kazbek	18	40	640	40
Mayka.	19	43	667	45

Note: Mean lifetime of dogs was 46 days.

The data in table 2 show that the dogs were irradiated at intervals of 2-3 days and the single doses ranged from 8.3 to 80 r. Table 3 gives data on the lifetime of the dogs.

As a result of multiple radiation exposure, all dogs developed a well-expressed form of acute radiation sickness. Table 3 shows that all dogs died in the period from the 37th to the 59th day from onset of irradiation. The average lifetime of the dogs was 46 days.

The first indications of illness in the dog Pamir appeared very early. Even after the fifth irradiation (total dose 101 r) there was diarrhea, although it stopped intermittently. The dog became listless and passive. Twenty-two days after onset of the irradiations the state of the other animals remained completely satisfactory. The first indications of illness in these dogs appeared when the total dose reached 223-269 r (after 10 irradiations): listlessness, decreased appetite and increased body temperature. Thereafter the condition of the dogs gradually deteriorated: there was an increase of listlessness and a blanching of the mucous membranes of the eyes and oral cavity.

Diarrhea appeared after the total doses reached about 421-480 r. On the days which followed (on the 30th-31st day) there were punctulate hemorrhages in the ocular conjuctivae and the mucous membrane of the mouth. A particularly sharp deterioration of the condition of the animals was observed toward the end of the period of irradiations (35th-38th day) and in some of the dogs soon after termination of this period: multiple hemorrhages appeared in the skin and subcutaneous cellular tissue of the torso and there were extensive confluent hemorrhages in the abdominal regions. In this same period there were increasing weakness and a sharp decrease of appetite, and finally the dogs refused food entirely. There were gangrenous changes in the mouth, edema of the snout and extensive hemorrhages in the upper lip and hemorrhaging in the muscles of the paws, with subsequent ulceration. Blood appeared in the feces.

The body temperature increased to 39.7-40.8°, and in individual dogs there was a temperature decrease with a subsequent increase in the terminal period (fig. 1). Beginning on the 30th day there was a gradual decrease in the respiration rate. The respiration rate decreased by half or even somewhat more (by 70 percent of the initial level). The loss of weight by the dogs was insignificant and amounted to only 7 percent of the initial weight. The animals died from a sharply expressed general weakness and from the progressive nature of the other symptoms of the illness. As an illustration we cite one history of the disease.

The Dog Pamir. During the period Aug. 5-Sept. 13 the dog was subjected to irradiation 18 times in single doses of 10-68 r. Total irradiation dose was 657.2 r.

The first indications of illness appeared very soon after the total dose had reached 101.2 r (5 irradiations, 10th day). Diarrhea appeared at that time. The general condition changed after the 12th irradiation (total dose 387.2 r, 25th day), and listlessness was noted. After the 13th irradiation (total dose 418.2 r, 28th day) the diarrhea was intensified. After the 14th irradiation (total dose 464.2 r, 31st day) the diarrhea became hemorrhagic and remained so to the dog's death. After the 15th irradiation (total dose 517.2 r, 33rd day) the dog's temperature rose to 39.9°, and on the days which followed, until death occurred, it remained high (40.1-42.0°). Blanching of the mucous membranes was manifested sharply after the 16th irradiation (total dose 582.2 /188 r, 37th day). After the 17th irradiation (total dose 629.2 r, 39th day) the condition of the dog was critical and there was hemorrhagic gingivitis, edema of the upper lip, hemorrhagic diarrhea, hemorrhaging in the skin and the subcutaneous cellular tissue of the abdominal regions and hemorrhages in the shaved part of the skin. The dog died after the 18th irradiation after an increase of these symptoms (on the 41st day).

Autopsy Report. Multiple hemorrhages in the skin and in the subcutaneous cellular tissue and in the muscles between the bones. Necrotic tonsillitis. Extensive hemorrhages in the muscles of the heart and stomach and punctulate hemorrhages in the cortical layer of the kidneys. Individual small hemorrhages in the lungs and along the small and large intestines. Hemorrhagic ascites.

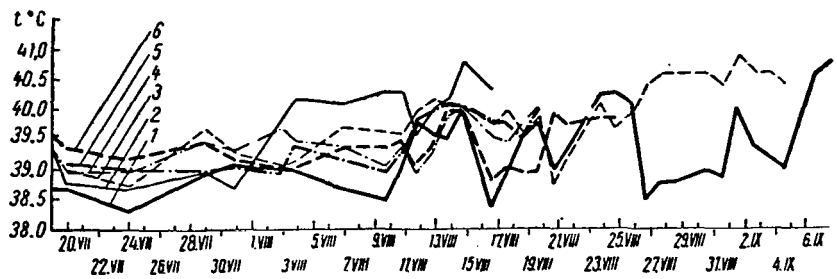


Figure 1. Body temperature changes in dogs. 1, No. 1198; 2, No. 1165; 3, No. 1186; 4, No. 1156; 5, No. 1196; 6, No. 1182.

In analyzing the clinical picture of radiation injury caused by multiple irradiation, it is necessary to note the appearance of symptoms of the illness at total doses of the order of 223-269 r. Thereafter, the development of the illness continues at a relatively rapid rate. We note a sharp manifestation of the hemorrhagic syndrome in these animals. Usually in the case of acute radiation sickness caused by a single exposure to the minimal absolutely lethal dose, the hemorrhagic manifestations are considerably less prominently expressed.

A corresponding evaluation was made of the state of the cardiovascular system. Changes of arterial pressure, the rate of propagation of the pulse wave and an electrocardiogram were recorded.

The pulse was recorded by a piezoelectric sensor attached to the artery of the tibia of the rear leg. The levels of maximum and minimum arterial pressure were determined at the time of decrease of pressure in the cuff from the appearance of the first pulse wave and the character of its dicrotic wave. The cuff was applied to the tibia of the rear extremity of a dog, somewhat above the sensor recording the sphygmogram. Pressure changes in the cuff were recorded by a pressure-mark apparatus which produced marks at each 10 mm Hg in the range from 20 to 190 mm Hg. In most of the experimental animals there was a decrease of both maximum and minimum pressure. The most clearly expressed hypotensive response was observed beginning with the 30th day after onset of radiation exposure. In a number of cases the minimum arterial pressure decreased to a considerable degree (by 50 percent of the initial level). However, the tone of the main arteries did not change significantly, as can be judged on the basis of data on investigation of the rate of propagation of the pulse wave. The most significant changes apparently occurred in the small arteries and arterioles. In addition, the decrease of arterial pressure could be attributed to a certain degree to weakness of the cardiac muscle.

Some indications of the possibility of weakening of the functioning of the myocardium in these animals are obtained from an analysis of the changes of the electrocardiogram. It was noted that there was some decrease of the voltage of most of the waves of the electrocardiogram, with the exception of the T-wave, which increased. The development of radiation damage was not /189 reflected significantly in the behavior of cardiac excitability. Nevertheless, in one dog there was an insignificant increase of atrioventricular conduction from 0.12-0.14 sec. In two other dogs there was a small increase of intraventricular conduction (ST interval) from 0.26 sec under normal conditions to 0.28-0.29 sec after irradiation. In these animals there was also a displacement of the ST interval below the isoelectric line by 1-2 mm in the standard abductions II and III. In addition, in most of the animals there was a noticeable decrease of the systolic index. However, on later days its value returned to normal, which probably was dependent on the change of frequency of cardiac contractions. There were a considerable decrease of the frequency of cardiac contractions and also a well-expressed respiratory arrhythmia. Toward the end of the observations the frequency of cardiac contractions returned to the initial values and even increased, which led to a decrease of the cardiac cycle.

Changes of the Peripheral Blood. After the first two irradiations there was an increase of the quantity of leukocytes by 6 percent of the initial level;

on the days which followed, a rather sharp decrease of their number began. After receiving total doses of the order of 184.5-200 r, the decrease attained 50 percent of the initial quantity of leukocytes. After the termination of the irradiations, the quantity of leukocytes decreased to 12 percent and before death decreased to 5-3 percent of the initial level. The number of erythrocytes was increased (up to a maximum of 125 percent) in the first 20 days and then their number decreased, and in individual dogs this decrease attained 37 percent of the initial quantity of erythrocytes.

The hemoglobin content in the first irradiation period varied at the level of the initial values, but in the second half of the period there was a decrease of hemoglobin in individual animals by 27 percent.

During the first 18 days of irradiations (total doses 170-200 r) the quantity of thrombocytes increased insignificantly (by 10 percent). However, beginning with the 10th day, their quantity decreased sharply. On the 27th day (total doses 360-387 r) the quantity of thrombocytes decreased by 50 percent of the initial level. On the days which followed, there was a slower decrease in the number of thrombocytes and by the end of the irradiation period it decreased to 25 percent of the initial level. Upon termination of the irradiations the number of thrombocytes in the individual dogs decreased to 8-5 percent (fig. 2).

Pathologic-morphological Investigation. At autopsy the principal indicators of damage in all dogs were extensive multiple hemorrhages localized in the skin and in the subcutaneous cellular tissue of the torso (especially extensive in the groin), in the soft tissues of the upper lip, the postpharyngeal space and radix linguae, in the muscles between the bones, in the lungs and in the heart. These hemorrhages were particularly prominent in the stomach, large and small intestines, mesenterium tenue and in the perinephric cellular tissue. Punctulate hemorrhages were discovered in the renal cortex and /190 in the wall of the urinary bladder. There was a degeneration of the parenchymatous organs and plasia of the bone marrow.

Pathologic-anatomic conclusions: in all dogs there was acute radiation sickness with a sharply expressed hemorrhagic syndrome.

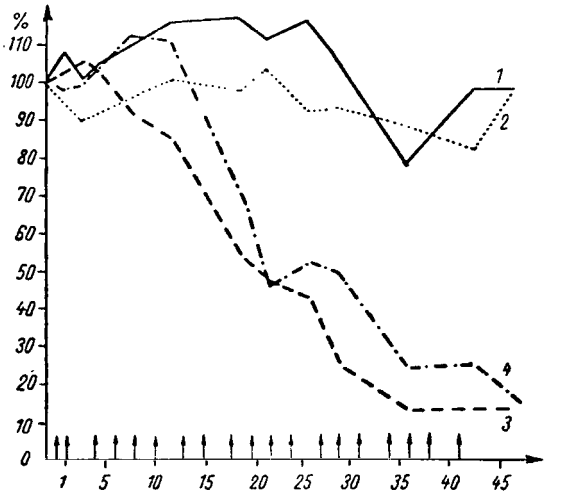
Experiment on Rats

X-ray irradiation of rats was carried out in the same rhythm and in the same doses as in the corresponding experiments with proton irradiation.

The conditions for irradiation of rats by X-rays were: RUM-3 apparatus, voltage 180 kv, filter 0.5 mm Cu + 1 mm Al, focal length 30 cm, dose intensity 60 r/min.

Two groups were used: group I--28 rats, group II--27 rats.

The rats of group I were subjected to 18-20-fold irradiation in single doses of 17-92.4 rad with a total dose of about 750 rad.



Days from beginning of experiment

Figure 2. Change of the quantity of erythrocytes (1), hemoglobin (2), leukocytes (3) and thrombocytes (4) in dogs in the case of fractional irradiation by X-rays (arrows).

In the course of the first two months from the beginning of the irradiations only one rat died (on the 43d day). The death rate for two months was 3.5 percent. Four rats were killed for histological study in each period: on the 64th-68th, 78th-82d and 91st-95th days.

During the next observation one other rat died, on the 198th day. /191
Thus, in 280 days of observation 2 rats died--a mortality of 12.5 percent.

In the rat which died on the 43d day after the beginning of irradiation there were poorly expressed manifestations of radiation sickness; the fur was dishevelled and there were small hemorrhages in the corners of the eyes and the mucous membrane of the nose, and diarrhea.

In the rat which died on the 198th day after beginning of irradiation there were no apparent indications of radiation damage.

Autopsy of the rat which died on the 43d day revealed distension of the intestine, effusion of serous fluid into the abdominal cavity, and an extensive hemorrhage in the ileocaecal region. In both rats the parenchymatous organs were degenerate. In the rat which died on the 198th day old hemorrhages were discovered in the lungs.

Thus, in the case of multiple irradiation by X-rays in doses of about 750 rad there are only individual cases of death of animals; clinical manifestations of radiation damage are poorly expressed.

The rats of group II were subjected to 20-fold irradiation in single doses: 15.5-111 rad and average total doses of 1,015 rad.

In the course of the first two months, six rats died in the period from the 32d to the 45th days. Mortality was 22.2 percent. Mean lifetime of the dead rats was 42.5 days. Four rats also were killed on both the 68th and 95th days.

Three other rats died at later times of observation: on the 76th, 239th and 248th day from the beginning of irradiations. The mortality of the rats of this group over a 280-day period therefore was 60 percent (among those surviving to this time).

In the rats dying during the first two months from the beginning of irradiations there were well-expressed manifestations of radiation sickness: a change in the behavior of the animals, dishevelled appearance, hemorrhages in the mucous membranes of the eyes and the tip of the nose, diarrhea and decrease of body weight. In the rats which died after a long time there were no apparent manifestations of radiation sickness; the rats died despite a state of apparent well-being.

At autopsy of the rats which died during the first two months it was found that there were hemorrhages in the lungs and in the small and large intestines; distension of the intestines; degeneration of the liver and kidneys and a decrease in the size of the spleen. In rats which died after a long time it was noted that there were degenerative changes of the liver and kidneys and old hemorrhages.

By comparing the weight dynamics of these groups of rats and of the control animals it can be seen that in the rats of both irradiated groups there was some lag in addition of weight, most clearly expressed in the rats of group II, irradiated by a large total dose. Such a lag was observed in the course of the first 30 days after the beginning of the irradiations. On following days the weight increment in the irradiated and control rats was identical, but later the irradiated rats increased in weight more rapidly than the /192 control rats.

The blood changes in the rats of both groups were approximately identical. Already after the third day of irradiation (total doses 97.5-168.6 r) there was a sharp decrease in the number of leukocytes (by 42-43 percent of the initial level). On the days which followed, the number of leukocytes decreased gradually. After the 14th irradiation (total doses 620.7-712 r) the number of leukocytes in the rats of group II had decreased to 30 percent of the initial level, whereas in the rats of group I (total doses 409-483.5 r) it was somewhat above 38 percent. After the termination of the period of irradiations the number of leukocytes began to increase gradually, and after 2 months there was a restoration of the number to the initial values (fig. 3).

