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TABLE OF CONTENTS

Page

Page

Brief Communications

Letters to the Editor

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On the Seventieth Birthday of Laureate of the Nobel Prize, Academician Nikolai Nikolaevich Semenov

INFLUENCE OF N. N. SEMENOV AND HIS SCHOOL ON THE DEVELOPMENT OF RADIATION BIOPHYSICS

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pp. 161-165

In modern science, the wide introduction of physical and chemical concepts into biology, which are vital for an understanding of the processes lying at the basis of vital phenomena, is being witnessed.

One of the most important divisions of physical chemistry is the theory of the kinetics of chemical reactions, which has now found wide use in the investigation of the processes that occur in biological substances and living cells. Biophysical kinetics pays much attention to the mechanisms of the regulation of reaction rates in living cells, the reactivity, inhibition, and to all the processes that bring about autoregulation of reactions in living systems. The possibilities of kinetic regulation of reaction rates determine such an important property of life as its adaptation to conditions of the external environment.

At the same time, any influences that disturb the regular occurrence of reactions and break down their coordination, converting their development from steady-state to nonsteady-state, cause disturbances of metabolism, pathology, while if they are irreversible, they also make existence impossible under such conditions.

Hence, investigations along this line have long been directed toward the study of the basic kinetic parameters of reactions that occur in living biological systems. Attempts to subsume processes that occur in biological systems under the existing kinetic types of reactions were made by many authors at the beginning of this century. The founder of modern chemical kinetics, Arrhenius, attempted to describe the course of certain immunological reactions from the standpoint of the concepts of classical kinetics. The school of Crosier*, developing these concepts, established that the activation energy E, determined for biological specimens according to the Arrhenius diagram, can serve as a characteristic of these reactions and can characterize their nature. A method

*[Transliteration]

of analysis and detection of the leading reactions in living functioning systems was constructed on this basis, and subsequently received wide development in biology.

However, researchers who attempted to use the concepts of the classical kinetics of Van't Hoff and Arrhenius to analyze the peculiarities of the kinetics of reactions arising in the case of injury of organisms by radiant energy and various toxic agents, encountered considerable difficulties. The reactions recorded in this case clearly did not fit into the classical types of monomolecular, bimolecular, and higher orders, while the values of the activation energy (μ) reached excessively high values, which varied with the temperature. In the 30's of this century, great revolutionary changes occurred in chemical kinetics. The studies of N. N. Semenov [1] on combustion and explosions disclosed the mechanisms of these chemical reactions and led to the construction of the theory of chain reactions and the development of new concepts -- chain reactions with branched chains. New peculiarities of the course of these reactions with time were revealed; the role of the kinetics of the formation of active intermediates --"promoters" of these reactions -- was also established. This theory made it possible to explain the cause of the lack of obedience of many reactions to the Arrhenius law, and the appearance of abnormally high values of the activation energy. An anomalous effect of the influence of the oxygen concentration upon the rate of these reactions (the theory of the upper and lower oxygen limit, at which the rates of oxidative reactions of the branched-chain type drop sharply) was detected and theoretically explained. N. N. Semenov established that the chain mechanism of the development of chemical transformations is far more widespread than was earlier thought. It was revealed that many reactions which, for example, were previously described as monomolecular reactions, were actually reactions that develop according to a chain mechanism.

The studies of this period (establishment of the theory of chain reactions), seem to have no relationship to biology at all; however, even then many theoretical biologists noted that the general principles of the course of biological reactions can be explained by the presence of chain processes (A. G. Gurvich, Kh. S. Koshtoyants, et al.), for example, the oligodynamic effect upon organisms of negligible quantities of metals, the phenomenon of acceleration of reactions in the excitation of the nerve, and the effects of the action of small doses of ultraviolet radiation. These considerations, of course, were speculative to a considerable degree and usually encountered objections that the rates of processes accomplished with such autoacceleration are scarcely possible in biological substrates.

The numerous investigations by N. N. Semenov's school of reactions of the chain and branched-chain type, occurring in the liquid-phase oxidation of hydrocarbons, represented an important approach to biological processes.

Even more interesting for biophysicists was the detection of a new type of reactions, developing in the liquid phase according to the laws of chain reactions in gas media, i.e., with autoacceleration;

however, this autoacceleration was far slower. Although in the first case, with chain ignition, it was a matter of fractions of a second, in the case of chain reactions occurring in the liquid phase, this process develops in minutes, hours, days, and even months. These reactions were entitled chain reactions with degenerate chains. It was established that oxidation reactions of this type develop mainly in liquid hydrocarbons and fats.

These investigations, especially the reactions with degenerate chains, attracted the attention of biophysicists. Twenty years before, in a study of the kinetics of the physicochemical changes in living tissues under the action of toxic substances -- dichlorodiethyl sulfide and bacterial exotoxins -- it was noted that the changes in the kinetics of the swelling of living tissues, which accompany poisoning by these substances, do not obey the law of the monomolecular reaction, as has been described by certain authors, and that an error was committed here, analogous to that which N. N. Semenov detected in an investigation of certain reactions previously assigned to the monomolecular type. It was established that under the action of the aforementioned agents, a more complex kinetic dependence is observed, characteristic of degenerate chain reactions with autoacceleration and an induction period [2]. The presence of such a chain process was also indicated by the high values obtained for the activation energy and the lack of obedience to the Arrhenius dependence. These observations, demonstrating the possibility of induction of a chain reaction under the action of botulinus, diphtheria, and tetanus toxins and dichlorodiethyl sulfide and its derivatives, were of interest in view of the fact that these substances exert a strong biological effect upon the organism in negligible concentrations, analogously to the action of X rays.

The widespread interest in the determination of the mechanisms of the action of ionizing radiations upon organisms, which has arisen in the last decades, has directed the efforts of researchers to the detection of reactions that can play a starter role in the radiation injury. The external characteristics of radiation injury indicated that in the primary mechanisms there may be induced chain reactions with an induction (in the biological terminology incubation) period.

The criteria established by N. N. Semenov's school for the analysis of chain reactions were used in the analysis of radiation influences, which, as is well known, induce a great biological effect.

One of the puzzling facts that was considered as a specific peculiarity of the radiation injury in radiobiology, was the "oxygen effect," discovered at the end of the 40's. This anomalous dependence of the injurious effect of radiation upon the oxygen concentration has been established by numerous authors on the most varied plant and animal organisms. When the oxygen concentration is lowered, the injurious action of ionizing radiations drops, and this indicates that an oxidative reaction is induced in the biosubstrate under the action of radiation. A peculiarity of the oxygen effect is the presence at high oxygen concentrations of a broad range, in which variation of the oxygen concentration has no effect upon the vulnerability of biological

specimens. At low concentrations, on the contrary, a sharp change in the radiation effect is observed as a result of a negligible change in the oxygen concentration. The nature of this dependence forced the assumption that the mechanism of the action of oxygen is the same as in the case of an anomalous dependence of the rate of oxidative branched chain reactions upon the oxygen concentration, discovered by Semenov and his school, i.e., it was necessary here to approach the viewpoint of the theory of oxygen limits, developed by Semenov. On the basis of these premises, it should have been expected that if the established threshold of injury with decreasing oxygen concentration is the lower limit for branched oxidative reactions, then there is also an upper limit when the oxygen concentration is increased. However, it is more difficult to detect this limit, since oxygen in increased concentrations itself exerts a strong toxic effect. There are organisms upon which an increase in the oxygen concentration has no harmful effect. The curve of the dependence of the injury of yeast cells upon the oxygen concentration within the range from 0 to 11 atmospheres under γ radiation has not only a lower limit, but also an upper limit, at which the injurious effect of radiation drops [2]. It was later demonstrated on plants that when they are injured by X rays at various oxygen concentrations (within the range from 0 to 70 atm), lower and upper limits also are always observed [3]. This was a weighty argument in favor of the appearance of a chain reaction in the cellular substrate during irradiation.

An analytical examination of the reactivity of various biological substrates, and the studies of N. N. Semenov's school on the induction of slowly developing chain reactions in fats (Émmanuél, Knorre, Lyaskovskaya, Burlakova [4]) forced the assumption that the most probable cell substrate for the development of chain reactions is the lipids, and precisely the structural lipids from which the cell membranes, mitochondria, and lysosomes are constructed, and which play an important role in the regulation of chemical processes in living cells. On the basis of this assumption, A. I. Zhuravlev [5] studied the behavior of the cell lipids on various lipid models and demonstrated that chain reactions, the rate of development of which has the typical concentration limits, depending upon the partial pressure of oxygen, actually arise in them.

A great step forward in the understanding of the kinetic peculiarities of reactions induced by radiation in the cell lipids, was the discovery, made by a number of authors both abroad and in the USSR, that an extremely negligible amount of peroxide compounds can be detected in lipids extracted from animal tissues, and after irradiation by ionizing radiations, this amount increases sharply. On the basis of the assumption that these peroxide compounds are intermediate products of chain reactions, V. Mal'ts*studied the kinetics of the variation of the concentration of these peroxides during radiation injury on the basis of an enormous fund of material (animal), and established that the concentration of these peroxides increases and falls in accord with the curve of variation of the concentration of the intermediate products, according to the theory of N. N. Semenov, for branched chain reactions.

*[Transliteration]

Further investigations along this line, conducted by E. B. Burlakova (N. N. Semenov's school) on irradiated animals, set the question of the possibility of the appearance of chain reactions in the lipids of organisms upon a firm foundation.

A great influence upon the development and elucidation of the mechanisms of the reactions that arise during irradiation was exerted by the theoretical premises and studies of N. N. Semenov's school on the problem of the inhibition of chain reactions. Investigations of the influence of inhibitors upon the induction period and kinetics of the accumulation of hydroperoxides in the oxidation of isopropylbenzene evidently gave a key for the interpretation of the processes of induction of oxidative chain reactions in the lipid structures of cells. The hypothesis arose that the basic role in the possibility of induction is played by a breakdown of the antioxidant systems, which protect organisms from spontaneous oxidation. Under the influence of these concepts, A. I. Zhuravlev et al. undertook a study of the antioxidant systems in the lipid phases of cells during irradiation by ionizing radiations and demonstrated that during irradiation there is a substantial consumption of the natural lipid antioxidants, and their amount appreciably and progressively decreases during the development of the effect of irradiation. Among biophysicists, the idea has arisen that the phenomenon of chemical protection -- decrease in injury by the introduction of certain substances into the organism -- is associated with an inhibition of the oxidative reaction, which normally develops in the lipid phases of cells at a very low level in a steady-state system, but when the antioxidants are broken down, occurs on a nonsteady-state basis and develops with autoacceleration, imposing an imprint upon the general dynamics of the injury. From this standpoint, a general property for all protective substances is antioxidant and antiradical activity. Numerous investigations have actually established a complete correlation between the protective effect and the ability of substances to inhibit oxidative reactions in lipids [6].

Taking the path of kinetic analysis of the reactions arising during irradiation in lipid phases, comprehensive investigations of the nature of the active products of radical and peroxide character that arise and multiply during the occurrence of chain reactions in the lipid phases of cells have been conducted. Yu. B. Kudryashov and his associates [7, 8] have investigated and isolated various products formed in the chain oxidation of biolipids. Moreover, they have succeeded in isolating from the lipids of irradiated animals a product containing fatty acid peroxides, which prove to be a "radiomimetic." They succeeded in demonstrating on biological specimens that this product acts upon cells to induce the phenomena characteristic of radiation injury and possesses the ability to induce reactions of oxidation in the biolipids, that is, they succeeded in bringing about what N. N. Semenov's school calls initiation of a reaction. This is evidence that this product is actually an intermediate product of the reaction and its "promoter."

N. N. Semenov's school considers radicals to be especially important in the development of reactions and reactivity; their

determination plays a great role in the analysis of the nature of a reaction. Biophysicists have also taken this route; however, they have encountered great difficulties here. The determination of radical states in living cells by the methods used by physicochemists is impossible.

Yu. P. Kozlov et al. [9] have succeeded in developing a method for determining radical states according to the polymerization of monomers, introduced into living cells. This has made it possible to trace the kinetics of the development of the radical chain reaction in cells after irradiation and to confirm its chain character. Yu. P. Kozlov and Urtile Sterike have succeeded in determining directly, by fractionation of cells in which polymerization of a carbon-14-labeled monomer (acrylamide) occurred during irradiation on different substrates, that the formation of radicals occurs in the lipid fractions.

Great aid in the analysis of radical formation during the irradiation of living cells and the possibility of following the kinetics of the reactions that occur after irradiation in living cells have been afforded by a study of ultraweak luminescence, which we have detected among all living cells. This radiation is due chiefly to the lipids and arises during their slow oxidation. Observations of ultraweak radiation, which we have conducted [10, 11], have indicated that in normal cells the oxidation reactions develop on a steady-state basis. When cells are damaged by various factors, including irradiation, the reaction leaves the steady-state system, and the chemiluminescence curve directly shows that it begins to develop with autoacceleration (degradation radiation), according to the kinetics of branched chain reactions. These data confirm the correctness of prerequisites based upon the chain theory.

The work of N. N. Semenov and his school has been responsible for the success of biophysicochemical investigations in the detection of the primary mechanisms of radiation injury and has laid the foundation for further trends along this line.

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INFLUENCE OF CYSTAMINE AND S,β-AMINOETHYLISOTHIURONIUM ON THE ACTIVITY OF DEOXYRIBONUCLEASE II IN RADIOSENSITIVE ORGANS OF RATS IRRADIATED AT DOSES OF 350 AND 100 R

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pp. 166-169

A reliable index of radiation injury of the organism, even in the case of its irradiation at sublethal doses is the increase in the deoxyribonuclease II activity in radiosensitive organs [1-7]. We know of two literature studies [8, 9] on the influence of radioprotective compounds upon the DNA-ase activity in the tissues of the irradiated organism, conducted on animals irradiated at doses close to the lethal.

In this work we studied the influence of cystamine and S,β-aminoethylisothiuronium (AET) on the activity of DNA-ase II in the spleen and in the thymus gland of rats irradiated at doses of 350 and 100 R.

PROCEDURE

In the experiments we used male white rats weighing from 180 to 220 g. Radiation sickness was induced by a single total irradiation by X rays at doses of 350 R ($LD_{25/30}$) and 100 R (sublethal dose). Conditions of irradiation: current strength 15 mA, voltage 180 kV, filters: 0.5 mm Cu and 1.0 mm Al, dose rate 12 R/min and 3.7 R/min for doses of 350 and 100 R, respectively.

The rats among whom the influence of radioprotective compounds upon the activity of DNA-ase II was investigated received intraperitoneal injections of solutions of these preparations directly before irradiation, in doses of 90 mg cystamine hydrochloride or 250 mg AET hydrobromide per kg of weight of the animal. The experimental rats were killed by decapitation on the first, third, and seventh days after the influence of ionizing radiation.

Samples of tissue were homogenized in the cold in double-distilled water (1:50 for the spleen and 1:100 for the thymus). The DNA-ase II activity in the homogenate was determined by the method of Allfrey and

Mirsky [10] in Kowlessar's modification [11]. The value of the enzymatic activity was determined according to the increase in the amount of low-molecular decomposition products of DNA, which were determined by the Dische color reaction in Burton's modification [12]. The color intensity was measured on the FÉK-M colorimeter with a neutral filter. The DNA-ase II activity was expressed in μg of DNA phosphorus, cleaved in three-hour hydrolysis, calculated on the basis of 1 mg of crude tissue.

The data obtained were treated by the method of variation statistics; variations were considered reliable at $p \leqslant 0.05$.

DNA-ase II Activity in Tissues of Irradiated Rats

(In μg Phosphorus of Cleaved DNA per mg of Crude Tissue)

Investigated Organ	Period after Irradiation (days)	Conditions of Experiment	Number of experimental animals	$M \pm m$	p*	In % of normal level
		IRRADIATION DOSE 350 R				
SPLEEN	—	Without irradiation	26	3,63±0,18	—	100
	1	Irradiation	16	6,69±0,56	—	184
		Irradiation + cystamine	12	4,30±0,40	<0,01	118
		Irradiation + AET	10	5,10±0,37	<0,02	140
	3	Irradiation	13	5,51±0,57	—	152
		Irradiation + cystamine	12	2,30±0,26	<0,001	63
		Irradiation + AET	13	3,06±0,44	<0,01	84
THYMUS	—	Without irradiation	21	2,80±0,07	—	100
	1	Irradiation	13	5,89±0,57	—	210
		Irradiation + cystamine	12	4,94±0,30	<0,01	175
		Irradiation + AET	10	4,45±0,39	<0,05	159
	3	Irradiation	13	4,65±0,58	—	166
		Irradiation + cystamine	12	3,10±0,31	<0,02	110
		Irradiation + AET	13	3,40±0,29	<0,05	121
		IRRADIATION DOSE 100 R				
SPLEEN		Without irradiation	26	3,63±0,18	—	100
	1	Irradiation	12	5,30±0,51	—	146
		Irradiation + cystamine	22	3,51±0,24	<0,01	97
		Irradiation + AET	12	3,98±0,32	<0,05	109
	3	Irradiation	17	4,46±0,53	—	123
		Irradiation + cystamine	11	2,60±0,40	<0,01	71
		Irradiation + AET	13	3,14±0,26	<0,05	86
THYMUS	—	Without irrariation	21	2,80±0,07	—	100
	1	Irradiation	12	4,40±0,32	—	157
		Irradiation + cystamine	12	2,70±0,16	<0,001	95
		Irradiation + AET	17	3,39±0,25	<0,05	121
	3	Irradiation	15	4,31±0,42	—	154
		Irradiation + cystamine	11	4,00±0,42	<0,6	142
		Irradiation + AET	13	4,02±0,35	<0,6	143

*Reliability of the measurements with respect to irradiated control.

RESULTS

As can be seen from the table, among the irradiated control animals the highest rise in the DNA-ase II activity in the investigated organs was detected one day after the influence of ionizing radiations. On the third day the enzyme activity still continued to remain at an increased level, but by the seventh day it was normalized.

On the basis of the data obtained, a study of the DNA-ase II activity under conditions of chemical protection was conducted on the first and third days from the moment of irradiation. The administration of radioprotective preparations promoted a weakening of the changes in the DNA-ase activity in the investigated organs. A pronounced effect of protection was obtained in the irradiation of rats at a dose of 350 R. Among the animals protected by cystamine, the DNA-ase II activity in the spleen and thymus were increased by only 18 and 75% of the normal level, respectively, one day after irradiation (with an increase of 84 and 110%, respectively, in the control). Analogous results were obtained during this same period after the administration of AET. On the third day after the influence of radiation, the favorable influence of protectors upon the DNA-ase II activity was even more pronounced. Among the animals irradiated at a dose of 100 R, the action of protective compounds was also distinctly manifested. Both preparations exerted a normalizing action upon the enzyme activity in the spleen, and on the first day in the rat thymus.

Control experiments studying the influence of radioprotective preparations upon the DNA-ase II activity among nonirradiated animals indicated that the administration of cystamine and AET to healthy rats without their subsequent irradiation does not influence the enzyme activity in the investigated organs.

DISCUSSION

Our experiments indicated that a substantial increase in the activity of DNA-ase II is observed in the spleen and thymus of irradiated rats. These results agree with the literature data. The increase in the nuclease activity undoubtedly is associated with simultaneously observed changes in the nucleic acid metabolism in the irradiated organism. There is now some basis for believing that the substantial decrease in the amount of DNA in the cells of radiosensitive tissues is a result of its breakdown under the action of DNA-ases. Hence, prevention of the increase in the DNA-ase activity after irradiation should play an important role in the restoration of nucleoprotein metabolism.

The results of this investigation show that under the conditions of prophylactic administration of cystamine and AET to animals, a distinct weakening of the increased DNA-ase II activity in the lymphoid organs is observed.

The studies of a number of authors [13-19] have established that

the prophylactic administration of cystamine and cysteamine promotes a normalization of the DNA content in the spleen during the first days after irradiation. There is every basis for attributing this effect of protection to the influence of protectors upon the enzymes that depolymerize DNA. However, the observed protective action of the sulfur-containing compounds upon the activity of DNA-ase II evidently is mediated and is determined primarily by a decrease in the damage to the intracellular structures [20].

In our experiments, the protective effect of the investigated preparations was distinctly manifested in the case of irradiation at doses of 350 and 100 R. Thus, on the basis of the data presented we may speak of the detection of protection with respect to certain biochemical indices in the case of irradiation both at lethal and at sublethal doses of ionizing radiation.

CONCLUSIONS

1. A total single irradiation of rats by X rays at doses of 350 and 100 R causes a substantial increase in the DNA-ase II activity in the spleen and in the thymus gland.

2. The prophylactic administration of cystamine and AET weakens the degree of increase in the DNA-ase II activity in the investigated organs of irradiated animals.

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DYNAMICS OF THE DISTRIBUTION OF FREE AMINO ACIDS IN THE WALLS OF THE GASTROINTESTINAL TRACT UNDER THE ACTION OF PENETRATING RADIATION

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pp. 170-173

The study of the protein and amino acid metabolism during radiation injury, and in connection with this, the study of the composition of free amino acids and the possibility of their redistribution among individual organs and tissues, are of great interest. In many pathological states, accompanied by a disturbance of the protein metabolism, the composition of the free amino acids in the organs is changed [1-3].

In this work we studied the composition of free amino acids in the walls of various portions of the gastrointestinal tract of non-irradiated and irradiated rabbits.

PROCEDURE

The experiments were conducted on male rabbits weighing 1700-2000 g. The animals were irradiated once on the GUT-Co-400 apparatus, focal length 60 cm, dose rate 10 R/min; summary dose 300, 500, 700, and 900 R. The animals were sacrificed five days after irradiation. Non-irradiated animals were investigated at the same time.

The animal received intravital perfusion of Ringer solution, warmed to 37°. After death, all the portions of the gastrointestinal tract were removed. The free amino acids were determined by the method of paper chromatography [4]. Chromatography was conducted on paper of Leningrad factory No. 2, "rapid" type. The solvent -- butanol-acetic acid-water (4:1:5) was passed through twice. After staining of the chromatogram with 0.5% ninhydrin solution in acetone, the spots of the amino acids obtained were eluted with a 0.005% copper sulfate solution in 70% ethanol (5 ml). The eluates, stained orange, were subjected to photometry against a 0.005% copper sulfate solution in 70% alcohol on the FÉK-M with a green light filter (530 mμ). The amino acid concentration

was determined on the basis of the value of the optical density according to calibration curves. The amount of amino acids was calculated in mg per 100 g of tissue.

From the results obtained it follows that each portion of the gastrointestinal tract is characterized by its own set of free amino acids. As an example, let us cite the composition of free amino acids in the wall of the stomach, jejunum, and transverse colon. From Table 1 it is evident that the presence of 17 free amino acids is determined in the wall of the rabbit stomach; the contents of histidine, glutamic acid with threonine, and valine with methionine are the greatest. In a comparison of the composition of free amino acids of the stomach wall and other portions of the gastrointestinal tract, a predominance of histidine can easily be noted. It is known that histidine is a precursor of histamine -- a powerful exciter of secretion of gastric juice, and it may be that the larger histidine content in the stomach wall is related to its role as a histamine precursor.

Table 1

Content of Free Amino Acids in the Wall of the Rabbit Stomach, mg/100 g of Tissue (Average of Five to Six Experiments)

Amino acids	Norm	Irradiation dose, R			
		300	500	700	900
Cysteine	6,0±0,2	17,6±0,5	8,6±0,3	39,9±1,2	6,0±0,2
Lysine	10,3±0,3	12,4±0,4	11,9±0,3	32,9±1,0	10,3±0,3
Arginine	11,9±0,4	39,9±1,2	18,3±0,6	28,9±0,8	25,4±0,8
Histidine	31,4±0,9	15,7±0,5	11,3±0,3	Traces	5,8±0,2
Aspartic acid	13,3±0,4	8,3±0,2	24,0±0,7	39,9±1,2	26,8±0,8
Serine	7,2±0,2	8,4±0,3	10,5±0,3	15,9±0,5	7,1±0,2
Glycine	9,3±0,3	9,4±0,3	10,0±0,3	58,2±1,8	15,8±0,5
Glutamic acid + threonine	55,6±1,6	28,3±0,9	55,6±1,6	129,4±3,8	103,4±3,0
Alanine	5,6±0,2	6,7±0,2	10,9±0,3	27,8±0,8	10,1±0,3
Proline	—	—	—	—	—
Tyrosine	5,6±0,2	Traces	—	Traces	Traces
Valine + methionine	21,0±0,6	Traces	Traces	Traces	Traces
Tryptophan	7,7±0,2	17,8±0,5	15,8±0,5	—	—
Phenylalanine	9,3±0,3	Traces	Traces	—	Traces
Leucine + isoleucine	13,5±0,4	19,8±0,6	13,5±0,4	15,4±0,5	Traces

Under the influence of an increasing dose of penetrating radiation, the free histidine content in the stomach wall drops catastrophically. The disturbance of the secretion of gastric juice and the appearance of achillin during radiation sickness may be substantiated in the light of these data.

Of great interest is the change in the content of aromatic amino

acids and valine with methionine in the stomach wall. These amino acids almost entirely disappear when the animal is irradiated at doses of 700-900 R. At the same time, the content of lysine, arginine, aspartic acid, serine, and glutamic acid increases two- to three-fold, while that of cysteine, glycine, and alanine increases almost seven-fold. At lower doses, an increase occurs only for arginine, and at a dose of 900 R, a high level is retained only for glycine, arginine, alanine, aspartic and glutamic acids. If we proceed from the biological significance of these amino acids and their participation in metabolic reactions, then the formation of urea and the transamination reactions should not only be retained, but should even be intensified during the development of the radiation injury. By the way, a direct determination of the blood urea, conducted in our laboratory, indicated that it increases in the stage of the disease under consideration.

Of all the investigated portions of the gastrointestinal tract, it is precisely in the jejunum that the maximum assimilation of amino acids is observed, with high activity of the enzymes contained in the wall (especially cholinesterase and dipeptidase). The free amino acid content in the wall of this portion of the intestines is the greatest. The only exception is histidine, if we compare its content with the stomach wall (Table 2).

Table 2

Free Amino Acid Content in the Wall of the Jejunum (mg/100 g of Tissue)

Amino acids	Norm	Irradiation dose, R			
		300	500	700	900
Unidentified spot	—	—	+	++	+++
Cysteine	5,4±0,1	28,4±0,8	10,0±0,3	7,4±0,2	11,6+0,3
Lysine	41,0±1,3	29,7±0,9	22,1±0,7	13,2±0,4	10,6±0,3
Arginine	34,9±1,11	55,4±1,6	52,1±1,5	42,8±1,3	36,9±1,1
Histidine	16,2±0,5	11,2±0,3	23,0±0,7	20,0±0,6	16,0±0,5
Aspartic acid	27,1±0,5	22,2±0,7	39,8±1,2	46,3±1,3	25,4±0,8
Serine	31,5±1,0	17,0±0,5	14,9±0,4	13,5±0,4	11,3±0,3
Glycine	35,8±1,2	25,0±0,7	22,9±0,7	20,6±0,6	—
Glumatic acid + threonine	89,1±2,8	90,6±3,0	175,6±5,7	151,8±4,6	122,3±3,8
Alanine	22,1±0,3	18,2±0,6	18,0±0,6	19,8±0,6	15,4±0,5
Proline	—	+	++	++	+++
Unidentified spot	—	+	—	++	+++
Tyrosine	6,6±0,2	5,7±0,2	5,6±0,2	4,8±0,1	3,3+0,1
Valine + methionine	60,8±1,8	18,3±0,6	22,3±0,7	18,3±0,6	8,4±0,3
Tryptophan	29,3±0,9	25,1±0,8	19,7±0,6	—	—
Phenylalanine	16,2±0,5	9,3±0,3	8,0±0,3	8,0±0,2	5,1±0,2
Leucine + isoleucine	40,0±1,2	26,1±0,8	11,9±0,4	8,7±0,3	6,4±0,2

Table 3

Content of Free Amino Acids in the Wall of the Transverse Colon
(mg/100 g of Tissue)

Amino acids	Norm	Irradiation dose, R			
		300	500	700	900
Cysteine	26,3±0,8	17,6±0,6	8,9±0,3	5,4±0,2	5,5±0,2
Lysine	17,8±0,5	17,0±0,5	8,4±0,3	7,0±0,2	4,4±0,1
Arginine	35,6±1,1	24,1±0,7	—	5,7±0,2	3,3±0,2
Histidine	10,3±0,3	15,0±0,5	—	4,8±0,1	2,6±0,1
Aspartic Acid	16,6±0,5	8,0±0,2	31,2±0,9	36,9±1,1	18,1±0,6
Serine	12,5±0,4	6,4±0,2	6,0±0,2	6,1±0,2	6,0±0,2
Glycine	19,7±0,6	17,1±0,5	15,8±0,5	12,8±0,4	11,5±0,4
Glutamic acid threonine	55,6±1,6	32,2±1,0	98,6±2,9	88,5±2,7	15,3±0,5
Alanine	10,9±0,3	12,1±0,4	12,0±0,4	12,0±0,4	9,3±0,3
Proline	—	—	—	—	+
Unidentified spot	—	+	+	++	+++
Tyrosine	6,2±0,2	—	—	—	Trace
Valine methionine	18,3±0,2	22,3±0,7	8,2±0,3	2,3±0,1	2,0±0,1
Tryptophan	13,6±0,4	—	—	—	+
Phenylalanine	2,6±0,1	1,6±0,1	1,0±0,1	0,8±0,0	Trace
Leucine isoleucine	4,4±0,1	5,4±0,2	5,3±0,2	5,8±0,2	2,1±0,1

During irradiation, the amounts of all the amino acids are reduced, with the exception of aspartic and glutamic acids, and partially histidine. Thus, the amino acid content in the wall of the jejunum is distinguished by certain peculiarities in the normal state and in the case of irradiation. From Table 3 it follows that in the jejunum, the free amino acid content is substantially lower than in the wall of the small intestine. Under the action of γ rays, the content of free amino acids drops, while the aromatic amino acids disappear entirely. Exceptions are glutamic and aspartic acids and alanine. The conservation of a high level only for these amino acids is a general principle.

Concluding our investigations, we can hypothesize that penetrating irradiation causes a disturbance of the metabolic processes to different degrees in the wall of different portions of the gastrointestinal tract.

CONCLUSIONS

1. It was demonstrated that 17 to 18 free amino acids are determined in the walls of all portions of the gastrointestinal tract; each portion is characterized by the predominance of only some of them.

2. The high content of histidine in the stomach wall is evidently due to the subsequent formation of histamine, while the sharp

decrease in the concentration of this amino acid may be attributed to a disturbance of the secretion of gastric juice after irradiation.

3. After the influence of penetrating radiation, the content of most of the free amino acids drops, which is most pronounced at a dose of 900 R; exceptions are glutamic and aspartic acids and alanine. This may be due to an intensification of the processes of transamination after irradiation.

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INFLUENCE OF TOTAL X RAY IRRADIATION UPON THE SOLUBLE PROTEINS OF THE SKELETAL MUSCLES

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pp. 174-178

The question of the changes that arise in the proteins of the muscle tissue of irradiated animals has been very little reflected in the radiobiological literature. Since the skeletal muscles comprise up to 40% of the body weight of mammals (and man) and more than 20% of the body proteins (with respect to the crude weight) are muscle proteins, it seems important to determine the degree to which the muscle proteins undergo changes under the influence of radiation.

In this work, in experiments on rabbits and white rats, we investigated the changes in the fraction of soluble proteins of the muscle tissue under the influence of total X ray irradiation. At the same time, we also investigated the changes in the thermal stability of various protein fractions of the muscles of irradiated animals, on the basis of the data of a number of authors, indicating that native protein may be converted to an unstable state by irradiation, which can be detected with the aid of supplementary influences of chemical agents, heating, etc.

Blood was taken from the animals 72 hours after irradiation at lethal doses (rabbits -- 4000 R, rats -- 1200 R); then they were killed, and part of the femoral muscle was taken for investigation. A sample of tissue was ground in liquid oxygen, and the soluble proteins were extracted from the muscle pulp with physiological saline. The preparations of extracts of the muscle proteins was performed according to G. Adjutantis in Kaplanskiĭ's modification [1]. The total protein content in extracts of the soluble muscle proteins was determined refractometrically; it was usually from 1.5 to 2.0% in the control rabbits, from 0.95 to 1.85% among the rats. In the irradiated animals, the protein content, as a rule, was closer to the lower level of the norm.

The electrophoretic separation of the proteins was conducted on paper, using two buffer solutions: veronal-acetate (pH 8.6 and μ 0.1)

and Tris buffer (pH 8.9 and μ 0.17). The rabbit muscle proteins were separated with veronal-acetate buffer, and the rat proteins with Tris buffer. The optimum conditions for the electrophoretic separation of proteins were the following: with veronal-acetate buffer, voltage 5 V/cm, at a current strength of 8.0 mA, and with Tris buffer -- 5-6 V/cm and 3.0-4.0 mA. The duration of electrophoresis was about 18 hours. The electrophoretograms were treated by the usual method, followed by densitometry and weighing of portions of the densitograms corresponding to the individual protein fractions. To determine the thermal stability, the muscle protein extracts of the control and irradiated animals were heated on a water bath for 30 min at t = 37, 56, and 60° (only the muscle extracts of rabbits were heated at t = 56°).

In the electrophoretic separation of unheated extracts of soluble muscle proteins of the control and irradiated rabbits, we determined the presence of five basic fractions moving toward the anode (which we numbered arbitrarily, beginning from the starting line), and a fraction of nonmobile proteins at the start (which we denoted as fraction zero). From Table 1 it is evident that in all the experimental variations, the greatest protein content among the control and experimental animals was found in the third fraction, in which about half of all the muscle proteins was concentrated. The least amount of protein was found in the fifth fraction. Heating at t = 37, 56, and 60° leads to disappearance of the proteins of the zero fraction in both groups of animals. Heating at t = 60°, moreover, also leads to the disappearance of the first fraction among the control and irradiated animals.

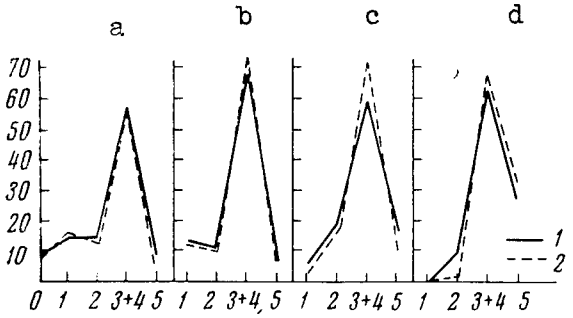

Fig. 1. Muscle proteins of rabbits. 1 -- control and 2 -- irradiated animals; a -- unheated extracts of soluble proteins; b -- extracts heated at 37°; c -- extracts heated at 56°; d -- extracts heated at 60°.

In a number of experiments, an additional fraction 4^a appeared; however, the relative amount of protein in this fraction was negligible; hence it was combined with the fourth fraction. In the experiments on heating of the muscle extracts at t = 56°, among the irradiated animals the third and fourth fractions, as a rule, merged into a single fraction.

Table 1

Soluble Muscle Proteins of Rabbits

Temperature	No. of experiments		Protein fractions													
			0		1		2		3		4(+4a)		3+4(+4a)		5	
	K	O	K	O	K	O	K	O	K	O	K	O	K	O	K	O
Without heating	5	6	8,4±2,18	7,5±2,31	13,8±2,05	15,9±3,28	14,5±2,74	13,3±2,01	46,5±2,76	46,7±3,50	10,2±1,06	10,3±0,50	56,7±3,40	57,0±3,32	6,6±0,77	6,2±0,80
37°	5	6	—	—	13,5±4,91	11,9±2,17	11,3±3,99	10,8±2,76	55,1±5,32	56,0±4,29	13,1±2,75	14,8±2,92	68,2±7,90	70,8±5,06	7,1±1,38	6,4±0,96
56°	5	5	—	—	5,9±3,80	2,7±1,67	18,8±2,10	16,8±3,42	—	—	—	—	59,1±5,70	70,2±4,39	16,2±1,42	10,1±1,35
60°	4	6	—	—	—	—	9,9±4,98	2,1±2,13	44,1±7,46	43,7±5,51	—18,8±2,22	21,7±7,60	62,8±7,23	65,4±5,23	27,2±11,26	32,5±5,15

К — контроль, О — опыт.

K -- control; O -- experimental.

Table 2

Soluble Muscle Proteins of Rats

Temperature	No. of experiments		Protein fractions													
			0		1		2		3		4		5		6	
	K	O	K	O	K	O	K	O	K	O	K	O	K	O	K	O
Without heating	9	13	5,2±0,71	6,5±1,09	9,9±0,99	10,6±0,94	13,2±1,35	9,6±0,94	40,0±2,21	37,8±1,37	16,7±1,42	18,6±1,27	14,1±1,95	15,7±1,31	0,4±0,36	1,9±0,69
37°	8	9	—	0,8±0,45	12,9±1,17	10,9±1,81	11,1±1,57	11,9±1,45	31,0±3,13	45,6±3,35	27,6±2,29	18,1±1,91	16,1±3,35	12,6±1,54	1,4±0,91	—
60°	9	13	—	—	21,1±2,68	13,2±2,61	21,2±3,14	16,9±2,24	28,4±3,02	36,0±3,08	20,1±4,03	22,7±2,40	6,3±2,20	11,1±2,62	3,0±1,99	—

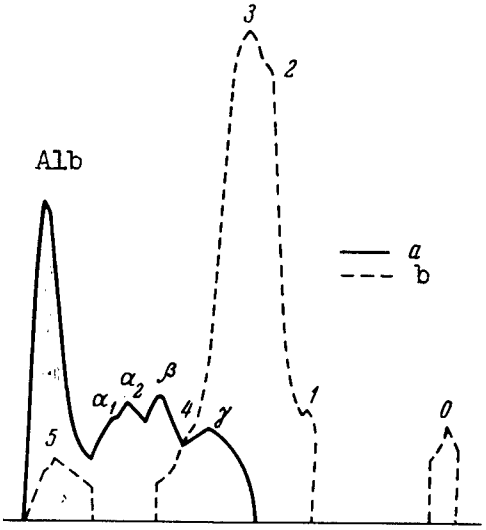

Fig. 2. Comparison of protein fractions of soluble muscle proteins with rabbit blood serum proteins. a -- protein fractions of blood serum; b -- muscle proteins.

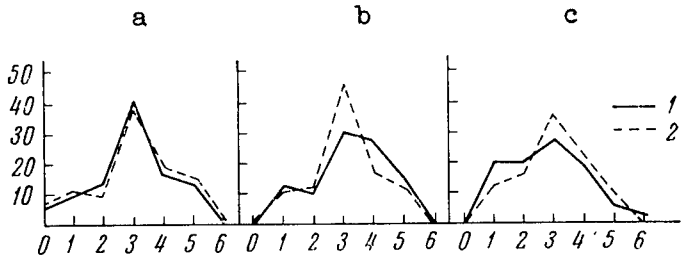

Fig. 3. Muscle proteins of rats. 1 -- control and 2 -- irradiated animals; a -- unheated extracts of soluble proteins; b -- extracts heated at 37°; c -- extracts heated at 60°.

This was responsible for the additional placement of the proteins of this fraction in the form of a sum in Table 1.