The number of erythrocytes increased somewhat (by 5-13 percent of the initial level) in the first 30 days, but by the end of the period of irradiations had decreased from the initial level by 22-26 percent. Later the number of erythrocytes increased and varied at the level of 85-90 percent of the initial values.

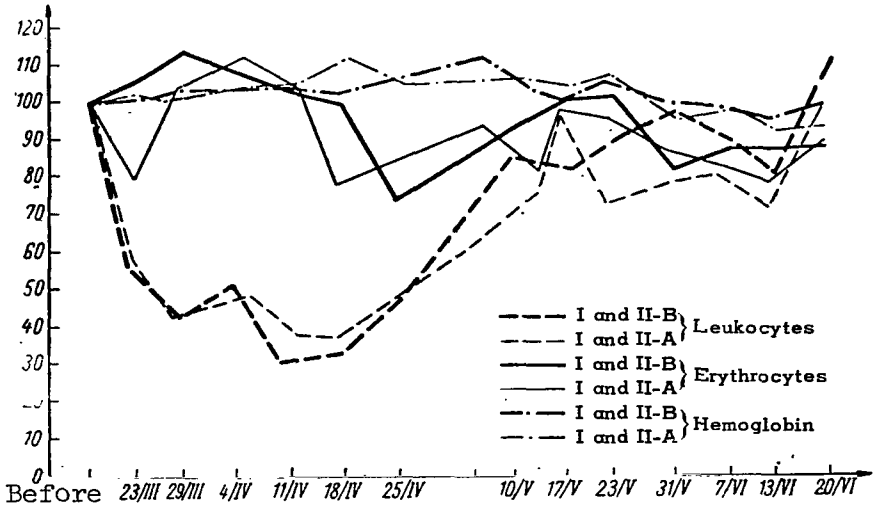


Figure 3. Change of quantity of erythrocytes, hemoglobin and leukocytes (in percent) in rats fractionally irradiated by X-rays in doses of 750 and 1,015 r. I and II - A - 750 rad: I and II - B - 1,115 rad.

The quantity of hemoglobin in rats of both groups also experienced identical changes. At the time of irradiations there was gradual small increase (by 7-12 percent), but in the course of the second month there was grad- /193 ual decrease to the initial level and even somewhat lower (to 94-96 percent of the initial level).

Thus, multiple (18-20-fold) exposure to X-rays in single doses of 15-110 r with intervals of 1-2 days and a total dose of about 750 r led to the development of a light form of radiation sickness with the death of individual animals. In the case of a total dose of 1,015 r there was a well-expressed picture of radiation damage with the death of 22 percent of the animals in 2 months and a loss of 60 percent when deaths at considerably later times are taken into account. Changes in the blood of rats of groups I and II were approximately identical.

CHAPTER 9. COMPARATIVE ANALYSIS OF BIOLOGICAL EFFECT OF PROTON RADIATION WITH ENERGY OF 510 MEV /194

N. G. Darenskaya, M. P. Domshlak, Yu. G. Grigor'yev, Yu. G. Nefedov, V. A. Razorenova and N. I. Ryzhov

In this study the determination of the relative biological effect of proton radiation with an energy of 510 Mev was made with a comparison of the collected data with materials characterizing the effectiveness of X- and γ-radiation. The admissibility of the comparison was ensured by adherence to spatial uniformity of the distribution of X- and γ-radiation in experiments made on dogs and white rats. This, in accordance with our former data, permits an analysis relating the effectiveness of proton radiation to the effectiveness of both X- and γ-radiation.

Comparative characterization of the effectiveness was based on percentage survival and on the basis of a comparison of morphological changes in the organs and tissues and also from the depth and intensity of changes of individual physiological functions in animals subjected under identical conditions to the effect of proton, X- and γ-radiations.

Comparison of Data Obtained in Experiments on Dogs Injured by Proton (510 Mev) and X-ray (180 kv) Fractional Irradiations

In the preceding chapters we have described the clinical manifestations of radiation injuries in dogs caused by fractional multiple proton and X-ray exposures. In a comparison of the radiation injuries caused by the effect of a flux of protons with an energy of 510 Mev and X-radiation we found uniformity of clinical manifestations, changes of the indices of the morphological and biochemical picture of the peripheral blood and bone marrow, changes of the cardiovascular system and also numerous pathologic-anatomical changes detected in macro- and microscopic investigations. Table 1 gives data on the survival time of dogs subjected to fractional irradiation by proton (510 Mev) and X-ray (180 kv) radiations. /195

Table 1 shows that the death of animals irradiated by X-rays began somewhat earlier (on the 37th day) in comparison with dogs irradiated by protons (41st day). However, all the dogs irradiated by protons died in a shorter span of time, all between the 41st and 49th day. In the group of dogs irradiated by X-rays there is a large variation in the time of death (37th-59th day); this apparently can be attributed to individual differences in the animals of this group. Comparison of the mean lifespan reveals that it does not differ appreciably (43.3 days under the influence of protons and 46 days when irradiated by X-rays).

TABLE 1. COMPARATIVE DATA ON LIFETIME OF SIX DOGS INJURED BY PROTON (510 MEV) AND X-RAY (180 KV) FRACTIONAL IRRADIATIONS

No. of dog	Proton radiation (510 Mev)			X-ray irradiation (180 kv)		
	Total dose, rad	Time of death after beginning of irradiation, days	Mean lifetime, days	Total dose, rad	Time of death after beginning of irradiation, days	Mean lifetime, days
1	649	41	43.3	613	37	46.0
2	661	45		667	45	
3	632	41		632	56	
4	637	41		637	59	
5	657	49		657	40	
6	660	43		640	40	

All these data indicate that there are no appreciable differences in the injurious effect of proton (510 Mev) and X-ray (180 kv) radiations.

Comparative data on the development of clinical manifestations of injury (times of development and degree of their severity) in dogs irradiated by proton (510 Mev) and X-ray (180 kv) radiations are given in table 2.

By comparing the clinical manifestations of the radiation sickness of dogs, developing as a result of fractional exposure, it can be seen that after X-ray irradiation the symptoms of the illness were manifested somewhat earlier and developed more gradually than after irradiation by protons.

There is development of an acute form of radiation sickness with a sharply expressed hemorrhagic syndrome as a result of fractional proton and X-ray irradiation. It should be taken into account that the values of the doses (613-667 r) leading to the development of acute radiation sickness and the death of the dogs as a result of fractional X-ray irradiation exceed by a factor /196 of 2 (for a given duration and rhythm of irradiation) the value of the minimum absolutely lethal dose of single irradiation (350 r).[1] At the same time, the mean lifetime of the dogs after fractional irradiation by X-rays (46 days) is greater than the mean lifetime in the case of single irradiation (43.8 days).

[1] 350 r is the minimum absolutely lethal dose for dogs subjected to uniform irradiation from different sides.

TABLE 2. COMPARISON OF PRINCIPAL CLINICAL MANIFESTATIONS OF RADIATION SICKNESS IN SIX DOGS INJURED BY PROTON (510 MEV) AND X-RAY (180 KV) FRACTIONAL IRRADIATIONS

Clinical symptoms of illness	Proton radiation (510 Mev)			X-ray radiation (180 kv)		
	Frequency of appearance	Time of appearance	Degree of severity	Frequency of appearance	Time of appearance	Degree of severity
Decrease of motor activity	6	33d	III	6	30th	III
Worsening of appetite	6	35th	III	6	31st	III
Increase of body temperature	6	38-40th	III	6	24-31st	III
Blanching of mucous membranes	6	35th	II	6	27th	II
Diarrhea	3	40th	II	6	26-36th	III
Appearance of hemorrhages:						
in mucous membranes	3	38th	I	5	33-38th	I-II
in skin and subcutaneous cellular tissue	3	38th	II	4	33-36th	II-III
in gastrointestinal tract	2	40th	II	4	26-33d	
Nectrotic changes in mouth	5	38-40th	III	4	36-33d	II-III

The changes in the state of the cardiovascular system during proton and X-ray irradiations were of an identical character.

Loss of weight after proton and X-ray irradiations was insignificant and attained only 5-7 percent in relation to the initial level.

Comparison of changes in the peripheral blood shows that after the /197 first X-ray irradiations of the dogs there was a small increase of the quantity of leukocytes, hemoglobin and thrombocytes. No such an increase was noted after proton irradiation. However, in the latter case, beginning on the 7th-10th day and continuing to the 20th-22d day, there was appreciable restoration of the quantity of leukocytes. As a result, over a considerable period there were appreciable differences in the content of leukocytes in the blood of dogs irradiated by X-rays and protons (fig. 1).

As a result of the proton irradiations, the quantity of thrombocytes began to decrease even after the first exposures, whereas in the dogs subjected to X-ray irradiation the intense decrease in the quantity of thrombocytes began only after the ninth exposure (fig. 2).

The change in the quantity of erythrocytes during the period of the irradiations was dissimilar in the two groups. The decrease was expressed more clearly in dogs irradiated by protons (fig. 3).

It is interesting to note that in the animals injured by proton radiation there was no total devastation of the bone marrow and disappearance of leukocytes from the peripheral blood (2-3 days before death), as often occurred during X-ray irradiation of animals with a similar clinical injury. This fact

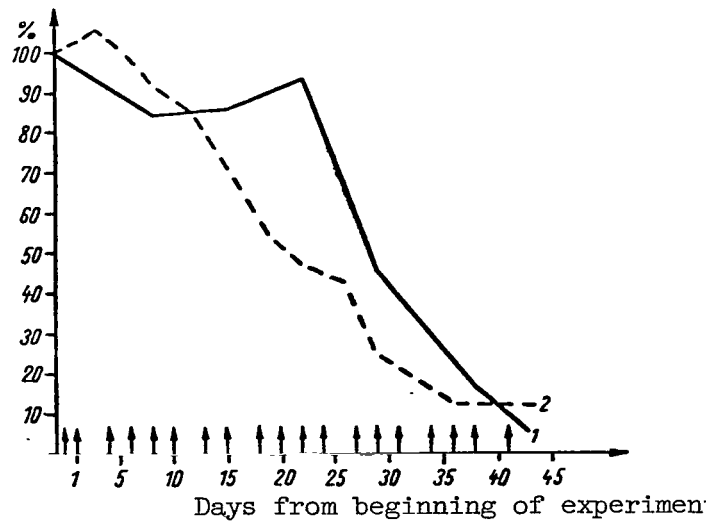


Figure 1. Dynamics of change of number of leukocytes in dogs during fractional proton (1) and X-ray (2) irradiations, noted by arrows.

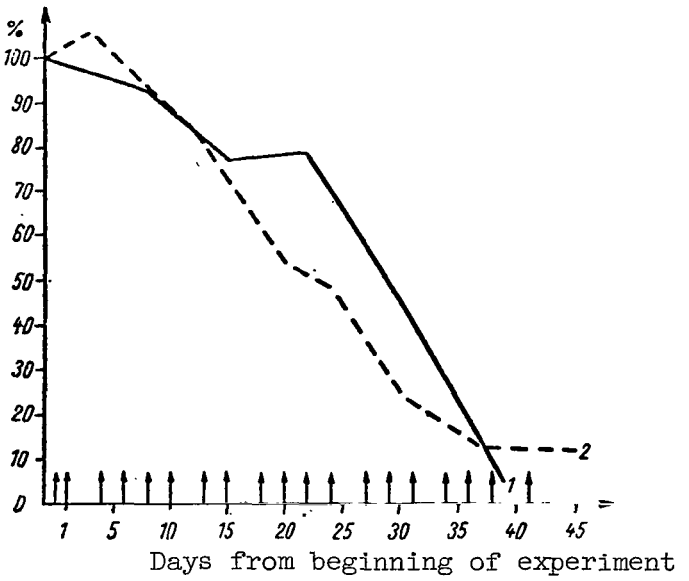


Figure 2. Dynamics of change of number of thrombocytes in dogs during fractional proton (1) and X-ray (2) irradiations, noted by arrows.

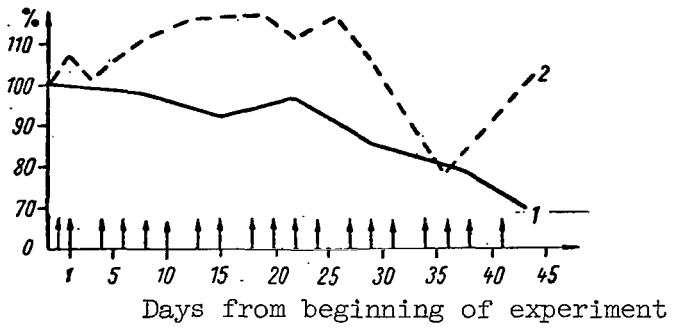


Figure 3. Dynamics of change of number of erythrocytes in dogs during fractional proton (1) and X-ray (2) irradiations, noted by arrows.

apparently indicates some specific effect of protons on the blood-generating function of the bone marrow. At the same time, it can in no way serve as /198 a basis for concluding that protons exert a lesser influence on the bone marrow than do X-rays. The fact that there is no total devastation of the bone marrow on the eve of death is evidence that the death of the animals apparently is unrelated to the disruption of blood generation, but possibly is caused by the sharp development of a hemorrhagic syndrome and the appearance of hemor- /199 rhages in vitally important organs and tissues.

By analyzing data from pathologic-morphological investigations it also is possible to note a uniformity of the changes. However, certain differences were found. In the case of proton irradiation, as mentioned previously, the hemorrhagic syndrome was very clearly expressed. Hemorrhages were numerous and were observed in the subcutaneous cellular tissue and in all the internal organs. Very frequently they were localized in the subdural space of the brain.

In summarizing the histological data on investigation of the central nervous system of animals which died after proton irradiation, it is necessary to note the similarity to the pathological processes in the central nervous system of dogs with acute radiation sickness caused by general X-ray irradiation. The distinguishing characteristic of the pathological process in the central nervous system of dogs injured by protons is a predominance of the destructive process in the intracerebral vessels, and as a result, more frequent hemorrhages and necroses than in dogs subjected to X-ray irradiation in adequate doses. It also should be noted that during irradiation with protons (510 Mev) there were no sharply expressed destructive changes of the cellular elements of the liver and kidneys.

In comparing the clinical manifestations of radiation sickness developing during fractional and single irradiation it is possible to note some peculiarities in the development of the terminal period of the sickness. For example, as a result of the extraordinarily strong manifestations of the hemorrhagic syndrome the death of the animals frequently occurred very rapidly when there was a relatively high content of leukocytes in the peripheral blood and a poorly defined suppression of blood generation in the bone marrow, when the marrow contained an adequate quantity of cellular elements of the myeloblastic and erythroblastic series. There were relatively shallow and poorly expressed changes in the state of the protein fractions of the blood serum. In other words, by the time of death the animal organism revealed no phenomena of intense disintegration of tissues and sharp disruptions of the functional properties of the organs ensuring synthesis and regulation of blood proteins. When there were relatively less expressed changes in the content of leukocytes and the leukopoietic function, there was a somewhat greater depth of the injury of the erythroblastic material and also changes in the content of thrombocytes, more sharply expressed than during X-ray irradiation.

Returning to a comparison of the effect of fractional proton and X-ray irradiations, it is necessary to admit that despite these peculiarities, there were no detectable significant differences in the effect of fractional proton and X-ray irradiations. This permits us to assume that in the case of fractional exposure to proton (510 Mev) and X-ray (180 kv) irradiation there /200
is an identical effectiveness with respect to the immediate results of injury of dogs. Accordingly, the coefficient of the relative biological effect of proton radiation for the studied conditions can be assumed equal to unity.

At the same time, taking into account the determined peculiarities of the effect of protons, it is impossible to exclude differences which could appear in the case of exposure to lesser doses.

Comparison of Data Obtained in Experiments on Rats Injured by Fractional Proton (510 Mev) and X-ray (180 kv) Irradiations

After a comparison of data obtained during the fractional irradiation of rats (in doses of 750 and 1,015 rad) certain differences were found. Whereas in the case of proton irradiations during the first 60 days from the onset of irradiation not a single rat in either group died, in the case of exposure to X-rays 3.1 percent of the rats in the first group died during this period and 21 percent in the second group. Thus, with respect to the death of rats the X-rays were somewhat more effective. The greater effectiveness of X-ray irradiation also was confirmed by later observations. After a long period (280 days after beginning of exposure) a heavy loss of rats was also observed in the group of animals subjected to X-ray irradiation (table 3). The clinical manifestations of illness were expressed clearly only in the animals which died much later.

TABLE 3. COMPARATIVE DATA ON LIFETIME OF RATS EXPOSED TO FRACTIONAL PROTON (510 MEV) AND X-RAY (180 KV) IRRADIATIONS

Proton radiation (510 Mev)				X-ray radiation (180 kv)			
Total dose, rad	Number of animals	Animals dying by 60th day, in %	Animals dying by 280th day, in %	Total dose, rad	Number of animals	Animals dying by 60th day, in %	Animals dying by 280th day, in %
750	29	0	6.6	750	28	3.1	12.5
1015	29	0	19.8	1015	27	22.2	60.0

The character of the changes of the peripheral blood was approximately identical. It is possible to note a somewhat greater depth of the decrease of the quantity of leukocytes after X-ray irradiation (fig. 4).

Comparison of the derived data on the injury of rats indicates a somewhat greater effectiveness of the X-rays. The coefficient of relative /201 biological effect of fractional proton irradiation under the studied conditions (duration and rhythm of the exposure, dose levels, etc.) can be assumed equal to 0.8.

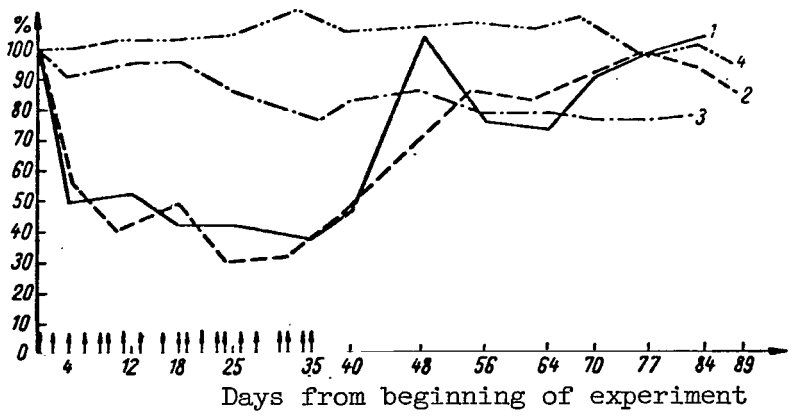


Figure 4. Dynamics of change of number of leukocytes (1 and 2) and erythrocytes (3 and 4) in rats during fractional X-ray (2 and 4) and proton (1 and 3) irradiations, noted by arrows (dose 1,115 rad).

Comparison of Data Obtained in Experiments on Dogs Injured by Single Proton (510 Mev) and Gamma-(Co^{60}) Radiations

For comparative purposes we used 6 groups of dogs (6, 8, 26, 10, 7 and 5 dogs) which were subjected to exposure of γ-radiation in the following doses: 250, 300, 350, 400, 600 and 700 r (table 4), which in all cases led to the development of acute radiation sickness, ending with 100 percent death of /202 the animals. The injuries caused by single γ-radiation of Co^{60} have been described in detail, and therefore this paper gives only brief information, for the most part concerning the times and frequencies of appearance of individual symptoms of radiation sickness and the depth of changes of certain indices of the peripheral blood.

A comparison of the clinical picture of radiation injuries, the dynamics of change of the indices of certain physiological functions and data from pathologic-morphological investigations reveals that in animals subjected to proton and γ-radiations there is an almost total uniformity of the principal manifestations of acute radiation sickness. There are some differences, primarily in the times of appearance of various symptoms and also the depth and intensity of changes of individual indices.

Comparison of the frequency, times of development and degree of severity of the principal clinical symptoms of radiation sickness reveals that despite identical frequency, somewhat earlier and more clearly expressed manifestations of injury are observed in the animals after proton irradiation (table /203 5). The principal clinical symptoms (increase of body temperature, diarrhea, appearance of hemorrhaging in the mucous membranes, skin and subcutaneous cellular tissue and in the gastrointestinal tract) after proton irradiation in a dose of 250 rad appear almost at the same times as after γ-irradiation in a dose of 350 r (table 6).

TABLE 4. COMPARATIVE DATA ON INJURIOUS EFFECT OF SINGLE PROTON (510 MEV) AND γ-RADIATION EXPOSURE OF DOGS

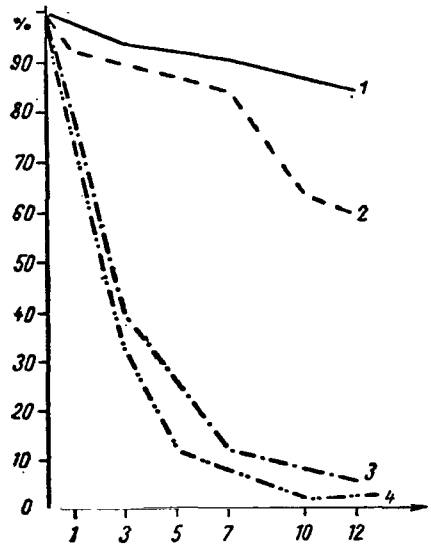
Protons				γ-radiation			
Dose, rad	Number of animals	Number dying	Mean lifetime, days	Dose, r	Number of animals	Number dying	Mean lifetime, days
250	5	2	14	250	6	3	20
400	5	5	11.4	300	8	7	14.8
550	5	5	7.6	350	26	26	14.2
				400	10	10	13.2
				600	7	7	9.9
				700	5	5	9.1

After single proton irradiation in a dose of 400 rad the principal symptoms of injury appear on an average of 2.4 days earlier than after a single γ-irradiation in a dose of 400 rad (table 7).

A similar tendency is also noted in a comparison of the mean times of the appearance of symptoms after single proton irradiations in a dose of 550 rad and γ-irradiation in a dose of 700 r (table 8). On the whole, the comparison of the times of the onset and the gravity of manifestation of the clinical symptoms under the studied conditions indicates a greater biological effect from proton radiation.

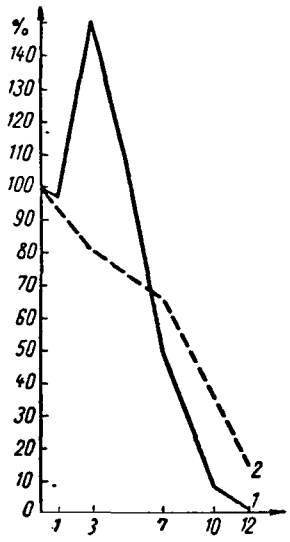
The same conclusion can be drawn by analyzing the results of comparisons of changes in the peripheral blood. As a rule, all the indices (quantity of erythrocytes, leukocytes, thrombocytes, etc.) indicate that the depth and /206 intensity of the changes were more clearly expressed after proton irradiation (figs. 5 and 6).

These data indicate that the degree of clinical manifestations of radiation sickness in dogs injured by γ-radiation in a dose of 700 r (630 rad) was less than in dogs injured by proton irradiation in a dose of 550 rad. If we use as a point of departure values accepted with a certain reserve, indicating that they cause an equal degree of injury, the coefficient of relative biological effect of single proton irradiation under the studied conditions can be 1.14 (630:550).



Days from beginning of experiment

Figure 5. Dynamics of change of number of leukocytes (3 and 4) and erythrocytes (1 and 2) in dogs after single proton (2 and 4) and γ-irradiations (1 and 3) in a dose of 400 rad.



Days from beginning of experiment

Figure 6. Dynamics of change of number of thrombocytes in dogs after single proton (1) and γ-irradiations (2) in a dose of 400 rad.

TABLE 5. PRINCIPAL CLINICAL MANIFESTATIONS OF RADIATION SICKNESS OF DOGS INJURED BY PROTONS (510 MEV) AND γ-RAYS

Clinical symptoms of sickness	Proton radiation (510 Mev)			γ-radiation of Co^{60}		
	400 rad (5 dogs)			400 r (10 dogs)		
	Frequency of appearance	Time of appearance, days	Degree of severity	Frequency of appearance	Time of appearance, days	Degree of severity
Decrease of activity	5 dogs	7.6	III	10 dogs	10.5	III
Worsening of appetite	ditto	6	III	ditto	11.1	III
Temperature increase	ditto	7.6	II-III	ditto	11.2	II-III
Blanching of mucous membranes	ditto	6.8	II-III	ditto	10.6	II-III
Diarrhea	4 dogs	11	II-III	8 dogs	11.5	II
Appearance of hemorrhages:						
in mucous membranes	1 dog	11	II	ditto	12	II
in skin and subcutaneous cellular tissue	2 dogs	7	II	4 dogs	12.2	II
in gastrointestinal tract	3 dogs	11	II-III	7 dogs	12.7	II-III

TABLE 5 (CONTINUED)

Clinical symptoms of sickness	Proton radiation (510 Mev)			γ-radiation of Co^{60}		
	550 rad (5 dogs)			700 r (5 dogs)		
	Frequency of appearance	Time of appearance, days	Degree of severity	Frequency of appearance	Time of appearance, days	Degree of severity
Decrease of activity	5 dogs	4.6	III	5 dogs	5.8	III
Worsening of appetite	ditto	2.6	III	ditto	4.6	III
Temperature increase	ditto	5.0	III	ditto	6.6	III
Blanching of mucous membranes	ditto	3.6	III	ditto	7.2	II-III
Diarrhea	4 dogs	6.8	II-III	ditto	7.6	II
Appearance of hemorrhages:						
in mucous membranes	ditto	6.6	II-III	1 dog	7.0	I-II
in skin and subcutaneous cellular tissue	2 dogs	7.6	II	ditto	7.0	I
in gastrointestinal tract	5 dogs	6.8	III	5 dogs	7.6	II-III
Mean times of appearance of objectively recorded symptoms			-	-	7.1	-

TABLE 6. COMPARATIVE CHARACTERISTICS OF SOME INDICES OF THE PERIPHERAL BLOOD IN DOGS AFTER SINGLE PROTON IRRADIATIONS IN A DOSE OF 250 RAD AND SINGLE γ-IRRADIATIONS (Co^{60}) IN A DOSE OF 350 R

Time of analysis, days	Quantity of leukocytes, 1,000/mm^3		Quantity of erythrocytes, 1,000,000/mm^3	
	Proton radiation (510 Mev)	γ-radiation (Co^{60})	Proton radiation (510 Mev)	γ-radiation (Co^{60})
Before irradiation	11.15 ± 1.23	11.1 ± 0.46	7.54 ± 0.48	6.3 ± 0.07
3d	5.35 ± 1.01	5.6 ± 0.2	6.44 ± 0.49	6.15 ± 0.13
7th	2.31 ± 0.18	1.5 ± 0.13	6.60 ± 0.36	5.9 ± 0.15
10-12th	2.95 ± 0.67	0.47 ± 0.06	6.01 ± 0.20	5.3 ± 0.22
19th	1.33 ± 0.37	-	3.69 ± 0.74	-
30th	5.41 ● 0.70	-	4.54 ± 0.67	-
45th	12.61 ± 0.80	-	6.03 ± 0.23	-

Time of analysis, days	Hemoglobin content, Sali method		Reticulocyte content, %		Thrombocyte content, 1,000/mm^3	
	Proton radiation (510 Mev)	γ-radiation (Co^{60})	Proton radiation (510 Mev)	γ-radiation (Co^{60})	Proton radiation (510 Mev)	γ-radiation (Co^{60})
Before irradiation	90 ± 5.5	83 ± 1.4	5	6.9 ± 0.42	189 ± 17	335 ± 15
3d	86 ± 6.8	81 ± 1.5	0	0.23 ± 0.1	249 ± 31	286 ± 19
7th	73 ± 3.6	77 ± 1.9	0	0	123 ± 12	143 ± 17
10-12th	73 ± 3.4	67 ± 2.6	0	0	15 ± 5	11 ± 3
19th	50 ± 12.7	-	3	-	9 ± 4	-
30th	53 ± 6.9	-	31	-	-	-
45th	75 ± 6.2	-	31	-	207 ± 88	-

TABLE 7. COMPARATIVE CHARACTERISTICS OF SOME INDICES OF THE PERIPHERAL BLOOD IN DOGS AFTER SINGLE PROTON IRRADIATIONS IN A DOSE OF 400 RAD AND SINGLE γ-IRRADIATIONS (Co^{60}) IN A DOSE OF 400 R

Time of analysis, days	Quantity of leukocytes, 1,000/mm3		Quantity of erythrocytes, 1,000,000/mm3	
	Proton radiation (510 Mev)	γ-radiation (Co^{60})	Proton radiation (510 Mev)	γ-radiation (Co^{60})
Before irradiation	11.75 ± 1.19	8.9 ± 0.58	7.65 ± 0.35	6.6 ± 0.15
3d	3.78 ± 0.52	3.6 ± 0.30	6.86 ± 0.10	6.2 ± 0.24
7th	0.93 ± 0.24	1.1 ± 0.27	6.49 ± 0.40	6.0 ± 0.20
10-12th	0.26 ± 0.09	0.45 ± 0.1	4.91 ± 1.25	5.6 ± 0.35