Fig. 1 presents a graphical depiction of the protein content (in percent) in individual fractions among the control and irradiated rabbits in all the experimental series. The investigation of the muscle proteins was paralleled by an electrophoretic separation of the proteins of the rabbit blood serum. Fig. 2 presents typical densitograms of the blood serum and muscle proteins of a control (non-irradiated) rabbit. It was found that the fifth fraction of the muscle

proteins is situated precisely at the place corresponding to the serum albumins, fractions three and four correspond to the β- and γ-globulins, while the first and second fractions are situated behind the γ-globulins, closer to the start.

In the separation of unheated extracts of rat muscle proteins, three protein (anodic) fractions and a zero fraction of nonmobile proteins at the start were detected. Data on the muscle proteins of rats are presented in Table 2. Just as for the rabbits, in the rats the greatest protein content in the muscles was found in the third fraction, while the least protein content was detected in the sixth fraction. Heating the extracts at t = 37° leads to a disappearance of the zero-fraction in the control rats, while in the irradiated animals this fraction was retained in individual experiments. Heating at t = 60° leads to a complete disappearance of the zero-fraction, both among the control and among the irradiated animals.

A qualitative difference of the muscle proteins of the control and irradiated rats was detected only in the sixth fraction, detected most often among irradiated animals in the electrophoretic separation of unheated extracts. Heating at t = 37 and 60° leads to a complete disappearance of the sixth fraction among the irradiated animals. Reliable quantitative changes in the protein content among the control and irradiated animals are observed in the second fraction in experiments without heating of the extracts, while in the case of heating at t = 37°, they are observed in the third and fourth fractions (among the irradiated animals, there is an increase in the protein in the third fraction, and a decrease in it in the fourth). Fig. 3 graphically depicts the protein content (in percent) in individual fractions of the control and irradiated rats (according to average data). The serum proteins of the rats were separated on paper (using Tris-buffer) into five to six fractions. The fractions of rat muscle proteins are arranged with respect to the protein fractions of blood serum in approximately the same manner as in the experiments on rabbits (the fifth and sixth fractions correspond approximately to the serum albumins, the third and fourth fractions to β- and γ-globulins, while the first two fractions lie somewhat closer to the start.

Single experiments on control and irradiated dogs indicated that the soluble proteins of the cardiac muscle are electrophoretically separated into five to six basic fractions. The greatest amount of protein was also detected in the third fraction (up to 46°), and the least protein in the sixth fraction. Veronal-acetate buffer [6] and Tris-buffer were used to separate the proteins. A number of authors succeeded in detecting from three to five fractions of muscle proteins on paper in electrophoretic separation [2-7]. According to the literature indications [2], the fifth fraction, possessing the greatest electrophoretic mobility, may be identified with myoalbumins, the fractions with medium electrophoretic mobility -- the third and fourth -- with myoglobin, and the relatively nonmobile first fractions evidently represent myogens.

The literature contains single and ambiguous observations with

respect to the incorporation of labeled amino acids into the muscle proteins of normal and irradiated animals.

Investigations conducted on surviving muscle slices did not indicate any distinct difference in the nature of the incorporation of S^{35}-methionine into the muscle proteins of irradiated animals. In the case of subcutaneous injection of S^{35}-methionine into rats on the third day after irradiation (at lethal doses), a decrease was observed in the incorporation of methionine into the muscle proteins. Similarly, the administration of C^{14}-alanine to mice on the fourth day after irradiation (800 R) revealed a substantial decrease in the incorporation of alanine into the muscle proteins, with a simultaneous increase in its incorporation into the liver. Thus, the authors of [8] indicated that the relative intensity of the renewal of proteins in the muscle tissue is reduced during irradiation.

Our investigations indicated that under the influence of total X ray irradiation, negligible changes arise in the proteins of the muscle tissue, which could be detected when muscle protein extracts were heated. Moreover, it was found that the rat muscle proteins are less resistant to heating than the muscle proteins of rabbits. It was also found that the proteins of the rat spleen are less thermoresistant than the proteins of the rabbit spleen. This fact may be explained if we consider that according to the data of many authors [9-12], the proteins of various animal species react differently to denaturing influences.

Experimental investigations on plant and animal specimens have established that the thermostability of the protein components is of vital importance in the thermostability of the organism, and can even enter into the species characteristics [10, 11]. Species differences in protein stability have been established with respect to various denaturing influences [12]. The difference in the thermostability of different animal tissues from one species evidently can also be explained by a difference in the thermostability of the tissue proteins [11].

According to the literature data, the denaturation of the tissue protein complexes under the experimental conditions can be accomplished at temperatures corresponding to the normal body temperature of a given animal, the organ of which was subjected to heating. Thus, denaturation of the rat liver proteins in vitro begins at only 35°, while the body temperature of rats is $34\text{-}35^{\circ}$ [13]; denaturation of the muscle proteins of birds begins at 42°, while the body temperature of birds is $41\text{-}43^{\circ}$ [14]. Thus, it has been established that protein complexes possess greater thermostability under intravital conditions than in vitro, which is evidently associated with the corresponding regulatory adaptations of the organism.

The data obtained lead to the conclusion that the muscle proteins are rather resistant with respect to radiation influences, and this, in all probability, lies at the basis of the low radiosensitivity of muscle tissue.

CONCLUSIONS

1. The paper electrophoretic separation of soluble muscle proteins reveals the presence of five basic protein fractions among rabbits and six fractions among rats.

2. A different thermostability of individual protein fractions has been established among control and irradiated animals.

3. The muscle proteins of rabbits are more thermolabile and more resistant to penetrating radiation than the muscle proteins of rats, which indicates a species specificity of the muscle proteins.

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EFFECTS OF NEUTRON IRRADIATION UPON THE PROCESSES OF TISSUE METABOLISM

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pp. 179-184

The biological effects of neutron irradiation, which exhibits a number of specific features that distinguish it from other types of radiation, still remain unstudied. Most researchers indicate a relatively great biological effectiveness (RBE) of neutrons in comparison with X rays and γ rays. On the average, the RBE is equal to 10 [1]; however, this is particularly arbitrary, since when different indices of biological effectiveness are used, such as fertility, sterilizing action on males, cataractogenic action, hematological indices, survival rate, and others, the RBE ranges from 3 to 34 [1-3]. Kuzin [4] cites data on the RBE of neutrons, published in recent years for various organisms and criteria of evaluation, and the results obtained in the laboratory that he heads, and indicates that this value ranges from 1 to 87. When large doses, inducing a rapidly developing effect, are used, the RBE of neutrons is either equal to or somewhat less than 1. The biological effectiveness of neutrons increases somewhat during chronic irradiation. The RBE values are high (6, 10, and more) when the effect is evaluated according to long-term results. The RBE values differ especially in work with large and small doses of irradiation. The lower the dose and the longer the exposure time, the higher the RBE becomes. However, a dose-effect comparison is insufficient for an understanding of the mechanisms of the specific action of neutrons. The specific influence of neutrons upon various aspects of metabolism must be investigated.

The task of this investigation was to study the influence of neutron irradiation at relatively low doses upon certain aspects of the carbohydrate-energy and protein metabolism in the central nervous system, skeletal muscles, and liver.

The experiments were conducted on sexually mature white rats

weighing from 170 to 250 g. Total neutron irradiation was conducted on the ninth channel of the IRT-2000 reactor of the Academy of Sciences Belorussian SSR, specially equipped for the irradiation of biological specimens. Gamma rays, thermal and partially fast neutrons were filtered out with the aid of a filter, consisting of boron carbide -- 4.5 cm -- and iron -- 50 cm, introduced into the gate valve. The flux was measured by L. N. Uspenskiĭ and I. V. Filyushin with an instrument of the 54 C-5 type. The basic contribution to the total flux (~92%) and to the measured dose (~95%) is made by neutrons whose energy lies within the range 0.04 MeV $\leqslant$ En $\leqslant$ 1.35 MeV. The contribution of neutrons with E $\leqslant$ 0.3 MeV to the total flux is equal to 58%, those with E $\geqslant$ 0.3 MeV -- 42%. A moderator with ϕ 300 mm ensured a variation of the detector sensitivity corresponding to the variation of the maximum biological dose in the human body according to the calculations of Snyder and Neufeld. On the basis of the measurement and corresponding calculations, the total dose is 136 rem/hour, at a reactor power of 1000 kilowatts.

The dose of neutron irradiation for rats according to the calculated data is ~13 rad. The rat was irradiated in a plexiglas container outside the gate valve, and it received the indicated dose for 60 min. Subsequently the animals were kept in a vivarium on the standard diet. Investigations were conducted on the 15th and 30th days after irradiation. All the results obtained were compared with those for the control intact animals.

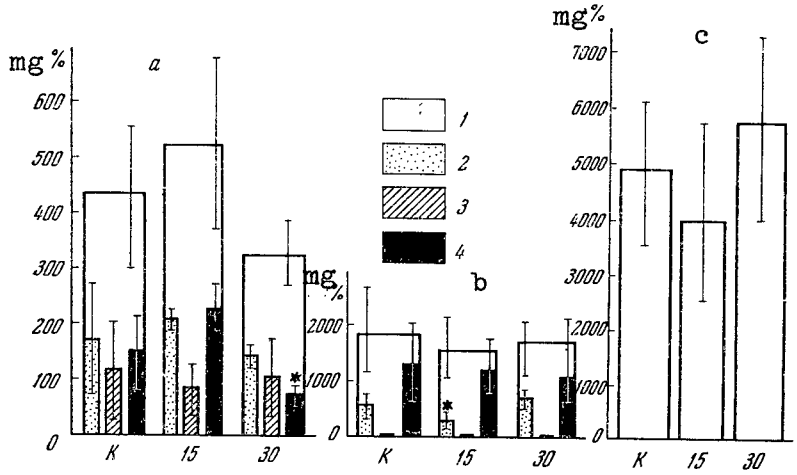

Fig. 1. Variation of tissue glycogen (mg % per dry weight of tissue) after neutron irradiation. a -- cerebral cortex; b -- skeletal muscles; c -- liver; 1 -- total glycogen; 2 -- free glycogen; 3 -- glycogen-lipid fraction; 4 -- glycogen-protein fraction; K -- control; 15,30 -- days after irradiation; * -- significant variations.

In selecting the neutron irradiation dose, we attempted to have it approximate an X ray irradiation dose of 40 R, the influence of which upon the processes of carbohydrate-energy and protein metabolism of the central nervous system and other tissues were studied in detail in [5]. The RBE of ~3, used for the conversion, is arbitrary.

We studied the changes that occur after total neutron irradiation at a dose of ~13 rad in the content of glycogen and its fractions in the tissue of the cerebral cortex and skeletal muscles -- free glycogen, protein-bound glycogen, lipid-bound glycogen, as well as the total glycogen content in the liver. The following were studied: the content of intermediate phosphorus-containing products of the decomposition of carbohydrates -- glucose-1-phosphate (G-1-P), glucose-6-phosphate (G-6-P), fructose-6-phosphate (F-6-P), fructose-1,6-diphosphate (F-1,6-DP), phosphotrioses and phosphopyruvic acid (PPV), and energy-rich adenosinetriphosphoric (ATP) and creatinephosphoric acid (CP) in the tissues of the cerebral cortex and skeletal muscles; the activity of the enzymes of carbohydrate-energy metabolism, phosphorylase (code No. 2, 4, 1, 1), amylase (code No. 3, 2, 1, 1), succinate dehydrogenase (code No. 1, 3, 9, 1) [6], and the intensity of tissue respiration of the cerebral cortex and skeletal muscles; the content of certain soluble proteins in the cerebral cortex, cerebellum, spinal cord, and liver [6]. The content of protein fractions was studied according to the method of Mirsky and Pollister [7], in the modification of Petterman and Lamb [8] and Kravchinskiĭ [9]. The proteins extractable by 0.14 M NaCl were studied, chiefly the cytoplasmic ribonucleoproteins (RNP -- fraction A); the proteins extractable by 1M NaCl, primarily the nuclear deoxyribonucleoproteins (DNP -- fraction B) and fraction C -- alkali-soluble proteins were studied. All the material was treated by the method of variation statistics for an interpretation of the results of the investigations, and a number of arbitrary correlation coefficients were calculated.

The changes in the content of glycogen and its fractions after total neutron irradiation at a dose of ~13 rad in the tissues of white rats, expressed in mg per 100 g of dried tissue, are cited in Fig. 1. In the cerebral cortex, after a certain increase (by the 15th day after irradiation) in the content of the biologically most important glycogen-protein fraction, by the 30th day there is a substantial (up to 46.4% of the control) and significant decrease in it. In the muscle tissue, a statistically confirmed drop in the free glycogen content is detected only by the 15th day after irradiation.

Neutron irradiation at a relatively low dose changes the direction in the processes of glycogen biosynthesis and decomposition, which is evidenced by changes in the ratios of the individual glycogen fractions with respect to its total content in the tissues and the ratio of free glycogen to the bound fractions, which we calculated (Table 1). As can be seen from the material cited, there are substantial changes in the relative protein- and lipid-bound glycogen content in the brain and muscle tissue, and by 30 days after irradiation there is an inhibition

Table 1

Experimental series and time after irradiation	Glycogen fraction, % of total glycogen taken as 100			Ratio of free to bound glycogen
	Free	Lipid bound	Protein bound	
Cerebral cortex				
Control	39,1	33,7	27,2	0,64
15 days	39,3	44,9	15,8	0,64
30 »	44,7	20,3	35,0	0,8
Skeletal muscles				
Control	28,6	70,3	1,1	0,40
15 days	18,7	80,7	0,6	0,22
30 »	42,5	56,5	1,0	0,73

of the processes of biosynthesis of the bound forms of glycogen. This is evidenced by the increased values of the relative free glycogen content and the higher ratio of free glycogen to its bound fractions (protein- and lipid-bound) than in the control.

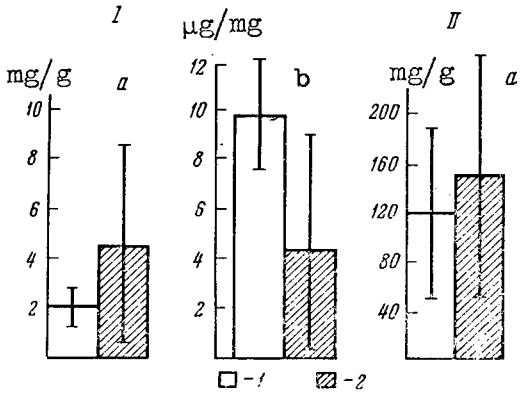

Fig. 2. Changes in tissue phosphorylase and amylase activity after neutron irradiation. I -- brain; II -- skeletal muscles; a -- phosphorylase (according to decrease in phosphorus in mg per g of tissue); b -- amylase (according to increase in reduction in μg per mg of protein); 1 -- control; 2 -- 15 days after irradiation.

The data cited in Fig. 2 on the change in the phosphorylytic activity of the phosphorylases of the cerebral cortex and muscles and the amylase activity of the brain are evidence of a discoordination in the processes of glycogen metabolism by 15 days after neutron irradiation. Data on the state of the intermediate phosphorus-containing

products of the carbohydrate metabolism are cited in Table 2. As can be seen from the table, by 15 days after irradiation of white rats at a dose of ~13 rad, there are no significant changes in the content of intermediate phosphorus-containing products of the decomposition of carbohydrates in the cerebral cortex and skeletal muscles. A drop in the phosphotriose content in the brain tissue to 155% with respect to the control and in the PPV to 60% of the control is not confirmed in a statistical treatment of the material. For the analysis of these data we calculated the series of correlation ratios cited in Table 3.

Table 2

Experimental series and time after irradiation	Cerebral cortex				Skeletal muscles			
	Number of experiments	$x \pm m$	% of control	t_{05}	Number of experiments	$x \pm m$	% of control	t_{05}
GLUCOSE-1-PHOSPHATE								
Control	10	1,0±0,20	100	—	10	1,0±0,16		—
15 days	10	1,0±0,12	100	0	10	0,8±0,11	80	1,05
GLUCOSE-6-PHOSPHATE								
Control	10	1,42±0,21	100	—	10	1,79±0,17		—
15 days	10	1,45±0,20	102	0,10	10	1,71±0,20	96	0,32
FRUCTOSE-1,6-DIPHOSPHATE								
Control	10	3,64±0,70	100	—	10	17,86±1,47		—
15 days	10	2,69±0,80	74	0,86	10	14,82±1,83	83	1,29
PHOSPHOTRIOSES								
Control	10	0,18±0,08	100	—	10	0,26±0,12		—
15 days	10	0,10±0,05	55	0,90	10	0,28±0,13	108	0,11
PHOSPHOPYRUVIC ACID								
Control	10	1,56±0,37	100	—	10	1,72±0,51		—
15 days	8	0,93±0,43	60	1,05	10	1,48±0,31	86	0,41

Table 3

Conditional ratios	Cerebral cortex		Skeletal muscles	
	Control	15 days after irradiation	Control	15 days after irradiation
G-1-P/G-6-P	—	—	0,55	0,46
G-6-P/F-1,6-DP	0,39	0,53	0,10	0,11
F-1,6-DP/phosphotrioses	2,02	2,69	68,85	51,71
Phosphotrioses/PPV	0,11	0,11	0,15	0,19

Table 4

Experimental series and time after irradiation	ATP/CP	
	Cerebral cortex	Skeletal muscles
Control	1,29	0,95
15 days	1,21	1,45
Control	1,05	0,93
30 days	0,95	0,92

Table 5

Experimental series and time after irradiation	Ratio of fraction A to B			
	Cerebral cortex	Cerebellum	Spinal cord	Liver
Control	0,27	0,30	0,25	0,30
15 days	0,28	0,29	0,28	0,29
30 days	0,25	0,22	0,25	0,25

By 15 days after neutron irradiation, the conversion of G-6-P to F-1,6-DP in the cerebral cortex and the conversion of F-1,6-DP to phosphotrioses are inhibited. The rate of conversion of phosphotrioses to phosphopyruvic acid in the cerebral cortex remains at the level of the control under these conditions.

A different character of the disorders is detected in the skeletal muscles. With an unchanged rate of formation of F-1,6-DP and G-6-P, the rate of formation of G-6-P from G-1-P increases, and the rate of formation of PPV is inhibited.

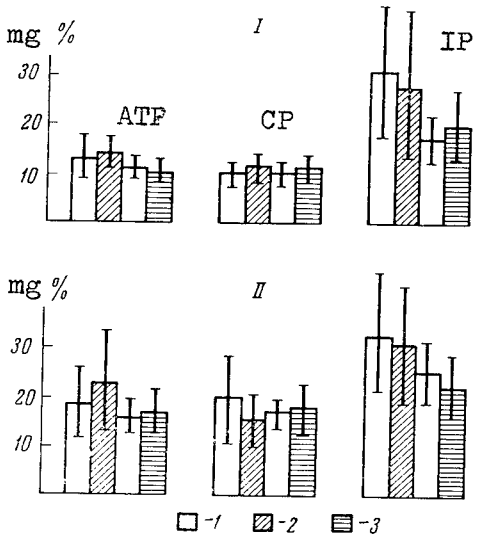

Fig. 3. Changes in ATP, CP, and inorganic phosphorus (IP) of the tissues (mg %) after neutron irradiation. I -- cerebral cortex; II -- skeletal muscles; 1 -- control; 2 -- 15 days; 3 -- 30 days after irradiation.

Fig. 3 presents data characterizing the state of the energy reserves of the cerebral cortex and skeletal muscles. In the brain tissue, the ATP and CP content is unchanged 15 and 30 days after irradiation; the ATP/CP ratio also remains unchanged (Table 4).

In the skeletal muscle, the ATP content increases somewhat by

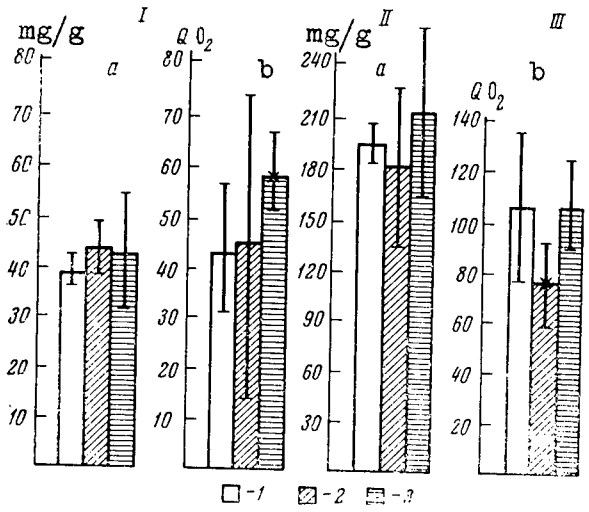

Fig. 4. Changes in succinate dehydrogenase activity and rate of respiration of tissues after neutron irradiation. I -- cerebral cortex; II -- liver; III -- skeletal muscles; a -- succinate dehydrogenase (mg formazan per g of tissue); b -- tissue respiration (QO_2); 1 -- control; 2 -- 15 days; 3 -- 30 days after irradiation; * -- significant variations.

the 15th day, which is also confirmed by the corresponding correlation coefficient. By 30 days after irradiation, the ATP and CP contents in the muscles and their ratio remain within the limits of the control values.

The stability of the energy metabolism in brain tissue after total neutron irradiation of animals is evidenced by the activity of succinate dehydrogenase and the absorption of oxygen (QO_2) (Fig. 4). However, for the values of QO_2, an increase in the dispersion of the figures is noted 15 days after irradiation, and a certain increase in them, lying within the limits of the control, on the 30th day after irradiation. The respiration rate of the muscle tissue is somewhat inhibited 15 days after irradiation.

Fig. 5 presents data on the protein content in the cerebrum, cerebellum, and spinal cord and liver. Total neutron irradiation of white rats at a dose of ~13 rad somewhat stimulates protein biosynthesis in the central nervous system and liver. Protein biosynthesis of fractions A and B increases in the same direction by the 15th day after irradiation, which is evidenced by the ratio of fraction A to fraction B (Table 5). In the cerebellum and liver, by 30 days after irradiation, the rate of protein biosynthesis of fraction B lags somewhat behind protein biosynthesis of fraction A. Noteworthy is the decrease in the dispersion of the figures for proteins of fraction A of the cerebellum and liver by the 15th and 30th days after irradiation

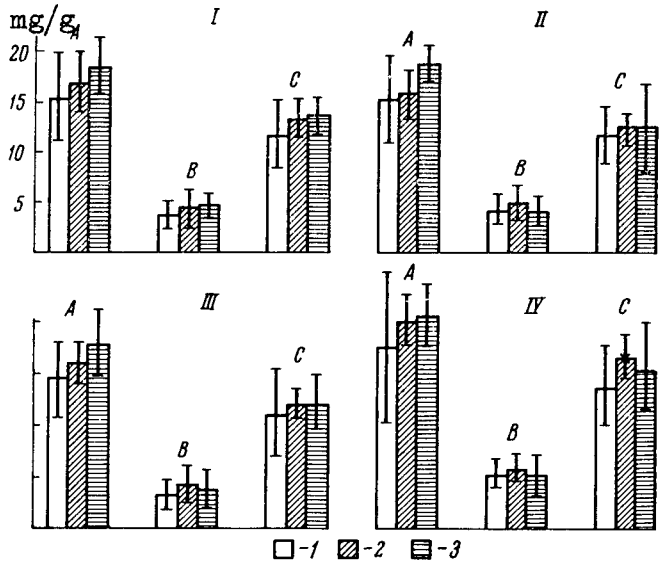

Fig. 5. Changes in tissue protein fractions (mg per g of tissue) after neutron irradiation. I -- cerebral cortex; II -- cerebellum; III -- spinal cord; IV -- liver; 1 -- control; 2 -- 15 days; 3 -- 30 days after irradiation; A, B, C -- fractions, respectively; * -- significant changes.

and for the proteins of fraction C of all portions of the central nervous system and liver by the 15th day after irradiation.

As it follows from the data, total neutron irradiation at a dose of ~13 rad changes the direction in the processes of carbohydrate-energy and protein metabolism of the central nervous system, skeletal muscle, and liver. The presence of these changes on the 30th day after irradiation confirms the possibility of long-term responses of the organism to neutron irradiation.

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ON THE BIOLOGICAL EFFECTS OF POLONIUM-210

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pp. 185-193

The literature contains only single studies on the biological effects of polonium-210. In these works, data are cited on the effects of Po^{210} upon the carbohydrate metabolism, immunobiological reactivity, function of the kidneys, liver, composition of the peripheral blood, and organs of vision [1-9].

This investigation cites data on the influence of Po^{210} within a broad range of doses upon the lifetime of animals, the composition of the peripheral blood, weight, and frequency of tumoral and non-tumoral forms of pathological processes in the long-term periods.

MATERIAL AND METHODS

The experiments were conducted on 704 white rats weighing 170 ± 2 g. The age of the rats at the time of use in the experiment was 2.5-3 months. A neutralized solution of polonium-210 chloride was administered to the rats once intraperitoneally in amounts of 0.06, 0.12, 0.24, 0.48, 0.96, 2.0, 4.0, and 8.0 microcuries per rat, which corresponded at the time of administration to 0.00035, 0.0007, 0.0014, 0.0028, 0.0056, 0.0117, 0.0236, 0.047 microcurie per gram of weight of the animal.

The values of the doses (LD_{50}) inducing the death of 50% of the animals in 30, 60, 120, 240, 360, and 480 days were determined with confidence intervals for $p = 0.05$ to evaluate the biological effectiveness of Po^{210} according to the method of Miller and Tainter [10]. In the calculations, a correction for death of the animals in the control was introduced when necessary. In addition, the average lifetime of the rats was determined, both in the control and in the experimental groups of animals that received various quantities of polonium-210. The frequency and nature of the long-term effects, depending upon the sex of the animal and amount of Po^{210} activity introduced, were judged according to the results of autopsy and a pathomorphological investigation. The doses of radiation created by Po^{210} in the organs and

tissues of the animal, as well as the "average" doses were calculated according to the method proposed in [11]. The "average" doses were calculated for the case of uniform distribution of Po^{210} in the animal organism. In this case radioactive decay and excretion of polonium-210 with the urine and feces were considered. The calculations indicated that the levels of ionization doses in the spleen are 20 times as high as the "average" dose, in the kidneys ten times as high, in the blood five times as high, and in the bone marrow, lungs, testes, and liver 2.7, 2.6, 2.5, and 2 times as high, respectively, while in the bones, muscles, and brain they were 1.3, 2.4, and 2.6 times lower. In the calculations it was assumed that the distribution of Po^{210} in the tissues and organs is uniform.

When Po^{210} is introduced in a dose of 1 microcurie/g, the "average" dose rate varies from 21.5 to 230 rad during the first 100 days of the experiment, and on the average, considering the creation of a dose equal to 12 krad in 100 days, it may be assumed equal to ~120 rad per day. These data lay at the basis of the quantitative analysis of the experimental results on the action of Po^{210}.

RESULTS AND DISCUSSION

The dynamics of the death of male and female rats after the administration of various amounts of Po^{210} are presented in Fig. 1, showing that the experimental females die out more slowly than the controls during the first seven to nine months after the administration of Po^{210} in amounts of 0.00035-0.0028 microcurie/g of weight, while the males die out more slowly than the controls during the first 10 to 12 months after the administration of 0.0028-0.0056 microcurie/g.

During this period, an "average" tissue dose equal to 4-34 rad is accumulated in the females, and 17-34 rad in the males. During later periods, the differences in the death rate at first are effaced, but later (which is distinctly detected 18 months after the beginning of the experiment), the experimental animals die out more rapidly than the controls at all the doses of polonium-210 used. Table 1 gives the values of LD_{50} for various periods after intraperitoneal injection of Po^{210}.

It is noteworthy that the value of the chronically effective dose ($LD_{50/480}$) of Po^{210} is an order of magnitude (14-fold) lower than the acutely effective dose ($LD_{50/30}$). In this respect, the action of Po^{210} differs substantially from external radiation sources (X rays, γ rays, neutrons and protons), of course, in the case of a single influence of them, which is characterized by a small difference between the acute and chronic effective doses. Thus, for example, after a single influence of fast neutrons, the value of $LD_{50/30}$ (408 ± 30 rad) differs by only 40% from $LD_{50/420}$ (299 ± 80 rad) [12]. This observation indicates that in contrast to a single irradiation by external radiation sources, under the influence of polonium-210, a substantial

Table 1

Values of LD_{50} (With Confidence Intervals for p = 0.05) for Various Periods After Parenteral Administration of Polonium-210 to Rats

Period, days	Dose, microcurie/g of weight of rat (♂ + ♀)	'Average' dose of radiation accumulated by death of 50% of rats, rad	Period, days	Dose, microcurie/g of weight of rat (♂ + ♀)	'Average' dose of radiation accumulated by death of 50% of rats, rad
30	0,0272±0,0016	120	240	0,0087±0,0013	105
60	0,0240±0,0021	180	360	0,0060±0,0011	74
120	0,0169±0,0017	180	480	0,0019±0,0005	23

fraction of the animals die not only in the acute, but also in the subacute and chronic stages of the injury.

When the acute and subacute ($LD_{50/60}$ and $LD_{50/120}$) effective amounts of Po^{210} were administered, the females proved less resistant to the toxic effect of polonium than the males. The values of $LD_{50/30}$, $LD_{50/60}$, and $LD_{50/120}$ for the males proved equal to 0.0294 ± 0.0031, 0.0228 ± 0.0036 and 0.0169 ± 0.0027 microcurie/g, and for the females, correspondingly, 0.0235 ± 0.0040, 0.0186 ± 0.0025 and 0.0132 ± 0.0024 microcurie/g. The levels of LD_{50} for the later periods are the same for the animals of both sexes. Thus, for example, $LD_{50/240}$ for the males is equal to 0.0085 ± 0.0016 microcurie/g, for the females 0.0066 ± 0.0016 microcurie/g; $LD_{50/360}$ -- 0.0054 ± 0.0013 and 0.0066 ± 0.0016 microcurie/g and $LD_{50/480}$ -- 0.0022 ± 0.0006 and 0.0019 ± 0.0009 microcurie/g, respectively.

Together with an estimate of the values of LD_{50} for various periods, the average lifetime of the animals at a given dose, the so-called value of LV_{50} may also be used as a criterion of injury.

Fig. 2 presents data characterizing the dependence of the average lifetime of rats on the amount of introduced activity. The time versus effect curve has a typical S-shape. Moreover, it is distinctly evident that appreciable shortening of the average lifetime of the rats is detected at doses of polonium-210 equal to and higher than 0.0028 microcurie/g.

As was indicated above, for a quantitative evaluation of the experimental results we calculated the values of the ionization doses created by Po^{210} in various organs and tissues of the animal. The calculations indicated that when polonium is administered in amounts of 0.047, 0.0236, 0.0117, 0.0056 and 0.0028 microcurie/g of weight, by the time of death of 50% of the animals, an "average" radiation dose equal to 146.5, 152, 140, 67.5, and 34.5 rad, respectively, has been accumulated. When Po^{210} is administered in amounts of 0.046, 0.0236,

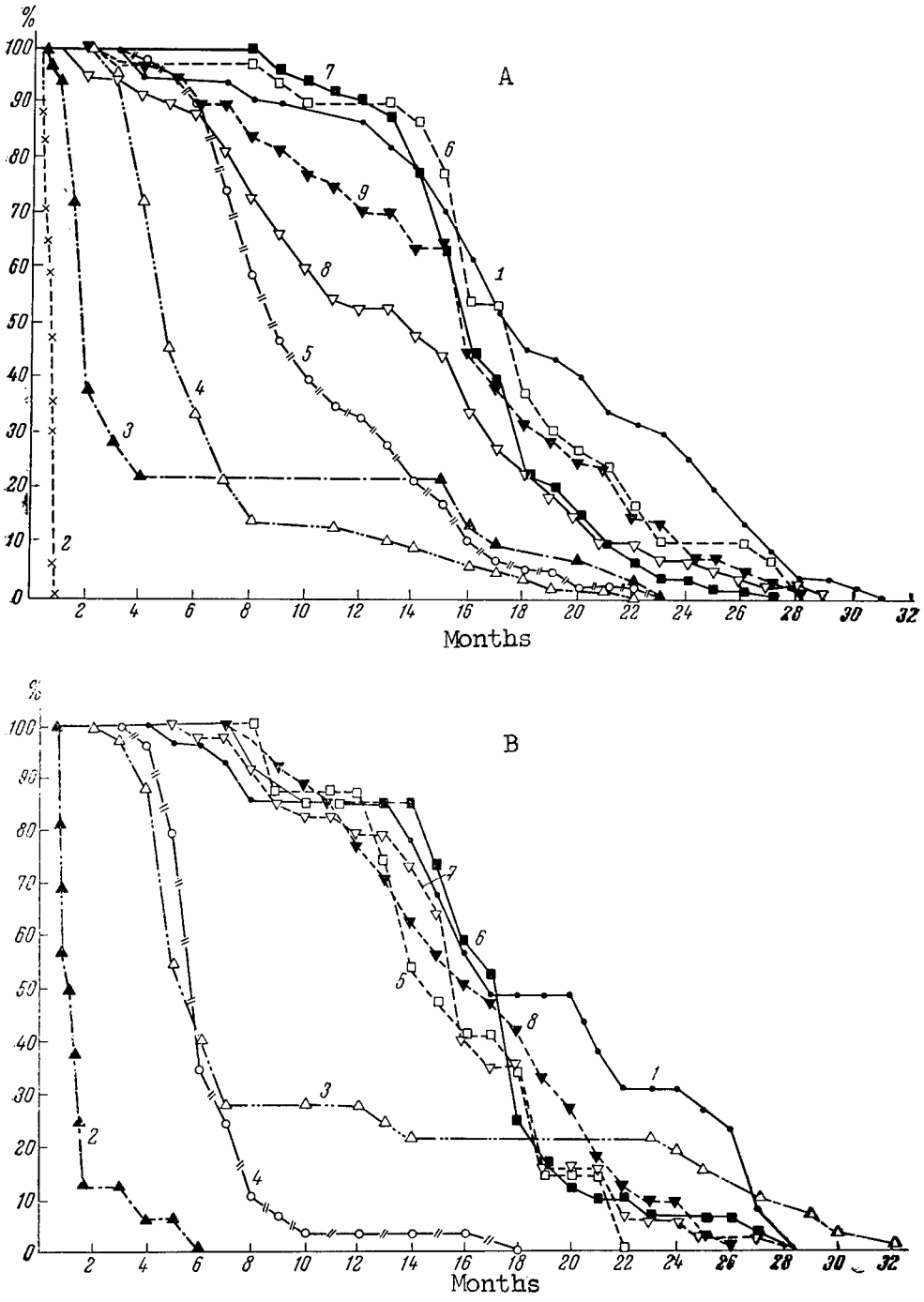

Fig. 1. Dynamics of the dying-off of female (A) and male (B) rats after administration of various amounts of polonium-210. A: 1 -- control; 2 -- 0.047; 3 -- 0.0236; 4 -- 0.0117; 5 -- 0.0056; 6 -- 0.0028; 7 -- 0.0014; 8 -- 0.0007; 9 -- 0.00035 microcurie/g. B: 1 -- control; 2 -- 0.0236; 3 -- 0.0117; 4 -- 0.0056; 5 -- 0.0028; 6 -- 0.0014; 7 -- 0.0007; 8 -- 0.00035 microcurie/g.

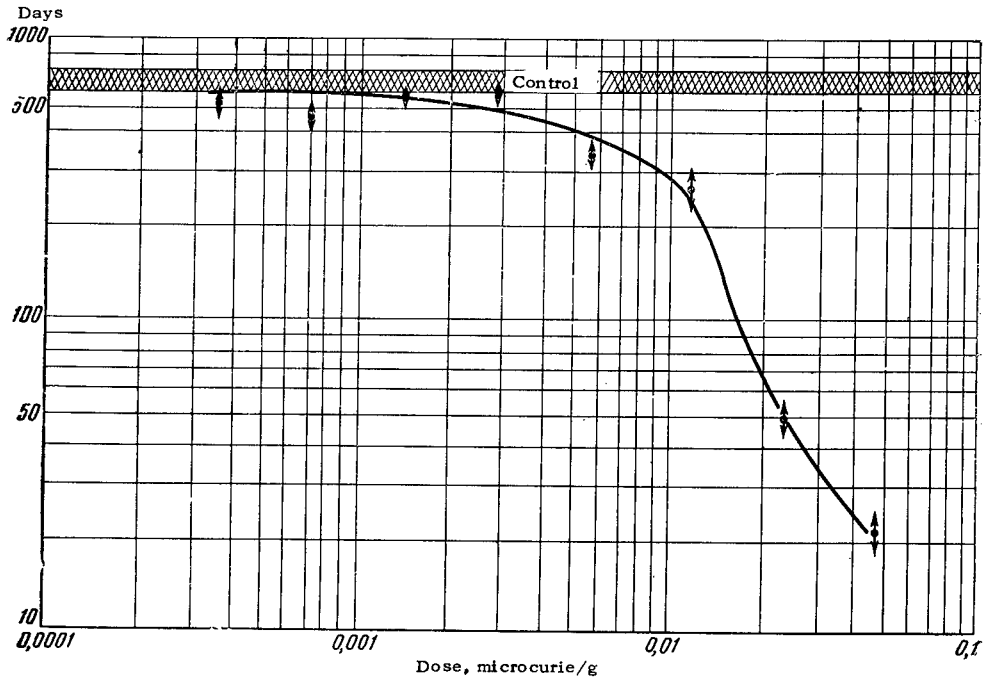

Fig. 2. Dependence of the average lifetime of rats on the dose of polonium-210.

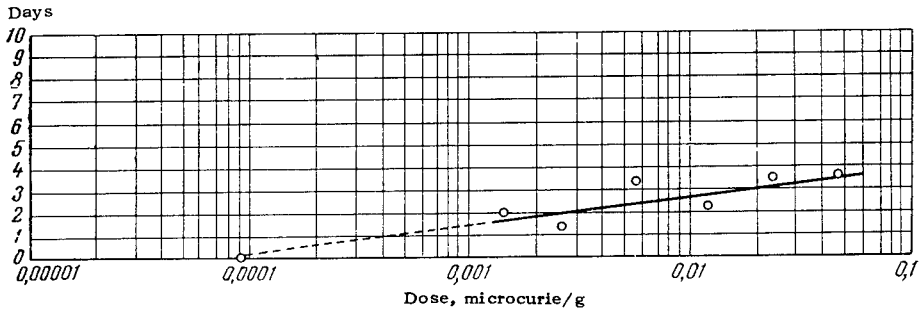

Fig. 3. Degree of shortening (days) of lifetime of rats per rad of accumulated "average" dose.

and 0.0117 microcurie/g, 50% of the animals die on the 21st, 49th, and 261st days after accumulation of practically identical "average" radiation doses (140-152 rad). Moreover, as the dose rate decreases, the lifetime of the animals increases substantially.