Time of analysis, days	Hemoglobin content, Sali method		Reticulocyte content, %		Thrombocyte content, 1,000/mm3	
	Proton radiation (510 Mev)	γ-radiation (Co^{60})	Proton radiation (510 Mev)	γ-radiation (Co^{60})	Proton radiation (510 Mev)	γ-radiation (Co^{60})
Before irradiation	85 ± 2.5	94 ± 2.5	6	5.9 ± 0.6	171 ± 19	338 ± 14
3d	83 ± 1.4	91 ± 3.3	0	0.6 ± 0.4	257 ± 45	274 ± 32
7th	68 ± 6.6	88 ± 2.3	0	1.18 ± 0.11	84 ± 19	224 ± 20
10-12th	58 ± 3.4	80 ± 2.8	0		14 ± 8.5	48 ± 19

TABLE 8. COMPARATIVE CHARACTERISTICS OF INDICES OF PERIPHERAL BLOOD IN DOGS AFTER SINGLE PROTON IRRADIATIONS IN A DOSE OF 550 RAD AND SINGLE γ-IRRADIATIONS (Co^{60}) IN A DOSE OF 700 R

Time of analysis, days	Quantity of leukocytes, 1,000/mm^3		Quantity of erythrocytes, 1,000,000/m^3	
	Proton radiation (510 Mev)	γ-radiation (Co^{60})	Proton radiation (510 Mev)	γ-radiation (Co^{60})
Before irradiation	9.52 ± 1.65	10.2 ± 1.86	7.03 ± 0.46	6.60 ± 0.51
3d	3.38 ± 1.29	1.72 ± 0.36	6.85 ± 0.34	6.70 ± 0.38
7th	0.38 ± 0.06	0.23 ± 0.20	5.92 ± 0.30	5.90 ± 0.27

Time of analysis, days	Hemoglobin content, Sali method		Reticulocyte content, %		Thrombocyte content, 1,000/mm^3	
	Proton radiation (510 Mev)	γ-radiation (Co^{60})	Proton radiation (510 Mev)	γ-radiation (Co^{60})	Proton radiation (510 Mev)	γ-radiation (Co^{60})
Before irradiation	97 ± 4.2	85 ± 4.0	7 ± 0.8	9.4 ± 2.6	276 ± 30	318 ± 38
3d	98 ± 3.6	78 ± 4.2	0	0.2 ± 0.12	279 ± 58	243 ± 28
7th	87 ± 6.9	70 ± 1.87	0	0	90 ± 22	61 ± 16

Comparison of Data Obtained in Experiments on Rats Injured by Single Proton (510 Mev) and Gamma-(Co^{60}) Radiations

Determination of the coefficient of relative biological effect was done by comparison of the doses of proton (510 Mev) and $\gamma(Co^{60})$ radiations, which led to a 50 percent and 100 percent death of the animals in a period of 30 days. The study was made on pubescent rats (males and females) irradiated by protons in doses of 330, 400, 420, 470, 520, 620, 730, 850 and 950 rad[1] and γ-rays in doses of 100, 200, 300, 400, 500, 600, 800, 900 and 1,000 r. /207

The results of these investigations revealed that the biological effect of proton radiation with an energy of 510 Mev was somewhat less than the effect of the γ-radiation of Co^{60}. In the case of proton irradiation, a 50 percent and 100 percent death rate of the animals is observed at doses of 580 and 830 rad, respectively. To attain the corresponding effect with γ-irradiation of Co^{60} the necessary doses are about 440 and 630 r. Comparison of the half-lethal and minimum absolutely lethal doses reveals that under the studied conditions the coefficient of relative biological effect of single proton irradiation of rats can be assumed equal to 0.75.

In conclusion it is worth noting the special differences observed in the coefficients of relative biological effect of protons for small and large animals. These differences are rather large. They can apparently be attributed to the fact that during the irradiation of small animals (due to their small size) there apparently are no appreciable changes in the energy loss of particles. At the same time, during the irradiation a considerable quantity of particles is absorbed completely in the body of the dogs. As a result, in those tissues where the final parts of the proton paths lie there is a high ionization density which is known to sharply intensify the biological effect. The size of the animals also can have a certain importance in the case of so-called nuclear "additions" to the relative biological effect; these occur when animals are irradiated by protons with energies greater than 100 Mev.

[1]The clinical picture of the sickness and the mortality of the animals are discussed in Chapter 4.

CHAPTER 10. PROPHYLAXIS AND THERAPY OF RADIATION INJURIES CAUSED BY PROTONS /208

Yu. D. Balika, A. F. Bibikova, B. I. Lebedev, V. D. Rogozkin and K. M. Znamenskaya

Results of Use of Therapeutic and Protective Agents

The data available at the present time indicate that in the course of space flights, made under complex radiation conditions, radiation injuries can occur. As previously mentioned, in this case the principal harmful radiation factor is the high-energy proton. The development of methods of prophylaxis and therapy of radiation injuries is, therefore, one of the important problems, when considering radiation safety of space flight.

It should be noted that in recent years considerable experimental data have accumulated on the effectiveness of some methods of prophylaxis and therapy of radiation injuries caused by irradiation of animals by γ- and X-rays.

Decreased sensitivity of biological objects to these forms of radiation can be achieved, in particular, by a change of the external conditions of existence of the animal organism, causing the development of hypothermia or hypoxia. It has been demonstrated that it is effective to use methods of shielding of individual parts of the body or their ischemia by means of temporary incomplete binding of the vessels supplying the most radiosensitive organs (such as ischemia of the bone marrow by temporary binding of the extremities).

There are many experimental and clinical data on the therapeutic effectiveness of transplantation of bone marrow in radiation sickness. However, the use of these protective and therapeutic methods under space flight conditions is quite impractical. It is far more promising to use pharmacological (chemical) prophylaxis and therapy.

As has been established in a large number of investigations (refs. 1 and 2), an increase of the resistance of the organism to the effects of ionizing radiation can be achieved by the pre-irradiation injection of substances of many classes of compounds (amines, aminothiols, isothiuronium compounds, cyanides, nitriles, etc.). It also has been established that postirradiation admin- /209
istration of agents intended for the prevention of infectious complications, changes in the blood-generation system and impairments of strength and permeability of the vessels, as well as for the normalization of conditions in the central nervous system and metabolism can moderate the course of radiation sickness.

Despite the existence of a large number of agents for chemical prophylaxis and therapy of radiation injuries, the problem of their practical use still is far from solved. There is inadequacy of information on the comparative effectiveness of different prophylactic and therapeutic agents for use in acute and, especially, subacute and chronic forms of radiation sickness. Methods of prophylaxis and therapy for radiation injuries caused by γ- and X-rays during multiple (chronic) exposure have not been sufficiently developed. In general, there are no data on the effectiveness of therapeutic and prophylactic agents in the case of proton irradiation of large animals. The sessions of the Biological Division of the Academy of Sciences USSR, held in 1961-1962, devoted to the results of investigations in space biology, revealed that this problem has not yet received adequate attention.

With this in mind, we carried out our investigation for finding therapeutic agents and prophylactic techniques for radiation injuries which can arise under space flight conditions in two stages.

In the first stage of formulation of comparative investigations, it was planned to select the most convenient and effective means for protection and therapy in the case of exposure to γ- and X-rays.

In the second stage, the principal problem of the work was to determine the effectiveness of the selected agents for cases of multiple and single irradiation of animals by protons with energies of 510 Mev.

In making the investigations the emphasis was on the search for such protective and therapeutic agents and complexes, which would be effective with enteral injection and could be used as self-assistance or administered by a victim's associates.

The investigations of the first stage of the work were made on 180 white mice weighing 18-21 g and 19 dogs weighing 9-14 kg. The mice were irradiated once with γ-rays from an EGO-2 cobalt apparatus with a total dose of 600 r with a dose intensity of 313-324 r/min. The dogs were subjected to multiple irradiation in a 12-tube X-ray apparatus with a total dose of 30-40 r 4 times a week for 30 days. Total irradiation dose was 560 r. Dose intensity was 63.8 r/min.

In the experiments on mice a study was made of the comparative effectiveness of the following known preparations affording radiation protection: cystamine, S-beta-aminoethylisothiuronium BrHBr, para-aminopropiophenone, /210 spasmolytin, 5-oxytryptamine, 5-methoxytryptamine, a combination of cystamine and amygdalin, S-beta-aminoethylisothiuronium BrHBr and para-aminopropiophenone. The observation tests in the experiments on mice were: (1) clinical state; (2) change of body weight; (3) survival rate and lifespan.

Maintenance and feeding of the control and treated mice were the same. Observation of the animals after irradiation was accomplished over a 30-day period. The results of the investigations revealed that of all the studied protective agents the most effective was 5-oxytryptamine by intramuscular injection 10-15 min before irradiation, and a combination of cystamine and amygdalin by enteral injection an hour before irradiation (table 1). These agents led to an increase of the survival rate of the animals by 40 percent.

TABLE 1. RESULTS OF USE OF PROTECTIVE AGENTS IN MICE IN CASE OF ACUTE RADIATION SICKNESS CAUSED BY SINGLE IRRADIATION BY γ-RAYS

Protective agent	Dose of matter, mg/kg	Number of animals	Results of experiments by 30th day		Mean lifespan, days
			Survived	Survival rate, %	
Cystamine	400	20	4	20	14.5
Cystamine + amygdalin	(Ts) 200 (A) 20	20	8	40	13.6
S-beta-aminoethylisothiuronium BrHBr (AET)	500	20	5	25	14.8
Para-aminopropiophenone (PAPF)	50	20	6	30	11.4
Spasmolitin	25	10	2	20	14.5
S-beta-aminoethylisothiuronium BrHBr + para-aminopropiophenone (AET + PAPF)	(AET) 500 (PAPF) 50	20	7	35	9.2
5-methoxytryptamine	100	10	3	30	14.4
Serotonin (5-oxytryptamine)	25	10	4	40	16.4
Control	-	30	0	0	11.9
Biological control	-	20	20	100	-

Note. All agents with the exception of 5-oxytryptamine and 5-methoxytryptamine were introduced orally an hour before irradiation; 5-methoxytryptamine and 5-oxytryptamine were injected intramuscularly 10-15 min before irradiation.

Furthermore, it was found that, in the protected animals there were considerably fewer impairments in the clinical state and little change in body weight. In particular, the body weight of those mice protected by cystamine and amygdalin on the 8th day averaged 2.4 g more than in the control mice.

Investigations of dogs subjected to repeated irradiation were directed/211 to the comparative study of the effectiveness of three variants of the complexes, which were developed first in 1959-1960 by V. P. Borisov and V. D. Rogozkin, using a model of acute radiation sickness. The first complex consisted of a number of therapeutic agents. These included vitamins of groups B, P and C, folic acid, a hemotopoietic and antibiotics (terramycin or streptomycin with penicillin in cases with increase of temperature to more than 39.5°). The second complex included these same therapeutic agents, and as a prophylactic measure there was preliminary (prior to irradiation) acclimatization to low oxygen content. The third complex consisted of prophylactic agents, cystamine with amygdalin and the therapeutic agents of the first complex.

The acclimatization of the dogs to low oxygen content before irradiation was in a pressure chamber.

The effect of the first complex was studied on 3 dogs, the second on 6 dogs and the third on 4 dogs. The control group included 4 dogs.

The observation tests in the experiments on dogs were: (1) clinical state, with data on temperature and weight; (2) data on the peripheral blood; (3) pathologic-anatomical data.

The animals were observed over a 9-month period after irradiation. As a result of the investigations it was established that the third therapeutic complex (table 2) yields a definite therapeutic effect. In addition to a favorable influence on the survival rate and lifespan, this therapeutic /212 complex attenuated the clinical manifestation of the sickness and changes in the blood system. In particular, this could be seen from the changes in the quantity of leukocytes (fig. 1).

Thus, the first stage of work in the experiments on mice, irradiated by γ-rays (LD_{100}) and with acute radiation sickness, revealed that the most effective radioprotective agents were 5-oxytryptamine and a combination of cystamine and amygdalin.

The experiments of the first stage on dogs, with multiple irradiation (LD_{100}), determined an effective protective-therapeutic plan and created the basis for carrying out the second-stage investigations.

The principal objective of the second stage of the work was checking the effectiveness of a number of known radioprotective agents in the case of single irradiation of animals by protons, and the development of a protective-therapeutic complex for multiple irradiation with high-energy protons (510 Mev). The experiments were made on 177 white rats weighing 180-240 g, 80 white mice weighing 18-21 g and 15 dogs weighing 6.6-16.5 kg. The animals were irradiated

TABLE 2. RESULTS OF USE OF THERAPEUTIC AGENTS IN DOGS EXPOSED TO MULTIPLE IRRADIATION BY X-RAYS

Therapeutic complexes	Number of animals	Survived to 65th day	Mean lifespan, days
First--therapeutic agents (vitamins of groups B, P, C, folic acid hemotopoietic and antibiotics)	3	0	38
Second--acclimatization to oxygen starvation before irradiation in course of 1 month + therapeutic agents of first complex. . . .	6	0	36
Third--cystamine, amygdalin an hour before irradiation before each exposure + therapeutic agents of first complex.	4	2	41
Control	4	0	28

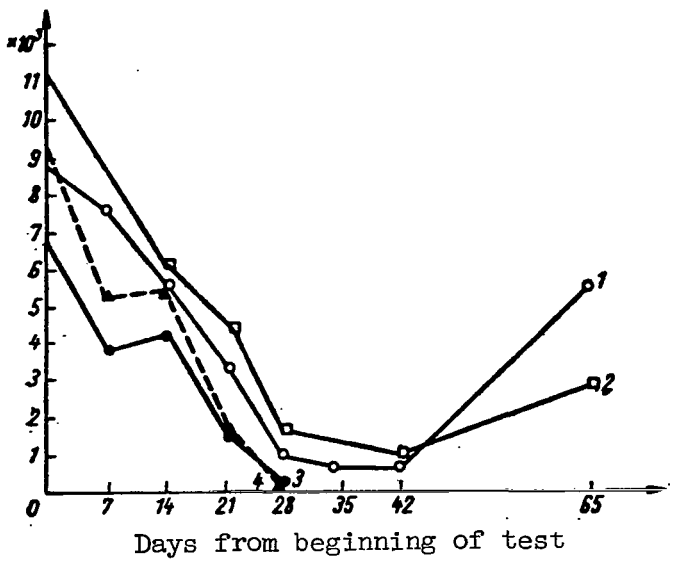


Figure 1. Change of quantity of leukocytes (thousands per 1 mm^3) in treated (1 and 2) and control (3 and 4) dogs irradiated by X-rays.

in the synchrocyclotron. The mice were irradiated with a dose of 375 rad and the rats with a dose of 412-1,050 rad. The dose intensity was 1-1.5 rad/sec.