Fig. 3 presents data characterizing the degree of shortening of the average lifetime of the rats (in days) per rad of accumulated "average" dose, after the administration of from 0.0014 to 0.047

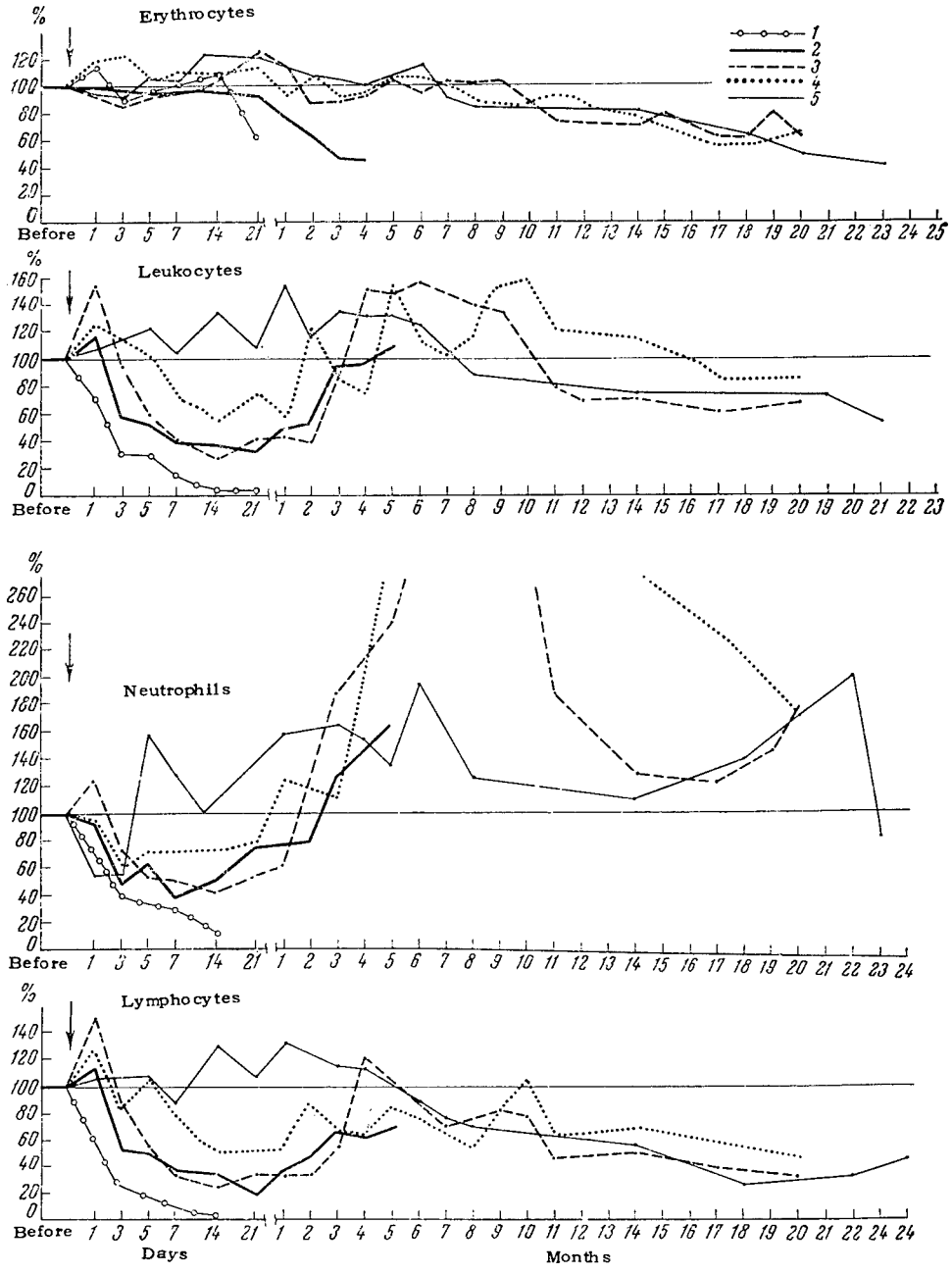

Fig. 4. Change in the composition of the peripheral blood among rats after administration of various quantities of polonium-210. 1 -- 8.0; 2 -- 4.0; 3 -- 2.0; 4 -- 0.96; 5 -- 0.06 microcurie per rat.

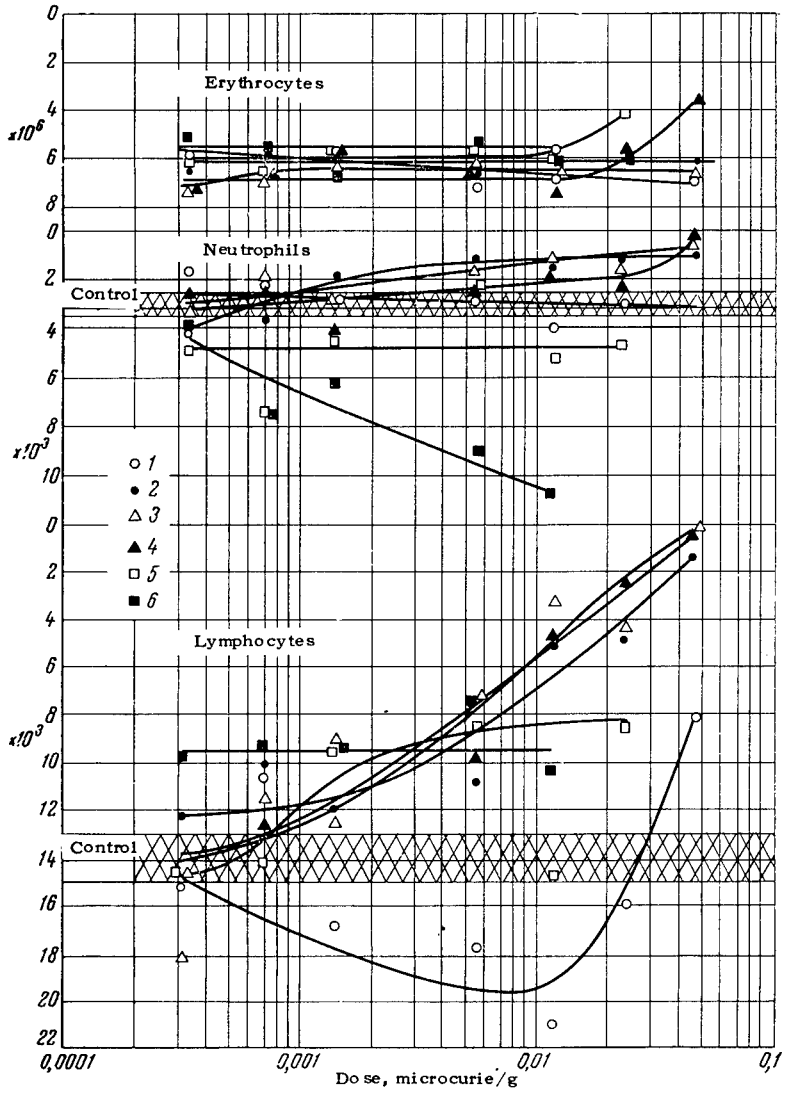

Fig. 5. Shape of dose-effect curves for erythrocytes, neutrophils, and lymphocytes among rats at various periods after administration of polonium-210. 1 -- one day; 2 -- seven days; 3 -- 14 days; 4 -- 21 days; 5 -- 120 days; 6 -- 240 days.

microcurie of Po^{210} per gram of weight of the rat. From this figure it is evident that when the amount of introduced activity is decreased by 33-fold (from 0.047 to 0.0014 microcurie/g), and the dose rate is correspondingly reduced, the degree of shortening of the lifetime per rad of "average" accumulated dose decreases by only 1.75-fold (from

3.68 to 2.1 days per rad of "average" dose). This fact indicates a good summation and poor restoration of damages caused by polonium-210 (an α emitter). However, it would be incorrect to assert that there are no processes of recovery and compensation after injury by an α emitter (Po^{210}), which has been proposed by a number of researchers [13]. Actually, the degree of shortening of the lifetime of the rats per rad of accumulated "average" dose drops with decreasing dose rate, which of course may be the result of the presence of recovery processes and incomplete summation of the damages. If we assume that there are linear relationships between the degree of shortening of the lifetime per rad of accumulated dose and the amount of activity introduced (or dose rate), not only for the investigated dose range, but also for a broader level of them, then by extrapolation we can determine the amount of Po^{210} activity and the dose rates that do not cause shortening of the rat lifetime. Such extrapolation (see Fig. 3) shows that when Po^{210} is administered in amounts of 0.00008 microcurie/g, no shortening of the lifetime of the rats per rad of accumulated "average" dose will be observed. When the indicated amount of Po^{210} penetrates into the organism, the "average" dose rate is ~0.01 rad/day. During the lifetime of the rat, when 0.00008 microcurie/g of Po^{210} has penetrated, an "average" radiation dose equal to ~1 rad is accumulated, while the amounts in the spleen and kidneys are correspondingly 20 and 10 times as great.

Fig. 4 presents the changes in the composition of the peripheral blood of the rats. The number of erythrocytes changed negligibly up to the 14th day. Subsequently, at doses of 4.0 and 8.0 microcurie per rat, the number of erythrocytes dropped by 40 and 60% after 21 days and four months, respectively. Noteworthy is the decrease in the number of erythrocytes 18-20 months after the introduction even of smaller amounts of Po^{210} (0.06 microcurie per rat).

Fig. 5 presents the dose-effect curves for the erythrocytes, lymphocytes, and neutrophils, detected at various periods of observation. It is distinctly evident that the shape of the dose-effect curve differs for these indices and varies substantially with time. Changes appear most rapidly in the lymphocytes: on the seventh, 14th, and 21st days at the height of radiation sickness, the number of lymphocytes drops in proportion to the dose; the dose-effect curve is S-shaped. The "average" radiation dose inducing a 50% reduction of the lymphocytes on the seventh to 21st days in comparison with the initial level is ~20 rad, and about 60 rad in the bone marrow. During the late periods (240th day), for all the doses of polonium-210 used, moderate lymphopenia is detected, which does not depend upon the amount of activity introduced. The dose-effect curve is linear.

The dose-effect curve is no less complex for the neutrophils. On the seventh, 14th, and 21st days, the dose-effect curve for the neutrophils is S-shaped; the number of cells drops with increasing polonium-210 activity. During the late periods (240th day), on the contrary, a sharp increase in the number of neutrophils in the blood

is detected, in proportion to the introduced activity, and the dose-effect curve becomes linear.

The changes in the red blood cells are comparatively slowly detected. The dose-effect curve is S-shaped (21st and 120th days) or linear (7th, 14th, 240th days).

These facts are evidence that the shape of the dose-effect curve for the hematogenic system, possessing a high regenerative potential, depends not only upon the radiosensitivity of the corresponding blood cells, but also upon the periods of observation, and for the same indices it may vary substantially with time.

In the rats that died during the late periods, tumoral and non-tumoral forms of long-term effects develop, including cirrhosis of the liver, nephrosclerosis, dyshormonal disorders, hyperplasia of the anterior pituitary, thyroid gland, as well as tumors of the kidneys, large intestine, testes, prostate gland, adrenals, subcutaneous tissue, pituitary, thyroid gland, mammary glands, and uterus (Table 2).

Cirrhosis of the liver arises in 8-18% of the rats when polonium-210 is administered in doses from 0.0007 to 0.0056 microcurie/g. The greatest frequency of cirrhosis (in 60% of female rats) is detected after the administration of Po^{210} in a dose of 0.0056 microcurie/g (radiation dose equal to ~0.15 krad). Nephrosclerosis arises in 20-100% of the females and in 20-80% of the males that received from 0.0007 to 0.0056 microcurie/g intraperitoneal Po^{210}. The frequency of nephrosclerosis increases corresponding to the increase in the amount of incorporated Po^{210}. The increased activity of the parathyroid glands, hyperplasia of which was observed in an average of 16% of the rats poisoned by Po^{210}, may also play a role in the genesis of nephrosclerosis induced by polonium-210, together with the direct action of α radiation upon the renal parenchyma. In the chronic phase of the injury, dyshormonal disorders arise in the animals. Here it is a matter of hyperplasia of the anterior pituitary on account of the proliferation of chromophobic cells among the males and females and of the appearance of dyshormonal proliferates in the mammary glands (mastopathy) and mucosa of the uterus (polyposis) in these animals, especially among the females. Among the males, dyshormonal proliferates were represented by mastopathy, diffuse and focal hyperplasia of the prostate gland.

In 40% (86 out of 215) of the rats injured by polonium-210, tumors of the kidneys, large intestines, testes, prostate gland, uterus, adrenals, and subcutaneous tissue arose, which were absent among the control animals (see Table 2). The greatest frequency of kidney tumors was detected among the male and female rats after the administration of polonium-210 at a dose of 0.0028 microcurie/g (in 31.5% of the rats, dose equal to ~0.31 krad). Kidney tumors were also detected among the rats in the case of higher amounts of Po^{210} (from 0.0117 to 0.0056 microcurie/g; dose 1.31-0.63 krad). The minimum tumoral dose for the kidneys after injury by polonium-210 evidently lies at a level of 0.1 krad.

Tumors of the large intestine arose after the administration

Table 2

Number of Tumors (%) of Various Types and Localization in Rats After Parenteral Administration of Various Amounts of Polonium-210 (From 0.00035 to 0.0236 Microcurie/g)

Type and localization of tumor	Number of animals					
	Control			Experimental		
	Males	Females	Males and Females	Males	Females	Males and Females
	16	19	35	144	71	215
Subcutaneous sarcoma	0	0	0	1,4	0	0,93
Liver tumors	6,2	0	2,9	2,1	1,4	1,86
Kidney tumors	0	0	0	6,3	8,5	7,0
Tumors of small intestines	0	0	0	5,6	2,8	4,7
Leukemia	6,2	5,3	5,7	4,2	7,1	5,1
Tumors of pituitary	0	15,8	8,6	3,5	9,9	5,6
Tumors of thyroid gland	0	10,5	5,7	13,2	9,9	12,1
Tumors of adrenals	0	0	0	1,4	1,4	1,4
Tumors of ovaries	—	5,3	—	—	5,6	—
Tumors of mammary glands	0	10,5	5,7	0,7	18,3	6,5
Tumors of uterus	—	0	—	—	8,5	—
Tumors of testes	0	—	—	4,9	—	—
Tumors of prostate gland	0	—	—	1,4	—	—
% of animals with tumors	12,5	31,6	22,8	39,6	41,0	40

both of comparatively high (0.0236-0.0117 microcurie/g) and low (0.0014-0.0007 microcurie/g) levels of Po^{210}. Tumors of the testes (in 3.7-10% of the rats) were detected among the animals that had received polonium-210 in amounts from 0.0056 to 0.0007 microcurie/g (dose equal to 0.18-0.05 krad).

Neoplasms of the glands of internal secretion dominated among the animals that had received smaller amounts of polonium-210. The minimum of the doses that we studied -- 0.00035 microcurie/g Po^{210} -- practically did not increase the frequency of tumors of the endocrine and sex organs: in the experimental group -- in 13 rats out of 46 (28%) that survived for more than 200 days, and in the control, in 8 rats out of 35 (22%). The frequency of pituitary and thyroid tumors among the experimental females was the same as in the control; among the males it was significantly higher (see Table 2). This fact indicates that Po^{210} is also able to induce tumors of the glands of internal secretion. However, this effect is not detected among the females, possibly as a result of the high spontaneous frequency of these tumors among them. It is known that the frequency of spontaneous tumors of the glands of internal secretion is two to three times as high among females as among males [14].

The high toxicity of polonium-210 indicates a need for expanding

investigations of the biological effects of small amounts of this element.

CONCLUSIONS

1. The values of LD_{50} for 30, 60, 120, 240, 360, and 480 days in the case of intraperitoneal administration of polonium-210 to rats are equal to 0.0272, 0.0240, 0.0169, 0.0087, 0.0060, and 0.0019 microcurie/g of weight of the animal.

2. In acute and subacute forms of injury by polonium-210, the females proved more sensitive than the males, while in the long-term periods the differences are effaced.

3. The shape of the dose-effect curve for erythrocytes, neutrophils, and lymphocytes depends upon the period of observation and may vary in character.

4. In the long-term periods, the rats develop nontumoral (cirrhosis of the liver, nephrosclerosis, dyshormonal disorders) and tumoral forms (neoplasms of the kidneys, large intestine, testes, prostate gland, subcutaneous tissues, pituitary, thyroid and mammary glands, uterus) of long-term effects.

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INFLUENCE OF FRACTIONATED IRRADIATION ON THE CHOLESTEROL CONTENT IN THE BLOOD SERUM IN GUINEA PIGS AND DOGS

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pp. 194-201

In recent years, investigations of the problem of the change in sensitivity of an animal to repeated influences of ionizing radiation have been rather widely represented in the radiobiological literature [1-8]. An increase in the radioresistance as a result of preliminary irradiation may be considered established. The response of the organism in this case varies depending on the size of the dose of the preliminary influence, the summary dose, the interval between irradiations, as well as the biological specifics of the specimen.

The task of this work was to observe the change in the cholesterol content in the blood serum of guinea pigs and dogs during fractionated irradiation by X rays.

The experiments were conducted on male guinea pigs weighing 300-400 g and mongrel male dogs weighing 17-23 kg. The animals were subjected to total irradiation on the RUM-11 apparatuses at a voltage of 180 kV, current strength 15 mA. Filters of 0.5 mm Cu + 1 mm Al were used in the irradiation of the guinea pigs, focal length 40 cm, dose rate 43.6 R/min. The dog was irradiated on two apparatuses with bilateral arrangement of the tubes; filter 0.5 mm Cu, focal length 100 cm, dose rate 14.4 R/min. The indices of the degree of injurious action of radiation were the survival rate of the animals, lifetime, and change in the cholesterol content and leukocytes in the blood of the animals. Blood was taken for analysis from the ear vein of the guinea pigs, and from the femoral vein among the dogs. The cholesterol content in the blood serum of the dogs was determined by the Neuschlosz method [9], and among the guinea pigs by the Levchenko method [10]. The cholesterol determination and leukocyte count were performed three to four times before irradiation, and 30 min, 24 hours, 3, 5, 7, 9, and 14 days after irradiation. In the dogs, additional determinations were made at various

intervals up to the death of the animals. The results of the investigation of the cholesterol content in the experiment on guinea pigs were treated by the method of variation statistics. The guinea pigs were observed for a 30-day period, the dogs for 45 days.

RESULTS OF THE INVESTIGATIONS

Experiments on guinea pigs. Groups of guinea pigs were subjected to a single total irradiation at doses of 150, 200, 300, 400, 500, and 600 R. In another series of experiments, the animals were subjected to total irradiation at doses of 30, 50, 100, and 500 R, and after 10 days at a dose of 200 R (Tables 1, 2). A single irradiation at doses of 500 and 600 R caused the death of all the animals; in this case the average lifetime did not exceed 10 days. With decreasing dose of the influence, the number of surviving animals and lifetime increased. At equal doses of irradiation, the number of surviving animals was greater in the case of fractionated irradiation than in the case of a single influence. Preliminary irradiation at doses of 30 and 150 R had no effect upon the increased radioresistance of the animals. In the case of a single total irradiation at doses of 200, 300, 400, 500, and 600 R, the cholesterol content was reduced on the seventh day, and on the 3th to 14th days after the influence at doses of 400 and 500 R (Table 3). In the case of irradiation at a dose of 600 R, an increase in the cholesterol was noted on the first to fifth days after irradiation. Irradiation at doses of 200 and 300 R was accompanied by a fluctuation within normal limits. Under the conditions of preliminary irradiation at doses of 30, 50, 100, and 150 R, and repeated irradiation at a dose of 200 R, fluctuations in the cholesterol level not exceeding the upper and lower normal limits were observed. Together with a change in the indices of survival and the cholesterol content, observations were made of the change in the number of leukocytes in the peripheral blood of the experimental animals (Fig. 1). We see that on the third day after preliminary irradiation at doses of 30, 50, 100, and 150 R, a decrease in the number of leukocytes was noted among the animals of all the experimental groups; in this case the degree of leukopenia depended upon the dose used. Repeated irradiation at a dose of 200 R was accompanied by a decrease in the number of leukocytes from the first to 11th days in all series of the experiments, reaching 20% of the initial level, just as in the case of repeated irradiation at the same dose. Thus, in spite of the fact that the initial leukocyte level before the repeated irradiation was lower than in the control groups in all the experimental variations, the degree of leukopenia and restoration was the same in the experimental and control series, i.e., preliminary irradiation according to the scheme used promoted an increase in the survival rate of the guinea pigs in only two variations of the experiment. No changes in the cholesterol content were noted in the case of fractionated irradiation.

Table 1

Influence of Various Doses of Single X Ray Irradiation Upon the Survival of Guinea Pigs

Irradiation dose, R	No. of animals in experiment	Survived	Died	Survival, %	Average lifetime, days
150	8	3	5	37	27
200	10	2	8	20	19
300	14	2	12	8	14
400	18	1	17	5,8	10
500	14	0	14	0	10,7
600	14	0	14	0	7

Table 2

Influence of Fractionated Irradiation on the Survival of Guinea Pigs

Dose of preliminary irradiation, R	Repeated dose, R	No. of animals	Survived	Died	Survival, %	Average lifetime, days
30	200	12	3	9	25	21
50	200	12	9	3	75	18
100	200	12	6	6	50	54
150	200	12	3	9	25	27
Control, 200 R	—	10	2	8	20	19

Experiments on dogs. The animals were divided into three groups differing in scheme of irradiation (Table 4). The doses of repeated irradiation were varied, just like the intervals between them. The animals of the first group were irradiated at a dose of 70 R; in this case one dog died after 17 days, the second suffered an acute form of radiation sickness, and after 53 days was irradiated again at the same dose, and after another 30 days at a dose of 800 R. Altogether this animal survived 94 days from the time of the first irradiation; the summary dose was 2200 R. The animals of group II were irradiated twice at a dose of 300 R with a 54-day interval, and after 38 days, again at a dose of 800 R. One dog survived 106 days with a total radiation dose of 1400 R, while the second was additionally irradiated at a dose of 884 R after another 30 days and survived a total of 152 days with a total radiation dose of 2284 R.

In the third group, fractionated irradiation was carried out at doses of 50 + 100 + 200 + 300 R with seven-day intervals; then, after two

weeks the animals were irradiated four times at a dose of 300 R with 14-day intervals, and 46 days later, a dose of 700 R was used for one dog, and then after 35 days, 800 R. The animal died on the 166th day, with a total X ray irradiation dose of 3350 R. For the second dog, the total dose was 2850 R, and the animal died on the 178th day from the time of the first irradiation.

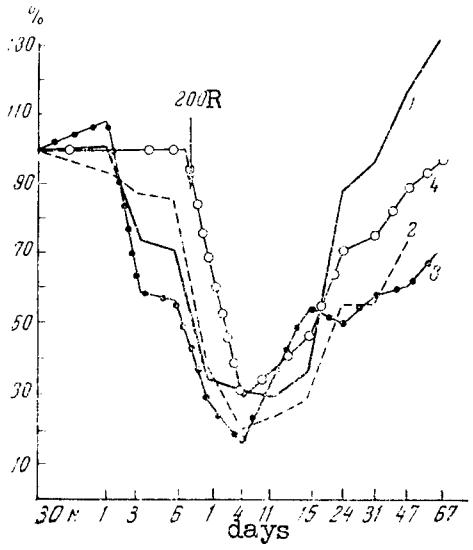

Fig. 1. Change in the number of leukocytes in guinea pigs after fractionated irradiation. Along X-axis -- days after irradiation; along Y-axis -- leukocytes, % of initial level. Irradiation doses: 1 -- 30 R, 2 -- 50 R, 3 -- 100 R, 4 -- control 200 R.

Consequently, in the case of fractionated irradiation, dogs tolerate doses up to 3350 R; in this case the lifetime of the animals increases five to ten-fold in comparison with the control dogs, irradiated once at a dose of 700 R. It should be mentioned that the lower the initial dose of fractionated irradiation (30, 50, 100 R), the greater the summary dose of radiation tolerated by the animal with a greater lifetime (Table 4). Figure 2 presents data on the change in the leukocytes of the peripheral blood during fractionated irradiation. Among the animals of all the experimental series, leukopenia was noted, the depth of which depends upon the radiation dose used. After each repeated irradiation, a tendency for restoration of leukocytes was noted; hence the leukocytic curve seemed to reflect a wave-like course of the reparative processes in the organs of hematogenesis. In the case of a single irradiation at a dose of 700 R (the animal died), the increase in the cholesterol level noted 30 min after irradiation by the 15th day had reached figures 2.5-times as great as the normal concentration (Fig. 2).

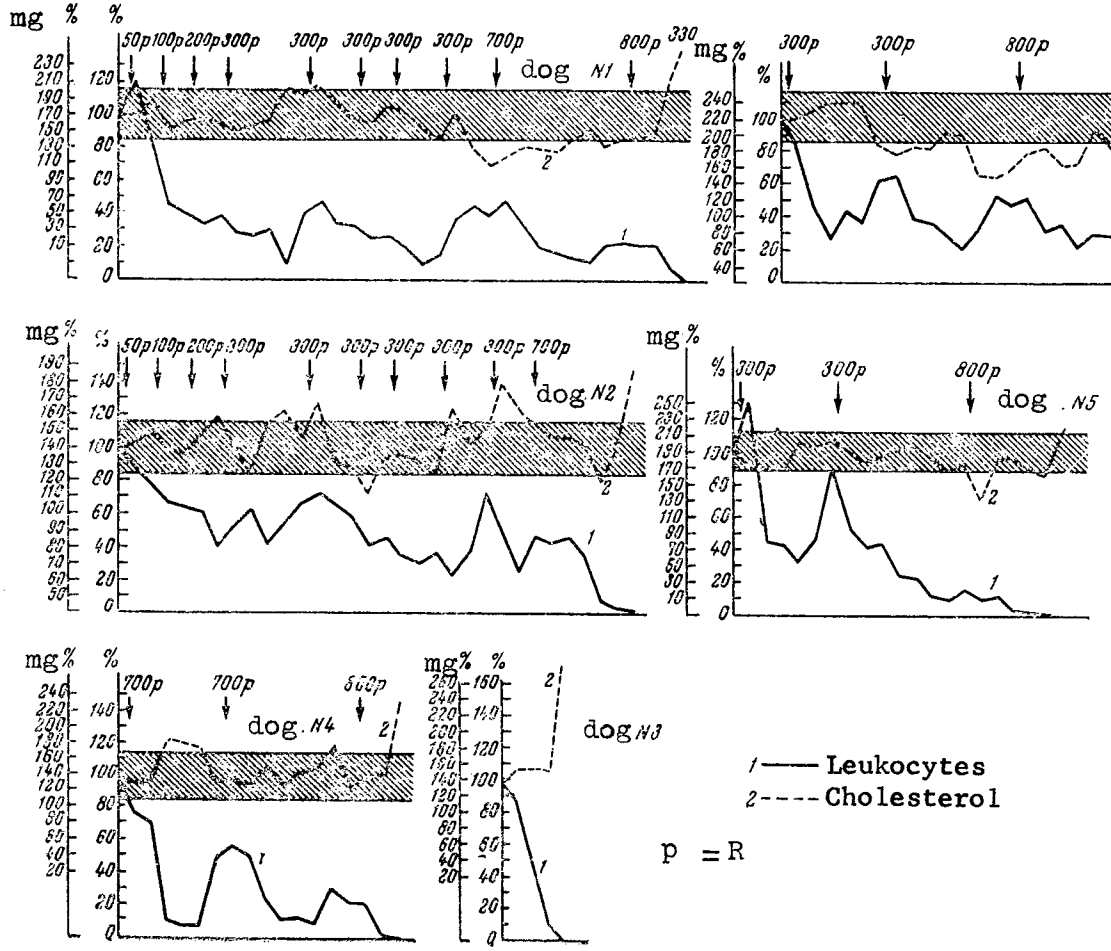

Fig. 2. Change in amount of cholesterol and leukocytes in the blood of dogs after fractionated irradiation. Along X-axis -- days after irradiation; along Y-axis -- cholesterol, mg%; leukocytes, % of the initial level: 1 -- leukocytes, 2 -- cholesterol.

Table 3

Cholesterol Content in the Blood Serum of Guinea Pigs

In the Case of Single Irradiation

Group of animals	Cholesterol in mg% of norm	Irradiation dose, R	Time after irradistion, days						
			30 MIN	1	3	5	7	10	14
			Cholesterol content						
I	84±6,4	200	82±17	73±3,4	72±4,2	74±3	73±3,4	75±4,3	72±3,8
II	82±5,3	300	84±4	82±3,8	79±6	74±4,2	73±3,8	76±4,3	75±4,5
III	81±3,8	400		86±3,2	89±6.2		66±3,6 T=2,9	77±7,4	71±7,4
IV	81±3,8	500	86±5	82±5	64±5,5 T=2,5	66±5,5 T=2,5	63±5,2 T=2,4		59±9,8 T=2,2
V	94±9,8	600		121±5,6 T=2,4		120±4,3			

In the Case of Fractionated Irradiation

Group of animals	Cholesterol in mg% of norm	Dose of preliminary irradiation R	Time after irradiation, days										
			1	3	5		30 MIN	1	3	5	7	9	14
I	84—4,3	30	72±5,7	71±4,3	73±4,1	Repeated irradiation dose 200 R	77±3,8	80±4,3	78±5,4	76±4,2	71±6,2	75±3,8	74±6,2
II	80±5	50	73±3,4	71±4,6	72±4,8		78±4,3	82±7	76±6,1	74±3	73±4,1	72±4,3	76±3,8
III	82±4,3	100	75±3,6	74±5,3	71±4,8		76±4,8	74±6	74±3,5	72±4,2	75±6,2	74±3,6	78±5,5

Table 4

Scheme of Fractionated Irradiation of Dogs

Dog No.	Group	Weight, kg	Irradiation dose, R	Interval, days	Irradiation dose, R	Interval, days	Irradiation dose, R	Interval, days	Irradiation dose, R	Interval, days	Irradiation dose, R	Lifetime, days	Total dose, R
3	I	17	700	—	—	—	—	—	—	—	—	17	600
4		20	700	53	700	30	800	—	—	—	—	94	2200
5	II	18	300	54	300	38	800	—	—	—	—	106	1400
6		23	300	54	300	38	800	30	884	—	—	152	2284
1	III	21	50 + 100 + 200 + 300 with 7-day interval			14	300×4 with 14-day interval	46	700	35	800	166	3350
2		21						49	300	24	700	178	2850

Under analogous experimental conditions, in dogs that had survived acute radiation sickness, an increase in the cholesterol was observed in the period from seven to 21 days; then normalization set in. Secondary irradiation at the same dose was accompanied by a fluctuation of the cholesterol within the normal limits, and only by 24 days was an increase noted. In the following irradiation at a dose of 800 R (summary dose 2200 R), the cholesterol content was again increased. Three to four days before death, both dogs exhibited high cholesterol values. In the second series of experiments, after each successive irradiation according to the scheme used, (Table 4), a decrease in the cholesterol level of the animals was noted, especially pronounced in Dog. No. 6 (to 60% of the normal concentration). No increase in cholesterol was observed before the death of the animals. In the dogs of group III, the cholesterol content was within normal limits until a total dose of 650 R was obtained. Then, just as in the second series of experiments, each successive irradiation was accompanied by a decrease in the cholesterol level, especially pronounced in dog No. 1, which survived for 166 days with a summary dose of 3350 R. Three to four days before death, this group of dogs exhibited a sharp increase in the cholesterol level.

An analysis of the data obtained shows that preliminary

repeated irradiation of dogs at sublethal doses increases the resistance of the animals to a subsequent influence of radiation, which was expressed in our experiments in an increase in the tolerable dose of radiation to 2200-3350 R, with an increase in the lifetime to 178 days. It should be mentioned that the resistance to repeated doses and the lifetime in these experiments depended upon the scheme of preliminary irradiation (size of the doses and intervals between them). The use of doses of the order of 30 and 50 R, with a gradual increase in them, led to the fact that the animals withstood doses of up to 3350 R, with a simultaneous increase in the lifetime. In the case of fractionated irradiation, the animals differed little from the healthy animals in external appearance and behavior. Only when a summary dose of more than 2000 R was reached, were transitory intestinal disorders noted among the dogs. In all three series of experiments, a decrease in the leukocyte content in the peripheral blood was observed among the dogs after each dose of fractionated irradiation; by the time of repeated irradiation, the leukocytes did not return to normal. Thus, before each repeated irradiation in the experimental series, the initial leukocyte level was below normal; however, the degree of leukopenia ultimately was the same in the experimental and control series, which is evidence (together with reparation processes) of an increase in the resistance of the organs of hematogenesis in the case of fractionated irradiation. A number of authors report on a decrease in the effect of injury to the peripheral blood in the case of a fractionated dose of irradiation [11, 12]. In the case of a single irradiation of dogs at a dose of 700 R (in the case of a lethal result), an increase in the cholesterol was noted on the first day in our experiments, with a sharp increase (to 300% of the norm) before the death of the animal. In those cases when radiation sickness was not accompanied by a lethal result, the cholesterol fluctuations were within normal limits. This pattern has been noted in the studies of other authors [13].

Noteworthy in our experimental material is the fact that in the case of fractionated irradiation, no sharp fluctuations of the cholesterol content are observed in the blood serum of the animals. It was noted that after each repeated irradiation, the amount of cholesterol is somewhat reduced (no increase is noted), and then is restored to normal. The decrease in the cholesterol of the dogs after repeated irradiations was accompanied by the survival of a larger summary radiation dose and an increase in the lifetime. Conversely, if a stable increase in the cholesterol for three to seven days was noted after irradiation, then the animals died. In the case of fractionated irradiation, in not one series was there a sharp increase in the cholesterol level. The possibility of development of increased resistance among the dogs is evidenced by the known data, which indicated that in preliminarily irradiated dogs and rats after the influence of a massive dose, there was no disturbance of the carbohydrate and protein metabolism. The sharp increase in the cholesterol content several days before death of the dogs, which we and a number of authors have noted, has not yet been sufficiently explained. It may be assumed that in severe radiation sickness and before the death of the animal, the assimilation of cholesterol and its utilization are impaired.

CONCLUSIONS

1. Fractionated irradiation of guinea pigs at doses of 50 + 200 R and 100 + 200 R at 10-day intervals promoted a 35-55% increase in the survival rate and an increase in the lifetime in comparison with the control group.

2. Repeated X ray irradiation in increasing doses promotes an increase in the radioresistance of the dogs: the animals survive summary doses four to five times as great as the lethal dose (3350 R), while the lifetime is increased five to ten-fold (92-178 days). The degree of radioresistance in this case varied depending upon the scheme of preliminary irradiation. In all the variations of tested doses, a decrease in the number of leukocytes was observed; in this case the degree of leukopenia corresponded to the value of the radiation dose. In the intervals between the repeated irradiations, in not one of the experimental variations did the leukocytic curve return to the normal level.

3. In the case of a single total irradiation of guinea pigs at doses of 400 and 500 R, a drop in the cholesterol level was noted on the third to 14th days. In the case of irradiation at a dose of 600 R, an increase in the cholesterol was observed immediately after irradiation and on the fourth day, without a return to normal up to the death of the animal. Fractionated irradiation was accompanied by fluctuations in the cholesterol level within the normal limits.

4. Total X ray irradiation of dogs at a dose of 700 R (lethal result) produces a sharp rise in the blood cholesterol 24 hours after the influence, lasting to the death of the animal. Under conditions of fractionated irradiation, no sharp fluctuations in the cholesterol content were observed. After each repeated irradiation, a labile equilibrium was established, with a tendency for deviation of the cholesterol concentration in the direction of a decrease from the norm.

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INFLUENCE OF A HIGH-FREQUENCY GAS DISCHARGE ON FREE RADICAL FORMATION IN AMINO ACIDS

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pp.202-205

The explanation of the primary mechanisms of the action of ionizing radiation upon biomolecules is now attracting the fixed attention of researchers. The method of electron paramagnetic resonance (EPR), with the aid of which free radicals arising in various molecular systems under the action of irradiation are studied [1, 5], etc. is widely used for this purpose. However, it should be noted that the observation of many radiation effects of this kind becomes possible only at sufficiently large doses ($\sim 1\text{-}2 \cdot 10^6$ R) of high-energy radiation, which considerably complicates the corresponding experiments. In addition, the investigations of the kinetics of free radical formation in irradiated material and the detection of certain optimum conditions of irradiation, leading to a maximum yield of free radicals, is also important for a number of allied fields of biological physics and chemistry -- the initiation of special chemical reactions, etc. Hence, searches for new and effective methods of influence have not yet lost their importance.

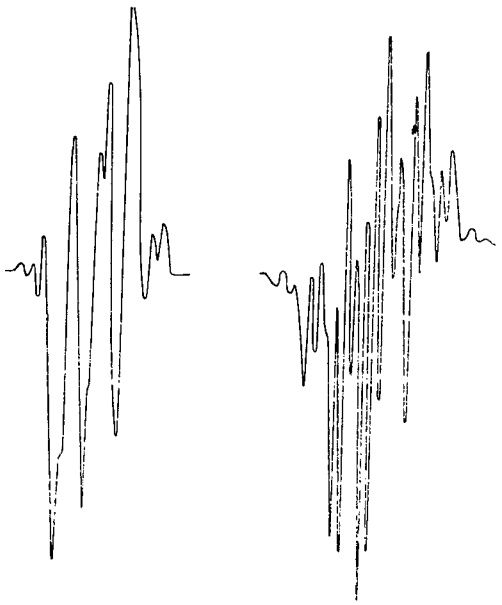
In view of this, the influence of a high-frequency gas discharge in the reduced pressure region upon the formation of free radicals in biological systems, first noted in the summaries of the report [6], proved extremely interesting: when substances are placed in a gas discharge plasma for a time of $\sim$ 10-15 min, the yield of free radicals is extremely substantial.

We conducted special experiments directed toward a comparative study of the effects of a gas discharge and the γ rays of Co^{60} upon free radical formation in amino acids. The amino acids investigated were placed in glass ampoules, which were sealed at an air pressure within the interval 10^{-1}-10^{-2} mm Hg. An electrodeless gas discharge in the ampoules was created with the aid of a Tesla high-frequency transformer with a continuous spectrum (energy maximum at a frequency of $\sim$ 1 Mc/sec). The experiments indicated that the EPR spectra for the 20 most important

amino acids (with the exception of valine) after the latter have remained in a gas discharge plasma for 10-15 min are entirely identical with the spectra of the same amino acids after the action of γ radiation at a dose of $1\text{-}2\cdot10^6$ R. Since the latter spectra have already been described in sufficient detail, it would be superfluous to cite them here. The EPR spectra of valine after the influence of a gas discharge and γ radiation are cited in the figure. It is quite evident that the free radical concentration after a comparatively short influence of a gas discharge approximately coincides with that in the irradiation of valine with an enormous dose of γ radiation -- 1.5 million R. However, in contrast to all the other amino acids, in valine treated with γ rays there is a greater splitting of several lines, which is not observed in the case of irradiation in a gas discharge. The monotypic character of the spectra obtained can be explained by the fact that the final cause of the observed cleavage of the bonds in both cases -- in the discharge plasma and in γ irradiation -- lies in particles or electromagnetic vibrations of the same energies. The latter may be represented as the result of a continuous "exchange" of energy of the original particles or photons in their interaction with the substance. The question of the dissipation of γ ray energy has been sufficiently completely studied [7]; it is also of primary interest to evaluate the energy spectrum of particles in radiations in the plasma of a high-frequency gas discharge. In this preliminary communication we did not attempt to give an exhaustive quantitative description of the phenomena that occur in the discharge; we were interested primarily in the qualitative aspect of the problem.

The activating effect of a low-frequency gas discharge upon the occurrence of chemical reactions in the gas phase has long been known. Its basic cause has also been disclosed -- a direct influence of free electrons and ions upon the molecules of the reacting substances: ionization by electron impacts, excitation of internal degrees of freedom of complex molecules during collisions, formation of various complex ions (ionic catalysis), etc. [8]. In this case, the deexcitation of energy quanta in the visible and near ultraviolet regions [9] plays a secondary role. However, if the substance studied is present in the solid state, the mechanism of activation indicated above essentially drops out in view of the rapid absorption of particles in the surface layer of the substance, and the cause of the strong influence of the gas discharge upon the free radical formation noted should be sought in the influence of the short-wave radiation generated in the high-frequency discharge.

The ionization potentials of the molecules of different gases are not the same, but they are all close in order of magnitude to ~ 10 eV. In order for the discharge to be able to exist for a long time, an equilibrium between the number of events of recombination, leading to the disappearance of free electrons in the plasma, and the number of events of ionization of the molecules and atoms under the action of collisions with the electrons vibrating in the high-frequency field, is necessary. Actually, in an electrodeless high-frequency discharge, the knockout of new electrons or the absorption of old ones on the walls plays an



a b

EPR Spectrum of valine after a 10-min stay in the plasma of a high-frequency gas discharge (a); EPR spectrum of valine irradiated at a dose of $1.5 \cdot 10^{-6}$ R (Co^{60})(b).

extremely negligible role [10]. Ionization may occur in the case when the average electron energy in the plasma (electronic "temperature") is close to the ionization potential (~ 10 eV). Thus, for an estimate of the intensity of the radiations arising, we can use the rather well developed theory of low-temperature plasma.

The generation of short (ultraviolet and X) rays in plasma is possible on account of two basic mechanisms -- deexcitation in the collision of ions with fast electrons (Bremsstrahlung) and deexcitation of energy quanta during recombination (recombination radiation). The exact quantitative theory of Bremsstrahlung has been developed by Sommerfeld [11]. In the high-frequency region, when T ~ 10 eV, for the spectral intensity $dQ_{(\varepsilon)}$ of the photon emission within the energy interval $d\varepsilon$, we can use an approximate quasiclassical expression [12]:

$$dQ_{(\varepsilon)} \simeq \frac{16\pi}{3\sqrt{3}} \frac{r_0^2 c^2}{137} n^2 \sqrt{\frac{2m}{\pi T}} e^{-\frac{\varepsilon}{T}} d\varepsilon,$$

where r_0 and m are the classical radius and mass of the electron ($r_0 = 2.82 \cdot 10^{-13}$ cm); c is the velocity of light; n is the electron density in

the plasma (usually $n \sim 10^{11} - 10^{13}$ units/cm^3). From this it is evident that the bulk of the energy is emitted in the ultraviolet portion of the spectrum when $\varepsilon \sim T \sim 10$ eV (for comparison, let us indicate that the energy of the photons of the visible region of the spectrum is contained within the interval from 1.5 to 3 eV). Furthermore, the total intensities of Bremsstrahlung and combination radiation are approximately comparable when $T \sim 30$ eV [12]. At lower temperatures, the intensity of the recombination radiation exceeds the intensity of the Bremsstrahlung. Moreover the recombination radiation spectrum is shifted toward shorter wave lengths, of the order of the ionization potential, i.e., of the order of T. An exact calculation of the spectral intensity in this case is hindered by the fact that when $T \sim 10$ eV, the stepwise recombination, i.e., capture of electrons not on the ground level, but on the excited levels of the atoms, can no longer be neglected. However, the available estimates quite definitely indicate that the maximum energy of recombination radiation falls in the far ultraviolet or X ray region of the spectrum (see, for example, the results for hydrogen-like plasma in [13, 14]).

The total energy of all the radiation per electron in the plasma at an average electron temperature of ~ 10 eV, is equal to $\sim 10^{-24}$ erg·cm^3/sec [14]; assuming $n \simeq 10^{12}$ units/cm^3, we obtain for the radiation energy of a unit volume of the plasma a value of ~ 1 erg/sec, which is quite sufficient for an extremely strong influence of a gas discharge upon a substance placed in the plasma. Thus, the results of our experiments find a qualitative theoretical explanation.

CONCLUSIONS

1. A high-frequency gas discharge is a highly effective influence promoting the formation of free radicals in amino acids: a 10-15 min action of it is equivalent to irradiation by γ rays at a dose of $1\text{-}2 \cdot 10^6$ R.

2. The EPR spectra of the 20 most important amino acids, irradiated by γ rays or in a gas discharge, coincide in form. Valine is an exception to this rule.

3. The high effectiveness of the discharge is explained by the action of short-wave radiation -- ultraviolet and X rays -- generated in the plasma upon the amino acids.

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EFFECTS OF CERTAIN PEROXIDES AND IONIZING RADIATION ON MICE

A. S. Popov

pp. 206-209

Our earlier investigations were directed toward determining the role of organic peroxides in the radiobiological effect at the cellular level. The problem of this work was to compare the deleterious effect of γ rays and certain peroxides on mammals and to compare the results obtained with our previous data.

Similar studies were conducted previously by certain authors. Feinstein and associates subjected mice to the influence either of X rays at a dose of 800 R or of hydrogen peroxide at a dose of 340 mg ($1 \cdot 10^{-2}$ mole) per kg of weight (LD_{50}) and arrived at the conclusion that H_2O_2 may play a substantial role in the radiation injury [1]. The most extensive comparative study of the effects of radiation and a whole series of peroxides upon mice was conducted by Horgan and Philpot et al. [2-4]. They investigated the lifetime, survival rate, and change in weight of the animals under the isolated and combined action of the indicated agents, and with the use of radioprotectors. The above-mentioned authors believe that there is little similarity between the effects of radiation and injected lipid peroxides. In contrast to these researchers, we decided to use nonlipid peroxides: hydrogen peroxide, urea peroxide, and succinic acid peroxide.

PROCEDURE

Hydrogen peroxide was purified by distillation under vacuum in quartz apparatus. Urea peroxide (peroxohydrate) was synthesized according to the method described in Shvitser's manual [5]. We produced succinic acid peroxide according to the method cited in Guben's book [6]. When dissolved in water, they are immediately hydrolyzed, forming one molecule of succinic and one molecule of persuccinic acids; the latter, being a hydroperoxide, is the active product in solution [7]. The enumerated compounds were stored in a vacuum desiccator, in a refrigerator. Their solutions were prepared ex tempere, and the concentration was determined by iodometric titration.

The indicated peroxides were dissolved in triple-distilled water, and their solutions of various concentrations were administered once intraperitoneally in amounts of 0.3 ml per mouse. The noninbred white male mice, weighing 20-25 g, were from one lot and were kept on the usual laboratory feed. The mice were divided into groups of 10 animals of the same weight in each. The control mice were injected with pure water. Another group of animals was subjected to total irradiation by the γ rays of Co^{60} at a dose of 700 R (LD_{100}) on the EGO-2 apparatus at a dose rate of 263 R/min. For a month after the influence, the death rate, lifetime, and change in weight of the animals were recorded. The reliability of the differences of the average lifetime was determined according to the Wilcoxon criterion, which was the best for the case under consideration.

RESULTS

The death rates and lifetimes of the mice are presented in the table. It can be seen that the toxic concentrations of each peroxide lie within relatively narrow limits, and that succinic acid peroxide is 100 times as poisonous as H_2O_2 and urea peroxide, the toxicities of which are approximately the same. It was found that the average lifetime in all experiments with peroxides, both in the case of high and in the case of low death rate, is far shorter than in the irradiated animals. This difference is reliable in all cases but one. In this variation (injection with H_2O_2 at a dose of $1.5 \cdot 10^{-2}$ mole/kg), the possibility of random error is far greater than 10%.

Effects of Radiation and Certain Peroxides on Mice

Lethal agent	Dose of peroxides, mole/kg	No of mice		Average lifetime, days	Reliability of difference of lifetime from that in the case of irradiation	
		Used in experiment	Died		Wilcoxon criterion	Possibility of error, %
Succinic acid peroxide	$6{,}63 \cdot 10^{-4}$	10	10	1,4	0	<0,00
	$3{,}35 \cdot 10^{-4}$	10	3	1,0	0	1,22
	$1{,}73 \cdot 10^{-4}$	10	1	3,0	0	4,44
Hydrogen peroxide	$2{,}55 \cdot 10^{-4}$	10	10	1,3	0	<0,00
	$1{,}88 \cdot 10^{-2}$	10	6	3,3	2	0,26
	$1{,}5 \cdot 10^{-2}$	10	3	5,3	6	≫10
Urea peroxide	$2{,}38 \cdot 10^{-2}$	13	13	2,5	2	0,02
	$2{,}12 \cdot 10^{-2}$	10	7	2,3	0	0,04
Gamma rays, 700 R	—	10	8	10,04	—	—
Control (injection of water)	—	6	0	—	—	—

However, an attentive consideration of the results of this case does not permit unreserved belief in the variation statistics. Actually, three out of 10 mice died; moreover, one died on the first day, another on the second, and the third not until the 13th. In none of the variations of all in which the animals received peroxides was such a late death observed. Death did not occur later than the sixth day. The mice that survived this period soon recovered entirely, and in external appearance, weight, and behavior in no way differed from the controls. Evidently the mice that died on the 13th day died for some other reason. Moreover, the effects of the two agents compared are biologically nonequivalent in this case. Eight out of ten irradiated animals died, while only three of the same number of mice subjected to the action of H_2O_2 died. In the variations with approximately the same death rate, the lifetime of the animals poisoned with peroxides was three to four times shorter than the irradiated animals. Thus, there is no similarity in this index between the action of radiation and that of the peroxides that we tested upon mammals.

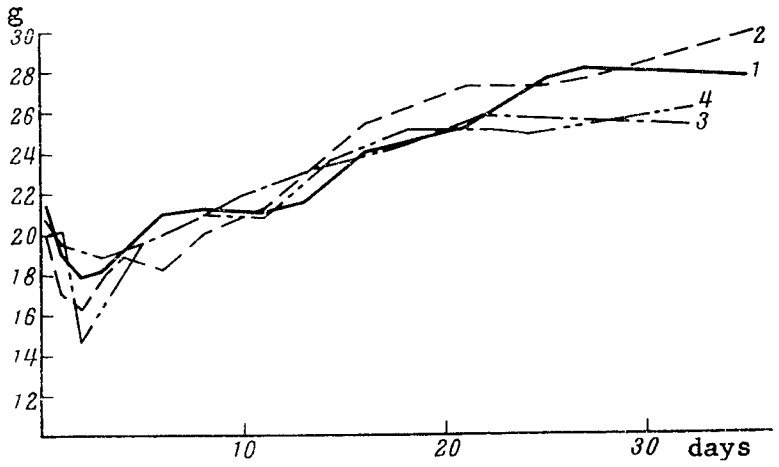

Fig. 1. Changes in weight of mice after intraperitoneal injection of hydrogen and succinic acid peroxides. 1 -- H_2O_2, $1.9 \cdot 10^{-2}$ mole/kg; 2 -- H_2O_2, $1.5 \cdot 10^{-2}$ mole/kg; 3 -- succinic acid peroxide, $3.35 \cdot 10^{-4}$ mole/kg; 4 -- succinic acid peroxide, $1.75 \cdot 10^{-4}$ mole/kg. Along Y-axis -- average weight of surviving animals, g; along X-axis -- days after injection.

A comparison of the changes in weight of the experimental animals over a period of a month leads to an analogous conclusion (Figs. 1 and 2). These results are evidence that the average weight in the irradiated group is appreciably decreased, and after some fluctuations is then stabilized at a level correspondingly lower than the original level. At the same time, when peroxides are administered, the weight of the mice,

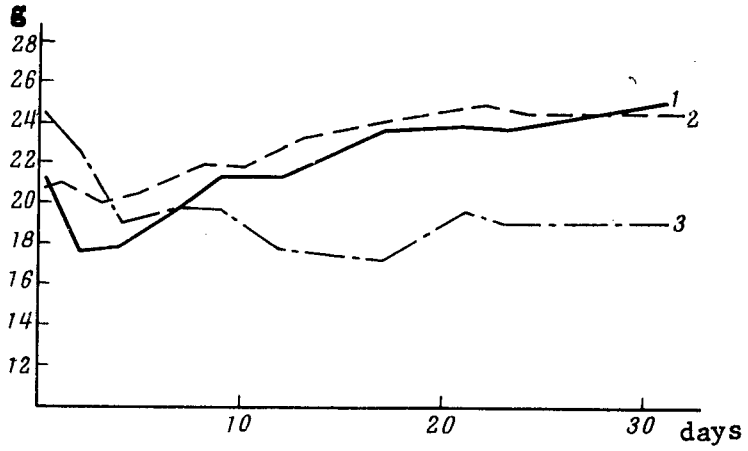

Fig. 2. Changes in weight of mice after irradiation or after injection of urea peroxide. 1 -- Urea peroxide, $2.12 \cdot 10^{-2}$ mole/kg; 2 -- control (injection of water); 3 -- γ irradiation, 700 R. Along axes the same as in Fig. 1.

after a more or less profound drop on the first days, rapidly returns to the initial level, and subsequently increases just as in the control, and sometimes even somewhat more.

DISCUSSION

Thus, our experiments confirmed the data of Horgan and Philpot, that the action of various peroxides on mammals differs greatly from the radiation influence, and demonstrated the correctness of this conclusion not only for lipid peroxides, but also for hydrogen peroxide and urea peroxide, which were not studied by the above-mentioned authors. Both in their work and in ours, succinic acid peroxide was tested. The results obtained differ somewhat only in the magnitude of the toxic doses. In the experiments of Philpot and Roodyn, nine micromoles of this peroxide per mouse corresponded to LD_{50}, while in our experiments 6.9 micromoles (conversion to one mouse of a dose of $3.35 \cdot 10^{-4}$ mole/kg) caused an approximately 70% death rate. Such a difference is easily explained by the fact that these researchers used females of the R line while we used noninbred males. An analogous explanation is applicable to the comparison of our data with the work of Feinstein and co-authors.

The absence of similarity between the effects of peroxides and radiation upon higher animals is evidence of unsubstantiation of the idea that ionizing radiation injures the organism through the formation of some sort of universal toxin. Philpot and his associates attempted to demonstrate that the role of such a toxin may be played by squalene peroxide or autooxidized linoleic acid, but were unsuccessful. Evidently

the toxemia detected in radiation sickness cannot be related to peroxides. Probably the role of peroxides is limited to the initial stage of the radiation sickness -- injury of the cells of the radiosensitive organs. An indirect confirmation of this opinion is the fact that, according to our data, the comparative toxicities of the various peroxides were quite different for mammals and unicellular organisms. In experiments on Paramecia, the most effective of several peroxides tested was succinic acid peroxide, but it proved least lethal for mice [8]. In the experiments of Philpot and associates, this peroxide occupied second place, yielding in toxicity only to autooxidized linoleic acid.

The mechanism of the action of peroxides in the case of intraperitoneal injections remains unclear. It is known that succinic acid peroxide causes necrotic changes of the internal organs close to the site of administration, while there are no morphological disorders in the distant tissues [9]. On the other hand, a definite portion of the peroxides evidently penetrates into the blood, traveling along the vessels of the mesentery and intestines, and then penetrates into the liver, in which, as is well known, approximately 40% of the catalase of the entire organism is concentrated. Probably toxic doses of hydrogen and urea peroxides are two orders of magnitude greater than the dose of succinic acid peroxide precisely because the first two compounds are readily decomposed by the liver catalase, while the latter substance is very weakly decomposed by this enzyme [8, 10]. Moreover, succinic acid peroxide may influence the Krebs cycle according to the principle of metabolite replacement.

CONCLUSIONS

1. The effects of ionizing radiation and hydrogen, urea, and succinic acid peroxides upon mice were studied. It was found that the lethal doses of succinic acid peroxide are 100 times lower than the dose of hydrogen and urea peroxides.

2. It was shown that there is no similarity in the effects of radiation and peroxides on the lifetime and change in weight of the animals. It was established that the comparative toxicities of certain organic peroxides for mammals and protists are quite different. Succinic acid peroxide is most toxic for mice and least for Paramecia.

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DISTURBANCE OF THE REGULATION OF SECRETORY ACTIVITY OF THE STOMACH DURING BLOOD LOSS IN ANIMALS SUFFERING FROM ACUTE RADIATION SICKNESS OF MEDIUM SEVERITY

M. G. Mikaelyan

pp. 210-213

The task of this work was to study the disturbances of the regulation of secretory activity of the stomach after the combined action of irradiation and blood loss upon the organism. For this purpose we had one experimental group of dogs, subjected to irradiation with subsequent bloodletting, and two control groups. In the first control group, the dogs were subjected only to irradiation, and in the second only to bloodletting. Each group consisted of nine dogs: three dogs with isolated stomach according to Pavlov, three according to Heidenhain, and three esophagotomized with gastric fistula according to Basov. Raw chopped meat (200 g) was used as the food stimulus. The gastric juice was investigated each hour after feeding, followed by determination for the four hours of the experiment. The free and total acidity were determined in the gastric juice by the method of titration, and the enzymatic activity was determined according to Mett's method. In all the experiments we determined the latent period of secretion and nature of the curve of secretion of gastric juice according to hours.

The animals were subjected to a single total irradiation at a dose of 600 R, with RUM-11 apparatus, under the following conditions: voltage 187 kV, current strength 15 mA, filter 0.5 mm copper + 1 mm aluminum, skin-focal length 100 cm, dose rate, measured in air, 11 R/min.

Bloodletting was performed in amounts of 4% of the body weight of the animal from the femoral artery, which was exposed under novocain anesthesia.

RESULTS AND DISCUSSION

In the dogs with isolated stomach according to Pavlov, a strong inhibition of secretion, lengthening of the latent period, and decrease in food excitability were noted in response to the food stimulus in the

first days after irradiation and blood loss. Between the third and fifth days, a short period of negligible hypersecretion with spontaneous liberation of juice was observed in dogs Nos. 53 and 54, again giving way to hyposecretion, the development of which progressed to the death of the animals (12-15 days). In dog No. 55, transitory (on the second day) hypersecretion was immediately replaced by prolonged wave-like periods of hyposecretion and a normal level of secretion, against a background of an improvement of the condition of the animal (Table 1). The latent period of secretion of gastric juice was shortened in the case of hypersecretion and lengthened in the case of hyposecretion. In all the dogs, a change in the secretion occurred in both phases of the secretory period, but it was especially pronounced in the first phase, which is evidence of a disturbance primarily of the conditioned reflex mechanism of regulation of secretion.

On the dogs subjected only to irradiation, a wave-like alternation of hyper- and hyposecretion was noted, with a corresponding change in the acidity and digestive power, as well as a disturbance of the latent period of secretion of juice and type of the secretion curve (Table 1). From the data cited in Table 1, it is evident that a decrease in the amount of juice with corresponding decrease in acidity and enzymatic activity, was observed in the dogs subjected only to bloodletting as well. Among these dogs, a change in the latent period of secretion of juice and in the type of secretion curve was also noted. However, these changes were only temporary and were rapidly restored; the latent period of secretion was normalized first, and then the quantity and quality of the secretion.

The data obtained on dogs with denervated stomach according to Heidenhain give evidence of profound changes in the neurohumoral regulation as well (Table 2).

Comparing these data with the data obtained on isolated stomachs according to Pavlov, we note that both in the case of combined injury and in the case of irradiation alone a stronger disturbance of the gastric secretion is observed among the denervated dogs than among dogs with the inervation of the stomach preserved. In the same dogs, comparatively great secretion of juice in response to food stimulus is observed during the first hours of secretion; the secretory period is greatly shortened, the juice obtained contains more mucous, and perversion of the acid-forming function of the gastric glands is frequently observed.

A disturbance of the conditioned reflex regulation of the stomach activity is also evidenced by the result of experiments on esophagotomized dogs. After the combined influence of irradiation and bloodletting in the dogs, a weakly expressed, wave-like variation of the amount of the gastric juice secreted was observed (Table 3). A lengthening of the latent period, decrease in the food excitability, shortening or lengthening of the secretory process, appearance of spontaneous juice, and perversion of the type of secretion curve with a shift of the secretion maximum from the first hours of the experiment to the middle hours were noted. These changes progress with the development of radiation sickness

Table 1

Change in Gastric Secretion in Dogs with Isolated Stomach According to Pavlov

Conditions of experiment	Dog No	Initial background	Amount of juice (ml) obtained from the isolated stomach in 4 hrs after offering of 200 g of meat													
			Days of investigation													
			1	2	3	5	7	10	12	15	20	30	40	50	65	80
Irradiation + Bloodletting	53	24,0	6,5	17,0	31,0	10,3	3,5	2,0	2,0	Dog died on the 12th day						
	54	32,0	6,8	13,6	28,0	38,0	21,8	10,4	4,5	1,6	Dog died on the 15th day					
	55	46,3	2,0	59,5	18,5	43,5	19,5	9,2	—	14,0	35,0	27,0	17,5	36,2	47,0	25,5
Irradiation	56	28,5	35,0	30,0	32,3	29,7	11,0	9,5	6,0	9,0	6,0	32,5	29,5	33,3	13,5	10,0
	57	34,6	24,8	45,6	39,7	32,8	40,7	47,8	13,4	8,6	43,9	49,8	34,2	26,0	32,7	19,7
	58	16,3	28,5	36,3	37,0	24,6	17,0	8,3	4,8	13,2	1,5	Dog died on the 20th day				
Bloodletting	40	30,2	5,8	15,0	20,7	22,5	23,0	20,2	30,0	35,8	32,1	28,3	32,4	—	—	—
	41	40,0	20,1	13,5	22,0	27,8	29,2	28,2	34,5	34,5	42,0	39,0	38,0	—	—	—
	68	14,7	5,1	6,6	8,2	7,0	12,0	8,5	11,8	13,1	14,5	13,7	16,0	—	—	—

Table 2

Change in Secretion in Dogs with Denervated Stomach According to Heidenhain

Conditions of experiment	Dog No	Initial background	Amount of juice (ml) obtained from denervated stomach in 4 hrs after offering of 200 g of meat													
			Days of investigation													
			1	2	3	5	7	10	12	15	20	30	40	50	65	80
Irradiation + Bloodletting	59	37,4	5,0	41,5	57,2	50,5	32,5	26,2	25,2	22,2	29,0	18,9	15,5	27,0	38,5	40,5
	60	42,0	6,5	48,0	43,8	30,0	18,0	12,0	3,0	Dog died on the 12th day						
	61	36,0	2,5	14,0	25,2	14,0	4,5	3,0	3,0	Dog died on the 15th day						
Irradiation	62	57,0	68,0	49,0	40,2	48,5	22,1	17,0	10,7	28,3	20,5	14,2	118,5	26,5	18,8	
	63	12,3	18,7	15,3	6,4	5,1	7,2	5,0	2,3	12,0	17,0	18,0	8,6	24,0	17,0	20,5
	64	16,3	12,0	8,7	24,9	20,6	18,9	14,6	5,6	5,3	7,2	1,9	Died on the 29th day			
Bloodletting	46	39,0	8,0	18,2	20,1	22,2	24,3	19,3	22,3	22,5	30,0	35,5	38,0			
	47	14,6	3,6	5,2	6,4	5,3	7,4	4,3	8,3	13,2	15,0	14,1	15,4			
	69	26,4	3,5	4,2	7,7	8,0	10,5	12,7	16,8	22,7	27,1	28,1	24,8			

Table 3

Change in Gastric Secretion in Esophagotomized Dogs

Conditions of experiment	Dog No	Initial background	Amount of juice (ml) obtained from the stomach after sham feeding											
			Days of investigation											
			1	2	3	5	7	10	12	15	20	30	40	50
Irradiation + Bloodletting	48	61,5	18,8	46,1	54,4	57,3	18,8	10,5	5,0	Dog died on the 12th day				
	49	106,6	13,1	80,4	98,3	90,6	30,0	40,4	12,1	6,2	Dog died on the 15th day			
	50	40,6	18,1	42,1	30,3	10,4	6,2			Died on the seventh day				
Irradiation	51	120,0	137,6	127,0	114,0	92,0	120,3	120,0	60,0	30,0	12,0	Died on the 16th day		
	52	110,0	134,4	140,7	143,0	130,2	116,0	110,0	80,0	55,0	60,0	60,0	120,0	46,0
	67	80,0	91,2	86,2	84,0	70,3	30,3	4,5	8,0	4,0	Died on the 16th day			
Bloodletting	33	93,3	17,5	26,0	56,4	69,0	99,0	87,0	—	97,0	104,0	56,5		
	34	108,0	40,0	56,3	70,6	90,0	96,3	70,0	100,7	100,0	102,0	93,0		
	65	34,0	10,3	12,4	16,5	12,4	17,9	12,7	—	22,4	30,8	42,4		

up to the death of the animals.

The results obtained in dogs that were only esophagotomized on the whole resemble the same changes in dogs with isolated stomach, subjected only to irradiation (Table 3).

The decrease in the quantity and quality of juice, lengthening of the latent period of its secretion, and change in the shape of the secretion curve during the first five days were also observed in dogs subjected only to bloodletting, but from the seventh day on, a gradual normalization of all the indices investigated was noted.

The results of the investigations show that under conditions of radiation sickness of medium severity and blood loss, the secretory function of the gastric glands undergoes a serious disturbance, related to a considerable degree to a disturbance of the regulation of the conditioned reflex and neurohumoral mechanisms. At the height of acute radiation sickness, these disturbances are probably accompanied by morphological changes in the secretory cells of the stomach itself, and of the local nerve processes.

It should be mentioned that bloodletting, performed 25-30 min after irradiation, aggravated the course of radiation sickness, which also led to early death of the animals, while in the case of irradiation alone, (600 R), a substantial fraction of the animals remained alive, while in the group of animals subjected only to bloodletting, no cases of death were observed.

CONCLUSIONS

1. Bloodletting in a volume of 4% of the body weight of the animals causes a transitory decrease in the quantity and quality of gastric juice secreted, as well as a change in the latent period of its secretion. In this case both the conditioned reflex and the neurohumoral regulation of secretion are disturbed.

2. A single total irradiation at a dose of 600 R causes a wave-like variation of the gastric secretion and latent period of secretion of juice. Periods of hyper- and hyposecretion are observed. In this case primarily the conditioned reflex regulation of the activity of the gastric glands is impaired.

3. Irradiation, followed by bloodletting, causes a more profound disturbance of the regulation of secretory activity of the gastric glands, both in the first -- conditioned reflex, and in the second -- neurohumoral phases of the secretory period.

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DISTURBANCE OF THE STRUCTURE AND FUNCTION OF THE ISLETS OF LANGERHANS OF THE PANCREAS DURING RADIATION SICKNESS INDUCED BY RADIOACTIVE STRONTIUM

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pp. 214-218

The purpose of this investigation was to study the functional changes, microscopic and submicroscopic changes in the islet apparatus of the pancreas during chronic radiation sickness, induced by Sr^{89} and Sr^{90}.

EXPERIMENTAL SECTION

The experiments were conducted on 125 white rats and 25 guinea pigs. Radioactive strontium in the form of the salts $Sr^{89}Cl_2$ and $Sr^{90}Cl_2$, existing in equilibrium with Y^{90}, was introduced into the abdominal cavity in 1 ml of physiological saline in a dose of 0.32 microcurie/g once, or twice with a month interval.

The animals were killed at the following periods: after 30 min, one hour, two to five days following the first injection, during the first month, and then at 15-30 day intervals all the way up to 240 days in the more long-term periods. Fixation in 10% neutral formalin and in Bouin's, Gelmi's, and Shabadash's fluids was used to investigate the tissue under a light microscope. The slices were stained with aldehyde-fuchsin according to Gelmi in Dŷban's modification [1], with azane according to Heidenhain, impregnated with silver according to Foote, and stained for RNA according to Brachet and for DNA according to Feulgen. In certain cases the slices were stained with methylene blue to determine the physicochemical properties of the RNP of the mitochondria of the α- and β-cells [2]. The sugar content in the blood was determined according to the method of Hageron and Jensen.

Several hours after penetration into the organism, radioactive strontium is concentrated in the bones. Uniform irradiation of the

organism occurs only during the first hour. In the first hour the amount of energy absorbed per gram of the pancreas after the administration of Sr^{90} was about 0.9 rad, and in one day 3.7 rad; after the administration of Sr^{89} the values were 0.4 rad and 1.8 rad, respectively. In the first five days, the amount of absorbed energy increased by 1.2 rad/day after the administration of Sr^{90} and 0.5 rad/day after the administration of Sr^{89}. In the subsequent periods, the amount of absorbed energy increased by tenths of a rad per day after the administration of Sr^{90} and hundredths of a rad after the administration of Sr^{89}. The total amount of energy absorbed in the first month was 14.2 rad for Sr^{90} and about 7 rad for Sr^{89}. In the long-term periods, the irradiation dose rate decreased in the animals that had received Sr^{89} and remained at a constant level (about 0.1 rad/day) in the animals that received Sr^{90}.

After 30 min to one hour following administration of Sr^{90}, pericapillary edema developed in the pancreas; the nuclei of the α- and β-cells were polymorphic; fine vacuoles developed in the cytoplasm of many cells. After one day, the signs of edema were a maximum. Individual degeneratively changed cells appeared. On the third to fourth days, the polymorphism of the cells and their nuclei was intensified. On the seventh to 12th days, individual cells died; foci of micronecrosis appeared. On the third to seventh days, degeneration of the α- and β-cells were sharply pronounced, especially in the small islets. In the long-term periods (160-240 days), together with degeneration, signs of proliferation of the cells were detected, especially along the periphery of the islets.

From the 30th day on, an increase in the number of reticular collagen fibers and a thickening of the argyrophil substance of the connective tissue were detected, especially around the islets and their capillaries.

In one islet of healthy guinea pigs, there were an average of: 84% β-cells, 14% α-cells, 2% D-cells. In rats: 73.5% β-cells, 24% α-cells, 2.5% D-cells. The functional index β/α was equal to 6.2 for guinea pigs ($m = \pm 1.11$) and 2.7 for rats ($m = \pm 0.37$).

One of the earliest reactions of the islet apparatus to the penetration of Sr^{90} into the organism was degranulation of the β-cells and a decrease in the blood sugar level. During the first hours after the administration of Sr^{90}, the number of α-cells decreased by 10-16%. The functional index in rats increased to 4.0 ($m = \pm 0.4$, $p < 0.001$). Normalization of the ratio of α and β cells occurred by the end of the first day (24 hrs), and during the period of 3--12 days the number of α cells in the islets of rats and guinea pigs even somewhat increased (the functional index in guinea pigs was equal to 2.77 ($m = \pm 0.92$) and in rats 2.1 ($m \pm 0.52$)). After 4-28 days there was some normalization of the ratio of β and α cells and the number of granules in them was somewhat increased. On the 85th day the number of β cells increased. During the following days, the number of α and β cells was at the level of the upper normal limit. In the β cells, partial or complete degranulation was observed. In the long-term periods (160-240 days), the

islets contained many different forms of β cells with large or small nuclei.

Some decrease in the amount of DNA was detected in the nuclei of the β- and α-cells and the capillary endothelium on the third to sixth days. By 35th day, the amount of DNA detected in the endothelial nuclei was somewhat increased, in the nuclei of the α- and β-cells there was a concentration of DNA around the nucleoli and nuclear membranes. In the more long-term periods (70-150 days), the amount of DNA in many α- and β-cells was smaller than in the excretory cells. On the 180th to 240th days, in most of the nuclei of the α- and β-cells, there was somewhat less DNA than in the normal state. One to 20 days after the administration of Sr^{90}, the RNA content in the islet cells was somewhat decreased; after 20 to 30 days, the amount of RNA was appreciably increased in individual islet cells, and in almost all the cells after 100-240 days. After 107 days, the mitochondrial RNP of the α- and β-cells is detected at pH 2.4, the nuclear DNP at pH 2.6, while in the normal state they are detected at pH 3.2.

During the first month after the administration of Sr^{89}, changes occurred in the islets similar to those that developed after the administration of Sr^{90}; however, they were less pronounced. In the subsequent periods, qualitative differences appeared in the development of individual processes: the processes of regeneration after the administration of Sr^{89}, as a rule, ended in almost complete restoration of the islet structure, with few degenerative cells; the proliferation phenomena were less pronounced.

After repeated administration of isotopes, there were quantitative and qualitative differences both in the early and in the long-term periods. In response to secondary administration of Sr^{89} and Sr^{90}, edema was less pronounced, but it disappeared more slowly than after primary administration of the isotope. The number of hemorrhages increased; degeneration of the α- and β-cells was more pronounced, and changes in the walls of the capillaries and small vessels occurred to a greater degree.

In the more long-term periods after the administration of Sr^{89} and Sr^{90}, proliferation of the epithelium of the ducts and islet cells was observed. However, after the administration of Sr^{89}, proliferation of the islet cells frequently ended in the formation of new ducts and islet cells, while after the administration of Sr^{90}, cells with a changed structure more often arose as a result of proliferation.

On the 49th and 157th days, a test was made in individual experimental animals to determine the reactivity of the organism to sugar loading. For this purpose, the animal twice received 150 mg of glucose in 1 ml of water through a probe into the stomach, and the blood sugar level was determined at various periods after each administration.

It was established that normalization of the blood sugar after the administration of glucose is decelerated in the experimental rats; moreover, after a repeated administration of glucose there was a secondary increase in the blood sugar, which is evidence of functional insufficiency of the islet apparatus.

There are numerous data on the submicroscopic structure of the incretory islet cells of healthy animals [3-8]. We studied the submicroscopic structures of the β-cells and capillaries of the islet apparatus only in animals that received Sr^{90} in the form of single injection. The nuclei of the β-cells are among the most vulnerable organoids.

After 30 min to one hour following the administration of Sr^{90}, there is a loosening and frequently disappearance of the outer nuclear membrane and thickening of the inner nuclear membrane. During this period, there was a thickening of the chromatin and nucleolar structure.

Most of the mitochondria became wounded and vacuolized; the arrangement of the cristae was disturbed, and part of the cristae were decomposed. Sometimes there was an abrupt thickening of the mitochondria. The ergastoplasmic membranes lost their proper arrangement, became thickened, and some became fragmented. The total number of RNP grains decreased. The number of specific granules in the β-cells decreased; their polymorphism developed; the distinctness of the contours was lost, and the electron density was reduced. The pericapillary spaces were dilated as a rule during this period; specific granules of endocrine cells and many fine particles about 200 Å in size were frequently found in them.

Two to four days after administration of the isotope there was a gradual increase in the number of specific granules in the β-cells; their contours were indistinct, while their electron density was lowered. Beginning with the fourth to 12th days, many nuclei of the β-cells had no outer membrane; the inner membrane was thickened, and the amount of lumpy, dense chromatin at its surface increased; there was an enlargement and thickening of the particles of the finely dispersed chromatin fraction. The nucleoli increased in size and frequently took the form of a loose ball, consisting of thick interlaced strands. In the cytoplasm of the β-cells, unusually shaped stretched out homogeneous mitochondria appeared. The pericapillary spaces, as a rule, were constricted during these periods. The basal membranes were somewhat thickened. The pavement endothelium of the capillaries was also dense and thickened.

After 20-42 days, the nuclear membrane of the β-cells was most often single-contoured, and wide (up to 500 Å) pores were visible in it. The chromatin in the nuclei was frequently arranged in the form of large lumps around the nucleoli, which were enlarged during this period, but became denser and seemed homogeneous. Most of the mitochondria of the β-cells were circular shaped, loose, sometimes vacuolized (Fig. 1). The ergastoplastic membranes were most often smooth; the RNA grains varied in size and were freely arranged in the matrix. The Golgi apparatus consisted of five to six circular vesicles, surrounded by loose membranes. The number of specific granules in the β-cells varied, and their dimensions ranged from 0.1 to 0.5 μ. During these and subsequent periods, large round granules appeared in certain β-cells (Fig. 2).

On the 70th to 120th days, the outer membrane around many nuclei of the β-cells again became dense. In most of the nuclei the chromatin was fine grained and distributed uniformly over the nucleus (Fig. 3). During this period the polymorphism of the mitochondria was especially

pronounced. The ergastoplastic membranes were frequently arranged randomly. The RNA grains, as before, possessed varied shapes and densities. In certain β-cells, unusual formations appeared: circular or elongated cisternae, surrounded by loose membranes, on the surface of which were arranged grains about 200 Å in size (Fig. 3). Very often such cisternae

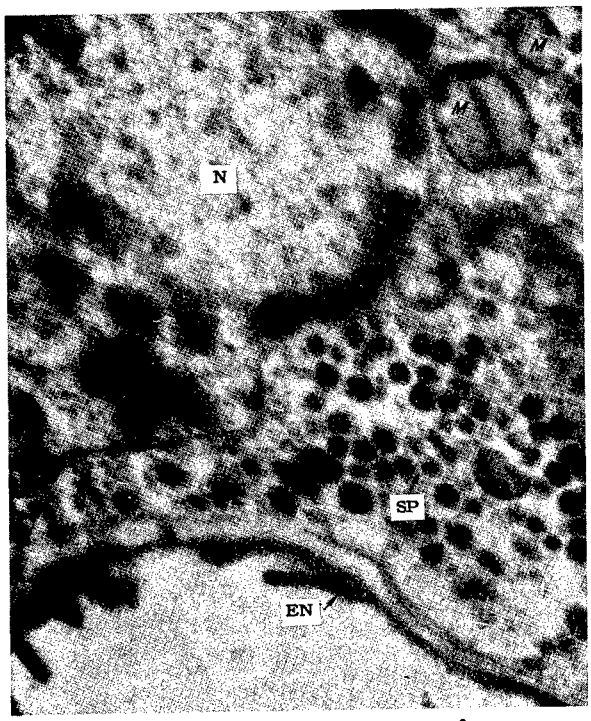

Fig. 1. Portion of capillary wall and β-cells of guinea pig on the 22nd day after administration of Sr^{90}. EN -- endothelium of capillary; M -- mitochondria; N -- nucleus of β-cells; Sp -- specific granules. Mag. x20,000.

were arranged in the pericapillary portion of the β-cells. During these periods the Golgi apparatus was frequently represented by vesicles, surrounded by two or three concentric membranes, or consisted of numerous tiny vesicles, forming a coiled chain (Fig. 2).

The number of specific granules in the β-cells varied; many large irregular-shaped granules, varying in density, appeared. The pericapillary spaces were sometimes filled with connective tissue fibers during these periods (Fig. 4).

DISCUSSION OF RESULTS

The administration of radioactive strontium during the early periods caused degranulation of most of the β-cells and a decrease in the blood sugar level. The changes in the β-cells may be explained by a decrease in the blood sugar content only during the first six to seven hours, i.e., during the time that the insulin penetrated into the blood

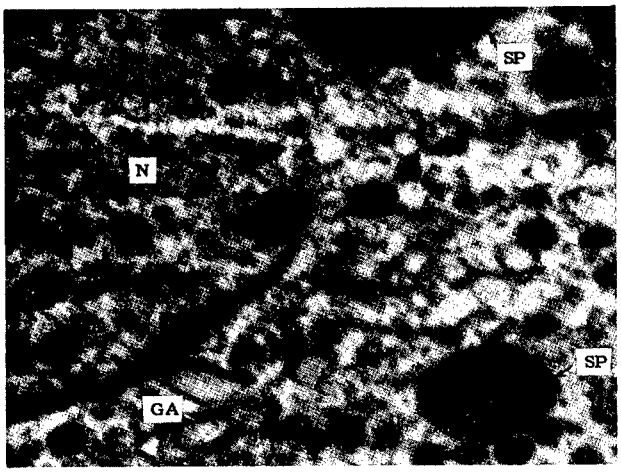

Fig. 2. Portion of β-cell of a rat on the 100th day after administration of Sr^{90}. GA -- Golgi apparatus. Remaining notations as in Fig. 1. Mag. x12,000.