In the experiments on small laboratory animals a study was made of the effectiveness of cystamine, S-beta-aminoethylisothiuronium BrHBr, para-aminopropiophenone, 5-methoxytryptamine, S-beta-aminoethylisothiuronium BrHBr in combination with para-aminopropiophenone and cystamine with amygdalin. The observation tests in the experiments on mice and rats were: (1) clinical state; (2) change of body weight; (3) survival rate and lifespan; (4) total quantity of blood-generating cells of the bone marrow (in rats).

Maintenance and feeding of the control and treated animals were /213 identical. The animals were observed for 60 days.

The results of the investigations on rats and mice revealed that in acute radiation sickness, caused by irradiation with protons with energies of 510 Mev, as in the case of irradiation with γ-rays, the greatest effect was produced by 5-oxytryptamine and a combination of cystamine with amygdalin (table 3), whereas 5-methoxytryptamine and a combination of S-beta-aminoethylisothiuronium BrHBr with para-aminopropiophenone were ineffective (table 3).

The changes in the blood-generating system were minimal in the treated animals, which had been given prophylatic injections of S-beta-aminoethylisothiuronium BrHBr and cystamine in combination with amygdalin.

Second-stage Experiments on Dogs. These were made for improving the protective therapeutic complex found to be effective in X-ray irradiation (third therapeutic complex). In addition, this therapeutic complex included serotonin (5-oxytryptamine) as a protective and therapeutic means and prednisone for combating postponed specific changes observed at the time of proton irradiation.

The irradiation of the control and treated dogs was in the synchrocyclotron by protons with energies of 510 Mev. There was multiple exposure, three /214 times a week for six weeks. Table 4 gives the single and total doses of radiation. Before and after irradiation the animals were subjected to careful examination. The effectiveness of the therapeutic complex was judged on the basis of the following indices: (1) clinical state; (2) hematological data on the peripheral blood; (3) morphological composition of the bone marrow, obtained by puncture of the sternum; (4) serotonin content in the blood.

It was necessary to determine the serotonin in the blood of the dogs, because our earlier investigations had revealed the great importance of a change in the serotonin level in the pathogenesis of radiation injuries. In particular, it was established that in the bodies of dogs and monkeys with an acute form of radiation sickness a progressive decrease of the quantity of serotonin in the blood sets in, accompanied by the hemorrhagic syndrome. It also was taken into account that the dynamics of serotonin in the blood are unknown in the case of proton irradiation, and the study of this index can serve as the basis for a more rational use of therapeutic means, especially serotonin. The determination of serotonin in the blood was done in our experiments by the biological method, based on the capability of this substance to cause spasms of the smooth muscles. This method involves the preliminary extraction of serotonin from the blood by acetone, with subsequent testing of samples on an isolated segment of the colon of a rat. This method now has been well developed. A number of investigators (ref. 1) have noted the high sensitivity of this method.

TABLE 3. RESULTS OF USE OF PROTECTIVE AGENTS IN RATS WITH ACUTE RADIATION SICKNESS CAUSED BY SINGLE IRRADIATION BY PROTONS WITH ENERGIES OF 510 MEV

Name of agent	Dose of matter, mg/kg	Irradiation dose, rad	Number of rats	Survived to 60th day
S-beta-aminoethylisothiuronium BrHBr + para-aminopropiophenone (AET + PAPF)	(AET) 100 (PAPF) 50	555	15	0
5-methoxytryptamine	75	508	15	0
Cystamine + amygdalin	(Ts) 360 (A) 20	508	9	2
Cystamine	300	430	4	0
5-oxytryptamine (serotonin)	50	430	10	5
Para-aminopropiophenone (PAPF)	100	412	10	4
S-beta-aminoethylisothiuronium BrHBr (AET)	200	412	10	5
Control		430-555	20	0
Control		412	8	2

Note. All agents with exception of 5-oxytryptamine and 5-methoxytryptamine were introduced orally 1 hour before irradiation; 5-oxytryptamine and 5-methoxytryptamine were introduced intramuscularly 10-15 min before irradiation.

TABLE 4. DOSES OF IRRADIATION OF DOGS WITH ENERGIES OF 510 MEV

Group of animals	Number of animals in group	Single minimum, maximum and mean irradiation dose (in sequence)	Total minimum, maximum and mean irradiation dose (in sequence)	Mean duration of period of irradiation, days
Treated	9	17.1 57.2 34.3	642.6 652.2 651.2	43
Control	6	11.9 63.0 34.5	622.0 667.0 646.2	41-43

Prior to beginning the experiment all dogs were divided into two equal groups on the basis of biological, clinical and biochemical investigations. Dogs with a low content of serotonin in the blood were not used in the experiment, because our prior investigations had revealed that in such animals there is decreased resistance to ionizing radiation.

The group of treated animals consisted of 9 dogs and the control /215 group of 6. The dogs weighed 7-16 kg. The animals were observed over a 4-month period.

Results of the observations revealed that multiple irradiation of dogs by protons leads to development of early changes in the clinical condition. Already by the 10th-28th day there were clinical manifestations of radiation sickness in the control animals--temperature increase, blanching of the visible mucous membranes, a decrease of food excitability and pulpy stool. In most dogs all these phenomena were unstable and disappeared after 1-3 days, but they appeared again after 5-7 days. Such a picture was observed until the 35th-37th day, i.e., almost to the end of the period of irradiations. Beginning on the 35th-37th day a picture of acute radiation sickness with a severe hemorrhagic syndrome began to appear in all control dogs. By the 39th day there was a rather sharp decrease in the number of thrombocytes (almost to zero) in the peripheral blood, total disappearance of reticulocytes and a decrease of leukocytes to 500-2,000 cells/mm^3. At the same time there was a decrease of the quantity of hemoglobin and erythrocytes (on the average by 25-30 percent).

By the 38th-45th day the serotonin content in all control dogs had decreased to zero. All control dogs died in the period between the 41st and 49th day after the beginning of irradiations. At autopsies a picture of acute radiation sickness was revealed, which was accompanied by aplasia of the blood-generating organs and a severe hemorrhagic syndrome.

In the clinical picture of the disease the treated dogs differed sharply from the control dogs. The principal clinical manifestations of the sickness in the treated dogs developed later and were more weakly expressed. This can be seen on the basis of many indices. For example, a very severe general condition was observed in 3 of the 6 control dogs and in 2 of the 9 treated dogs. Five of the control dogs and 1 treated dog refused food completely. Repeated vomiting and bloody stool were observed only in the control dogs. There were no clinical indications of radiation sickness at all in 4 of the treated dogs. There were no such cases among the control dogs. The treated dogs also differed from the control dogs in the course of the hemorrhagic syndrome. For example, in 5 of the 6 control dogs there were very severe hemorrhagic phenomena, which progressed rapidly. In most (5) of the treated dogs there were only individual hemorrhages, which did not increase in size and which disappeared rapidly after several injections of serotonin. Pathologic-anatomical data indicate the hemorrhagic syndrome was more clearly expressed in the control animals. By the time of death of all control dogs (49th day), only one had died in the treated group. By the 65th day (i.e., by the time of final disappearance of clinical changes characteristic of acute radia- /216 tion sickness) 7 of the 9 treated had survived (table 5).

TABLE 5. SURVIVAL RATE AND LIFESPAN OF CONTROL AND TREATED DOGS IN CASE OF MULTIPLE IRRADIATION BY PROTONS WITH ENERGIES OF 510 MEV

Group of animals	Number of animals	Survived to 65th day	Mean lifespan, days
Treated	9	7	51
Control	6	0	43

With respect to data on the peripheral blood, during almost the entire period of irradiation the treated dogs differed little from the control dogs.

The changes in the bone marrow of the dogs also were almost identical. However, it should be noted that in most of the treated dogs there was a small increase in the number of young forms of erythroblastic cells. In the treated dogs lesser changes were observed in the mitotic activity of the red cells.

By the 38th day (i.e., by the time of onset of death of the control dogs), there was a small increase in the total quantity of erythroblastic or myeloidal cells in the bone marrow of some of the treated dogs. There was a stable decrease in the number of these elements in the control dogs.

The pattern of changes of the quantity of serotonin in the blood of treated and control dogs was similar. In the control animals the serotonin level decreased to zero by the end of the irradiation (38th-45th days), and this coincided with the period of death. In the treated animals there also was a decrease in the quantity of serotonin, but the dogs lived for approximately 20 days with serotonin equal to zero, and their condition was satisfactory. This obviously can be attributed to the fact that the injected serotonin helped to normalize the resistance of the blood vessels, since the hemorrhages which developed in the dogs then disappeared. The effect was brief, which apparently was caused by a rapid destruction of the injected serotonin by monoaminoxidase, and was also caused by the fact that in all irradiated animals there was very strong thrombocytopenia.

Prior to the decrease of the quantity of serotonin in the blood to zero in the treated dogs, we observed a brief but considerable increase; in some dogs it attained the initial level and even exceeded it. Such an increase of the quantity of serotonin coincided with the beginning of its injection into the treated dogs. It should be noted that the treated dogs, having the maximum increase of serotonin in the blood in the irradiation process, easily withstood the acute radiation sickness.

In the control animals we also observed a brief increase of the quan- /217
tity of serotonin, but it was less clearly expressed and appeared considerably earlier--on the 15th day (table 6).

It was also noted that the control dogs which survived the longest time after total disappearance of serotonin from the blood had the most clearly expressed hemorrhagic syndrome. Two to 6 days after the decrease of the quantity of serotonin in the blood to zero, all control dogs died (table 7).

TABLE 6. PERIODS OF INCREASE OF SEROTONIN IN BLOOD OF DOGS AND TIME OF DEATH (RESULTS BY THE 65TH DAY)

Number of dog	Group of animals	Periods of increased serotonin in blood, days	Increase of quantity of serotonin in blood, %		Time of death, days
6	Control	8th	30.8		41st
1062	"	15th	79.3		43d
1057	"	15th	29.7	Mean	41st
1084	"	22d	320.0	157.3	49th
		15th	293.3		
1128	"	29th	326.6		41st
1049	"	None	0		45th
1091	Treated	29th	152.5		Lived
1006	"	29th	113.8		"
5	"	22d	506.6		"
		29th	780.0		
1050	"	8th	23.0	Mean	
890	"	29th	414.3	274.5	"
1119	"	29th	163.5		"
963	"	None	0		54th
883	"	None	0		48th
1085	"	None	0		Lived

TABLE 7. PERIODS OF DECREASE OF CONTENT OF SEROTONIN IN BLOOD OF DOGS

Number of dog	Group of animals	Period of decrease of serotonin content to zero, days	Lifespan after decrease of serotonin content to zero, days	Degree of severity of hemorrhagic phenomena
6	Control	38th	3	III
1057	"	39-40th	2	III
1128	"	39-40th	2	III
1049	"	43d	2	II
1062	"	38th	5	III
1084	"	43d	6	III
883	Treated	39th	10	I
963	"	43d	11	II

By the 65th day after beginning of irradiations 7 of the 9 treated dogs had survived, whereas all 6 animals in the control group had died. It was known /218 from experience in work with multiple X-ray irradiation that by the 65th day there is usually a stable restoration of clinical and hematological indices in the surviving dogs.

The treated dogs surviving after proton irradiation were also in good condition by the 65th day. They were in good spirits, active and had high food excitability and normal stool.

The first indications of restoration appeared in the peripheral blood of most of the surviving animals. In these animals the number of reticulocytes increased to 3-5 percent and the number of thrombocytes to 26,000-94,000/ mm^3. On the basis of these indications it could be assumed that the treated animals were in a period of restoration.

However, contrary to the expectations based on the results of experiments with the chronic effects of X-radiation, following irradiation with these same doses by protons, there was no onset of proper restoration of the blood system. For example, observations of treated dogs after a considerable time lapse (100th day) revealed that in 6 of the 7 surviving animals there was severe aplastic anemia. The use of hemotopoietics and prednisone in these dogs did not exert a well-expressed therapeutic effect, and by the 110th day 6 of the treated dogs died. The period of postponed changes transpired favorably in only one treated dog, whose irradiation occurred while she was pregnant. The dog gave birth 7 days after a 42-day period of irradiation. The restorative processes transpired most intensively in this animal, and by the 120th day the indices of the peripheral blood were very close to the initial data observed before irradiation.

Thus, the investigations of the second stage of the work made it possible to determine a number of means for carrying out prophylaxis and therapy in acute radiation sickness in its severe stage, caused by multiple irradiation of dogs with protons having an energy of 510 Mev.

Summarizing the results of our investigations, it can be stated that experiments on small laboratory animals permitted a relative evaluation of the effectiveness of presently known means for radiation protection. It was established that in the case of single irradiation with both γ-rays and with protons with energies of 510 Mev the maximum effect is obtained by use of 5-oxytryptamine with intrasmuscular injection 10 to 15 min before irradiation and a combination of cystamine with amygdalin by enteral injection an hour prior to irradiation.

The clearly expressed protective effect of S-beta-aminoethylisothiuronium BrHBr during proton irradiation also merits attention. The approximately identical prophylactic influence of a number of safeguards against radiation, when animals are irradiated by γ-rays and high-energy protons, to a certain degree is evidence of the unity of the mechanisms of radiobiological effect of these radiations.

Considering these experimental data, it is reasonable to carry out /219 investigations in the formulated problem in two stages: orientation experiments on animals with protective and therapeutic preparations, first with γ- and X-ray irradiation, and then with proton irradiation. However, final conclusions concerning the effectiveness of radiation protective and therapeutic means can be drawn only after direct experiments with irradiation of animals by protons. Our experiments with dogs demonstrated this. The protective-therapeutic complex, whose effectiveness was established on an experimental basis during the multiple irradiation of dogs by X-rays, exerted a well-expressed therapeutic influence in the case of multiple proton irradiation as well. Using the protective-therapeutic complex, consisting of cystamine and amygdalin, vitamins of the B, C and P groups, folic acid, hemotopoietics and serotonin, the majority of the treated dogs (7 of 9) easily withstood the radiation sickness in its extremely severe stage, which developed after multiple irradiation of the animals by protons. In this period (to the 50th day) all control dogs died. Up to the 65th day clinical manifestations of radiation sickness in most of the treated dogs were minimal.