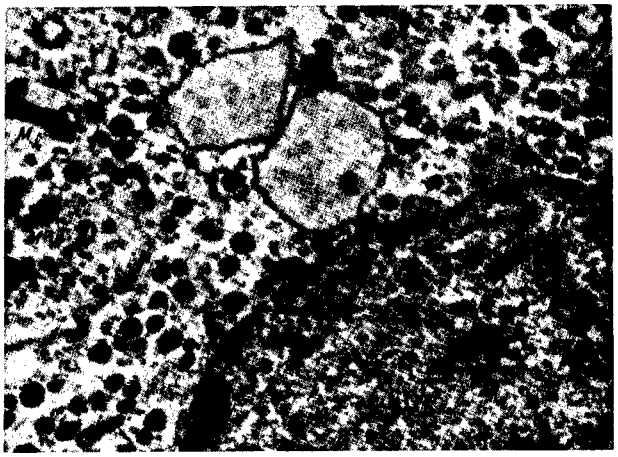

Fig. 3. Portion of β-cell of a rat on the 100th day after administration of Sr^{90}. Mag. x20,000.

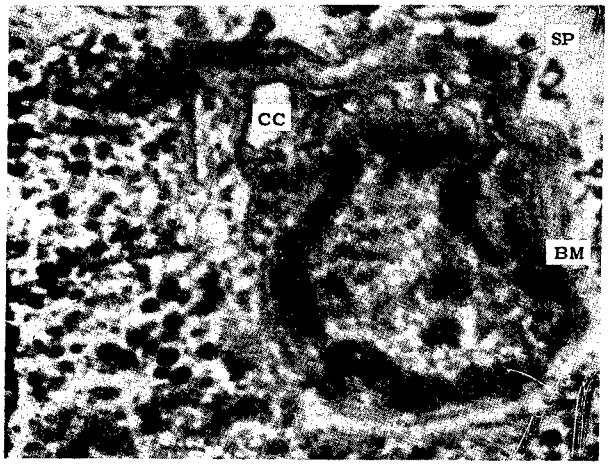

Fig. 4. Capillary from islet of the rat pancreas on the 100th day after administration of Sr^{90}. BM-- basal membrane; CC -- cavity of capillary. Mag. x13,000.

operates [9]. Subsequently the β-cells are degranulated; consequently, the reduced blood sugar level during this period depends not upon hyperfunction of the β-cells, but possibly on a certain decrease in the number of α-cells. After one to 12 hours, there was an appreciable increase in the number of α-cells, and this evidently promoted normalization of the blood sugar content. On the 12th to 30th days, the ratio of β- and α-cells approached the original ratio; their function may have been partially restored, as a result of which the blood sugar level remained within normal limits. The periodic increase in the number of β-cells during the long-term periods and the unusual response to sugar loading are evidence that there is no complete normalization of the function of the incretory cells. Such functional and structural changes might occur as a result of a disturbance of metabolic processes. The amount of DNA in the nuclei, especially in the long-term periods, was reduced: the macromolecular properties of DNA were changed, which is indicated by the enlargement and thickening of the chromatin lumps, the appearance of DNP and RNP in the nuclei at more acid pH values.

During the early periods, the amount of RNA decreased, while in the subsequent periods it increased somewhat, however, the properties of RNA were appreciably changed: it participates in the formation of large and dense cytoplasmic and nuclear grains of RNP. The structure and arrangement of the ergastoplasmic membranes, the basic function of which is to separate the enzyme and the substrate, are disturbed [10]; the structure of the Goldi apparatus is changed, and changes occur in the structure of the nucleoli, which, as is well known, are the basic

regulators of protein synthesis in cells [11, 12].

The data cited permit us to assume that at a definite state of radiation sickness there are irreversible changes in the β-cells, in the submicroscopic structures participating in the processes of oxidative phosphorylation, associated with the enzyme systems and protein synthesis; there are changed in the organoids and granules, in which insulin synthesis is usually accomplished, and it is accumulated. These structural changes may lie at the basis of the functional insufficiency of the islet apparatus during radiation sickness.

Another factor responsible for the insufficiency of the islet apparatus may be a disturbance of the permeability of the capillaries as a result of the progressive thickening of the basal membranes, pavement endothelium, and the appearance of elements of connective tissue in the pericapillary spaces.

CONCLUSIONS

1. The microscopic, submicroscopic, and functional changes described permit us to believe that in the long-term periods of radiation sickness in rats, signs of a light form of diabetes appear.

2. In the long-term periods, there is a disturbance of the process of tissue regeneration in the islet apparatus: proliferation of β-cells with unusual structure, cells of the epithelium of the ducts, and vigorous development of connective tissue are observed. During the process of pathological regeneration, atypical submicroscopic structures and organoids appear very early in the β-cells.

3. At the early stages of radiation sickness, the injurious action of β radiation is proportional to the amount of absorbed energy; in the long-term periods, the disturbance of regeneration of the islet apparatus is definitely dependent upon the level of chronic irradiation and depends less upon the amount of energy absorbed at the first stage of radiation sickness.

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INFLUENCE OF PLUTONIUM-239 ON THE FREE RIBONUCLEOTIDE METABOLISM IN THE RABBIT LIVER

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pp. 219-223

Readily hydrolyzed compounds of plutonium, penetrating into the blood stream, are deposited in the liver in substantial amounts [1-3], causing morphological changes of this organ [4]. We should have expected that the disturbance of the normal liver structure should be preceded by substantial changes in the basic biochemical processes that occur in this tissue. In the case of subacute and chronic injury by plutonium nitrate in experimental animals, the liver alkaline phosphatase activity is substantially increased [5], the intensity of urea synthesis is reduced [6], the amount of serum albumins is decreased, and the activity of the blood aminotransferases is increased [6]. Together with the inhibition of a number of processes specific for the liver, the rate of nucleic acid synthesis in this organ increases sharply after the introduction of plutonium [7, 8]. Most of the metabolic disorders detected arise before the existing cytological changes.

Considering the compulsory participation of the nucleoside di- and triphosphates, as well as a number of nucleotide derivatives, in most of the processes that occur in the liver (synthesis of nucleic acids, lipids, carbohydrates, glycolysis, synthesis of various proteins, formation of urea, and numerous other processes that occur with an energy expenditure), we investigated the free nucleotide metabolism of the liver at various periods after the administration of plutonium nitrate.

EXPERIMENTAL SECTION

The experiments were conducted on gray rabbits of both sexes, weighing 2.0-2.5 kg. The animals received a single intravenous injection of a solution of plutonium nitrate at pH ~ 2.0 in a dose of 7 microcuries/kg. The nucleotide content and intensity of the incorporation of radioactive phosphorus into these compounds were investigated one and 15 days, one, two, and six months after administration of the plutonium solution. Four to six animals were used at each period; five

rabbits served as the control.

To study the intensity of the metabolism of the nucleotide phosphate groups, the control and experimental rabbits received intravenous injections of a neutral solution of $Na_2HP^{32}O_4$, in a dose of 300 microcuries/kg of weight, 15 minutes before they were killed. The animals were killed by exsanguination under barbamil narcosis. The liver was frozen with dry ice, and all the subsequent treatment was performed at 0-5°. The nucleotides were isolated according to Kotel'nikova's method [9] and separated on the ion exchange resin Dowex I (x4200-400 mesh) in the formic acid form, as described in [10].

Inorganic tissue phosphorus was determined according to the method of Berenblum and Chain [11]. The intensity of the incorporation of P^{32} into nucleotides was expressed in the form of the relative specific activity (inorganic tissue phosphorus was taken as the precursor).

The data are presented in the form of arithmetic means with the error of the mean. The differences between the control and experimental values were considered reliable at the level of significance $p \leqslant 0.05$. The ionization doses were calculated on the basis of data on the plutonium content in the liver according to Morgan's formula [12].

One day after poisoning with plutonium nitrate, 92.1% of the amount of the radioelement introduced was detected in the livers of the rabbits. On the following days, the plutonium content in the liver decreased somewhat, but after six months it still comprised 47.7% of the introduced dose. As a result of such prolonged retention of the radioelement, the liver was subjected to substantial irradiation by high-energy α particles (5.16 MeV). The total ionization dose in the liver after one day was already 64.6 rad; after one month it was equal to 986 rad, and after six months to 5326 rad.

Data on the content of nucleotides of various degrees of phosphorylation in the rabbit liver are cited in Table 1.

One day after the administration of plutonium nitrate, the nucleoside polyphosphate content in the liver was distinctly reduced. The ADP, ATP, and GTP concentrations were 66.3, 20, and 41.3% of the control values, respectively. After two weeks, the nucleoside phosphate level was close to normal. After a month the ATP and GTP contents in one gram of liver were again considerably reduced (approximately two-fold). The lowest polyphosphate level was detected three months after the administration of plutonium, when the ATP and GTP concentrations were only 10.7 and 18.5% of the control, respectively. The ADP concentration was halved. The same low level of nucleoside polyphosphates was also found six months after the administration of the radioelement. As for the nucleoside monophosphates, their content in the livers of the experimental animals was higher in most periods than in the normal state. A day after the administration of plutonium, the concentrations of GMP, UMP, and IMP were equal to 145, 152, and 241% of the corresponding data for the control animals. After two weeks, the nucleoside monophosphate level did not differ significantly from the control values, while after a month there was a repeated increase in the content of almost all the nucleoside monophosphates. Three months after administration of the

Table 1

Content of Free Ribonucleotides in the Rabbit Liver at Various Periods After Injection of 7 Microcuries/kg Plutonium Nitrate (Micromoles per g of Crude Weight)

Substance	Control, (5)**	1 day, 64.6 rad, (4)	15 days, 420 rad, (4)	1 month, 986 rad, (5)	3 months, 2856 rad, (4)	6 months, 5326 rad, (6)
AMP	1,885±0,306	2,270*±0,218	2,120±0,051	2,350 ±0,113	2,735 ±0,305	3,193*±0,152
ADP	1,301±0,041	0,861*±0,108	1,204±0,125	1,258 ±0,094	0,620*±0,074	0,707*±0,104
ATP	0,945±0,123	0,189*±0,029	0,666±0,101	0,476*±0,084	0,101*±0,022	0,135*±0,020
GMP	0,145±0,018	0,211*±0,011	0,167±0,024	0,238*±0,019	0,262*±0,019	0,357*±0,019
GDP	0,092±0,011	0,106 ±0,027	0,154	0,114 ±0,007	0,059 ±0,007	—
GTP	0,092±0,015	0,038*± ,006	0,063±0,005	0,048*±0,002	0,017*±0,007	0,020*±0,002
UMP	0,221±0,044	0,335 ±0,021	0,236±0,035	0,311 ±0,042	0,314 ±0,020	0,365*±0,027
UDP	0,140	0,032			0,016	0,031
IMP	0,108±0,017	0,261*±0,050	0,209±0,053	0,279*±0,061	0,197 ±0,042	0,285*±0,035
CMP	0,050±0,005	0,035*±0,003	0,036±0,003	0,048 ±0,004	0,049 ±0,005	0,060 ±0,004
CDP-choline	0,043±0,003	0,031 ±0,003	0,036±0,006	0,030 ±0,006	0,026*±0,002	0,051 ±0,007
NAD	0,197±0,028	0,164 ±0,007	0,251±0,007	0,241 ±0,004	0,204 ±0,048	0,168 ±0,013
UDPAG	0,061±0,005	0,081 ±0,005	0,061±0,013	0,059 ±0,010	0,063 ±0,006	0,083 ±0,028
UDPG	0,033±0,007	0,053 ±0,010	0,034±0,006	0,035 ±0,007	0,049 ±0,002	0,120 ±0.025
UDPGA	0,236±0,020	0,159*±0,020	0,256±0,040	0,341 ±0,035	0,190 ±0,028	0,328 ±0,041

*Differences between data of control and experimental groups are significant, $p \leq 0.05$.
**The number of investigated animals is indicated in parentheses.

radioelement, the concentrations of AMP, GMP, and IMP were 145, 181, 142, and 183%, respectively. An even higher level of nucleoside monophosphates was detected after six months, when the concentrations of these substances were equal to 165-264% of the corresponding control. The increase in the nucleoside monophosphate content may be associated both with dephosphorylation of the corresponding polyphosphates and with decomposition of the ribonucleic acid in the tissue.

No regular changes in the NAD content and carbohydrate derivatives of UDP were detected in the livers of the experimental rabbits.

Data on the incorporation of labeled phosphorus into the nucleotides, presented in Table 2, show that the synthesis of liver nucleoside polyphosphates of the experimental animals was practically unchanged. We might note only an increase in the relative specific activity of GDP, which was 57% higher than the norm after one day, and exceeded the control by 32% after one month. One day after the

Table 2

Relative Specific Activity of Free Nucleotides in the Rabbit Liver After Injection of 7 Microcuries/kg Plutonium Nitrate

Substance	Norm, (5)**	1 day, 64,6 rad (4)	15 days, 420 rad, (4)	1 month, 986 rad, (5)	3 months, 2856 rad, (4)	6 months, 5326 rad, (6)
AMP	0,056±0,011	0,087*±0,006	0,072 ±0,006	0,064 ±0,005	0,054 ±0,004	0,060 ±0,011
ADP	0,837±0,100	1,004 ±0,024	0,912 ±0,030	0,871 ±0,046	0,904 ±0,031	0,804 ±0,073
ATP	1,601±0,169	1,909 ±0,133	1,716 ±0,066	1,746 ±0,141	1,855 ±0,147	1,207 ±0,201
GMP	0,025±0,005	0,022 ±0,004	0,030 ±0,009	0,015 ±0,004	0,023 ±0,002	0,012*±0,001
GDP	0,666±0,060	1,046*±0,169	0,733	0,883*±0,052	0,788 ±0,150	0,628
GTP	1,145±0,284	1,042	1,075 ±0,129	1,469 ±0,312	—	1,022 ±0,256
UMP	0,022±0,004	0,023 ±0,004	0,016 ±0,003	0,016 ±0,002	0,042* ±0,008	0,015 ±0,002
UDP	0,409	0,481	—	—	0,433	0,263
IMP	0,071±0,008	0,099*±0,006	0,077 ±0,022	0,060 ±0,008	0,072 ±0,008	0,063 ±0,012
CMP	0,010±0,002	0,019 ±0,006	0,014 ±0,002	0,013 ±0,004	0,026 ±0,005	0,010 ±0,001
CDP-choline	0,232±0,017	0,096*±0,014	0,102* ±0,020	0,114*±0,015	0,120*±0,008	0,054*±0,013
NAD	0,008±0,000	0,015*±0,001	0,008 ±0,001	0,010 ±0,001	0,009 ±0,001	0,014
UDPAG	0,131±0,014	0,174 ±0,015	0,153 ±0,019	0,186*±0,019	0,264*±0,023	0,260 ±0,054
UDPG	0,152±0,018	0,228	0,193 ±0,019	0,232*±0,024	0,364*±0,019	0,158 ±0,035
UDPGA	0,070±0,008	0,110*±0,014	0,096	0,087 ±0,012	0,113*±0,011	0,062 ±0,006

*For notations see Table 1.

administration of plutonium, radioactive phosphate was incorporated into AMP and IMP somewhat more intensively than in the normal state. During the subsequent periods of investigation, the activities of most of the nucleoside monophosphates did not differ significantly from the control values.

Noteworthy is the substantial decrease in the relative specific activity of CDP-choline, which was approximately half the normal value after one day, two weeks, one and three months, and was a quarter of the normal level after six months.

In most periods of the investigation, a substantial increase in the incorporation of radioactive phosphorus into the carbohydrate derivatives of uridine diphosphate was observed. After one month the activity of these substances was 25-54% above the norm, while after three months it exceeded the control values by 60-140%.

DISCUSSION OF RESULTS

Our data show that the plutonium deposited in the liver causes a disturbance of the free ribonucleotide metabolism. An especially significant change was noted in the content of nucleoside triphosphates. After only

one day, when the total ionization dose was equal to 64.6 rad, the concentrations of ATP and GTP were distinctly reduced. This may have been the initial response of the liver to the administration of a substantial dose of the radioelement. After a month (ionization dose 986 rad) the nucleoside triphosphate level was approximately halved, while after three and six months, when the ionization doses reached 2856 rad and 5326 rad, respectively, the content of these substances in one gram of tissue was five to ten times lower than normal. As for the nucleoside triphosphate synthesis, this process evidently occurred at the same rate in the livers of the experimental animals as in the control rabbits. In not one of the investigated periods was any significant decrease in the intensity of incorporation of P^{32} into the nucleoside triphosphates noted.

A substantial decrease in the concentration of one substance or another, with unchanged rate of synthesis, may occur only in the case of a greater consumption of it than normal. In our case, the decrease in the level of nucleoside triphosphates was probably due to a substantial degree to an intensification of the RNA and DNA synthesis, i. e., processes in which the nucleoside triphosphates serve as plastic material and energy donors. DNA synthesis in the rabbit liver is 3.5 times as intensive as normal one month after the administration of plutonium nitrate at a dose of 7 microcuries/kg, while after three months the specific activity of RNA is increased 2.5-fold, and that of DNA 5.5-fold [7].

The impression is created that in the case of subacute injury by plutonium, metabolism in the rabbit liver is shifted in the direction of such basic anabolic processes as DNA and RNA synthesis. It may be assumed that the further progression of anabolism, with catabolic processes inhibited, should lead to the development of tumors [13, 14]. Actually, in some of the animals that received the same dose of plutonium, four to five months after administration of the radioactive element, regenerative hepatomas and adenomas of the bile ducts were detected [4].

The sharp decrease in the nucleoside triphosphate pool that we detected in the early periods of plutonium injury may cause a serious disturbance of the coordination of metabolic processes in the liver, since the constant formation and sufficient level of substances with high energy bonds is one of the most important conditions for normal vital activity of the cells. Thus, inhibition of CDP-choline synthesis in the livers of the experimental animals may be attributed to a deficiency of ATP in the tissue. The change in the metabolism of CDP-choline, which necessarily takes part in phospholipid synthesis [15], may lead to a disturbance of this process, and is probably the cause of fatty distrophy of the liver [4].

It may be assumed that a deficiency of nucleoside triphosphates in the rabbit liver after the administration of plutonium nitrate is one of the factors responsible for the disturbance of such functions of this tissue as protein and urea synthesis [6].

The distinct increase in the rate of metabolism of carbohydrate

derivatives of uridine diphosphate in the livers of the experimental rabbits, beginning with one month after administration of the radioelement, is of interest. Since no simultaneous increase in the content of these substances was noted, evidently there was an intensified consumption of them. As is well known, carbohydrate derivatives of uridine diphosphate serve as cofactors in the synthesis of mucopolysaccharides [16]. Hence, their increased utilization indicates an intensification of the connective tissue metabolism and is a precursor of developing cirrhosis of the liver.

CONCLUSIONS

1. Plutonium nitrate, administered intravenously in a dose of 7 microcuries/kg, causes a substantial decrease in the nucleoside polyphosphate content in the rabbit liver. After one month, the ATP and GTP concentrations were approximately half the corresponding control values, while after three and six months they were nine and five times lower, respectively.

2. A distinct increase in the nucleoside monophosphate content was noted in the livers of the experimental animals.

3. In all periods of the investigation, a distinct decrease in the incorporation of radioactive phosphate into CDP-choline was detected, which may be the cause of the appearance of fatty dystrophy of the liver.

4. A certain increase in the rate of incorporation of P^{32} into the carbohydrate derivatives of uridine diphosphate occurred one month after the administration of plutonium, while after three months this process was 1.6 to 2.4 times as active as normal, which was accompanied by an intensification of the formation of connective tissue and the development of cirrhosis of the liver.

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DISTURBANCE OF THE ADAPTIVE POTENTIALITIES OF THE BLOOD-CLOTTING SYSTEM DURING RADIATION INJURIES

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pp. 224-227

The development of the hemorrhagic syndrome in acute radiation sickness, which is one of the basic causes of death of animals injured by ionizing radiation, is due to disturbances in the system of blood-clotting and changes in the vascular wall. The assertion of Allen, that an anticoagulant of the type of heparin appears in the blood after irradiation, has been refuted [1, 2]. During radiation sickness, the disturbance of blood-clotting is caused by inadequacy of the first phase of clotting, in connection with a deficiency of prothrombokinase in the blood [1, 3-7].

Injury to the clotting system of the blood and increased brittleness of the blood vessels create a threat of extensive hemorrhage. In the healthy organism, acute blood loss is accompanied by a response of increased blood clotting, promoting a cessation of hemorrhage [8-10]. Since radiation injuries may be combined with various types of traumas, it seems of practical importance to study whether such an adaptive response is preserved in an organism with a blood clotting system impaired as a result of radiation injury. On the other hand, a study of this question may cast light upon the role of prothrombokinase in the development of hypercoagulemia after acute blood loss.

EXPERIMENTAL SECTION

The experiments were conducted on 66 rabbits of both sexes, weighing from 2250 to 4150 g, on an empty stomach, without narcosis. Irradiation was performed on the RUM-11 apparatus at a voltage of 190 kV, current strength 10 mA, rate 9.5 R/min, filters 0.5 mm Cu and 1 mm Al, at doses of 800 and 1200 R.

The blood-clotting time was determined in the animals in a

Bazaron apparatus [14] with automatic thermoregulation; the numbers of thrombocytes and erythrocytes were determined by the method of direct count in a phase-contrast microscope, the thromboplastic activity according to Kudryashov and Ulitina [15], and the capillary strength according to the method of Borbeli [16]. All the investigations were conducted two to three times before irradiation at 10-day intervals, two weeks after irradiation, and also five and 30 min after bloodletting. Acute blood loss was induced by rapid bleeding of 25% of the blood from the femoral artery. Blood was collected for investigation before irradiation from the marginal vein of the ear, and after it from the femoral artery with silicone-coated cannulas.

The experimental results were treated statistically [17]. The reliability of the difference between the averages of the investigated indices was determined: 1) before irradiation and after irradiation; 2) before bloodletting and after bloodletting.

In the control series (10 rabbits), after bloodletting there was an abrupt acceleration of the blood clotting, which confirmed the results already described [11-13]. Five minutes after bloodletting, the clotting time decreased from the original 1334 to 486 sec ($p < 0.001$), and after 30 min to 651 sec ($p < 0.001$). This acceleration of blood clotting was not accompanied by any regular changes in the thromboplastic activity of the blood: in all cases the difference of the means was statistically insignificant.

Table 1

Influence of Acute Blood Loss on the Indices of the Blood Clotting System in Rabbits After Irradiation at a Dose of 800 R

Indices	Before irradiation	After irradiation		
		Before bloodletting	5 min after bloodletting	30 min after bloodletting
Clotting time, seconds	1334 * ±219,5	1732,7 ± 859	1263,8 ±705,1	1343,6 ±207,1
Thromboplastic activity, seconds	41,22 ± 5,3	86,84± 33,1	78,06± 22,5	65,59± 24,9
Thrombocytes, thous.	257 ± 52	243 ± 37	180 ± 54	164 ± 61
Erythrocytes, thous.	8170 ±423	7438 ±1523	5806 ±1706	5853 ±1414

*The figures were taken from the control series of experiments.

In five experiments of the second series, we investigated the influence of acute blood loss on the thromboplastic activity of the blood serum by the same method. The serum contains practically no thromboplastic activity, and both before bloodletting and after it, only traces of activity were detected.

In the third series of experiments, in 10 rabbits surviving after irradiation at a dose of 800 R (19 rabbits were used in the experiment),

after 14 days a substantial decrease in the thromboplastic activity was noted ($p < 0.002$), although the number of thrombocytes was not significantly changed (Table 1), i.e., just as in the investigation on rats [18], in our experiments on rabbits it was found that the inhibition of the thromboplastic activity of the blood may be caused by a qualitative, and not a quantitative change in the thrombocytes. Simultaneously with the drop in thromboplastic activity of the blood, the strength of the capillaries decreased by 49.93±14.37%.

Under the influence of bloodletting, although there was a shortening of the blood clotting time in the animals in seven experiments out of ten, in a statistical analysis, its shortening by 27.07% proved unreliable. The clotting time was an average of 77.54% of the initial value 30 min after bloodletting; the difference was also statistically insignificant. The changes in the thromboplastic activity, being so stereotypic after radiation injury, after blood loss, and in the healthy rabbits, did not exhibit any regular dynamics. The number of thrombocytes and erythrocytes increased regularly after blood loss.

Thus, radiation injury in a certain proportion of the animals prevents an increase in the blood clotting. This is evidently due to an inhibition of the thromboplastic activity in the organism. It is characteristic that the absence of hypercoagulemia is common among those animals in which the decrease in the thromboplastic activity was the greatest after irradiation. The results of these experiments differ from those that we obtained earlier on rabbits with the synthesis of prothrombin, thrombotropin, and other components of the prothrombin complex impaired under the action of dicoumarin [12]. A decrease in the prothrombin index below 20% did not disturb the response of increased blood clotting after bloodletting. For a more detailed verification of the hypothesis on the cause of the relationship between the response of hypercoagulemia and the prothrombokinase reserves of the blood, we conducted a fourth series of experiments on rabbits, in which the irradiation dose was increased to 1200 R. Nine out of 32 irradiated rabbits survived to the 14th day. Radiation injury was accompanied by a decrease in the number of erythrocytes and a substantial decrease in the number of thrombocytes and thromboplastic activity (Table 2). In comparison with normal animals, the blood clotting was slowed ($p < 0.001$). Just as in the preceding series of experiments, by the 14th day after irradiation the strength of the capillaries had decreased by 48.09% ($p < 0.001$).

Thus, in the case of a large irradiation dose, the drop in the thromboplastic activity is induced not only by qualitative, but also by quantitative changes in the thrombocytes. Among rabbits in this series bloodletting was not accompanied by an acceleration of the blood clotting while in most cases, 5 min after bloodletting even a small increase in the clotting time, 15% on the average, was noted.

The thromboplastic activity did not undergo any regular changes 5 min after bloodletting, while after 30 min, a tendency for an increase was detected. A similar tendency (of course, statistically unreliable) was also noted among healthy rabbits and in rabbits irradiated at lower

Table 2

Influence of Acute Blood Loss on the Indices of the Blood Clotting System in Rabbits After Irradiation at a Dose of 1200 R

Indices	Before irradiation	After irradiation		
		Before bloodletting	5 min after bloodletting	30 min after bloodletting
Clotting time, seconds	1334 *±219,5	4247±2914	4866±3157	4545±3266
Thromboplastic activity, seconds	60,6 ± 12,4	91,5± 30,5	96,0± 30,7	78,6± 18,6
Thrombocytes, thous.	424 ±106	266± 90	225 ± 82	204 ± 80
Erythrocytes, thous.	8376 ±442	6343± 718	5604± 848	5205± 940

*The figures were taken from the control series of experiments.

doses. Just as in the preceding series of experiments, the numbers of erythrocytes and thrombocytes decreased after bloodletting.

Thus, the increase in the thromboplastic activity detected 30 min after blood loss did not cause any increase in the number of formed elements of the blood.

CONCLUSIONS

Thus, injury to the clotting system of the blood, caused by ionizing radiation, is accompanied by an impairment of the adaptive potentialities of this system. When the prothrombokinase reserves of the organism are significantly reduced, the adaptive increase in the blood clotting in response to acute blood loss, which under normal conditions promotes a cessation of hemorrhage, disappears. The development of hypercoagulemia after acute blood loss with an intact blood clotting system includes a phase of thromboplastin formation as a necessary component. Hence, under conditions of a substantial insufficiency of this system, the appearance of the entire adaptive response is disturbed.

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BEHAVIOR OF STRONTIUM AND CALCIUM IN THE GOAT ORGANISM

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pp. 228-231

A study of the strontium and calcium metabolism in the human organism and in various animals [1-4] has indicated that together with great similarity in their behavior, there is also a substantial difference. In [5-7], it is noted that calcium, in comparison with strontium, is preferentially assimilated from the intestine, while the opposite picture is observed in the case of excretion with the urine.

Most of the available investigations have been devoted to the study of the principles of the behavior of strontium and calcium in the organism of laboratory animals and man, and substantially fewer to their behavior in farm animals. However, this question is of great interest, since farm animals are suppliers of food products and serve as a unique biological barrier between the external environment and man.

In this work we studied the comparative behavior of strontium and calcium in the lactating goat organism.

MATERIAL AND METHODS

The experiment was conducted on eight lactating goats three to four years of age, which were kept on a constant diet consisting of 1.5 kg of meadow hay, containing 5.3 g/kg of calcium and 1.9 g/kg of phosphorus, 0.5 kg of concentrates containing 1.5 g/kg of calcium and 3.2 g/kg of phosphorus, and 10 g of table salt, during a period of two months before the experiment.

The animals were divided into two groups (four goats in each group). The experimental animals received single administrations of a mixture of strontium-90 nitrate and calcium-45 chloride (with carrier) in a 1:2 ratio; the goats of the first group received 0.5 microcurie of strontium-90 and 1 microcurie calcium-45 per kg of body weight intravenously, while the goats of the second group received 5 microcuries strontium-90 and 10 microcuries calcium-45 per kg of body weight per os

The indicated methods of administration of the isotopes (intravenous and peroral) were used to determine the values of the descrimination of strontium with respect to calcium during assimilation.

The experiments lasted 24 hours in the first group, 72 hours in the second. During this time, the excreta (urine and feces) of the experimental animals were collected individually, and the milk was collected. All the animals were sacrificed at the end of the experiment.

Samples of blood, various bones of the skeleton (frontal bone, scapula, humerus, fifth and sixth thoracic vertebrae, fifth and sixth ribs), milk, urine, and feces were collected to determine the strontium-90 and calcium-45 contents. After preliminary preparation, the samples were ashed in a muffle furnace at a temperature of 600-700°. The ash residues obtained were exposed for two weeks for the accumulation of yttrium-90 to an equilibrium state with strontium-90, after which the activities of strontium-90 and calcium-45 were measured on the B-2 apparatus with a thin-window counter (1 mg/cm^2) T-25-BFL, according to the procedure proposed in [8]. In the calculation of the activity of calcium-45 in the samples, a suitable correction was introduced for radioactive decay. The error in the measurement of the activity of strontium-90 did not exceed 10%, and in that of calcium-45 -- 20%.

RESULTS AND DISCUSSION

An analysis of the data obtained indicated that the assimilation of strontium-90 and calcium-45 from the gastrointestinal tracts of the animals comprised 7 and 20% of the introduced dose, respectively. The excretion of strontium-90 and calcium-45 with the urine, feces, and milk in the case of intravenous and peroral administration of the isotopes is presented in Table 1.

Table 1

Excretion of Sr^{90} and Ca^{45} in Goats with Urine, Feces, and Milk

(in % of Introduced Dose)

Object of investigation	Method of administering isotope			
	Intravenous		per os	
	Sr^{90}	Ca^{45}	Sr^{90}	Ca^{45}
Urine	15,0	2,2	1,10	0,44
Feces	4,5	7,1	93,3	81,4
Milk	0,6	2,3	0,05	0,46

Table 2

Observed Ratios OR in Lactating Goats for Various Methods of Administration of the Isotopes

Link of biological system	Method of administering isotope	
	Intravenous	per os
Introduced activity — blood	0,42	0,22
Introduced activity — skeleton	0,40	0,24
Introduced activity — milk	0,14	0,08
Introduced activity — urine	4,8	3,0
Introduced activity — feces	0,40	—

From the table it is evident that the excretion of strontium-90 with the urine predominates over the excretion of calcium-45 for various pathways of administration of the isotope. Thus, in the case of intravenous administrations, the excretion of strontium-90 with the urine is seven times as great as that of calcium-45, while in the case of peroral administration it is 2.5 times as great. In the case of intravenous injection of the isotopes, 50% more calcium-45 is excreted through the gastrointestinal tract than strontium-90. The ratios of strontium-90 to calcium-45 in the milk, urine, and feces were determined up to the time of killing of the experimental animals, and after sacrifice of the animals, the ratios were determined in the blood and skeleton.

On the basis of the data obtained we calculated the observed ratios (OR), i.e., the ratios Sr^{90}/Ca^{45} of the sample to the Sr^{90}/Ca^{45} of the precursor. The numerical values of the observed ratios in various links of the biological system after intravenous and peroral administration of the isotopes are presented in Table 2. From the table it is evident that the observed ratios (OR) in the link introduced activity -- blood, introduced activity -- skeleton, and introduced activity -- milk in the animals of the first group are approximately twice as high as in the animals of the second group. The observed difference may be explained by the fact that calcium-45 is assimilated preferentially to strontium-90 from the gastrointestinal tract, i.e., there is a discrimination of strontium-90 with respect to calcium-45 in their passage through the intestinal wall, equal to 0.5.

In an analysis of the observed ratios obtained in the link introduced activity -- blood and introduced activity -- skeleton, in each series of experimental animals it was established that they are close. This evidently indicates the absence of discrimination of strontium-90 with respect to calcium-45 in the blood -- skeleton link. Analogous results were cited in [6, 7].

As can be seen from the data cited in Table 2, the OR skeleton -- diet and OR milk -- diet in the case of peroral administration of the isotopes are equal to 0.25 and 0.08, respectively. According to the data

of other authors [2, 5, 9, 10], the OR skeleton -- diet fluctuates within the range 0.17-0.5, while the OR milk -- diet ranges from 0.09 to 0.16. Thus, we establish that in lactating goats there is a discrimination of strontium with respect to calcium during assimilation, excretion with milk, and excretion with urine and feces.

In view of this, it was of interest to determine the fraction of activity of each discriminating unit in the total observed ration OR in the link diet -- organism and diet -- milk. The values of the contribution of each discrimination factor (DF) to the OR of organism -- diet were calculated according to the formulas cited below, proposed by Comar and coauthors [6].

The contribution of the discrimination of strontium-90 with respect to calcium-45 in the process of their assimilation from the gastrointestinal tract:

$$DF = \frac{100 - F_{Sr}}{100 - F_{Ca}},$$

in the case of excretion with the urine:

$$DF = \frac{(100 - F_{Ca}) - U_{Sr} \cdot \frac{100 - F_{Ca}}{100 - F_{Sr}}}{B_{Ca} + L_{Ca}},$$

in the case of excretion with the milk:

$$DF = \frac{B_{Sr}}{B_{Ca}} \cdot \frac{B_{Ca} + L_{Ca}}{B_{Sr} + L_{Sr}},$$

where F_{Ca} and F_{Sr} are the contents of calcium and strontium in the feces in percent of that which entered with the feed; B_{Ca} and B_{Sr} are the amounts of assimilated calcium and strontium in percent of that which penetrated with the feed; L_{Ca} and L_{Sr} are the amounts of calcium and strontium excreted with the milk, in percent of that which entered with the feed; U_{Ca} and U_{Sr} are the contents of calcium and strontium in the urine in percent of that which entered with the feed.

In the calculation of the OR of milk -- diet, instead of the value of the DF cited above for excretion with the milk, the DF of lactation -- milk is used; it is calculated according to the formula:

$$DF = \frac{L_{Sr}}{L_{Ca}} \cdot \frac{B_{Ca} + L_{Ca}}{B_{Sr} + L_{Sr}}.$$

As can be seen, the observed ratio OR is equal to the product of the corresponding contributions of each discrimination factor. The numerical values of the contributions of each discrimination factor to the total observed ratio OR and the observed ratio organism -- diet and milk -- diet are presented in Table 3. Comparing the calculated ratios in the diet -- organism and diet -- milk links with the corresponding

Table 3

Contributions of Each Discrimination Factor to the OR and Observed Ratios in Lactating Goats

Conditions of experiment	DF	OR	
		Calculated	Actual
In assimilation	0,35	—	—
In excretion with urine	0,8	—	—
In excretion with feces	1,03	—	—
In excretion with milk	1,0	—	—
Lactation – milk	0,32	—	—
Organism – diet	—	0,29	0,24
Milk – diet	—	0,09	0,08

observed ratios obtained experimentally, we can see that they are close.

An analysis of the contributions of each discrimination factor to the total observed ratio indicated that the basic contribution to the total discrimination in the diet -- organism link is made by the discrimination of strontium with respect to calcium in the process of their assimilation from the gastrointestinal tract, and the contribution of DF in the case of excretion with the urine is somewhat smaller, while in the OR of milk -- diet, the discrimination of strontium with respect to calcium in the process of their assimilation from the gastrointestinal tract and in the process of lactation make the basic contribution.

CONCLUSIONS

1. The behavior of strontium-90 and calcium-45 in the lactating goat organism differs: calcium-45 is better assimilated from the gastrointestinal tract into the blood and penetrates into the milk in larger quantities, while strontium-90 is preferentially excreted with the urine.

2. When the isotopes are administered per os, the observed ratio OR in the diet -- organism link is equal to 0.24, and that in the diet -- milk link 0.08.

3. The basic contribution to the total discrimination in the diet -- organism link is made by the discrimination of strontium with respect to calcium in the process of their assimilation from the gastrointestinal tract and in excretion with the urine, while that to the OR of milk -- diet is made by the discrimination of strontium with respect to calcium in the process of their assimilation from the gastrointestinal tract and in the process of lactation.

4. In the blood -- bone link, there is no discrimination of strontium-90 with respect to calcium-45.

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UNCONDITIONED REFLEXES OF THE PARIETAL AND VISCERAL PLEURA TO THE CARDIOVASCULAR SYSTEM IN HEALTHY AND IRRADIATED ANIMALS

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pp. 232-236

The need to study reflex influences from the pleura is dictated by practice, in particular, transthoracic surgery [1-3]. As has been shown by our experimental and clinical investigations and those of foreign authors, the visceral and parietal layers of the pleura are a powerful reflexogenic zone, exerting an influence upon the function of various organs and systems of the organism, frequently ending in pleuropulmonary shock [4-6].

Our investigations provided for a study of the nature of reflex influences from the visceral and parietal pleura upon the activity of the cardiovascular system before and after X ray irradiation of the animals. The data obtained were compared with the results of investigations of the reflexes from the vessels of the pulmonary circulation of the same animals in order to further clarify the question of the mechanism of the action of radiation.

PROCEDURE

The investigations were conducted in acute experiments on cats, using urethan narcosis. We investigated 30 animals. After opening the left chest cavity, respiration was maintained with the aid of an artificial respiration apparatus. The total blood pressure in the carotid artery was recorded with mercury and membrane manometers.

Definite portions of the parietal and visceral pleura were stimulated; a flat cotton wad 1.5-2 cm in diameter, moistened with $1:10^3$ and $1:10^2$ solutions of acetylcholine, were placed upon them. The stimulus was removed by removing the wad and wiping the given portion of the pleura with physiological saline. The animals were irradiated with X rays at a dose of 400 R at 205 kV, 15 mA, 0.5 mm Cu, 20 R/min, F = 50 cm. The reflexes from the pleura to the cardiovascular system were

investigated in all four periods of acute radiation sickness.

Vascular isolation of the pleura was not produced. Evidence of the fact that the observed changes in the activity of the cardiovascular system are caused by a reflex mechanism from a given reflexogenic field is the following: 1) the short latent period; 2) the beginning of the recovery period immediately after removal of the stimulus, 3) differences in the responses of the heart obtained in the case of stimulation of the pleura and in the case of control introductions of a stimulus into the general blood stream.

The results were evaluated by the method of variation statistics, using the Student index of significance. The probability of significance of the data on the change in the activity of the cardiovascular system under various influences was 99-99.9%.

EXPERIMENTAL SECTION

Stimulation of the receptors of both layers of the pleura by acetylcholine in intact animals is accompanied by substantial hypotension, comprising 45.5±10.4 mm of mercury when a $1:10^3$ solution of acetylcholine is used (Fig. 1, a, b, c,); when a $1:10^2$ solution is used the value of the depression is increased (Fig. 1, d). In the case of primary application of a $1:10^3$ acetylcholine solution, primarily a single phase depressor reaction is observed (Fig. 1, a, b); the influence of a $1:10^2$ solution most often leads to a two-phase decrease in the arterial pressure, when repeated and prolonged hypotension is observed after some recovery (Fig. 1, d).