The use of serotonin as an antihemorrhagic agent proved to be completely justified and to a considerable degree made it possible not only to decrease hemorrhagic phenomena, but also to prevent their occurrence in some animals. On the basis of these initially obtained data, serotonin can be considered a new effective agent for combating hemorrhagic phenomena during proton irradiation.

The picture of radiation damage caused by irradiation by protons is somewhat different from the effects of X-rays. Comparison of the clinical manifestations of radiation sickness caused by the multiple irradiation of dogs by these types of radiations in identical doses revealed that changes of the gastrointestinal tract and hemorrhagic phenomena are discovered sooner and are more clearly expressed in the case of proton irradiation than in the case of X-ray irradiation.

Comparison of the indices of the peripheral blood established that in the case of proton irradiation, in contrast to X-ray irradiation, there is a period of adaptation to multiple irradiation. This adaptation period was characterized by the absence of changes of the number of leukocytes (in some cases there was even an increase of the number of leukocytes) and thrombocytes. In addition, it was typical of the adaptation period that there was a period of some increase of the quantity of serotonin in the blood, which we feel is a manifestation of a compensatory mechanism. The periods of increase of serotonin in the blood of the irradiated animals were accompanied by improvement of their general condition. In most animals in which there was a clearly expressed increase /220 of the quantity of serotonin in the blood the period of survival was maximal.

Despite the occurrence of the period of adaptation with respect to a number of indices of the peripheral blood during proton irradiation, the changes of erythrocytes and hemoglobin set in somewhat more rapidly than with X-ray irradiation and reached a greater depth.

Judging from the hemotological changes, a more prolonged restorative period was observed in the treated dogs (fig. 2) in the case of proton irradiation than

in X-ray irradiation. However, this restoration was incomplete and development of aplastic anemia set in a considerable time after the radiation damage occurred. In its symptoms this anemia resembled Ehrlich's aplastic anemia. We feel that the aplastic anemia after proton irradiation developed as a result of profound impairments of nuclein metabolism. It is known from data in the literature (ref. 2) that the changes in nucleic acids and nucleoproteins in proton irradiation are greater than in X-ray irradiation.

The observations in the pregnant dog, in which aplastic anemia did not develop, indicate that particular attention should be given to problems of hormonal therapy.

On the basis of these investigations we obtained new data to introduce corrections into the therapy of radiation sickness caused by chronic proton irradiation. However, without further careful checking of the effectiveness of the new therapeutic measures, they can be recommended only on a preliminary basis, particularly since these measures should be supplemented by other new measures to prevent the development of grave effects of irradiation and aplastic anemia (as occurred in the treated dogs).

It is of particular interest, therefore, to consider data of pathologic-morphological investigations of treated dogs, in which acute aplastic anemia developed after multiple irradiation by protons.

Results of Pathologic-Morphological Investigations

The internal organs and tissues of 6 treated dogs were investigated. The autopsies of the animals were made at once or in the first hours after death. The autopsies revealed clear indications of hemorrhagic diathesis and dystrophic changes of the liver, kidneys, myocardium and other organs and also grave changes in the blood-generating organs. In addition, the treated animals revealed some peculiarities of the morphological picture in com- /222 parison with the control dogs. For example, hemorrhages were not so widespread, they were not found in all the organs and had small foci. It should be noted, however, that in these cases hemorrhages in the subpial space of the brain were encountered more frequently. In the treated animals it was rather uncommon to find complications from infection, especially necrotic anginas; dehydration of the tissues was expressed less clearly. At the same time, changes in the blood-generating organs were noted in these dogs, evidence of severe anemia.

Particular attention was devoted to the study of pathologic-morphological changes in the central nervous system. Microscopic examination of 5 animals revealed massive hemorrhages under the dura mater, with localization in the region of the base of the brain and the cerebral hemispheres. Macroscopic examination revealed severe anemia of the medulla, apparently caused by developing aplastic anemia.

The microscopically detected changes in the central nervous system were characterized by edema of the soft meninges, both at the surface of the brain and at its base (fig. 3). In places in the surface meninges there was slight

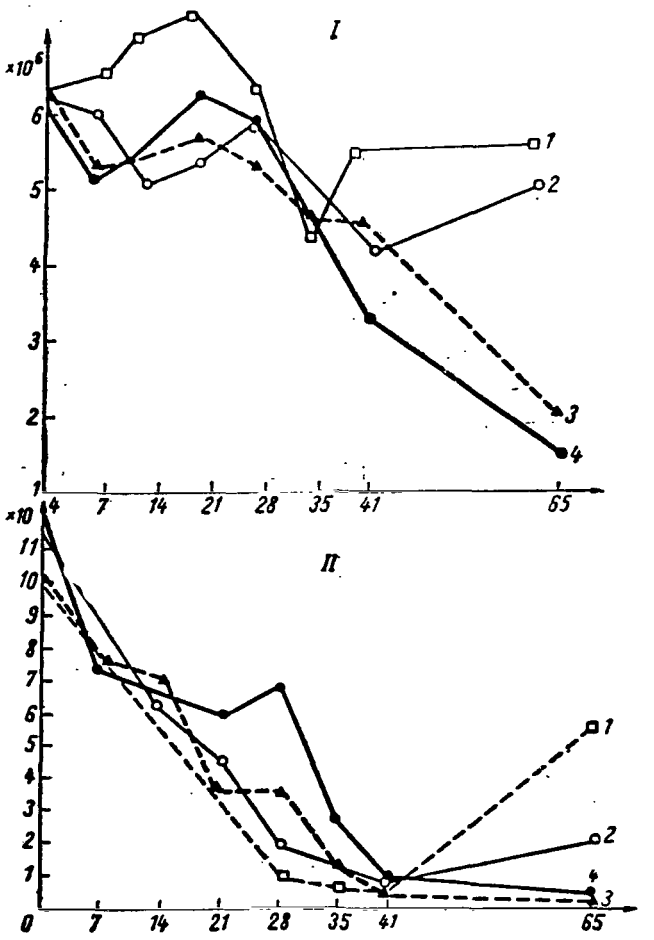


Figure 2. Change of quantity of erythrocytes (I in millions per 1 mm^3) and leukocytes (II in thousands per 1 mm^3) in treated dogs: 1 and 2 X-rays; 3 and 4 protons.

growth of collagenic tissue, grossly reflected in the meninges of the base of the brain and also in the soft meninges of the spinal cord. Many of the vessels of the soft meninges appeared distended, and their lumens were /223 filled for the most part by a homogeneous albuminous mass. Deep in the convolutions there were some fresh subpial hemorrhages. The changes of the intracerebral vessels essentially involved an extraordinary distension of the walls to the point of narrowing the lumens, but more frequently there was thinning and dystonia of the vascular walls with various abnormal forms of their lumens (fig. 4). In most cases there was expansion of the perivascular spaces around such vessels, with edematous disintegration of cerebral tissue over a great expanse. In the cortex and subcortical stem formations there were vessels (arteries and veins) of various sizes, whose lumens were filled in part with blood and in part with plasma (fig. 5).

The preserved cellular structures in the walls of some vessels appeared shriveled and atrophic. The lumens of the small vessels of the caudate nucleus sometimes were filled with pyknomorphic cast-off endothelium. Although the character of the pathological changes of the vessels of cerebral matter of all parts of the central nervous system is uniform, the degree of injury is dissimilar. The severest changes of the vessels occurred in the subcortical stem formations, especially in the caudate nucleus (wall of the cornu anteriorus of the lateral ventricle), hypothalamus, midbrain, tegument of the pons Variolii and medulla oblongata. A characteristic feature of the changes of the vascular system of these formations was a gross impairment of vascular tone, resulting in onset of a sharp collapse of the walls; the lumens of the fine vessels were devastated. The impairment of the tone of the vascular walls apparently facilitated their compression of the cerebral fluid accumulating in the perivascular spaces. The edema was not limited by the perivascular spaces, but was propagated to great parts of the cerebral matter, which appeared spongy and porous (fig. 6).

Gross disintegration was noted in the white matter of the subcortical stem sections, but there was some edematous softening and disintegration of the nerve fibers. The white matter of the brain stem appeared to be covered with fine and large pores (sometimes over extensive areas). Foci of necrosis with disintegration of cerebral matter were noted in some dogs at the base of the fourth ventricle and in the walls of the third and lateral ventricles (fig. 7). All these phenomena obviously are related not only to impairment of blood circulation, but also to impairment of the lymph-blood circulation. The pathological changes noted in the ependyma of the cerebral ventricles and in the soft meninges (pyknosis and necrobiosis of the cells of the ependyma, epithelium of the villi of the vascular plexi and proliferation of collagenic fibers of their stroma) indicate the impairment of the lymph-blood circulatory system.

Pathological changes of the nerve cells occurred in all parts of the brain. In character they did not differ from the structural changes in the cells /226 of the central nervous system of untreated dogs. In the treated dogs there was a somewhat clearer manifestation of the polymorphism of the nerve cells of the cortex of the hemispheres, but the principal difference was in the greater propagation and greater intensity of cellular pathology, which apparently can be attributed not only to the direct influence of protons on cellular structures, but also to the aplastic anemia developing in the dogs. The pathological changes in the nerve cells of the cortex involved mostly the distension of the nuclei, their degeneration (sometimes shriveled nucleoles of reduced size still are visible in such nuclei) and disintegration and dissolving of the cytoplasm. As a result of the dissolving of the cytoplasm it was possible to observe many "naked," extraordinarily distended nuclei with modified nucleoles (fig. 8). In each part of the cortex of the brain it was possible to observe widespread cytolysis (most frequently in the deep layers of the cortex); sometimes in such dying cells it was possible to detect nuclei with constrictions (fig. 9).

In places in the upper layers of the cortex there were shriveled nerve cells with pluglike appendices (fig. 10) and cells with ischemic modifications (fig. 11). In the cortex of the hemispheres there was some clearly expressed

Figure 3. Temporal cortex. Edematous separation into fibers of soft meninges. Staining with picrofuchsin. Magnification x 50.

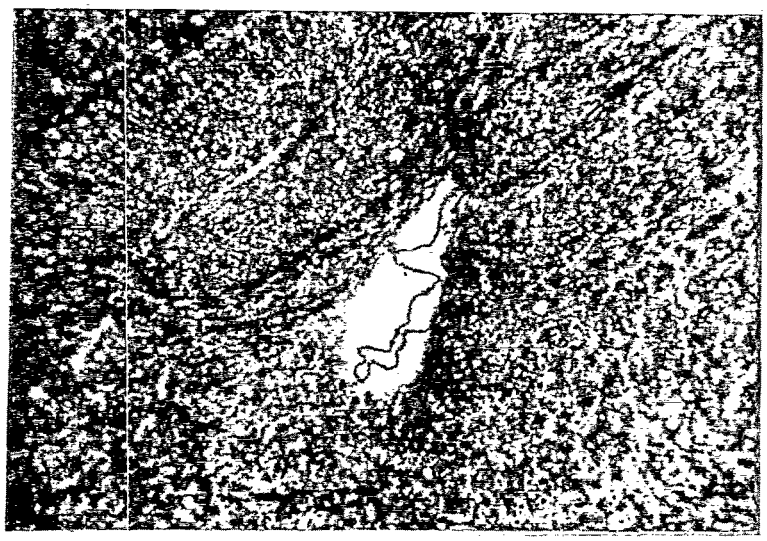


Figure 4. Pons Variolii. Distonia of vessel with perivascular edema. Staining with picrofuchsin. Magnification x 70.

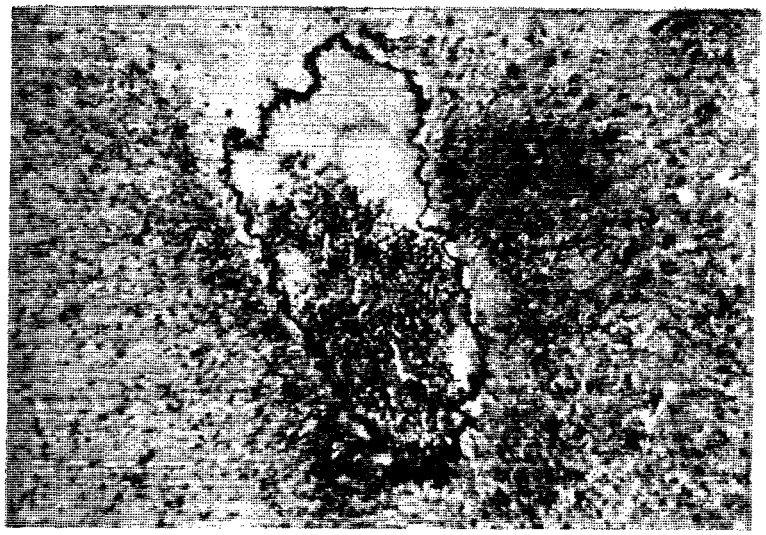


Figure 5. Hippocampal gyrus. Lumen of sharply dilatated vein is filled in part with blood and in part with homogeneous albuminous mass (plasma). Staining with picrofuchsin. Magnification x 100.

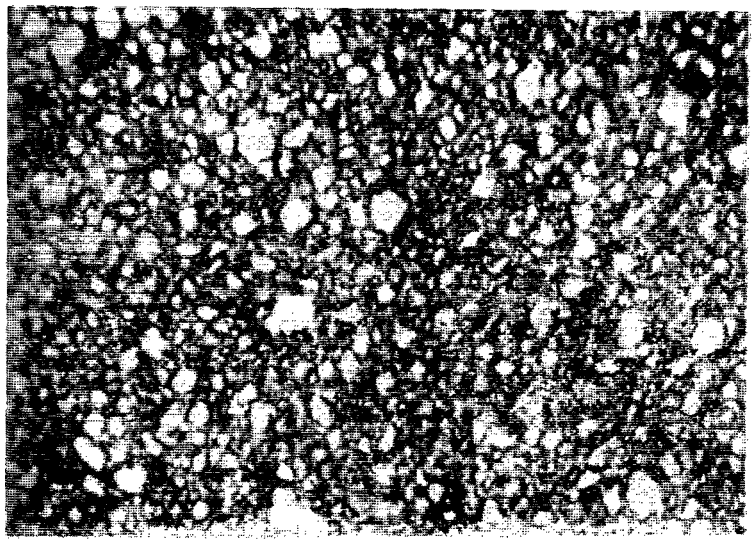


Figure 6. Pons Variolii. Porosity of white matter in reticular matter. Staining with picrofuchsin. Magnification x 200.

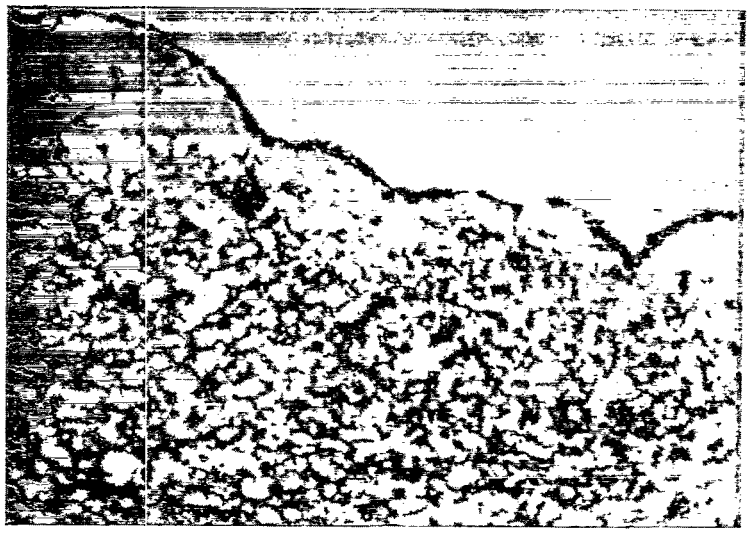


Figure 7. Necrosis with disintegration of cerebral matter in wall of fourth ventricle. Magnification x 200.

Figure 8. Straight convolution. "Naked" nuclei of nerve cells. Only remnants of cytoplasm with pathologically modified nucleoles. Staining with cresyl violet. Magnification x 900.

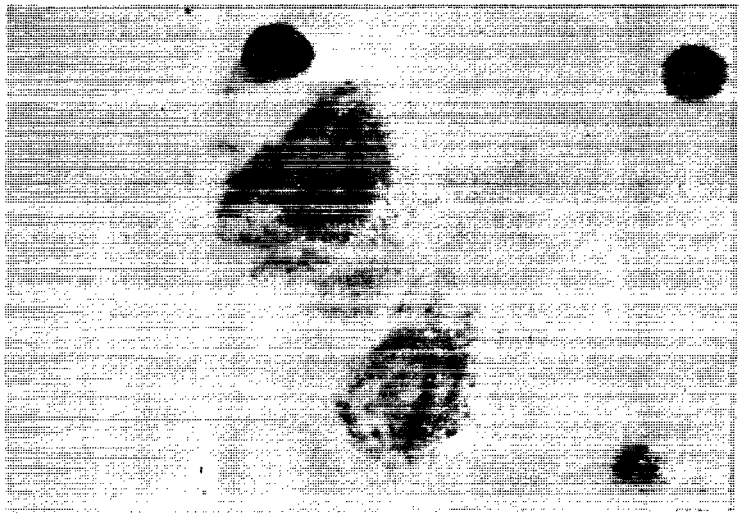


Figure 9. Temporal cortex. Cytolysis of nerve cells. Segmentation of nucleus. Staining with cresyl violet. Magnification x 1350.

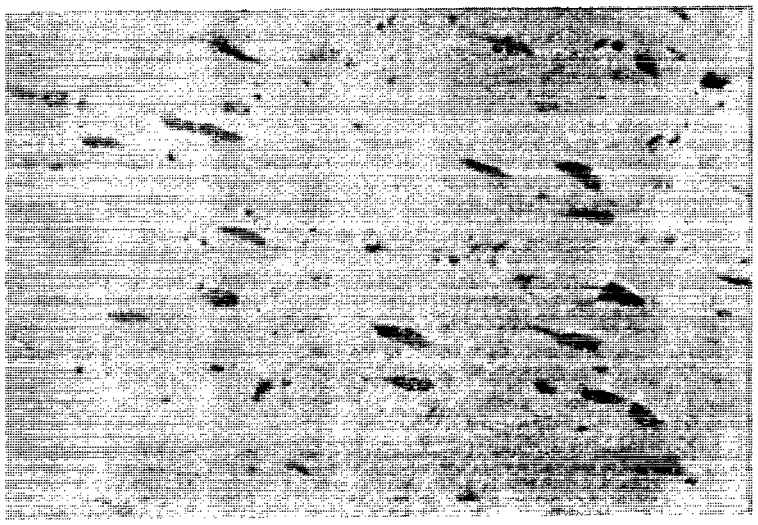


Figure 10. Temporal cortex. Shriveling and atrophy of large nerve cells. Pluglike appendices clearly shown in some cells. Magnification x 200.

Figure 11. Frontal cortex. Nerve cells with ischemic modification. Staining with hematoxylin-eosin. Magnification x 900.

pericellular edema. In the region of the motor apparatus there was necro- /228
biosis and an extraordinary distension of the appendices of some of the large pyramidal Betz cells. In all parts of the cortex of the hemispheres there were small foci of cellular obliteration of various sizes, still without impairing the cytoarchitectonics of the cortex, but there also were such foci of cellular shedding which led to disruption of cortical cytoarchitectonics--the straight gyrus, frontal, parietal and inferior temporal cortex (fig. 12).

The pathological changes in the nerve cells of the subcortical gray nodes were characterized by distension of the nuclei, hyperchromia and dissolving of the cytoplasm. Foci of shedding of the nerve cells were present in the caudate nucleus; somewhat less frequently they were encountered in the stem and pons. The nerve cells of the caudate nucleus, situated closer to the cornu anteriorus of the lateral ventricle, were particularly damaged. The total dissolving of the cytoplasm of some cells of the caudate nucleus was the cause of the formation of "naked" nuclei, sometimes very similar to the "naked" nuclei of the astroglia. In such cases a differential criterion can be a preserved nucleole or its remnants in the nucleus of a nerve cell.

Among the formations of the diencephalon particular attention should be given to the pathological changes of the nerve cells of the nuclei of the hypothalamic region, especially the neurosecretory neurons of the supra-optical nucleus. In these nuclei there were no cells whose structure did not /229
have deviations. The different stages of the pathological changes (from dissolving of the tigroid matter to death of the cells) were represented clearly in the supra-optical and periventricular nuclei in all animals. The death of many nerve cells is indicated by cellular remnants or debris scattered among

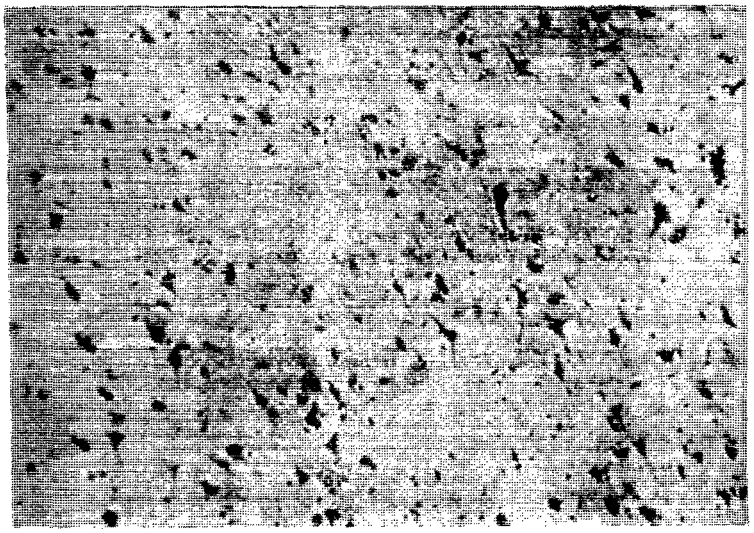


Figure 12. Frontal cortex. Foci without nerve cells. Staining with cresyl violet. Magnification x 100.

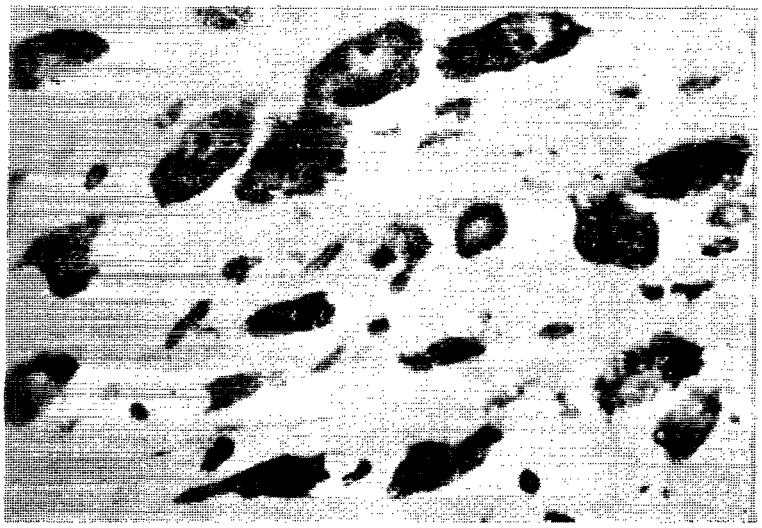


Figure 13. Subthalamic region (hypothalamus). Change of nerve cells of supra-optic nucleus. Cell fragments. Magnification x 540.

the remaining, but modified cells of the supra-optical nuclei (fig. 13). Central tigrolysis and accumulation of tigroid in the form of a basophilic column along the periphery of the body of some cells indicated the still reversible character of the changes. There were individual cells with vacuoles in

the cytoplasm, nearly normal structure but with a modified, scarcely detectable nucleus. The homogenization of many cells of the supra-optical and periventricular nuclei reflected a severer form of cellular pathology. In the cells of the central gray matter, surrounding the third ventricle, in the cells of the tuber cinereum and in the infundibulum there were changes characterized by shriveling and distension of nuclei and deformation of the nucleole. Less severe changes were observed in the nerve cells of the thalamus opticus.

The changes of the nerve cells of the midbrain, pons Variolii and medulla oblongata were not uniform, neither in character nor in intensity of the process. The most clearly expressed changes were observed in the cells of the cen- /230 tral gray matter, surrounding the cerebral aqueduct, partly in the cells of the bilobe, in the reticular matter of the midbrain, pons Variolii and medulla oblongata and partly in the cells of the nuclei of the fasciculus gracilis and fasciculus cuneatus, the cells of Edinger-Westphal nucleus and the posterior nucleus of the 10th pair. Diffuse injury of the cells was observed in the lower oliva. The occurring changes of the motor neurons had a selective character and essentially involved an extraordinary distension of the individual cells, but sometimes distension only of the nucleus and its hypochromia.

Among the motor neurons of the stem of the brain it was possible to observe shriveling and atrophy of individual cells. The motor neurons, therefore, were also involved in the pathological process, but their structure in comparison with the cells of the afferent and autonomic systems was more stable.

These data are in full accordance with data obtained in a histologic investigation of the central nervous system of dogs with acute radiation sickness caused by general exposure to X- and γ-rays. Various pathological deviations (from the initial stages to shadow cells) were noted in the cerebellum--in the layer of Purkinje cells (fig. 14). In places there was pyknosis and necrobiosis of the Purkinje cells over a considerable area. Pyknosis and necrobiosis of granular cells also were observed in the granular layer of the cerebellum (fig. 15).

In all parts of the brain and spinal cord, especially in the sub- /232 cortical stem formations, there were foci of demyelinization and disintegration of the myelinous nerve fibers (fig. 16).

In the treated dogs the reaction of the astroglia was manifested in focal proliferation in the gray matter of the subcortical stem formations, for example, in the caudate nucleus, in the tissue surrounding the cerebral aqueduct and at the base of the fourth ventricle, as shown in figure 17. In the white matter of the brain the changes of the astrocytes of a proliferative-degenerative character involved primarily the perivascular glia. In places it was possible to observe the disintegration of the vascular sheaths of the astrocytes (fig. 18). A gross disintegration of astrocytes was observed in the olivas of the medulla oblongata. When paraffin sections were stained with cresyl violet it was noted that there was hypochromia of the astroglia, whose size sometimes was greatly enlarged. Such nuclei were visible in virtually every field of view (fig. 19). With respect to changes of the oligodendroglia, they were

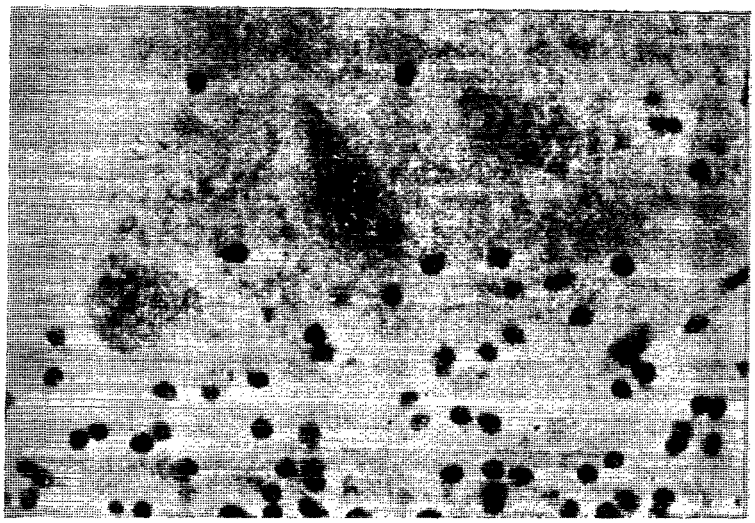


Figure 14. Cerebellum. Cytolysis of Purkinje cells. One cell with hyperchromatosis of nucleus. Pyknosis of nuclei of granular cells. Staining with cresyl violet. Magnification x 540.

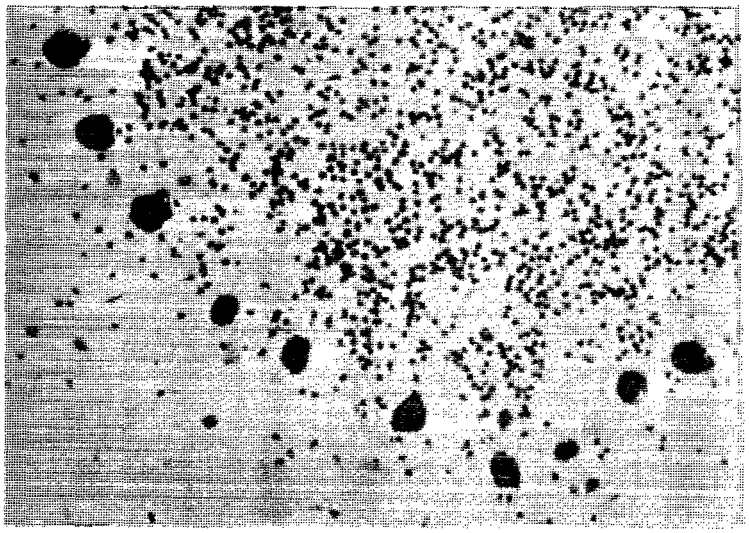


Figure 15. Cerebellum. Shriveling and necrobiosis of Purkinje cells. Staining with cresyl violet. Magnification x 200.