The time from the beginning of the reaction to the maximum decrease in the blood pressure in single-phase reactions and in the first phase to two-phase reactions is 13.7±2.7 sec; only in this case was it lengthened to 2 min.

The beginning of the recovery period depends upon the strength of the stimulus: when a $1:10^3$ acetylcholine solution is used, recovery begins against a background of its action immediately after depression has been completed, i.e., 10-30 sec after the beginning of the reaction (Fig. 1, a, b, c), while when a $1:10^2$ solution is used, normalization of the arterial pressure occurs chiefly only after removal of the stimulus, no matter how long it acted (Fig. 1, d). Repeated stimulation of one pleural layer or the other leads to a lengthening of the recovery period and to the appearance of wave-like reactions (Fig. 1, a, b). These changes also occur in the case of primary stimulation of one of the layers of the pleura, if a number of influences on the other were imposed before this (Fig. 1, b, c).

Thus, in spite of some morphological (structure, innervation) and physiological responses, both pleural layers represent a single powerful reflexogenic zone [7].

In part of the investigations (primarily in the case of application of a $1:10^2$ acetylcholine solution), a positive inotropic effect is observed, along with the beginning of depression of the blood pressure

which sets in somewhat later, and its recovery, which ends earlier (Fig. 1, d). Evidently the experimentally observed decrease in the arterial pressure should be considered chiefly as a result of vasomotor reactions, which arise from the receptors of the pleura and are primary. The intensification of the heart activity, on the other hand, is a secondary, compensatory response, and arises from the vascular reflexogenic zones.

After irradiation of the animals, there are definite changes in the nature of the conditioned reflexes from the pleura.

The reflex hemodynamic changes become more prolonged and inert: the period of drop in the blood pressure is lengthened, the recovery period (even for a $1:10^3$ solution) begins basically after cessation of the action of the stimulus and is delayed up to several minutes (Fig. 2, a, b). An increase in the number of two-phase reflex responses, which comprise one third ot the total number, is observed on account of their appearance even when a $1:10^3$ solution is used (Fig. 2, b).

An intensification of the work of the heart is observed more frequently and is more pronounced during hypotension than among the intact animals, i.e., after the action of radiation there is an even greater dissonance in the reflex responses of the heart and vessels (Fig. 2, a, b, d). The period of the height of acute radiation sickness is the most severe; normalization of the reflex activity is gradual, so that some deviations are still noted 1-1.5 months after irradiation, together with the normal responses (Fig. 2, c, d).

A comparison of the data obtained on the same animal under the influence of acetylcholine on the chemoreceptors of the pleura and pulmonary vessels indicated that the reflexes to the cardiovascular system from the vessels of the pulmonary circulation undergo more profound changes after irradiation than the reflexes from the visceral and parietal pleura [8, 9]. This permits us to conclude that in the case of total X ray irradiation of cats, the pleura and pulmonary tissue are injured differently, and that radiation exerts an influence not only upon the central, but also upon the peripheral nervous system [10--12].

CONCLUSIONS

1. The visceral and parietal layers of the pleura represent a single powerful reflexogenic zone, from which reflex influences can be imposed upon the activity of the cardiovascular system.

2. The depressor character of the reflex in the case of stimulation of the pleural receptors by acetylcholine is the result of vasomotor reactions, while the reflex changes in the cardiac activity are secondary and play a compensatory role.

3. After irradiation of animals with medium doses of X rays, the unconditioned chemoreflexes from the pleura to the cardiovascular system undergo changes, which are the result of a disturbance of the functional state of the receptors of the pleura and central nervous system.

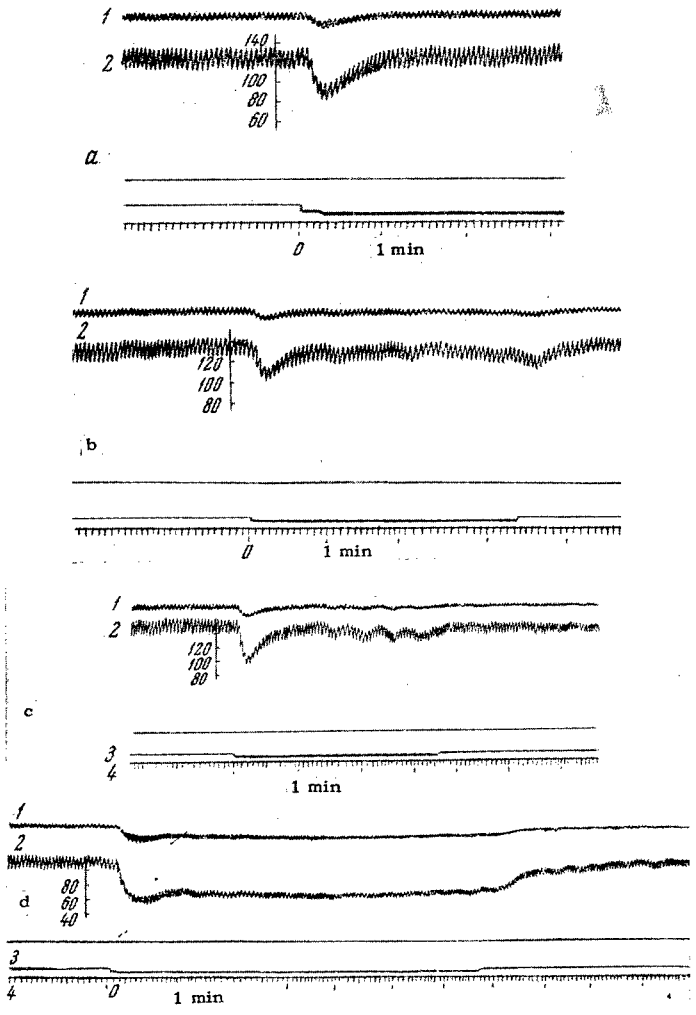

Fig. 1. a -- Experiment No. 171, nonirradiated animal; reflex changes in activity of cardiovascular system after application of a $1:10^3$ acetylcholine solution to the parietal pleura; b -- continuation, the same under an analogous influence; c -- continuation, the same after application of a $1:10^3$ acetylcholine solution to the visceral pleura; d -- continuation, the same after application of a $1:10^2$ acetylcholine solution to the visceral pleura. 1 -- Blood pressure in the carotid artery (membrane manometer); 2 -- the same (mercury manometer); 3 -- marking of introduction of stimulus; 4 -- time in 5 sec intervals. Scale -- value of blood pressure in mm of mercury.

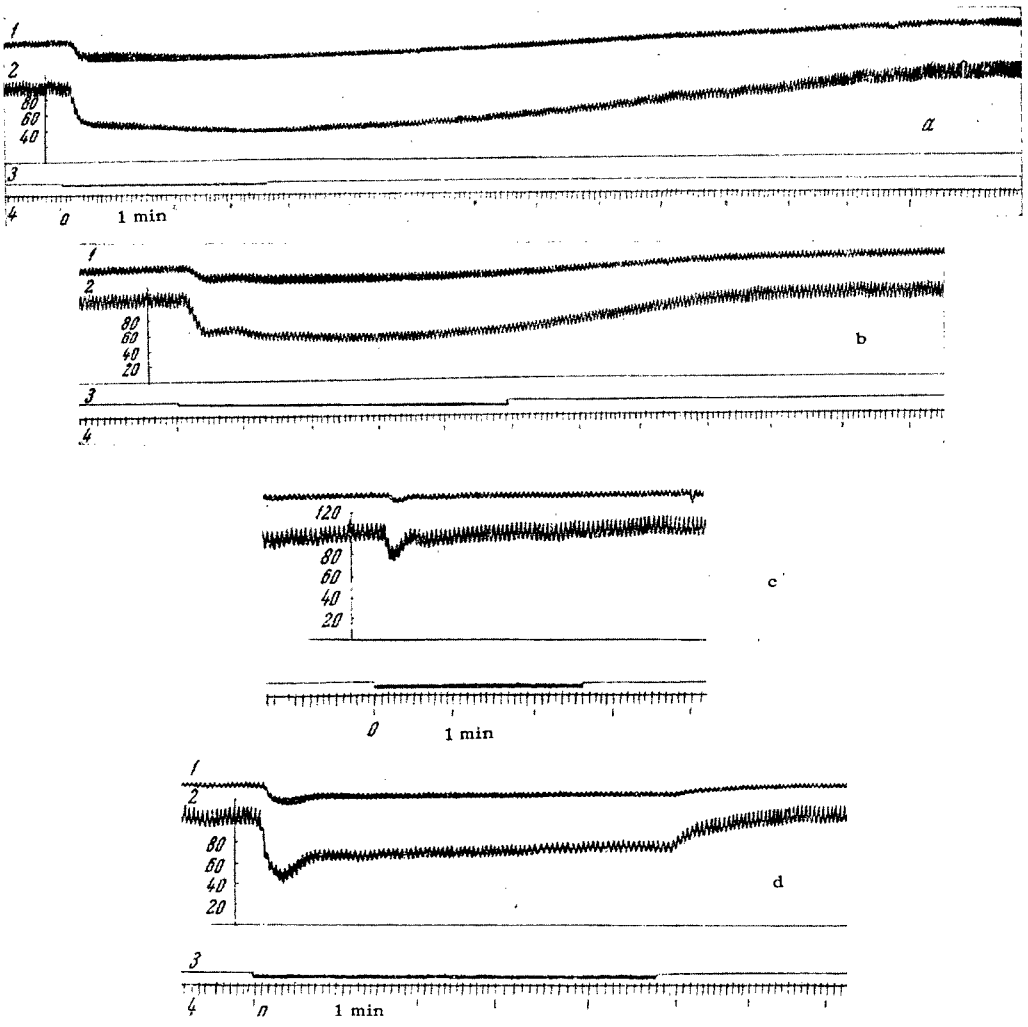

Fig. 2. a -- Experiment No. 173, irradiated animal (two days); reflex changes in the activity of the cardiovascular system after application of a $1:10^2$ acetylcholine solution to the parietal pleura; b -- experiment No. 172; irradiated animal (nine days); the same after application of a $1:10^3$ acetylcholine solution to the parietal pleura; c -- experiment No. 166, irradiated animal (44 days); the same after application of a $1:10^3$ acetylcholine solution to the visceral pleura; d -- experiment No. 166 (continuation), the same after application of a $1:10^2$ acetylcholine solution to the visceral pleura. 1, 2, 3, 4 -- the same as in Fig. 1.

4. The chemoreceptors of the pleura, as well as the vessels of the pulmonary circulation, respond differently to the effects of penetrating radiation; the greatest changes are undergone by the physiological peculiarities of the receptors of the pulmonary vessels.

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INVESTIGATION OF LOCAL AND LONG-RANGE INFLUENCES OF RADIATION UPON THE PROLIFERATIVE ACTIVITY OF THE BONE MARROW CELLS

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pp. 237-240

The inhibition of cell division is one of the manifestations of radiation injury to the hematogenic tissue, and a goodly number of investigations have been devoted to this question [1-7]. However, the mechanism of the decrease in an recovery of the mitotic activity of the cells in the hematogenic organs is rather complex and depends upon a number of factors, and with the abundance of variables, it is difficult to predict the nature of the change in the mitotic index after irradiation of such a mixed cell population as is, in particular, the bone marrow.

The task of the investigation was to study the mitotic activity of the bone marrow cells of irradiated mice under the action of absolutely lethal radiation doses upon the organism as a whole and in the case of its partial irradiation. For a comparison of the processes of change in the mitotic activity of the cells in various experimental variations we studied the dose curves for 200 and 400 R as well.

EXPERIMENTAL SECTION

The experiments were conducted on mice of both sexes of the C57Bl line, weighing 20-22 g. The animals were irradiated on the RUP-1 apparatus under the following conditions: 210 kV, 15 mA, filters 0.75 mm Al + 0.5 mm Cu, dose rate 50 R/min. Shielding was performed with a lead shield 5-8 mm thick. The animals received 0.2-0.5% of the total dose under the shield.

The following series of experiments were conducted: a) total irradiation of mice at doses of 200, 400, 700, and 1000 R; b) irradiation

of the body at a dose of 700 R, with shielding of one hind leg; c) irradiation of one hind leg at a dose of 700 R, d) irradiation of the excised small intestine at doses of 700 and 3000 R with shielding of the remaining portions of the organism. The animals were killed 2, 6, 24, 48, 72, 120, and 192 hours after irradiation, four to five animals in each period of investigation. The femurs of the irradiated and shielded limbs were fixed in Carnoy's fluid. The bone marrow was stained according to Feulgen, with final staining with light green. Squashes of the frozen preparations were prepared. The mitotic index was derived on the basis of a count of 1000-3000 cells, capable of division.

The number of dividing cells at all the radiation doses studied was sharply reduced in the first six hours after irradiation and fluctuated from 1 to 2%, exhibiting only a small dose dependence (Fig. 1). An increase in the mitotic activity was noted 24 hours after the influence at all the doses studied; the mitotic index still did not exhibit any significant differences by this period for the doses 400, 700, and 1000 R. Under the action of a dose of 200 R, the mitotic index exceeded the norm by this period. During the subsequent periods, the mitotic activity exhibited a sharp difference depending upon the irradiation dose. At a dose of 200 R, the mitotic index remained increased up to 72 hours, and then returned to normal.

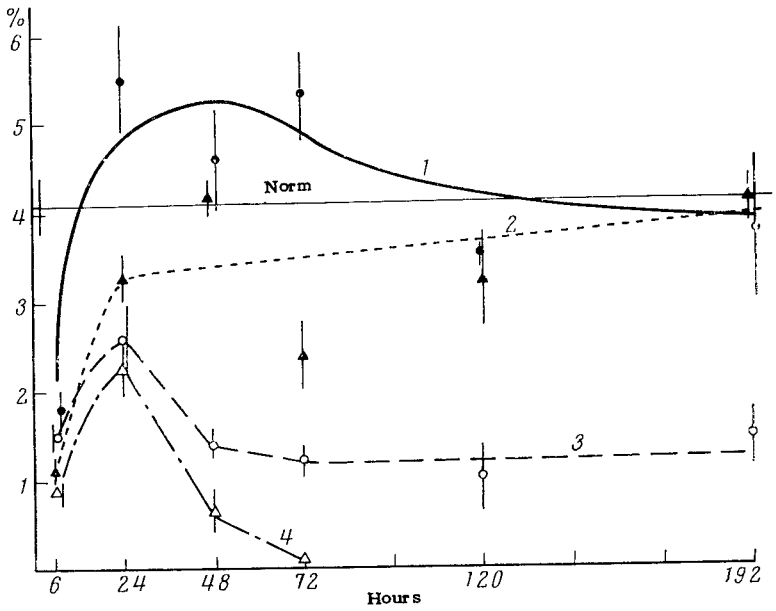

Fig. 1. Mitotic index of bone marrow cells of totally irradiated mice. 1 -- 200 R; 2 -- 400 R; 3 -- 700 R; 4 -- 1000 R; along X-axis -- time after irradiation, hours; along Y-axis -- mitotic index, %.

The number of dividing cells at a dose of 400 R gradually reaches the norm by the 8th day after irradiation, but in none of the investigated periods does it exceed the norm. Under the action of absolutely lethal doses of radiation, soon after the "abortive" rise in the mitotic activity, there is a sharp drop in it. Then at a dose of 700 R, the number of dividing cells is stabilized comprising 30-35% of the norm. The mitotic index of the bone marrow approaches zero by 72 hours after the influence of 1000 R. The results of an investigation of the long-range influences of radiation upon the mitotic activity of the bone marrow cells are presented in Fig. 2. It depicts the change in the mitotic index in the shielded bone marrow under the conditions of irradiation of other portions of the hematogenic system or intestines. Only in the early periods, i.e., two hours after irradiation in bone marrow cells not subjected to the direct action of radiation, is a decrease in the proliferative activity observed. Experiments on the irradiation of various portions of the body give evidence of nonspecificity of this reaction, since the inhibition of cell division occurs both during irradiation of the hematogenic system and during local irradiation of the small intestine.

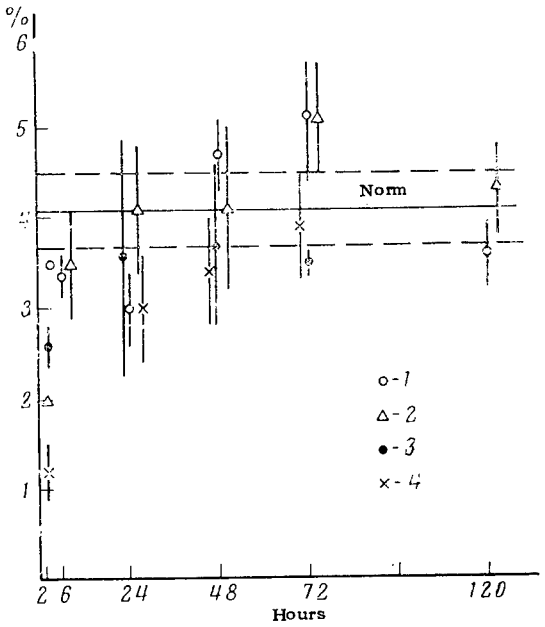

Fig. 2. Mitotic index of cells of the shielded bone marrow. 1 -- Irradiation of one hind leg at a dose of 700 R with shielding of other portions of the body; 2 -- irradiation of entire organism at a dose of 700 R with shielding of one hind leg; 3 -- irradiation of the small intestine at a dose of 700 R with shielding of other portions of the organism; 4 -- irradiation of the small intestine at a dose of 3000 R with shielding of other portions of the organism; along X-axis -- time after irradiation, hours; along Y-axis -- mitotic index, %.

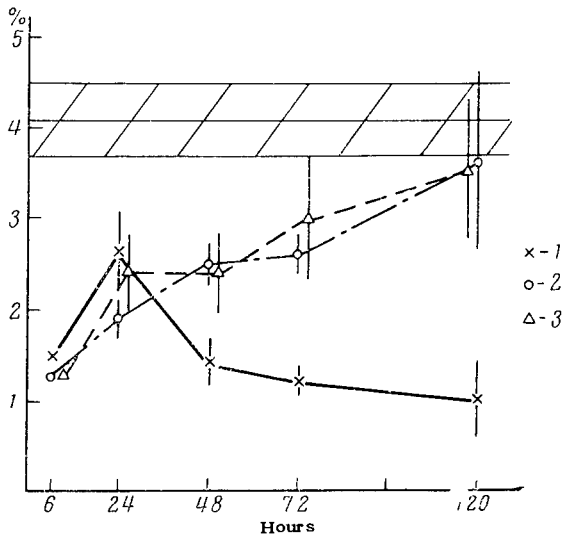

Fig. 3. Mitotic index of irradiated bone marrow. 1 -- Total irradiation at a dose of 700 R; 2 -- irradiation of one hind leg with shielding of other parts of the organism; 3 -- irradiation of entire organism with shielding of one hind leg; notations the same as in Fig. 2.

The action of radiation with shielding of one limb gives rise to a stronger long-range inhibition of the mitotic activity than irradiation only of one leg. The magnitude of the long-range effect also depends upon the radiation dose; irradiation of the small intestine at a dose of 3000 R causes a greater inhibition of cell division in the irradiated bone marrow than a dose of 700 R. However, this effect is detected only in the first two hours after irradiation. In the subsequent periods, the differences between the influences arising from the irradiated portions of the hematogenic system and intestines are expressed in the fact that in irradiation of the intestine, the mitotic index in the shielded bone marrow does not differ from the norm, while under the influence of radiation upon the hematogenic system, some activation of cell division is noted in its shielded portion after 48-72 hours.

The nature of the curves of the mitotic activity of irradiated bone marrow in experiments with shielding differs substantially from the analogous curve for totally irradiated mice (Fig. 3). Although in the first 24 hours after the influence at a dose of 700 R the mitotic index in all three experimental variations does not differ, after 48 hours the number of mitoses in the bone marrow of totally irradiated mice is lower and equal to 1.5%, remaining approximately at the same

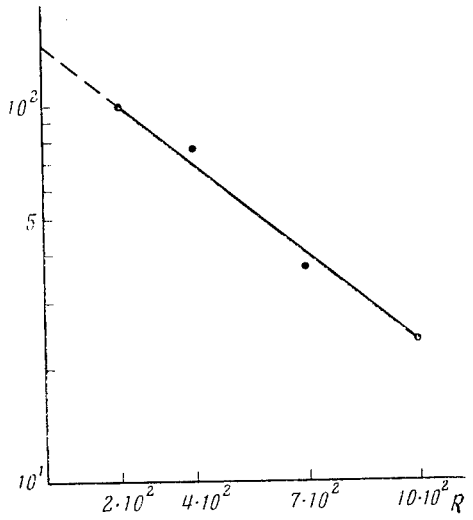

Fig. 4. Dependence of the number of dividing bone marrow cells on the radiation dose, % of norm. Along X axis -- irradiation dose, R; along Y axis -- average mitotic index for each investigated dose.

level all the way up to 170-192 hours. In the experiments with shielding of individual portions of the hematogenic system, the mitotic index in the irradiated bone marrow is gradually normalized. The increase in the mitotic activity does not depend upon the dimensions of the irradiated portion of the body.

DISCUSSION

Within the range of doses from 200 to 1000 R, during the first hours after the influence of radiation, there is no distinct dose dependence of the degree of inhibition of the mitotic activity of the cellular elements in the bone marrow. In the phase of the "abortive" rise in the mitotic activity, the dose dependence is also slight for absolutely lethal doses of radiation. In addition, if we take the mitotic index on the average from an eight-day period for each dose as the criterion, then the curve is exponential in character (Fig. 4), which may be used (together with other criteria [8]) to evaluate the dose absorbed by the organism in the case of total irradiation at doses from 200 to 1000 R.

An inhibition of the mitotic activity in the bone marrow also occurs under the long-range action of radiation. It evidently does not depend upon what tissue was directly irradiated, but depends upon the dimensions of the irradiated portion of the organism. The long-range effect is expressed only in an early and transitory inhibition of the

proliferative activity, while the long-range influence on the mitotic activity of the corneal epithelial cells of the mice sets in considerably later and is more prolonged [9]. The differences are evidently explained by the nature of the blood supply of these two types of tissues. The restoration of mitotic activity in the hematogenic tissue of totally irradiated mice depends upon the preservation of cells undamaged by radiation in the organism and increases with decreasing radiation dose. On the other hand, the activation of the cellular action in the irradiated bone marrow may occur on account of the presence of nonirradiated tissues in the organism, for example, the intestines [10]. However, the increase in the mitotic index in the irradiated bone marrow is substantially more intensive when nonirradiated portions of the hematogenic system are retained in the organism, which may be explained by migration of undamaged cells from the nonirradiated bone marrow. Thus, the intensification of the proliferative activity in the irradiated bone marrow is evidently due to hormone substances, arriving from the cells undamaged by radiation, and to migration of the cellular element from the shielded portions of the hematogenic system.

CONCLUSIONS

1. The degree of inhibition of the mitotic activity of the bone marrow cells exhibits a direct dependence upon the radiation dose (within the dose range 200-1000 R). During the first days, this dependence is weakly expressed, but it becomes distinct in the periods following irradiation.

2. The long-range effect of irradiation upon the proliferative activity of the bone marrow cells is transitory (two hours after the influence), depends upon the radiation dose, on the dimensions of the irradiated portion of the body, and has no tissue specificity.

3. The mitotic activity of irradiated bone marrow cells is rapidly restored when nonirradiated hematogenic tissue is present in the organism, which is explained by migration of undamaged cellular elements, as well as by the influence of humoral factors penetrating from the intact tissue.

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COMPARATIVE PHYSIOLOGICAL INVESTIGATION OF THE FUNCTIONAL STATE OF THE CENTERS OF SYMPATHETIC INNERVATION OF THE HEART UNDER THE ACTION OF IONIZING RADIATION

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pp. 241-245

A number of studies have established that after X ray irradiation the work of the heart is impaired [1-4], there are morphological and biochemical changes in the myocardium, coronary ganglia, and receptor apparatus of the heart [5-8]. In this case the hypothalamic influences [9] and influences from the vagus nerve centers upon the heart [10] are changed.

The task of this work was to study the functional state of the sympathetic centers exerting a tonic influence upon the activity of the heart, at various stages of acute radiation sickness, and in various species of animals in connection with species differences in the degree of expression of sympathetic influences upon the heart.

PROCEDURE

The investigations were conducted in chronic experiments on eight rabbits, nine cats, and five dogs and under conditions of acute experiments on 10 cats. The tonic influences on the heart along the sympathetic conductors were determined by cutting them off. For this purpose we used the blocking action of a sympatholytic -- dihydroergotamine, in chronic experiments, and extirpation of the stellate ganglia in acute experiments. The test for determination of the tone of the sympathetic centers was the degree of deceleration of the frequency of heart contractions (in percent of the initial level) to the intramuscular injection of dihydroergotamine (produced by Spofa Co.).

The cats and rabbits received dihydroergotamine in doses of 0.7 mg of the dry substance per kg of body weight in a 1:500 dilution (0.2% solution); the dogs received injections of 0.2 mg of the dry

substance per kg of weight in the same dilution. The pulse frequency was determined according to the electrocardiogram in the second takeoff with the animal unrestrained.

Before irradiation, three to four experiments were conducted on each animal with the administration of one of the indicated preparations. After irradiation, each animal received the preparation after 5-30 min and after 1, 3, 7, 14, 21, 28, and 35 days.

Total X ray irradiation of the animals was conducted on the RUM-11 apparatus under the following conditions: voltage 187 kV, current strength 15 mA, filters Cu 0.5 mm + Al 1 mm, focal length 60 cm for cats and rabbits, 100 cm for dogs; dose rate for cats 35.5 R/min, for rabbits 18.1 R/min, for dogs 13.8 R/min; the irradiation dose was 550 R for the cats, 1200 R for rabbits, and 700 R for dogs. In acute experiments, five cats were irradiated before operative intervention, while five served as a nonirradiated control. The material obtained was treated statistically.

EXPERIMENTAL RESULTS

The animals of different species were characterized by different degrees of slow-down of the heart contractions upon injection of the preparation: among the dogs, the decrease in the heart rhythm was an average of 44%, in cats 26%, and in rabbits 16%, which confirms the hypothesis of species differences in the degree of tonic accelerating influences on the heart [11, 12]. As a result of the radiation influence, severe radiation sickness developed in all the species of experimental animals. In two-month observations, two out of five dogs survived, three died; one out of nine cats survived, while eight died; three of the eight rabbits died, while five survived.

Several minutes after irradiation, changes were observed in the original heart rhythm. These changes were most pronounced in rabbits (29% increase in the heart beat frequency), less in cats (20%), and even less in dogs (17%) (Fig. 1).

Changes in the tone of the sympathetic centers were observed 30 min after the cessation of the influence in all species of experimental animals (Fig. 2, A). On the day of irradiation, there was a drop in the tone of the dogs and rabbits (Fig. 2, A). In the cats on the day of irradiation, the direction of the reaction was different: in six animals the response the the injection of dihydroergotamine was increased, while in three it was reduced (Fig. 2, A). At the subsequent periods of investigation, the reaction was in the same direction among all the animals of this group (Fig. 2, B).

The maximum changes in the test used among all the species of animals fell on the second day after irradiation. Although before irradiation the slowdown of the heart beat frequency was an average of 26% in the cats, one day after irradiation it reached 39%, while in dogs it decreased from 44 to 30%, and in rabbits from 16 to 6% (table).

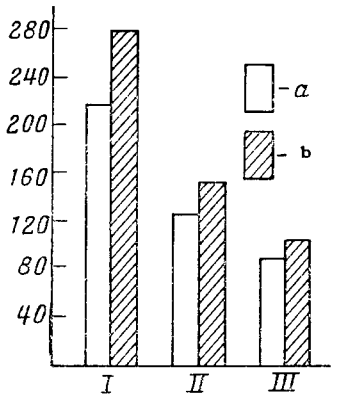

Fig. 1. Frequency of heart beat in rabbits (I), cats (II), and dogs (III). a -- Before irradiation and b -- after irradiation; along the vertical -- pulse frequency per min.

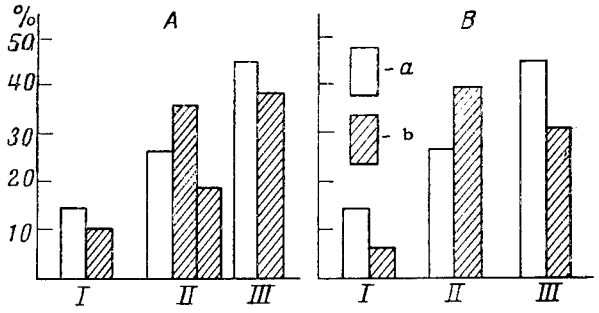

Fig. 2. Magnitude of the reaction to dihydroergotamine (slowdown of pulse in % of initial). I -- In rabbits; II -- cats; III -- in dogs; A -- on the day of irradiation; B -- after one day; a -- before irradiation; b -- after irradiation.

Change in the Response to Injection of Dihydroergotamine (According to Decrease in the Heart Beat Frequency in % of Initial Level) at Various Periods After Irradiation

Species of animals and number	Indices	Before irradiation	After irradiation (days)						
			Day of irradiation	1	3	7	14	21	28	35
Rabbits (8)	Heartbeat frequency	215	277	253	212	224	220	205	192	200
	Response to dihydroergotamine*	16	11	6	7	12	—	19	24	18
Cats (9)	Heartbeat frequency	125	150	155	143	136	180	180	—	150
	Response to dihydroergotamine*	26	29	39	27	18	16	25	—	26
Dogs (5)	Heartbeat frequency	85	100	70	84	75	80	65	80	67
	Response to dihydroergotamine*	44	38	30	34	29	35	35	32	30

*Degree of decrease in pulse frequency in % of initial level.

The dynamics of the change in the tone of the sympathetic centers of the heart during radiation sickness varied in the different species of animals (Fig. 3). In the preterminal period we observed a decrease in the sympathetic tone in dogs and cats, which agrees with the literature data [13].

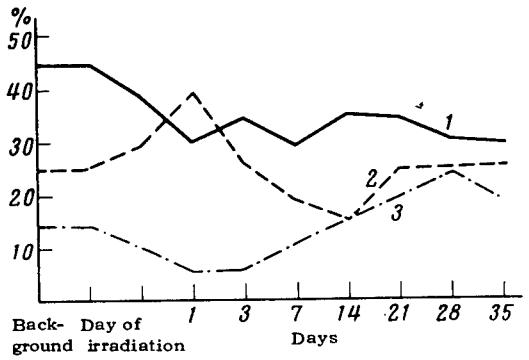

Fig. 3. Change in the degree of slowdown of the pulse in response to injection of dihydroergotamine at various periods of irradiation. 1 -- Dogs; 2 -- cats; 3 -- rabbits. Along horizontal -- experimental days; along vertical -- degree of slowdown of pulse in % of value of pulse before injection of preparations.

Removal of the stellate ganglia in a series of acute experiments caused an 11% slowdown of the pulse and a 17% drop in the blood pressure in the control cats, and a 22% slowdown of the heart beat frequency and 39% drop in the blood pressure among the irradiated animals. Thus, the data of acute experiments confirmed the data of chronic experiments on cats: the tone of the sympathetic centers of the heart increased 0.5 hour after the radiation influence.

A comparison of the results of our experiments with the results of Chang Ming-hua (Fig. 4) indicated that in rabbits and dogs the changes in the tone of the vagus nerve centers and the tone of the sympathetic centers mirror one another during the first three days after irradiation: an increase in the tone of the vagus nerve centers is promoted by a decrease in the tone of the sympathetic centers, while in cats an intensification both of the vagus and of the sympathetic influences upon the heart is observed during the same periods.

Treatment of the material obtained indicated that in dogs irradiation leads to a statistically reliable ($p > 95\%$) decrease in the heart beat frequency in percent of the initial level in response to the administration of dihydroergotamine (h.f.d.) in all the investigated periods of radiation sickness. In cats, according to the same index, significant changes were noted on the second day (increase in h.f.d.) and on the eighth day of radiation sickness (decrease in h.f.d.), and in rabbits on the day of irradiation (decrease in h.f.d.) and after 28 days (increase).

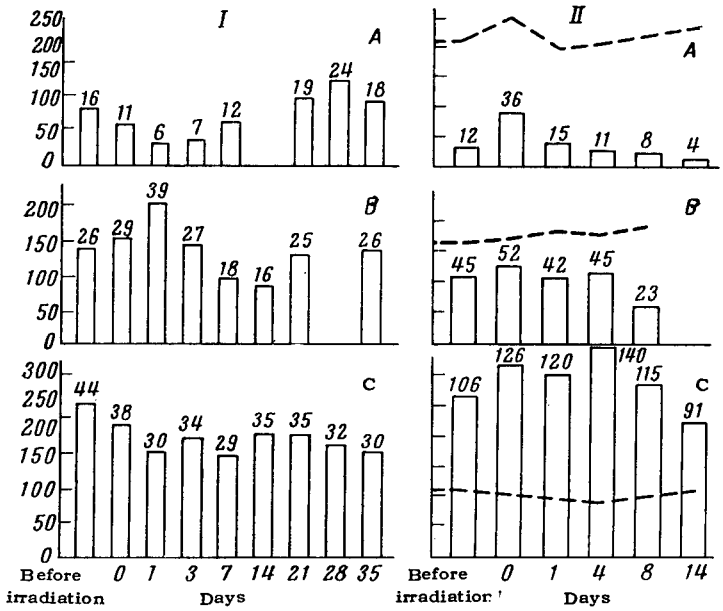

Fig. 4. Change in the heart beat frequency (%) in response to the injection of atropine (I -- from the work of Chang Ming-hua) and dihydroergotamine (II) at various stages of acute radiation sickness. A -- Rabbits, B -- cats, C -- dogs; along horizontal -- experimental days, along vertical -- heart beat frequency. Dotted lines -- change in heart beat frequency after irradiation; numbers above the columns -- magnitude of the reaction in % to the injection of atropine (I) and dihydroergotamine (II).

DISCUSSION

In our previous work, it was established that the functional state of the ganglia of the solar plexus is already changed 5-10 min after X ray irradiation of the animals [14].

The results of this investigation indicated that tonic accelerating influences on the heart in all species of experimental animals (cats, rabbits, dogs) are changed half an hour after the influence of ionizing radiation, and probably before. The dynamics of the changes in tone of the sympathetic centers differed in different species of animals: in cats the tone increased during the first three days after irradiation, while in dogs and rabbits it decreased.

However, as was shown by a comparison of the data of Chang Ming-hua [10] and our data, the dynamics of the changes in the tonic influences from the centers of the parasympathetic and sympathetic innervation of the heart after irradiation are of two types: of the

reciprocal type in rabbits and dogs, and of the type of mutual intensification in cats. In our opinion, this may be explained by species peculiarities of the animals. In the normal state, a dominance of the influences of the sympathetic centers upon the heart is observed in rabbits [11, 12], while a dominance of the parasympathetic centers is detected in dogs [15, 16]. After irradiation, they exhibit a similar nature of the changes in the functional state of the centers of vegetative regulation of the heart: an increase in the tone of the vagus centers is accompanied by a decrease in the tone of the sympathetic centers.

In cats, relative equalization of these influences may normally be assumed. After irradiation, the direction of the changes is the same in both systems.

In the mechanisms of the changes in the tone of the centers of sympathetic innervation of the heart, we may assume the participation of various portions of the sympathetic nervous system. Among these possibilities are morphological and biochemical changes in the myocardium, coronary ganglia, and receptor apparatus of the heart [5-8]. Under the action of ionizing radiation, as is well known, the afferent impulsation from various reflex zones is increased. These changes may also be due to functional changes in the peripheral ganglionic apparatus. As we demonstrated earlier, the excitability of the ganglia of the solar plexus is changed after irradiation [14].

Finally, changes in the tone of the sympathetic centers may be due to changes in the central portions of the sympathetic innervation of the heart: the hypothalamus [9] and cerebellum [9]. The participation of the reticular formation of the brain stem in the regulation of the tonic balance of the sympathetic and parasympathetic systems is also known [11, 18]. It is quite probable that one of the mechanisms of the change in the functional state of the sympathetic centers may be a direct or mediated action of ionizing radiation upon the reticular formation of the brain stem.

CONCLUSIONS

1. The tone of the coronary sympathetic centers (according to the change in the heart beat frequency in percent of the initial level to the injection of dihydroergotamine) is changed 30 min after X ray irradiation of animals with different balance of inhibitory and stimulatory influences upon the heart (cats, rabbits, dogs).

2. The maximum changes in the tone were observed in all species of animals one day after the action of ionizing radiation.

3. The dynamics and nature of the changes differed in different species of animals: in dogs and rabbits, a decrease in the tone was observed during the first three days after irradiation, and in cats an increase.

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FUNCTION OF THE RAT THYROID GLAND DURING REPEATED IRRADIATIONS AND ADMINISTRATION OF MERCAMINE

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pp. 246-249

The sensitivity of animals to the effects of radiation is related by some researchers to the level of basal metabolism in the organism, an indirect index of which is the functional activity of the thyroid gland [1]. Since preliminary irradiations with small doses of radiation changed the reactivity of the animal organism to the subsequent influences of ionizing radiations in lethal doses, a study of the thyroid function during repeated irradiations takes on special importance [2, 3]. On the other hand, it is known that one of the basic factors of the radioprotective action of mercamine is the decrease in the oxidative processes in the organism [4].

This investigation was conducted to determine the influence of repeated irradiations in conjunction with mercamine upon the functional activity of the thyroid gland. The work was conducted on male white rats 200-250 g in weight. A solution of radioactive iodine-131 was injected intraperitoneally, with an activity of 3 microcuries per rat. The standard corresponded to an activity of 1 microcurie.

The activity in the thyroid gland was determined according to the well-known method [5].

The pulses were counted 2, 6, 24, 48, 72, 96, and 120 hours from the time of injection of radioactive iodine. The time of count was 100 sec. Each count was repeated two to three times. In the case of a single irradiation with filtered X rays, a dose of 800 R was used. Preliminary irradiation was carried out in doses of 67 and 200 R 14 days before the repeated action of radiation at a dose of 800 R, which corresponded to $LD_{95/30}$, under the conditions of our experiments.

In a study of the influence of mercamine upon the thyroid function of the irradiated animals, the substance was administered in a dose of 150 mg/kg 15 min before irradiation. The control animals received injections of physiological saline in the same volume. The

radioactive iodine was injected an hour after irradiation. The average numbers of counts of each point in the experimental groups are represented in percent of the results of the determination obtained two hours after the administration of radioactive iodine-131 to control nonirradiated animals. The latter value was taken as 100%.

As can be seen from the table, in nonirradiated animals the absorption of radioactive iodine by the thyroid gland is rapid, and a maximum accumulation of it is reached 24 hours after the injection. After 48 hours there is a gradual, but rather intense elimination of iodine from the gland. After 72 hours, a little more than half the maximum accumulation remains in the gland.

Influence of Total Irradiation on the Absorption and Elimination of Radioactive Iodine-131 by the Thyroid Gland

Nature of group of animals		% of accumulation of I-131 with time (hours)						
		2	6	24	48	72	96	120
Nonirradiated		100	161±3,5	243±2,6	172±4,5	139±7,8	123±6,8	111±7,2
Irradiated, 800 R		81±2,1	114±3,0	129±3,9	148±3,5	137±4,2	138±4,9	123±6,3
Irradiated twice	67 p±800 R	65±7,0	83±5,3	84±4,4	92±4,0	88±3,7	81±7,9	58±5,3
	200 p±800 R	85±5,0	119±5,1	156±5,7	163±5,1	146±3,8	139±6,2	119±7,3

In the rats irradiated at a dose of 800 R, the absorption of radioactive iodine by the thyroid gland slows down. The maximum accumulation is reached 48 hours after the injection and comprises only 60% of the maximum accumulation of the control animals. The absorbed radioactive iodine is eliminated more slowly from the gland in comparison with the control animals. By the end of the observation period, in spite of the less intensive absorption, more iodine remains in the glands of the irradiated animals than in the control animals.

In rats irradiated at a dose of 800 R, two weeks after the preliminary irradiation, different results were obtained depending upon the dose of the preliminary irradiation. In the case of preliminary irradiation at a dose of 67 R, the absorption of radioactive iodine by the thyroid gland decreased even more. The accumulated iodine remained at the same level for a long period. The maximum accumulation corresponded to the period of the animals irradiated once, but the elimination of radioactive iodine from the gland was sharply inhibited.

In the case of preliminary irradiation at a dose of 200 R, data were obtained differing little from those corresponding to animals after a single irradiation. Certain differences lay only in the fact that iodine was more rapidly accumulated in the gland -- 24 hours after the

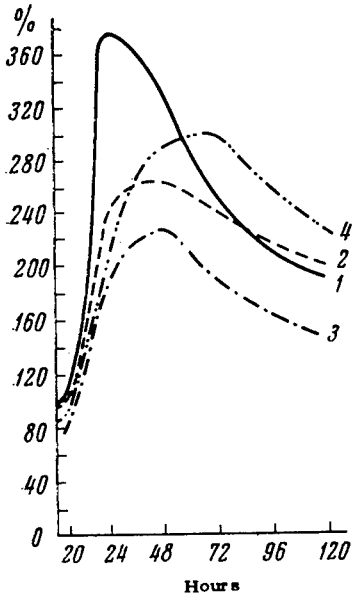

Fig. 1

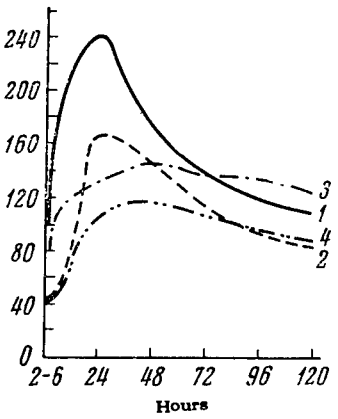

Fig. 2

Fig. 1. Effect of mercamine on the rat thyroid function after single and repeated irradiations: 1 -- nonirradiated animals with injection of mercamine; 2 -- animals irradiated once at a dose of 800 R with preliminary injection of mercamine; 3 -- two irradiations at a dose of 200 + 800 R with injection of mercamine before the second irradiation; 4 -- animals irradiated twice at a dose of 67 + 800 R with injection of mercamine before the repeated irradiation.

Fig. 2. Radioactivity of the thyroid gland in nonirradiated and irradiated rats with prophylactic injection of mercamine. Radioactivity of the thyroid gland: 1 -- control animals; 2 -- nonirradiated animals with injection of mercamine; 3 -- animals irradiated at a dose of 800 R with preliminary injection of mercamine.

influence, with a slight increase by the 48-hour period.

The results of an investigation of the effect of mercamine upon the thyroid function are presented in Figs. 1 and 2. When mercamine is administered to nonirradiated animals, a decrease in the incorporation of radioactive iodine into the thyroid gland is observed, but the nature of the absorption curve is retained.

In animals irradiated at a dose of 800 R with preliminary administration of mercamine, the absorption of radioactive iodine by the thyroid gland is also reduced in comparison with the control irradiated animals, but without injection of mercamine (Fig. 2).

In the group of rats with preliminary irradiation at a dose of 67 R, where the thyroid function was sharply reduced, in the case of prophylactic administration of mercamine before repeated irradiation, a sharp increase in the absorption of radioactive iodine was observed. At the point of maximum accumulation, i.e., after 48-72 hours, the amount of radioactive iodine in the gland was 60% higher in comparison with the group of animals irradiated twice, but without injection of mercamine.

In the case of prophylactic administration of mercamine to animals that received preliminary irradiation at a dose of 200 R, a decrease in the absorption of radioactive iodine by the thyroid gland is observed, in comparison both with animals irradiated once and with animals irradiated repeatedly, but without injection of mercamine (Fig. 1).

DISCUSSION

The results obtained on the decrease in the thyroid function after a single influence of a lethal dose of radiation are in full agreement with [6, 7], in which inhibition of the thyroid function under the action of lethal radiation doses and excitation of it at sublethal doses were demonstrated.

Preliminary irradiation changed the nature of the curve of absorption and elimination of radioactive iodine by the thyroid gland after repeated irradiation in comparison with a single irradiation at the same dose. The latter confirms the hypothesis [8] that each irradiation, even at low doses, changes the physiological state of the organism. There is no direct relationship in this case between the dose of preliminary irradiation and the level of functional activity of the thyroid gland. Such a lack of agreement may be due to many mechanisms. However, the most important is evidently a phase variation of the functional activity of the thyroid gland, caused by the action of various doses of preliminary irradiation.

A pronounced influence of prophylactic administration of mercamine upon the function of the thyroid glands of animals subjected to preliminary irradiation is evidence that a single irradiation of the organism at comparatively low radiation doses may entirely change the nature of the response to the action of a given pharmacological agent.

The results obtained in this investigation do not permit us to determine whether this is an indication of an intensification of the compensatory potentialities of the organism in connection with the use of mercamine, or, on the contrary, of their exhaustion.

CONCLUSIONS

1. After X ray irradiation of rats at a dose of 800 R, a decrease in the thyroid function is observed. The nature of the curve of absorption and elimination of radioactive iodine is thereby changed,

with a shift of the absorption maximum to a later period after the injection of iodine in comparison with nonirradiated animals.

2. In the case of repeated irradiation at a dose of 800 R, 14 days after a preliminary irradiation at a dose of 67 R, the absorption of radioactive iodine by the thyroid decreases even more. In the case of preliminary irradiation at a dose of 200 R, the absorption of iodine by the gland exceeds the level observed in animals irradiated once.

3. Mercamine, administered to nonirradiated animals and before a single irradiation at a dose of 800 R, leads to a decrease in the thyroid function; however, the nature of the curve of the absorption of radioactive iodine is unchanged.

4. The administration of mercamine before repeated irradiation at a dose of 800 R, after preliminary irradiation at a dose of 67 R, leads to a sharp increase in the thyroid function in comparison with the control repeatedly irradiated animals, without the administration of mercamine. In the case of preliminary irradiation at a dose of 200 R, the thyroid function is sharply inhibited in comparison with the control.

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REFLEXES FROM MECHANORECEPTORS OF THE UPPER RESPIRATORY TRACT DURING RADIATION SICKNESS

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pp. 250-253

It is known that the upper respiratory tract is an important reflexogenic zone, participating in the regulation of the activity of various organs and systems of the organism [1-10]. It is also known that the nervous system and especially its afferent and central portions are sensitive to penetrating radiation [11-13]. A phase variation of the sensitivity of the interoceptors and the magnitude and nature of the reflexes from them has been noted after irradiation, as well as a morphological change in these receptors during radiation sickness [11-20]. These changes in the afferent systems, in the opinion of Livanov, are among the factors that change the functional state of the central nervous system and are responsible for the development of radiation sickness [11].

In this work we determined the state of the reflex function of the upper respiratory tract during radiation sickness in 10 cats, in five on the third to seventh days after irradiation, and in five on the 13th to 16th days after irradiation. As a control, an analogous investigation was conducted on 10 nonirradiated cats. Irradiation was conducted once at a dose of 400 R on the RUM-3 apparatus under the following conditions: the cats were fixed in a position on the stomach with focal length 60 cm without tube, voltage 180 kV, current strength 20 mA, dose rate 23.4 R/min, filter 0.5 cm Cu + 0.5 cm Al. The presence of radiation sickness was confirmed by a 200-300 g drop in weight of the irradiated cats (about 1/8-1/10 of the initial weight) and by a two to three-fold decrease in the number of leukocytes. A morphological investigation of the bone marrow, conducted after each experiment, indicated substantial plethora and irritation of the bone marrow and necrobiosis of the hematogenic elements in the early period, and proliferation of the reticular elements in the later period.

The reflexes from the mechanoreceptors of the trachea, the sub-

and supraligamental spaces of the larynx and glottis upon respiration and blood pressure were investigated in cats under light urethan narcosis in acute experiments. Stimulation of the mechanoreceptors was accomplished by blowing up a little rubber balloon, connected to a rubber bulb and mercury manometer. The balloon was introduced into the trachea, larynx, and glottis through the head end of the trachea, cut between the seventh and eighth tracheal rings. The blood pressure was recorded in the carotid artery with a mercury manometer and membrane tonometer, while the respiration was recorded with the aid of a sleeve placed over the chest of the animal and connected to a Murray capsule. To judge the state of the general excitability of the central nervous system, we determined the threshold of stimulation of the femoral nerve by induced current.

In nonirradiated cats, stimulation of the mechanoreceptors of the trachea, the sub- and supraligamental space of the larynx and glottis caused primarily a pressor response of the blood pressure and a slowdown or brief stoppage of respiration. With increasing strength of the stimulus, these responses increased. A gradualness of the responses was noted, depending upon the area of stimulation. The most pronounced reactions were observed from the mechanoreceptors of the glottis, somewhat less from the mechanoreceptors of the supra- and subligamental spaces of the larynx, and the least from the mechanoreceptors of the trachea (Fig. 1). The threshold of stimulation of the mechanoreceptors of the glottis were lower, those of the mechanoreceptors of the supra- and subligamental spaces of the larynx somewhat higher, and the thresholds of stimulation of the mechanoreceptors of the trachea the highest (Table). Adaptation of the reflexes from the mechanoreceptors of the trachea, larynx, and glottis set in 40-60 sec after the beginning of stimulation. The threshold of stimulation of the femoral nerve was equal to 17-18 cm, the distance between the primary and secondary coils of the apparatus itself.

In cats on the third to seventh days after irradiation, stimulation of the mechanoreceptors of the trachea, sub- and supraligamental spaces of the larynx, and glottis induced a substantial slowdown or stoppage of respiration and two-phase reactions of the blood pressure (negligible depressor, alternating with substantial pressor), sometimes exceeding the action of the blood pressure in nonirradiated animals. The thresholds of stimulation of the mechanoreceptors of the trachea, larynx, and glottis were the same as in nonirradiated cats. An increase in the intensity of the stimulus did not cause any increase in the reactions of the respiration and blood pressure. There was no gradation of the reflexes depending upon the site of stimulation. Identical stimulation of the mechanoreceptors of the trachea, larynx, and glottis caused approximately the same reactions (Fig. 2). The adaptation of the reflexes and the thresholds of stimulation of the femoral nerve did not differ from the norm.

On the 16th to 17th days after irradiation, the reflexes from the mechanoreceptors of the trachea, larynx, and glottis upon respiration and the blood pressure were no longer detected in three cats,

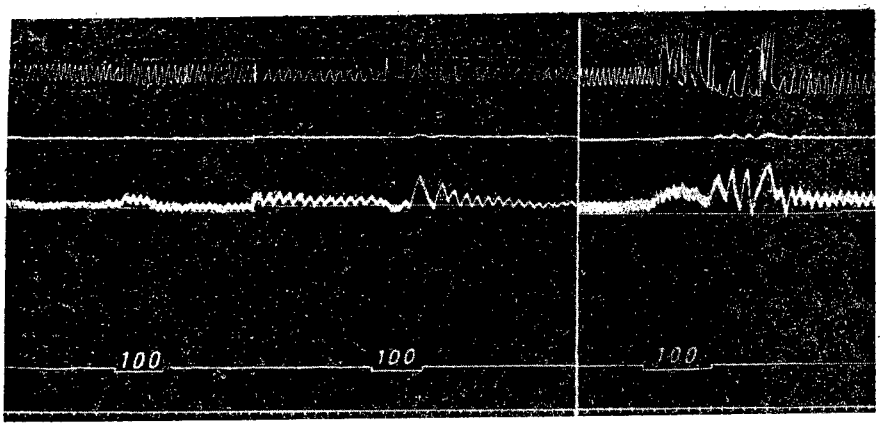

Fig. 1. Reflex changes in the respiration and blood pressure after stimulation of the mechanoreceptors of the trachea (left), supraligamental space of the larynx (in center), and glottis (right) with an intensity of 100 mm of mercury in a nonirradiated cat. Upper curve -- recording of respiration; second -- recording of blood pressure with a membrane tonometer; third -- recording of blood pressure with a mercury manometer; fourth -- marking of stimulation; fifth -- marking of time (5 sec).

Thresholds of Stimulation of Mechanoreceptors of the Trachea, Sub- and Supraligamental Spaces of the Larynx and Glottis in mm of Mercury, During Radiation Sickness

(According to Reaction of Blood Pressure)

Portions of upper respiratory tract	No of experiment and days of disease										Non-irradiated
	1/3	2/4	3/5	4/6	5/7	6/13	7/14	8/14	9/15	10/16	
Trachea	100	100	100	100	100	No reaction	No reaction	No reaction	120	No reaction	100
Subligamental space of larynx	80	80	100	80	80	120	No reaction	No reaction	No reaction	No reaction	80
Supraligamental space of larynx	60	60	60	60	60	100	No reaction	No reaction	120	No reaction	60
Glottis	50	40	40	40	40	120	No reaction	No reaction	140	No reaction	40

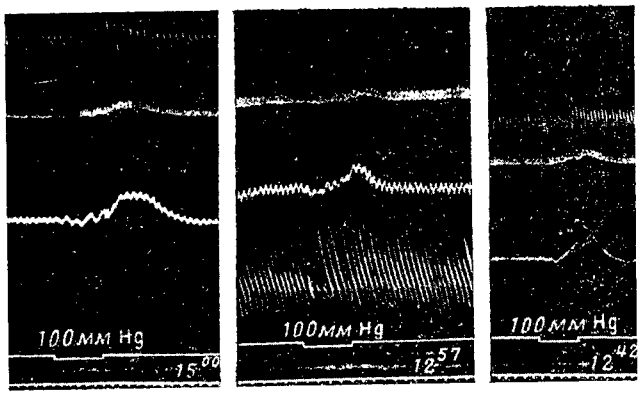

Fig. 2. Reflex changes in respiration and blood pressure after stimulation of mechanoreceptors of the trachea (left), supraligamental space of larynx (in center), and glottis (right) in a cat on the fifth day after irradiation. Notations the same as in Fig. 1. In the central figure: upper curve -- recording of blood pressure with a membrane tonometer; second -- recording of blood pressure with a mercury manometer; third -- recording of respiration.

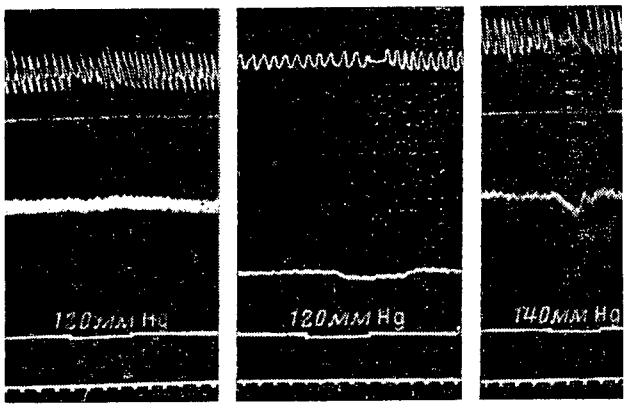

Fig. 3. Threshold changes in respiration and blood pressure after stimulation of mechanoreceptors of the trachea (left), and supraligamental space of the larynx (in center) with an intensity of 120 mm of mercury and of the glottis (right) with an intensity of 140 mm of mercury in a cat on the 15th day after irradiation. The notations the same as in Fig. 1. In center there is no recording of blood pressure with a membrane tonometer.

while in two they were very weakly expressed. Together with pressor reactions, depressor reactions were also encountered. An increase in the intensity of the stimulation caused no increase in the reactions. There was no variation of the degree of expression of the reactions depending upon the site of injury (Fig. 3). The thresholds of stimulation of the mechanoreceptors of the trachea, sub- and supraligamental spaces of the larynx, and glottis were sharply increased (see table). Adaptation of the reflexes set in 30-35 sec after the beginning of stimulation. The threshold of stimulation of the femoral nerve was equal to 12-13 cm.

From the work it may be concluded that during radiation sickness there is a disturbance of the reflex function of the upper respiratory tract, the nature and depth of which depend upon the stage of development of radiation sickness. During earlier periods of the course of the disease, there are negligible, finer changes in this function, evidently more related to a change in the excitability of the receptors of the upper respiratory tract than the corresponding centers. During later periods, a substantial inhibition of the reflex function of the upper respiratory tract arises, possibly due not only to a change in the excitability of the receptors situated in them, but also to a change in the excitability of the central nervous system as a whole.

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HISTOCHEMICAL CHANGES IN THE RAT ADRENAL CORTEX DURING ACUTE RADIATION SICKNESS

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pp. 254-258

The functional changes in the adrenal cortex during radiation injuries of the organism are of a nonspecific phase character [1-3]; the cortical substance reacts as a whole [4-7]. Histochemical methods have shown that after the influence of ionizing radiation upon animals, the content of lipids and cholesterol [2, 8], ascorbic acid [8, 9], and certain enzymes [10] in various zones of the glands changes. The dynamics of the content of steroid hormones, most fully characterizing the specific function of the adrenals, has received little histochemical study [9]. The purpose of this investigation was a comparative study by histochemical methods of the content of ketosteroids, lipids, and ascorbic acid in the cortical substance of the rat adrenals, during acture radiation sickness.

EXPERIMENTAL SECTION

The experiments were conducted on 127 male rats weighing 200-250 g. A single total X ray irradiation of the animals was performed on the RUM-11 X ray apparatus under standard conditions (187 kV, 15 mA, 40 cm, 0.5 mm Cu and 1 mm Al, 40.3 R/min) for total dose of 800 R (series I), and 1000 R (series II). The severity of radiation sickness was judged according to the relative drop in the weight of the animals and decrease in the number of leukocytes in the peripheral blood. The number of leukocytes dropped from an average of 21,450 per mm^3 to 1010 four days after irradiation in the animals of series (I) and to 500 in series (II). The death rate was 70% in series I (in 30 days), and 100% in series II (in seven to eight days). The rats were killed at the same time of day (from 1 to 3 p.m.). The adrenals were fixed by various methods, 5 min, 3, 6, 12, and 24 hours, 2, 3, 5, 7, 10, 14, 21, 30, and

60 days after irradiation. Ketosteroids were detected according to our modification of the method of Ashbell and Seligman [11], lipids according to Daddi and Ciaccio, and ascorbic acid according to Bacchus. Part of our investigations were made of preparations stained by the usual histological methods. The thickness of the zones of the adrenal cortex was measured with an AM9-2 ocular micrometer.

A quantitative evaluation of the ketosteroid content was performed with the aid of arbitrary values. For this purpose, the percent content of glandular cells, containing a small, medium, large, or very large amount of the hormone in the cytoplasm, and cells containing none of it at all, was determined in each zone of the cortex. Groups of cells containing the same amount of the hormone were designated as K_0, K_1, K_2, K_3, and K_4 in the further calculations. The percentages of each group of cells of the investigated zone were multiplied by the corresponding arbitrary coefficient (from 0 to 4), and the sum of the numbers thus obtained was multiplied by the thickness of the zone. The arbitrary quantity obtained characterizes the degree of hormone content in the zone at different periods after irradiation, considering: a) the intensity of incorporation of ketosteroids into cells, b) the percentage of cells containing various amounts of ketosteroid, and c) the thickness of the zone.

The arbitrary values characterizing the lipid content in the zones of the cortical substance were calculated analogously, in this case the coefficients of lipid content in each zone were determined according to the degree of average filling of the cells with sudanophilic droplets.

Table 1

Dynamics of the Change in the Ketosteroid and Lipid Contents in the Adrenal Cortex of Rats During Acute Radiation Sickness (in Arbitrary Units)

Time after irradiation	800 R		1000 R	
	Ketosteroids	Lipids	Ketosteroids	Lipids
Control	765±76	113±15	—	—
3 hrs	120±50	71±11	174±31	79±15
12 hrs	629±19	77±13	236±14	92±3
48 »	1487±67	41±19	161±123	34±9
3 days	—	—	1957±417	27±20
5 »	393±164	151±19	1471±44	119±100
7 »	58±78	194±28	79±38	186±10
10 »	1557±138	36±14	—	—
21 »	774±138	125±32	—	—
60 »	742±126	88±8	—	—

In the control animals, ketosteroids were detected in the form of red granules in the cytoplasm of the glomerular zone and chiefly in the outer portion of the fascicular zone. Lipids were situated in all zones, their content gradually decreased in the direction of the medulla (Fig. 1, a). The arbitrary quantitative data on the dynamics of the ketosteroid and lipid contents in the zones of the cortex in the control and irradiated animals are presented in Table 1 for the adrenal cortex as a whole and in Table 2 for the individual zones.

Table 2

Change in the Ketosteroid and Lipid Contents in Various Zones of the Rat Adrenal Cortex During Acute Radiation Sickness (800 R) (in Arbitrary Units)

Time after irradiation	Glomerular zone		Fascicular zone		Reticular zone	
	Ketosteroids	Lipids	Ketosteroids	Lipids	Ketosteroids	Lipids
Control	63	19	648	73	54	21
3 hrs	14	22	79	34	27	15
2 days	50	12	991	24	446	5
7 »	8	18	35	128	15	48
10 »	44	22	1117	14	396	0

During the first hours after irradiation at doses of 800 and 1000 R, a substantial decrease in the amount of ketosteroids and lipids in the adrenal cortex was noted, especially on account of the fascicular (Fig. 1, b), which is accompanied by a thinning of the zone of spongy cells and a thickening of the sudanophilic zone. Six to 12 hours after irradiation at a dose of 800 R, the hormone content in the cortex is restored (there is a parallel restoration of the usual ratio of the zones) and then increases gradually, reaching a maximum by the end of the second day. In series II, the ketosteroid content remains at a very low level (21%) during the first two days, after which their amount begins to be restored, reaching 256% by the end of the third day. Inclusions of hormones are detected in substantial amounts in all zones in these cases. Simultaneously with the accumulation of ketosteroids, there is a decrease in the amount of lipids, reaching 36% after two days in series I and 30% in series II.

In the further periods after irradiation, a secondary decrease in the ketosteroid content begins, leading after seven days to an almost total disappearance of the hormone from the cortex. Simultaneously there is an accumulation of fat in all the zones of the gland (Fig. 1, c). This period coincides with the period of maximum death of the animals after irradiation at a dose of 1000 R and the beginning of death of the animals irradiated at a dose of 800 R. In the animals of

series I, surviving up to 10 to 14 days, a substantial increase in the amount of hormone in the cells of this zone is observed (Fig. 1, d). The lipid content in the cortex at this time, with the exception of the glomerular zone, is sharply reduced. After 21 days, the content of ketosteroids and lipids in the cortical substance is normal.

Histochemical methods based on the ability of ascorbic acid to reduce silver nitrate do not permit a judgement of the intravital distribution of vitamin C in the adrenal gland, since in the process of treatment with the solvent there is a diffusion of vitamin into the deeper portions of the gland. Hence, the grains of reduced sugar that we detected in the cortical substance may serve only for a roughly tentative estimate of the vitamin content in the zones of the gland. In the fascicular zone in the control animals, an average of 10 to 15 grains are detected in one cell (thickness of slice 7 μ). Three hours after irradiation, in both series only individual fine grains can be found in individual cells. After two days, the number of grains is restored to 15-20, while on the seventh day only individual grains again appear. Ten days after irradiation, the number of silver grains again reaches 10-15. The vitamin C content in the cells of the fascicular and retinal zones in various periods after irradiation is shown in Fig. 2.

At the height of radiation sickness, circulatory (stasis, petechial hemorrhage) and necrobiotic changes (dissolution and pyknosis of the nuclei, etc.), already described by many authors [12, 13], were observed in the adrenal cortex.

DISCUSSION OF RESULTS

It was interesting to compare the curves that we obtained, pertaining to the content of ketosteroids and lipids in the adrenal cortex after irradiation, with the results of biochemical investigations, in which the dynamics of the content of these substances in animals was studied at various stages of acute radiation sickness. From a large number of literature studies, we decided to use the data of [6, 7] for comparison. The curves are cited in a comparable scale in Fig. 3. A comparison of the curves confirms the presence of two basic phases -- primary and secondary reactions -- in the change in the functional activity of the gland in the pathogenesis of radiation sickness.

The primary reaction, developing as a nonspecific response of the gland to a stress agent -- ionizing radiation -- [1, 14], is characterized by mobilization of ascorbic acid and lipids in the cortical substance during the first hours after irradiation [7, 9, 16, 15] and by an increase in the concentration of steroid hormones in the peripheral blood and urine [3, 5, 6], although a decrease in the corticosterone in the adrenal blood of rats after irradiation has been noted in the literature [17]. Our results agree with the aforementioned literature data, indicating increased activity of the adrenals in the primary reaction. The simultaneous decrease in the content in the

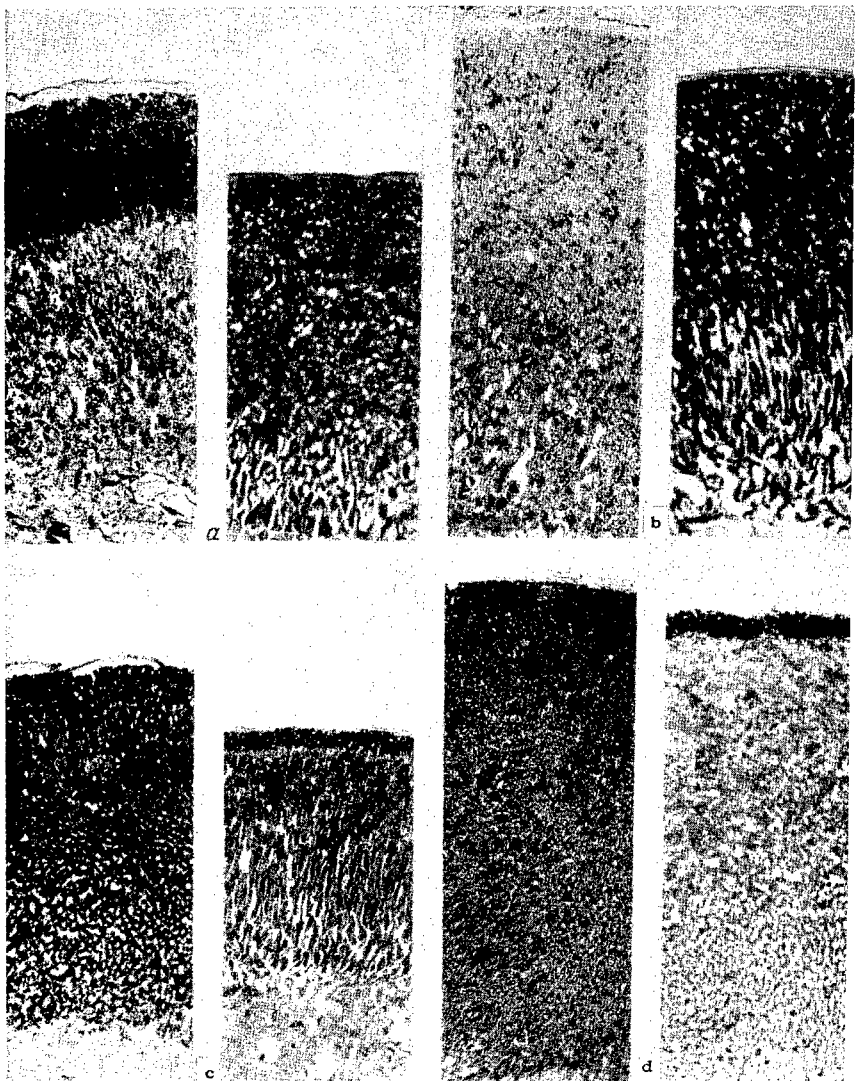

Fig. 1. Ketosteroids and lipids in the rat adrenal cortex during acute radiation sickness (800 R). a -- Control; b -- three hours after irradiation; c -- seven days after irradiation; d -- ten days after irradiation; upper row -- reaction to ketosteroids; lower row -- reaction to lipids; mag. x50.

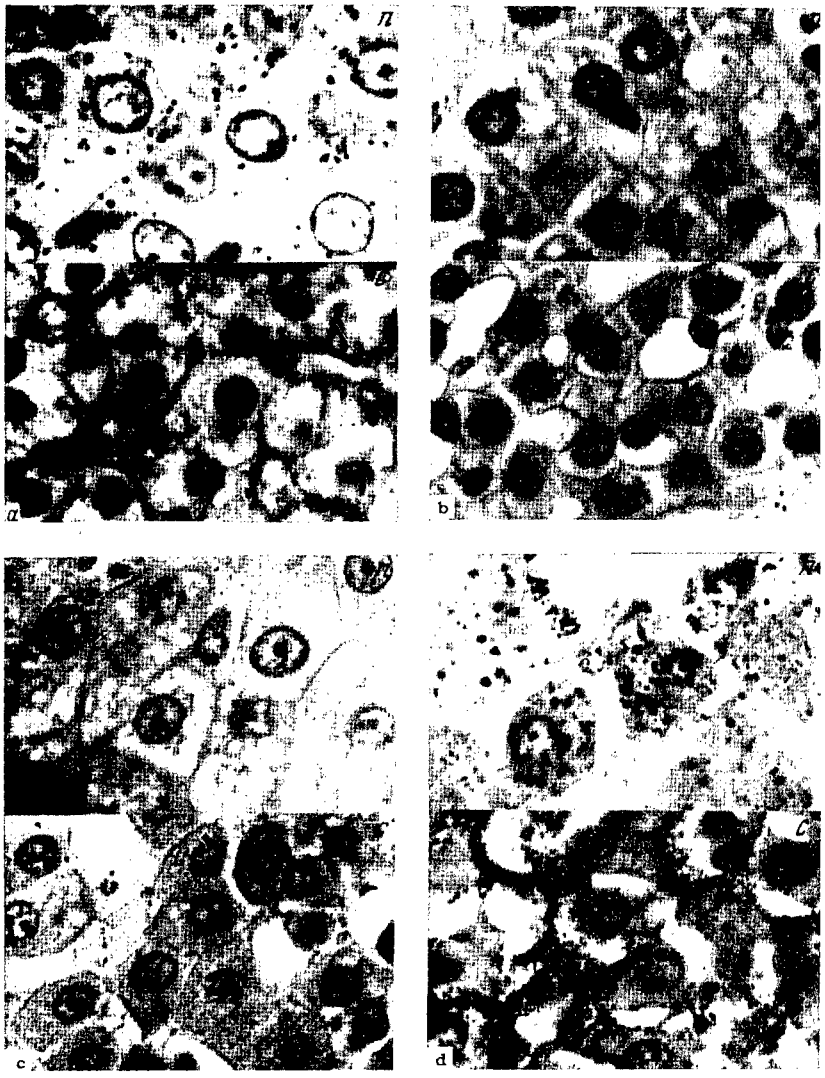

Fig. 2. Ascorbic acid in the rat adrenal cortex during acute radiation sickness (800 R). a -- Control; b -- three hours after irradiation; c -- seven days after irradiation; d -- 14 days after irradiation; n -- fascicular zone; c -- retinal zone; Bacchus reaction, mag. x1100.

cortex both of ketosteroids and of lipids is evidence of active synthesis and secretion of the hormone. The accumulation of hormones in the gland on the second and third days after irradiation above the normal level evidently is compensatory in character.

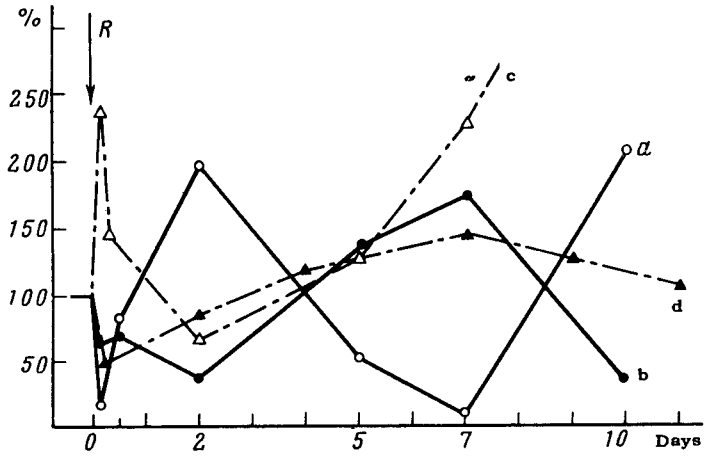

Fig. 3. Change in the ketosteroid and lipid content during acute radiation sickness. Along X-axis -- time after irradiation, days; along Y-axis -- content of the substance, % of control. The arrow with the letter R denoted the moment of irradiation; a -- ketosteroids, b -- lipids in the adrenal cortex of rats irradiated at a dose of 800 R (our data); c -- 17-hydroxycorticosteroid in the blood plasma of monkeys irradiated at a dose of 800 R (literature data [6]); d -- cholesterol in the adrenals of rats irradiated at a dose of 650 R (literature data [7]).

The literature data on the development of the secondary reaction of the adrenals as a response to a unique stress agent [1, 14] are extremely contradictory. A number of researchers observed a gradual decrease in the functional activity of the adrenal cortex several days after irradiation of animals at sublethal and lethal doses, and finally abrupt exhaustion of it [4, 9]. At the same time, other authors could not detect any signs of exhaustion of the cortical substance in dogs and rabbits irradiated by a lethal dose [5, 18]. Even a sharp increase in the concentration of 17-hydroxycorticosteroids in the blood not long before the death of the animal is described, which is evidence of a conservation of the reserve abilities of the cortical substance [6, 18]. In our experiments, as can be seen from Fig. 3, after two days a tendency appears for a gradual decrease in the ketosteroids and an increase in the amount of lipids in the cortical substance of the gland. Excessive accumulation of sudanophilic material, with an almost total absence of ketosteroids in the gland, observed on the seventh day after irradiation,

evidently indicates a disturbance of hormone synthesis from lipids [19, 20], possibly as a result of a disturbance of the extraadrenal system of regulatory mechanisms. The inhibition of the function of the gland at the height of radiation sickness is temporary: abundant accumulation of hormone with a simultaneous sharp decrease in the amount of lipids in the cortical substance in the animals surviving up to 10-14 days, but partly in the agonal state, is evidence not only of a maintenance of specific synthetic capability of the secretory cells, but also of a compensatory increase in it.

CONCLUSIONS

1. Histochemical changes in the content of ketosteroids, lipids, and ascorbic acid in the zones of the rat adrenal cortex during acute radiation sickness, caused by a single total X ray irradiation at a dose of 800 and 1000 R, are of a nonspecific phase character. The primary response of the gland, lasting for one to two days, is expressed in a mobilization of physiologically active substances. The secondary reaction, coinciding with the acute period of radiation sickness, is characterized by temporary reversible inhibition of corticoid synthesis, followed by a compensatory rise in their formation.

2. There is no parallelism in the content of individual physiologically active substances in the gland during acute radiation sickness in rats. An inverse proportionality, characterizing the process of synthetic activity of the secretory cells of the gland, exists between the levels of ketosteroid and lipid contents at various stages of radiation sickness.

3. In the functional respect, the cells of the fascicular zone, especially its outer portion, are the most active. Under conditions of increased hormone production, the cells of the reticular zone are also included in the process of secretion by compensation.

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IMMUNOLOGICAL REACTIVITY AND THE BLOOD PICTURE IN RATS AFTER A SINGLE PARENTERAL ADMINISTRATION OF TRITIUM OXIDE IN SMALL DOSES

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pp. 259-263

In spite of the great significance of the study of the state of the immunological reactivity of organisms injured by radioactive substances, by now a comparatively small number of studies devoted to this problem has been accumulated. There are single investigations studying the influence of such radioactive isotopes as strontium-90 [1], polonium-210 [2], calcium-45 [3], zinc-65 [4, 5], and certain γ emitters [6] upon the state of the natural immunity.

The purpose of this investigation was a prolonged (for 12 months) observation of the state of the natural immunity, the change in weight, and the blood picture among rats after a single parenteral administration of small doses of tritium oxide. The tritium was introduced intraperitoneally in doses of 0.01, 0.04, 0.08, 0.15, and 0.3 millicurie/g.

A total of 60 rats were under our observation; the rats were divided into five experimental groups of 10 rats in each group, according to the size of the dose; in addition, 10 rats comprised a control group.

The rats were examined twice before the administration of tritium oxide, then on the first, third, fifth, seventh, 10th, 20th, and 30th days after injury, and then each month for 12 months. The state of immunological reactivity was evaluated on the basis of a study of the quantitative and qualitative composition of the microflora of the skin and mucosa of the oral cavity.

We used the method of disk imprints, which we had developed [7], to determine the microflora of the oral cavity. Standard-sized disks of filter paper, after impregnation with secretion of the oral cavity, were ground in a mortar with 5 ml of physiological saline. Then 0.1 ml of this suspension was inoculated onto dishes with Endo's medium. The presence and number of colonies of E. coli -- the normal inhabitant of the intestines -- was determined specially. These bacteria are not encountered frequently in the mouths of healthy animals, and only in small amounts. The method of agar replica plates, proposed by Klemparskaya and Alekseeva [8], was used to investigate the skin microflora. A

modified Korostelev medium (with mannitol and bromothymol blue), upon which mannitol-decomposing microbes (i.e., more biochemically active) grow in the form of yellow colonies, was used as the nutrient medium.

Our investigations indicated that tritium oxide, administered to rats intraperitoneally in the indicated doses, did not cause death of the animals during the first two months after the injury. In the clinical picture of the injury, a slight listlessness could be noted, especially at doses of 0.15 and 0.3 millicurie/g. Among these animals the weight lagged. After one month, the weight began to return to the original level.

An investigation of the composition of the peripheral blood among the rats after the administration of tritium oxide in doses from 0.01 to 0.15 millicurie/g did not reveal any distinct changes. The number of erythrocytes among the experimental rats fluctuated within the range 6-9 million per mm^3 of blood, chiefly in the direction of the upper normal limits. Nor were any changes noted in the quantitative composition of the leukocytes. In the blood formula of the experimental animals, a periodic increase in the number of eosinophils was observed. A tendency toward an increase in the number of neutrophils was observed. No qualitative changes were detected in the formed elements of the blood. Such negligible changes in the blood may be explained by a phase of irritation of the organs of hematogenesis, which is observed under the action of small doses of ionizing radiation.

Distinct changes in the peripheral blood were detected among rats after the administration of tritium oxide in doses of 0.15 and 0.3 millicurie/g (Fig. 1). Thus, from the first to third days, a decrease in the number of erythrocytes, thrombocytes, and reticulocytes could be observed. From the first day of the injury, there was a decrease in the number of leukocytes. The maximum decrease in the number of leukocytes (to 60% of the initial data) was observed on the 10th day. Then their number was restored and reached the initial level by the end of the second month.