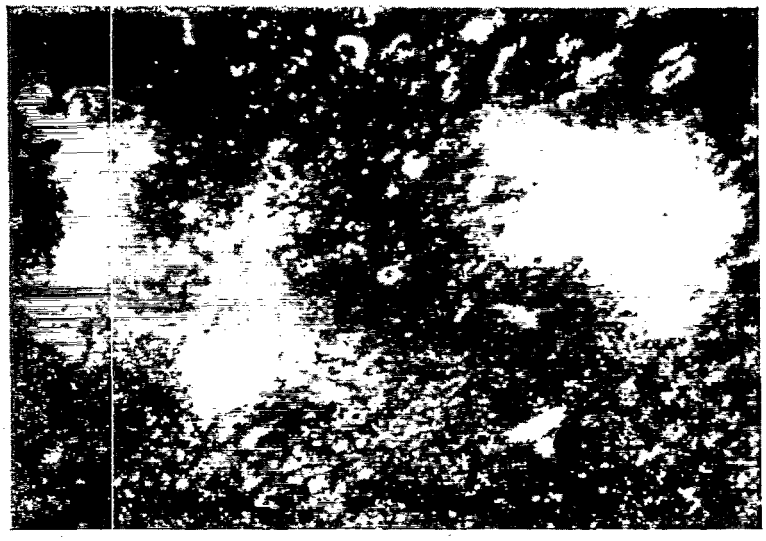


Figure 16. Medulla oblongata. Foci of demyelinization in corticospinal fascicles. Staining with hematoxylin (Kul'chitskiy type) by Sokolyanskiy method. Magnification x 70.

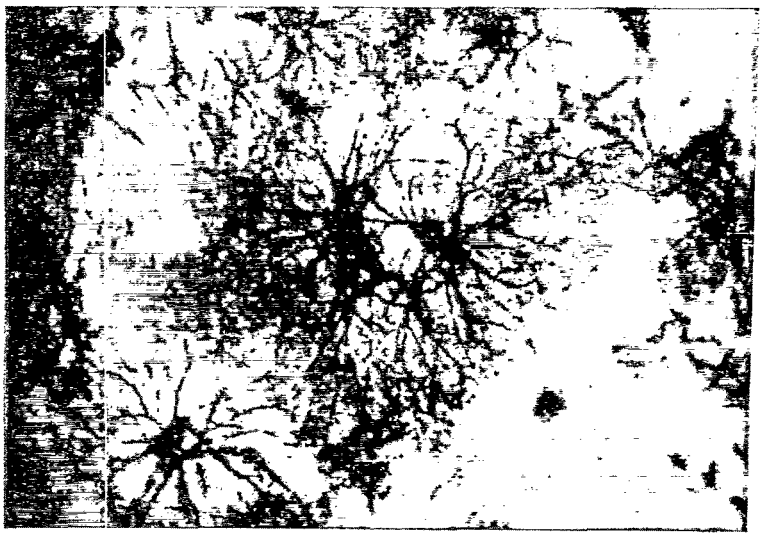


Figure 17. Caudate nucleus. Hyperplasia and hypertrophy of astrocytes. Staining by Kakhal method. Magnification x 450.

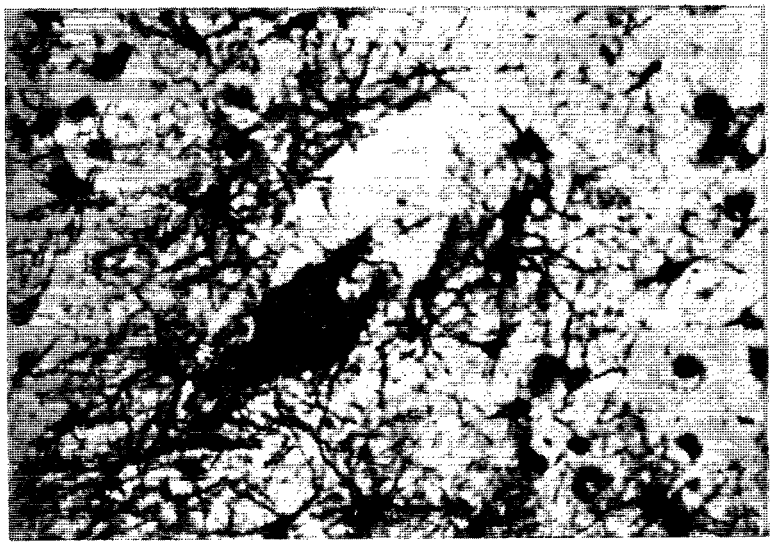


Figure 18. Subthalamic region. Hyperplasia of perivascular astrocytes. Staining by Kakhal method. Magnification x 280.

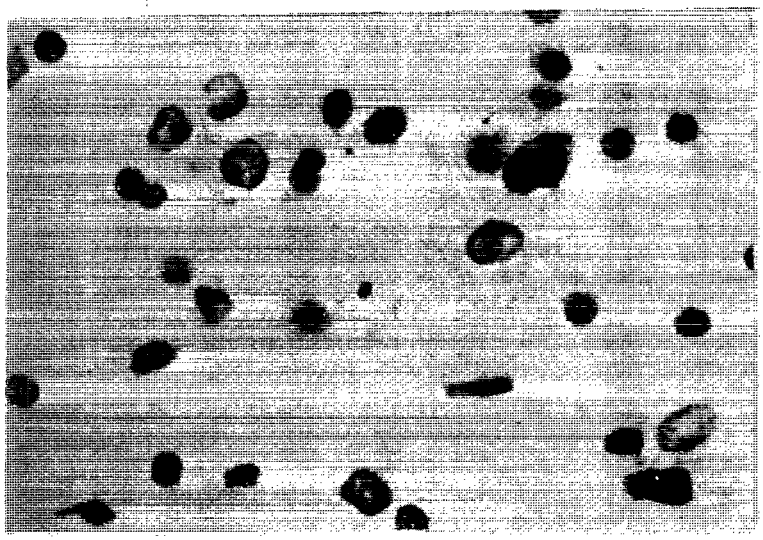


Figure 19. Frontal pole. Degeneration of astroglia (transparent naked nuclei) in white subcortical matter. Staining with cresyl violet. Magnification x 900.

characterized by pyknosis of some nuclei and a focal proliferation of drainage forms at the sites of the most severe edema of cerebral matter (fig. 20).

Experimental Histopathological Data. It should be noted that in the character of the pathological process developing in the central nervous system of the treated dogs there is great similarity to what was noted in the untreated animals during acute radiation sickness caused by multiple irradiation by protons. However, in the histopathological picture of the condition of the structures of the central nervous system of the treated dogs, there are also distinguishing characteristics mostly involving pathological changes caused by impairment of cerebral blood circulation. For example, in the treated dogs (those with a long life span) there was severe vascular dystonia with extensive perivascular edema, but intracerebral hemorrhages were encountered rarely, whereas in the untreated dogs hemorrhagic foci were encountered microscopically in almost every part of the brain and spinal cord, although in one of the dogs, irradiated with 690 rad, there was a macroscopically visible major focus of hemorrhaging in the cerebellum.

Apparently the absence of intracerebral hemorrhages in the treated dogs was due to the influence of therapeutic agents either directly on the wall of the vessels in the direction of improvement of their trophical systems, or through the higher regulatory centers, and as a result, in some period, exchange processes in the walls of the intracerebral vessels were impaired. The basis for this assumption is that intracerebral hemorrhages in the untreated dogs usually were caused not by an increase of the permeability of the vascular wall, but by its destruction (in many foci of hemorrhaging the walls of the vessels were indistinguishable). Obviously, in the treated dogs

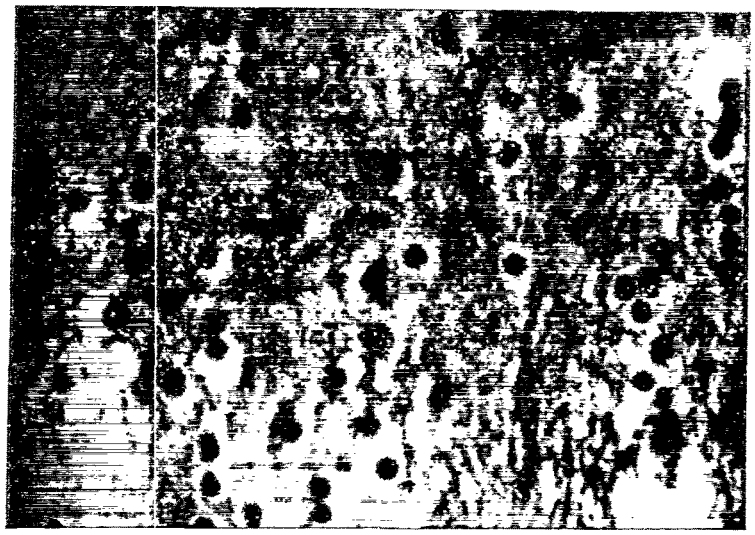


Figure 20. Frontal cortex. Drainage forms of oligodenroglia. Edema of white matter. Staining with hematoxylin-eosin. Magnification x 630.

the destruction of the cerebral vessels was prevented by therapeutic agents. However, the curative effect was temporary. The detected histostructural /235 impairments in the cerebral tissue indicate the involvement of nerve and glial structures, not only vessels, in the pathological process of dystrophic-necrobiotic character. At pathologic-anatomic autopsy of dogs severe expressed anemia of the brain was found, which corresponded to the microscopic picture, reflecting a severe, almost diffuse vascular dystonia, characterized by collapse of the walls of the vessels and less frequently by expansion of the lumens and their filling with a homogeneous rosy-albuminous mass.

It should be emphasized again that many of the changes detected in the central nervous system were the result of aplastic anemia developing in the treated dogs, which was diagnosed even while they were living and which was confirmed during autopsy of the animals.

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CONCLUSION /236

Analysis of available data on the possible sources of ionizing radiation in space has shown that high-energy (100-700 Mev) protons can represent a highly serious danger during space flights.

Taking this into account, the authors of this book carried out complex physical and biological investigations with the proton beam of the synchrocyclotron of the Joint Institute for Nuclear Research at Dubna. These investigations made it possible to determine the characteristics of high-energy protons and to discover ways for solving the problem of ensuring radiation safety during space flights.

The principal results of these investigations can be formulated as follows. It has been demonstrated that some well-known dosimetric methods (ionization, chemical, luminescent and others) can also be used for measurement of high-energy protons. Nuclear interactions of protons in the energy range 100-510 Mev make a small contribution to the measured absorbed dose. However, this contribution is important in evaluating the biological effect, considering the high linear energy losses of secondary particles.

Methods for investigations for protection from high-energy protons applicable to the proton beam of the accelerator were developed. It was established that in the case of normal incidence of a broad proton beam on a protective barrier the accumulation of secondary particles in it is extremely substantial, and this must be taken into account in its design.

Analysis of collected data on the radiation resistance of materials reveals that most of them retain their strength adequately for absorbed doses up to $3\text{-}4 \cdot 10^7$ rad.

Physical conditions were created for the irradiation of animals by high-energy protons; tissue doses were computed and an analysis made of the composition of radiation in the phantoms of animals. The biological effect of protons with energies of 126-510 Mev on large and small laboratory animals was investigated. It was established that as a result of multiple fractional irradiation by protons of these energies there will be acute radiation sickness with /237 a complex of symptoms of functional and morphological disturbances and changes characteristic of this disease. At the same time, the biological effect of the protons of the investigated energies is characterized by differences from the effect of γ- and X-ray radiations. In particular, the reaction of the peripheral blood and bone marrow at the early stages of the illness is less well-expressed in the case of irradiation by protons and does not have such obvious prognostic importance as in injuries caused by γ- and X-ray radiations. In later periods there are serious changes of the blood, even including complete aplastic

anemia; this apparently is the principal cause of the death of animals at these stages. It is very characteristic that the hemorrhagic syndrome is more clearly expressed under the influence of protons. This is manifested, in particular, by the presence of hemorrhages in all parts of the brain.

New experimental data were obtained on the influence of ionizing radiation on the activity of the vestibulary mechanism. Corresponding quantitative relationships were determined between the radiation dose and the biological effect.

During the determination of the relative biological effect of proton radiation it was established that the coefficient of relative biological effect of protons, with energies of 510 Mev in their total effect and in determination of lethal and 50 percent lethal doses the survival rate after 30-60 days is about 0.7 for rats and about 1.0 for dogs. However, observations of the results of proton irradiation after an extended period can introduce significant corrections, indicating an increase of this coefficient.

In the study of the development of lethal dominants in rats it was discovered that small proton doses are more effective biologically than corresponding doses of X-rays, whereas in the case of large doses an inverse dependence is observed.

The results of investigations of the therapeutic and prophylactic effectiveness of individual pharmacological materials and certain variants of protective-therapeutic complexes showed that the most clearly expressed protection is provided by 5-oxytriptamin and a combination of cystamin with amigdalin. A clearly expressed antihemorrhagic effect of 5-oxytriptamin during proton irradiation was established. An effective protective-therapeutic complex was developed, which consists of a minimum range of protective and therapeutic preparations used for the most part per os. The investigations on dogs revealed that this complex is extremely effective in the case of absolutely fatal acute radiation injuries developing as a result of multiple irradiations of dogs by protons. However, after long observation of the animals it was discovered that this protective-therapeutic complex did not prevent the animals from developing aplastic anemia a considerable time after irradiation, and this resulted in their death. For this reason it will be necessary in the future to develop effective methods for combating specific disorders developing a long time /238 after irradiation by high-energy protons.

The principal results of the experimental physical and biological investigations using the synchrocyclotron, reported in this book, are of definite importance for solution of the problem of ensuring radiation safety in space flight. At the same time it is entirely clear that these investigations only partially solve some aspects of this problem. Solution of the entire complex of practical problems of radiation safety in space flight requires that in the future there must be still greater expansion of both physical and biological experimental investigations of different types of corpuscular radiation in a broad range of energies.

Translated for the National Aeronautics and Space Administration
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