The same sharp decrease was also observed among the lymphocytes. The number of lymphocytes was restored to the initial level three months after the injury. By the sixth to seventh month, the number of lymphocytes increased by 40-60% above the initial data. A decrease in the absolute number of neutrophils was observed. Thus, on the 10th day their number had decreased by 45%. During the subsequent periods of observation, the number of neutrophils increased (to 400% on the seventh month). Sharp fluctuations were noted in the absolute number of eosinophils. Among the animals injured by tritium oxide at a dose of 0.3 millicurie/g, qualitative changes in the white blood cells were noted. They were manifested in increased fragmentosis, vacuolization and cytolysis of the lymphocytes and neutrophils. Qualitative changes were observed from the 15th day after the administration of tritium oxide and remained up to the sixth month of the observations.

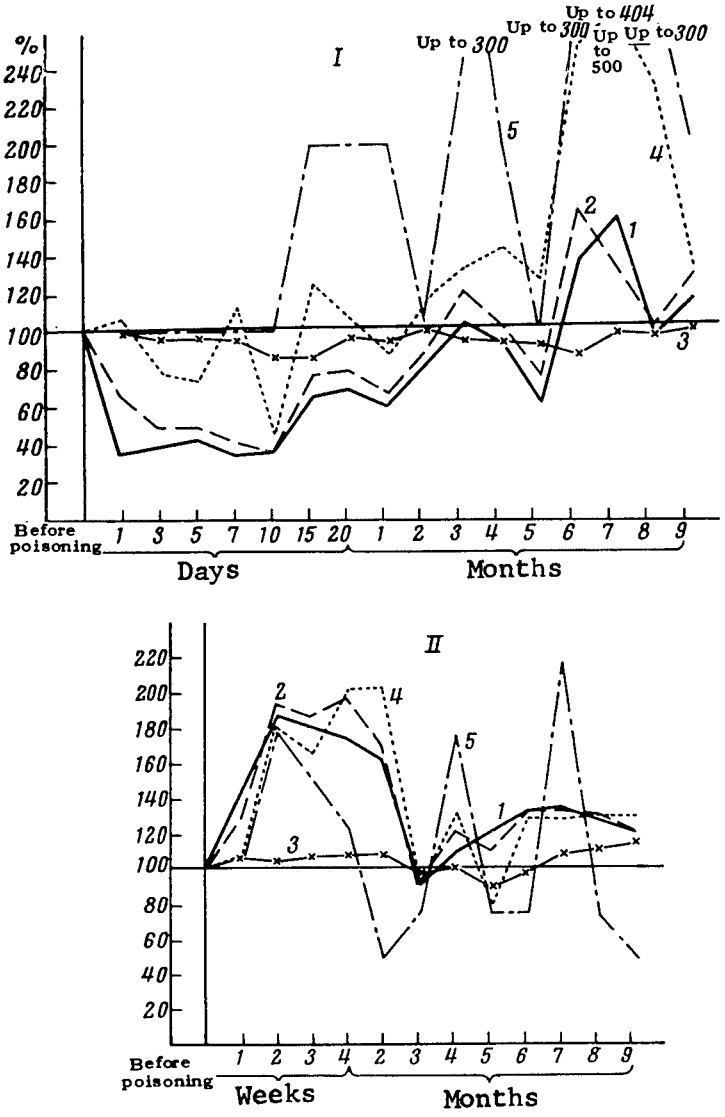

Fig. 1. Composition of the peripheral blood in rats. I -- After administration of tritium oxide at a dose of 0.3 millicurie/g; II -- in the control rats: 1 -- lymphocytes; 2 -- leukocytes; 3 -- erythrocytes; 4 -- neutrophils; 5 -- eosinophils. Along X-axis -- time after administration of tritium; along Y-axis -- percent of increase (or decrease) in the amount of formed elements of the blood in comparison with the initial amount, taken as 100%.

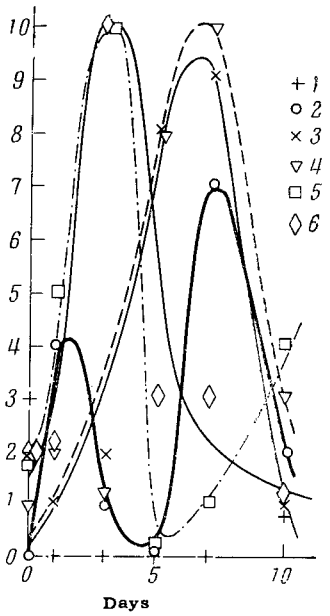

Fig. 2. Number of rats with the presence of E. coli in the mouth during the first 10 days after the administration of various doses of tritium oxide. 1 -- Control; 2 -- 0.01 millicurie/g; 3 -- 0.04 millicurie/g; 4 -- 0.08 millicurie/g; 5 -- 0.15 millicurie/g; 6 -- 0.3 millicurie/g. Along X-axis -- time after administration of tritium (days); along Y-axis -- number of rats with positive analyses.

Thus, our investigations indicated that tritium oxide in doses of 0.15 and 0.3 millicurie/g causes pronounced changes of the blood in rats, which are characterized by inhibition of hemopoiesis, followed by recovery. In addition to the change in the quantitative indices, qualitative changes are also observed on the part of the formed elements of the blood. The most pronounced changes in the state of the immunological reactivity among the injured rats were noted during the first month.

Intraperitoneal administration of tritium oxide caused a disturbance of the quantitative and qualitative composition of the microflora of the mucosa of the oral cavity. During the day immediately following the injury, E. coli is detected in the mouths of most of the rats (Fig. 2). Moreover, depending on the value of the dose, dysbacteriosis, associated with E. coli, was observed in a large number of animals even during earlier periods after the injury. Thus, at a dose of 0.01 millicurie/g, E. coli was not detected in the mouths of all the rats; on the seventh day after the injury it was found in seven rats out of 10. At a dose of 0.04 millicurie/g, the maximum number of

rats in which E. coli was detected in the mouth was also noted on the seventh day, but such rats were already nine out of ten; and on the fifth day, E. coli was isolated from the mouths of eight rats out of 10. At a dose of 0.08 millicurie/g, E. coli was isolated from the oral cavities of eight rats on the fifth day, and in all ten on the seventh day. At larger doses -- 0.15 and 0.3 millicurie/g, E. coli was already detected in the mouths of all the animals on the third day after the injury.

A computation of the analyses for the presence of E. coli in the mouth revealed that among the injured rats, E. coli is detected in a significantly greater percentage of cases in comparison with the control. This difference was more pronounced during the first 30 days after the injury. During the entire observation period, E. coli was detected among the injured animals 50 to 100% more often. During the first month after the injury, E. coli was found in three to four times as many cases.

Not only the presence of E. coli in the mouth, but to an even greater degree its quantitative content is of interest. For the intensity of inoculation of the extraneous flora of the oral cavity may characterize the degree of injury to the protective forces of the organism.

In the injured animals, in comparison with the controls, there were fewer analyses with a low content of E. coli and more analyses with a large content of these microbes. Moreover, a direct relationship was detected between the degree of inoculation of the oral cavity with E. coli and the dose of tritium administered.

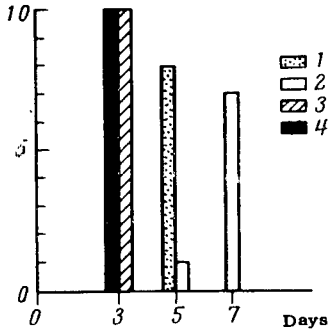

Fig. 3. Number of rats with the presence of solid growth of E. coli in inocula from the mouth during the first seven days after administration of tritium oxide in various doses (0.01-0.3 millicurie/g). 1 -- 0.04 millicurie/g; 2 -- 0.08 millicurie/g; 3 -- 0.15 millicurie/g; 4 -- 0.3 millicurie/g. Along X-axis -- time after administration of tritium (days); along Y-axis -- number of rats with positive analyses.

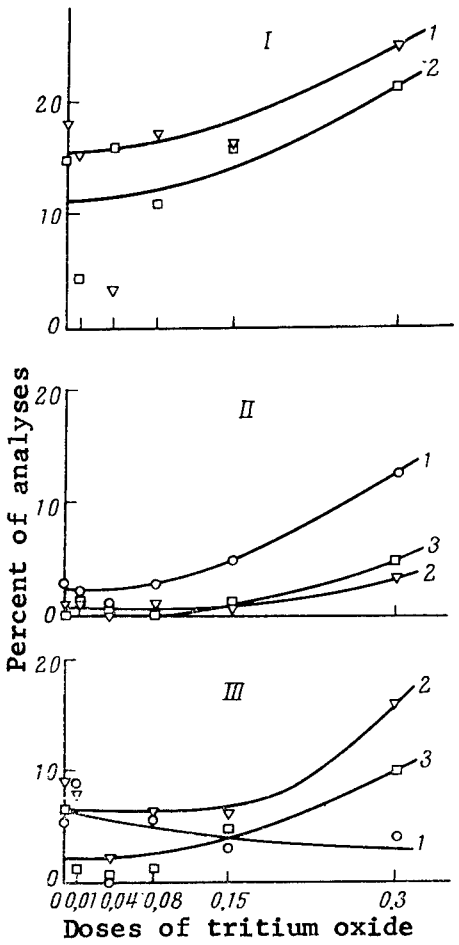

Fig. 4. Intensity of seeding of skin with microbes among rats injured by tritium in various doses (0.01-0.3 millicurie/g). Along X-axis -- doses of tritium oxide; along Y-axis -- percent of analyses. I -- Total number of microbes; 1 -- % of analyses with a large content of microbes (more than 100 colonies per plate); 2 -- % of analyses with the presence of solid growth. II -- Amount of E. coli: 1 -- % of analyses with increased microbe content (51-100 colonies per plate); 2 -- % of analyses with large microbe content (more than 100 colonies per plate); 3 -- % of analyses with presence of solid growth. III -- Amount of mannitol-decomposing cocci; 1-3 -- the same as in II.

In a number of cases, the seeding of the mucosa of the oral cavity with E. coli was so intensive among the injured rats that solid growth of a pure culture of these microbes was detected after inoculation

onto dishes. It was found (Fig. 3) that among the rats that had received tritium in a dose of 0.01 millicurie/g, massive seeding of the oral cavity with E. coli was never observed. At large doses of the influence, this phenomenon was observed; moreover, the frequency and time of its appearance were directly dependent upon the size of the tritium dose. Thus, at a dose of 0.04 millicurie/g, massive seeding of the oral cavity with E. coli occurred in eight rats on the fifth day after the injury; at a dose of 0.08 millicurie/g it was observed in one rat on the fifth day and on seven rats on the seventh day after the administration of tritium. When tritium was administered in doses of 0.15 and 0.3 millicurie/g, massive seeding of the oral cavity by E. coli had already occurred in all the rats on the third after the injury.

In a study of inocula obtained by replica plating from the skin of the tail, a count was made of the total number of colonies that grew and the presence and number of the colonies of E. coli and of mannitol-decomposing cocci were specially considered. An analysis of the materials obtained indicated that on the skin, just as in the oral cavity, there was an increase in the number of microbes among the rats after they were administered tritium oxide.

To determine the relationship of the dose of the influence to the intensity of seeding of the skin with microbes, an analysis was made of the frequency of detection of various degrees of seeding of the skin during the entire observation period, depending upon the dose of tritium oxide. Fig. 4 presents curves characterizing the number of analyses with a large content of microbes (more than 100 colonies grew on the plate) -- curve 1, and with a massive content (solid growth was detected on the plate) -- curve 2. The control indices were plotted on the vertical axis. In a consideration of these curves it is evident that there were more microbes on the skin of the injured rats than among the controls. With increasing tritium dose, the intensity of seeding of the skin with microbes increased.

The same pattern was also observed in a study of the intensity of seeding of the skin with E. coli and mannitol-decomposing cocci. Figure 4 presents curves reflecting the different degree of seeding of the skin with E. coli (graph II) and with mannitol-decomposing cocci (graph III). On both graphs, curves 1 characterize increased seeding (51-100 colonies of these microbes grew on the plate), curves 2 -- great seeding (more than 100 colonies grew on the plate), curves 3 -- massive seeding (solid growth of these bacteria was detected on the plate).

Thus, all the materials cited indicate that in the case of a single intraperitoneal administration of tritium oxide to rats in low doses, there is a disturbance of the immunological reactivity. The blood picture is changed, in the direction of an increase in its indices. The quantitative and qualitative composition of the autoflora of the mucosa of the oral cavity and skin of the tail is disturbed; in the mouth E. coli appears in large quantities, while the total number of microbes increases on the skin, chiefly on account of E. coli and mannitol-decomposing cocci. All the indicated changes are directly

dependent upon the dose of tritium introduced into the organism.

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ON THE TIME DISTRIBUTION OF THE DEATH RATE OF IRRADIATED ANIMALS

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pp. 264-267

The methods of quantitative analysis of the periods of death of irradiated animals, developed by Luchnik [1, 2], are very interesting and promising for a number of fields of radiobiology (pathogenesis of radiation sickness, effects of antiradiation agents, natural radioresistance) [2-4]. In connection with this, we consider it expedient to consider certain disputed questions of a methodological character.

Evaluating the time dynamics of the death of irradiated mice, N. V. Luchnik has repeatedly emphasized that the empirical distributions are not normal on account of the positive asymmetry and the highly real and very pronounced depression (negative excess). In confirmation of this, he cites data for mice of the X line. We doubled the calculations according to the formulas recommended by Fisher [5] and did not confirm the depression of the curve for X mice. This prompted us to treat N. V. Luchnik's data in greater detail (Table). The table shows that the distribution of time of death of a different line of mice -- M -- is characterized by a negative excess; however, it is quantitatively not large; no asymmetry of the curve was detected. On the basis of the experimental values of the mean and mean square deviations and a table of normal distribution, we calculated the theoretical curves, which we compared with the empirical distributions according to the χ^2 criterion [6] and graphically (Table, Fig. 1). For X mice on the whole, no statistically significant deviations from normality were detected, but on the 12th day the real death rate was significantly smaller than the calculated; the empirical death rate of M mice differed significantly from the normal curve -- exclusively on account of the peaks of death on the 8th and 17th days.*

*We should indicate that the data on the M line were based on material combined according to three groups differing in sex and dose rate; each of the indicated peaks occurred only in one group out of three.

Thus, the distribution of the death rate of irradiated mice with time according to individual indices (different for different series) may actually differ from the normal; however, these differences are quantitatively not large; there is no characteristic pronounced negative excess. We should agree with Luchnik [1] that in view of the great dispersion of the data (variation coefficient about 40%), the periods of death of individual animals have little value. However, the "conclusion of insufficient suitability of the average lifetime as a criterion for general injury ...." (p. 85) is too categorical. In the practice of mathematical statistics, a normal distribution is frequently used as a convenient and well developed model in those cases when the empirical distribution is statistically significant, but quantitatively does not differ sharply from the normal [6]. The calculations cited in the table and a graphical comparison of the theoretical and empirical distributions (see Fig. 1) give us the right to believe that a normal distribution quite satisfactorily approximates the dynamics of the death of irradiated mice of these series. Of course, there are also other data in the literature on the presence of substantial and statistically significant deviations from normality in the distribution of death of irradiated mice and rats with time. Probably the question analyzed should be resolved not in general, but concretely for given strains (series) of animals.

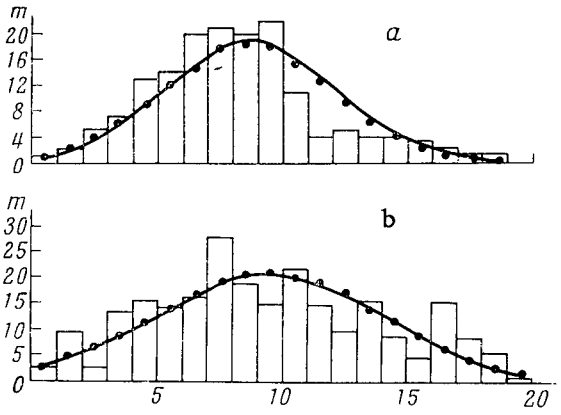

Fig. 1. Distribution of death rate of irradiated mice with time. Experimental data (columns) and calculated normal distribution (points and curve). Along X-axis -- time after irradiation, days; along Y-axis -- number of mice that died on a given day; a -- mice of line X; b -- line M.

N. V. Luchnik developed two methods for detecting the death rate peaks: a calculation of the probability of death on a given day (p_t) and a calculation of the increase in the probit of the death rate according to the logarithm of the time $\left(\frac{\Delta y}{\Delta \lg t}\right)$. The use of the logarithm

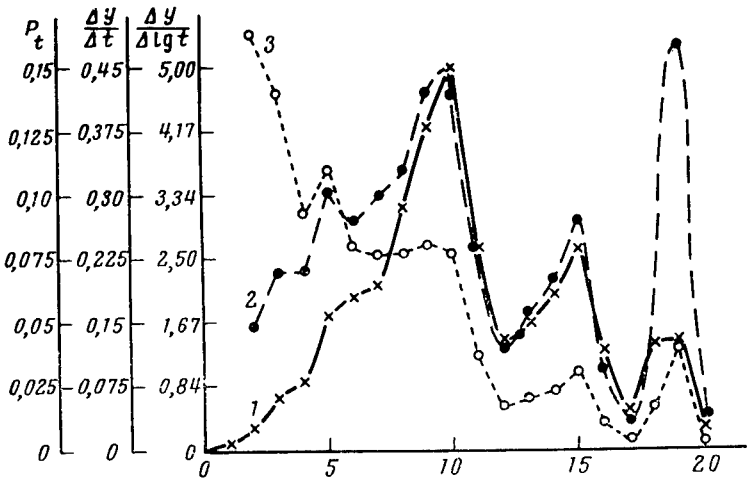

Fig. 2. Time peaks of the death of irradiated mice. Along X-axis -- time after irradiation, days;

1 -- P_t, 2 -- $\frac{\Delta y}{\Delta \lg t}$, 3 -- $\frac{\Delta y}{\Delta t}$

of the time actually might prove useful in view of the substantial dispersion of the data and the presence of a positive (left hand) asymmetry. In these cases, the logarithmic transformation frequently permits a conversion to the normal distribution [7]. However, our calculations (see table), indicated that in spite of a doubling of the coefficient of variation, the distribution of the death rate of X mice according to the logarithm of the time is no closer to the normal than the distribution with time; in a factor analysis, the differences from the log-normal curve proved highly significant: the distribution became right hand-asymmetric and was characterized by the presence of a positive excess. Hence, in this case the justification of the logarithmic transformation and the detection of death rate peaks according to the increase in the probit of the death rate according to the logarithm of the time is disputable.

We should like to turn our attention to the possibility of still another method of detecting peaks -- calculation of the increase in the probit of the death rate with time $\left(\frac{\Delta y}{\Delta t}\right)$, which in the case of one-day intervals of observation is equivalent to an increase in the probit. The death rate peaks detected by all three methods in X mice at a dose of 600 R are presented in Fig. 2; the relative heights of the peaks differ, but their positions with time coincide (except for the initial portion). This closeness of the results according to all three methods,

Distribution of Death Rate of Irradiated Mice with Time
(Up to 20 Days -- Analysis of Luchnik's Data)

Line	Dose, R	Mortality in 30 days, %	Expression of lifetime, days	Parameters of empirical distribution				Determination of deviations from normal distribution							
				No of mice that died, n	Average $\bar{x}$	Dispersion s^2	Coefficient of variation v	Evaluation according to factors				Evaluation according to χ^2			
								Asymmetry		Excess					
								g_1	P	g_2	P	χ^2	κ	P
M	500	75	Time	379	10,14	20,55	44,7%	0,1831	>0,2	—0,7667	<0,02	36,38	15	<0,002
X	600	74	»	221	8,588	11,36	39,2%	0,5633	<0,01	0,5097	>0,1	16,27	10	≈0,1
			Logarithm of time	221	0,8961	0,03774	21,7%	—1,109	<0,001	2,778	<0,001	13,21	10	≈0,2

based on different prerequisites, distinctly demonstrates the reality and stability of the time peaks of the death rate. Probit methods are frequently more sensitive in the detection of peaks, and justify themselves in practice in those cases when the position of the death rate peaks with time is basic [3]. However, not only the position, but also the relative height of the peaks is of interest, and it differs greatly for different methods of treatment.* This makes it desirable to select one of the methods on the basis of theoretical considerations, since the relative height of the peaks, according to N. V. Luchnik's concepts, should reflect the ratio of the different final causes of death of irradiated animals. For a given species of animals (possibly also for each strain and sex and different conditions of irradiation), we propose to determine the shape of the distribution of the death rate with time in normal and logarithmic scales and then to give preference to that one of the two probit methods $\left(\frac{\Delta y}{\Delta t} \text{ or } \frac{\Delta y}{\Delta \log t}\right)$ which is based upon a distribution closer to the normal law; otherwise, the deviations from normality (height of the peaks) will be artificially increased. N. V. Luchnik, widely using the probit method in view of its sensitivity [3], gives preference to a calculation of the probability of death; the latter method does not involve the assumption of a normal or any other theoretical distribution [2]. This questions does not seem entirely clear to us and will need discussion and additional accumulation of material.

It is noteworthy that the intervals between death rate peaks are approximately equal (three days on the average). This may be evidence of a rhythmic variation of the

*The differences according to probit methods are naturally due to the fact that when the increase in the probit is divided by the increase in the logarithm of the time (instead of the time), the relative height of the first peaks decreases, while that of the last increases.

ratio of processes of injury and compensatory reactions in the organism of irradiated animals; the periodic predominance of the former may lead to the development of a lethal result. This hypothesis agrees with Semenov's theory [8] of the existence of "critical periods" of acute radiation sickness and, in our opinion, does not contradict the interpretation of the peaks as a manifestation of the final causes of death. Actually, radiation injury to the organism may diverge not only at the very initial stage (directly after the physical and chemical processes) but may even develop for a long time as a general pathological process, giving offshoots or exacerbations in individual morphological or biochemical systems.

In conclusion we should like to pose the question of how to combine the presence of death rate peaks (Fig. 2) with relatively small deviations from the normal distribution (Fig. 1). It may be that the peaks are the vertices of individual normal distributions, each of which actually corresponds to a special cause of death. The individual distributions partially overlap [2] and form one common distribution; since the number of peaks is rather great (up to five-six), while the intervals between them are small (approximately three days), this general distribution may take a form approaching the normal. If we proceed from the assumption of the presence of a general pathological process with its final offshoots, we can expect the presence of a normal curve, upon which individual supplementary vertices (peaks) corresponding to the actual final causes of death, are superimposed.

A discussion of the problems noted in this report, with the participation of radiobiologists and mathematicians, seems highly advisable to us.

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SYNTHESIS OF INOSINIC ACID, INOSIN, AND HYPOXANTHINE IN THE PIGEON LIVER DURING THE EARLY PERIODS AFTER IRRADIATION AND ADMINISTRATION OF A RADIOPROTECTIVE AGENT

E. F. Romantsev, S. K. Khalikov

pp. 268-271

At the present time, a number of experimental data have been accumulated, permitting a representation of the general scheme of the synthesis of purine bases from the simplest precursors [1, 2, 3]. The question of the synthesis of purine bases under the action of ionizing radiation has been considerably less studied [4]. A number of investigations along this line have been conducted by Kritskiĭ et al. [5]. After total irradiation of pigeons at a dose of 2000 R, the author found that the synthesis of hypoxanthine, judging by the incorporation of C^{14} into it in C^{14}-1-glycine, in liver homogenates from the irradiated pigeons is increased 30 minutes after irradiation and is sharply reduced on the following days.

The content of adenylic, inosinic, guanylic, uridylic acids, hypoxanthine, and uracil in the bone marrow of rabbits was sharply reduced two days after local X ray irradiation at a dose of 2000 R [6].

It is known that radioprotective agents (protectors) influence the primary physicochemical and biochemical processes, ultimately weakening the course of the radiation injury among mammals [7, 8].

In connection with this, in this work we studied the influence of ionizing radiation and the protector β-mercaptopropylamine (MPA) upon the biosynthesis of purine bases in a pigeon liver homogenate, using 2-C^{14}-formic acid as the radioactive tracer.

The work was conducted on adult pigeons weighing 310-380 g. The inosinic fraction was isolated according to the method proposed by G. I. Kritskiĭ [3, 6]. The purine compounds were identified on a column [9]. The pigeons were irradiated totally with γ rays (Co^{60}), dose rate 188 R/min. Summary dose 3000 R. MPA was administered intraperitoneally in amounts of 200 mg/kg 10 min before irradiation of the pigeons. Each index presented in the graph was obtained on four to eight birds.

EXPERIMENTAL SECTION AND DISCUSSION OF RESULTS

The pigeon liver is an extremely convenient specimen for the study of the mechanism of purine synthesis in the normal state and after irradiation, since the purines and hypoxanthine formed do not undergo further oxidation or cleavage, which occurs in the tissues of other animals.

Figure 1 presents the incorporation of C^{14} from C^{14}-2-formic acid into the inosinic acid of the pigeon liver: I -- in the normal state; II -- 10, 30, and 60 min after irradiation of the pigeons at a dose of 3000 R; III -- 10 min after intraperitoneal administration of MPA to the pigeons; IV -- 36, 56, and 86 min after injection of MPA; V -- 10, 30, and 60 min after irradiation, but under the condition that MPA was administered 10 min before the beginning of irradiation.

It is known that C^{14}-formic acid is used in inosinic acid biosynthesis for the construction of the purine ring in the 2- and 8-positions, while most of the C^{14}-formic acid is detected, for example, in glutamine, serine, glycine, aspartic, glutamic, succinic, and fumaric acids and certain other compounds [3].

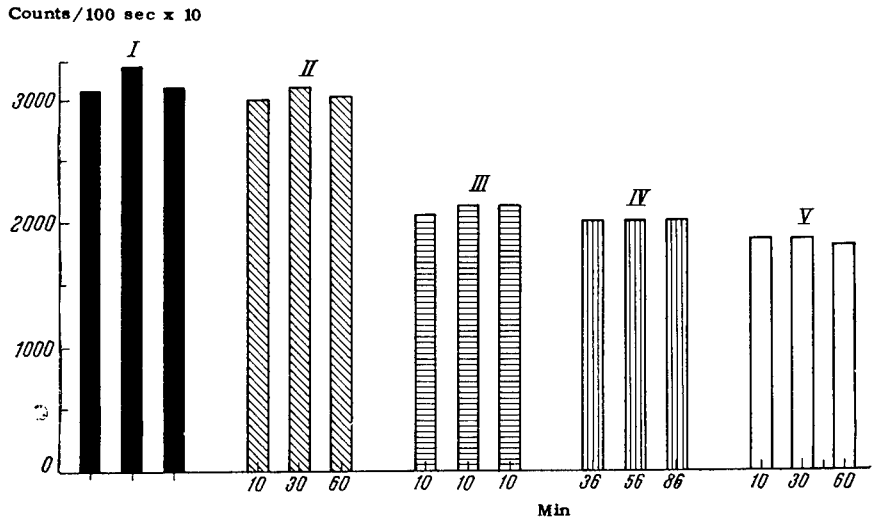

Fig. 1. Incorporation of C^{14} from C^{14}-2-formic acid into pigeon liver inosinic acid.

Under our experimental conditions, with the incubation medium used, about 12% of the entire C^{14}-2-formic acid added was used for the construction of inosinic acid.

The data cited in Fig. 1 (I) show good coincidence of three experiments conducted at different times. In the denaturation of the proteins of a perchloric acid homogenate before the addition of C^{14}-2-formic acid, no radioactivity was detected in the inosinic acid fraction,

which indicated an enzymatic character of the reaction that occurs (Fig. 1).

After irradiation of the pigeons at a dose of 3000 R, followed by incubation of the homogenate in a medium containing folic acid, we detected no decrease in the synthesis of inosinic acid in comparison with the control 10, 30, and 60 min after radiation injury. It is interesting to note that G. A. Kritskii noted a distinct, transitory intensification of the synthesis of inosinic acid in pigeon liver homogenates 30 min after irradiation at a dose of 2000 R, if glycine-1-C^{14}, incorporated into the 4- and 5-positions of inosinic acid, was used as the labeled precursor.

A comparison of our data with G. A. Kritskii's data indicates that 30 min after radiation injury, there is a selective disturbance of the synthesis of individual portions of the inosinic acid molecule. Ten minutes after the administration of MPA synthesis of inosinic acid in pigeon liver homogenates is distinctly inhibited (III). It is known that aminothiols of the MPA type exert a radioprotective effect if they are administered intravenously or intraperitoneally to mice, rats, and dogs 10-20 min before the beginning of irradiation. As we shall see later, the antiradiation effect of MPA on pigeons is manifested if this aminothiol is also administered 10 min before irradiation. Consequently, on the basis of our experiments we may conclude that after the administration of MPA, by the time of irradiation the synthesis of inosinic acid from the simplest precursor -- formic acid -- is distinctly inhibited (by 34% on the average).

Figure 1 (IV) presents data on the incorporation of C^{14}-formic acid into inosinic acid 36, 56, and 86 min after the administration of MPA. These data are an additional control for the following series of experiments (V), in which MPA was introduced 10 min before irradiation, lasting 20 min, and then the pigeons were decapitated 10, 30, and 60 min after the end of irradiation (36, 56, and 86 min, respectively, after the introduction of the aminothiols).

The data obtained indicate that 10 (III), 36, 56, and 86 minutes (IV) after the administration of MPA to pigeons, there is an inhibition of the incorporation of C^{14}-2-formic acid into inosinic acid. After preliminary administration of MPA, followed by irradiation of the pigeons (V), the synthesis of inosinic acid from formic acid after 10, 30, and 60 min remained practically at the same reduced level as in the case of the administration of the protector without subsequent irradiation.

In the following series of experiments, it was found that after two-hour incubation, about 4.2% of the C^{14}-2-formate added is used for the synthesis of inosin. Radiation and the addition of MPA exert the same influence upon the inosin synthesis as upon the synthesis of inosinic acid.

Irradiation and introduced protectors have practically the same effect upon hypoxanthine synthesis as upon the synthesis of inosinic acid and inosin.

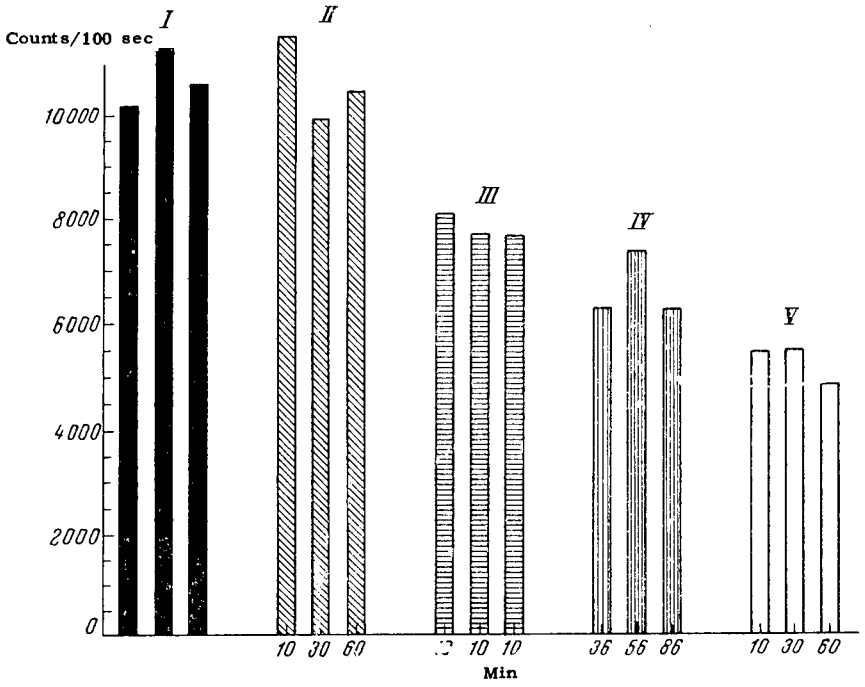

Fig. 2. Incorporation of C^{14} from C^{14}-2-formic acid into pigeon liver inosin.

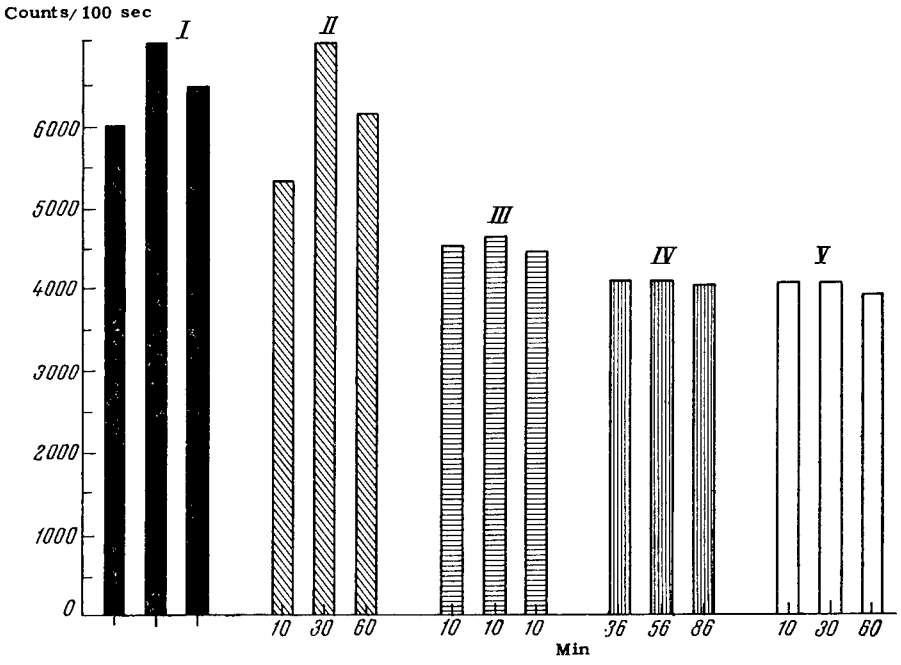

Fig. 3. Incorporation of C^{14} from C^{14}-2-formic acid into pigeon liver hypoxanthine.

There are a number of studies in which the influence of radioprotective aminothiols upon the synthesis and content of DNA in the cell was investigated. After the administration of MEA to half the animals, the DNA content in the liver was somewhat reduced [10, 11]. However, these data were evidence only of the end result of the action of the aminothiol. If different simple precursors were used to study DNA synthesis, then the influence of the protectors upon the synthesis depended not only on the chemical nature of the protector, but also on the chemical nature of the precursor itself. For example, when C^{14}-acetate was used as a label, the prophylactic administration of cysteine and glutathione led to an inhibition of the incorporation of the isotope into DNA and consequently indicated an inhibition of the process of synthesis [12]. If P^{32} was used as the precursor, then in analogous experiments cysteine exerted a stimulating effect upon the DNA synthesis [13]. Probably such a discrepancy may be due to the different nature of the action of protectors upon different intermediate stages of DNA synthesis. In our experiments MPA inhibited the synthesis of inosinic acid, inosin, and hypoxanthine from formic acid, evidently at one of the intermediate stages of the synthesis of purine compounds.

CONCLUSIONS

1. After the irradiation of pigeons in the absolute minimum lethal dose (3000 R) and incubation of a pigeon liver homogenate with C^{14}-2-formic acid, the synthesis of inosinic acid, inosin, and hypoxanthine is unchanged 10, 30, and 60 min after the radiation influence.
2. Intraperitoneal administration of MPA to healthy pigeons distinctly inhibits the synthesis of the purine compounds indicated above 10, 36, 56, and 86 min after the introduction of the aminothiol.
3. Preliminary (before irradiation) administration of MPA to pigeons reduces the synthesis of inosinic acid, inosin, and hypoxanthine to the same level as after the administration of the aminothiol alone without subsequent irradiation.

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COMPARATIVE EVALUATION OF THE PROTECTIVE EFFECTIVENESS OF POTENTIAL RADIOPROTECTORS -- BUNTE'S SALTS -- ON VARIOUS BIOLOGICAL MODELS

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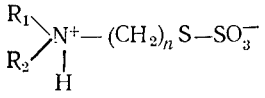
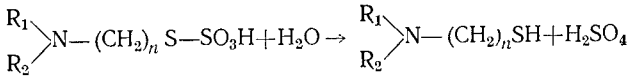
pp. 272-277

The search for radioprophylactic substances, exhibiting prolonged action, and capable of forming aminoalkylthiols in the irradiated organism, has led to the study of aminoalkylthiosulfuric acids (so-called Bunte's salts). Usually these substances exist in the form of "internal salts."



In recent years it has been found that aminoalkylthiosulfuric acids exhibit a radioprophylactic effect upon animals [1-2]. However, on chemical and other models used for rapid and preliminary evaluation of the effectiveness of radioprotectors, Bunte's salts have manifested no protective action [2-4].

It has been hypothesized [1, 2] that the radioprophylactic effect of aminoalkylthiosulfuric acids is manifested as a result of their hydrolysis in cells in vivo, with the formation of active aminothiols



In our previous studies, we proposed various models and methods for rapid preliminary selection of radioprophylactic substances [5-7]. In the studies mentioned, the radioprophylactic effect was evaluated directly with the aid of the so-called "radiation models," by irradiating yeast cells and erythrocytes with γ rays, and also indirectly,

by studying the inhibition of the radiomimetic effect with the aid of "radiomimetic models," or studying the decrease in the level of the stationary oxidation-reduction potential in the tissues of animals [5-9]. Using these models and methods in this work, we made a comparative evaluation of the protective effectiveness of Bunte's salts.

MATERIALS AND METHODS

The materials were male white mice, intact human erythrocytes, haploid yeast cells Zygosaccharomyces Bailii, and oleic acid solutions of β-carotene. The animals were irradiated on the RUM-3 apparatus (voltage 180 kV, current strength 15 mA, filters 0.5 mm Cu and 1 mm Al), at doses from 200 to 1000 rad. A total of 350 mice were used in the experiment.

The radioprotectors were administered intraperitoneally 25-30 min before irradiation. $\Delta\varepsilon h$ was measured on the LP-57 tube potentiometer with the aid of a smooth platinum needle electrode, which was implanted into the muscle of an animal fixed on a stand. The reference electrode was a saturated calomel half-cell, in contact with the muscle through a glass capillary, filled with a saturated solution of KCl in 3% agar-agar.

The yeast cells, erythrocytes, and oleic acid solution of β-carotene were irradiated in glass vessels on the GUT-Co-400 apparatus (dose rate 1 krad/min) at various doses (table). The radiomimetic -- oxidized oleic acid -- was prepared and added to the specimens just as was described in our previous work [9].

The initial solutions of the radioprotectors were prepared in physiological saline in the concentrations indicated in the table and were added to the investigated specimens 1-5 minutes before irradiation (or administration of the radiomimetic). The action of radiation and the radiomimetic upon the erythrocytes were evaluated by the method of erythrograms [7], and on yeast cells by the method proposed by Korogodin [10]. The coefficient of protection (table) served as the criterion of protective action of the preparations.

RESULTS AND DISCUSSION

The results of a comparative evaluation of the effectiveness of the preparations upon various models are summarized in the table. From the table it is evident that the preparations (Nos. 1-3): cysteamine, AET, and cystamine exert a good protective effect on all biological models, with respect to survival rate of mice after irradiation (700 rad), decrease in the stationary redox potential of muscle tissues, and protection of yeast cells and erythrocytes from radiation and a radiomimetic. Cysteamine and AET also manifest antioxidant properties, lowering the level of oxidation of β-carotene dissolved in oleic acid.

Comparative Evaluation of the Effectiveness of Preparations on Various Models

Radioprotectors	Preparation No	Chemical formula	Mice: Maximum tolerable dose of the preparation, mg/kg	Mice: Survival by 30 days after irradiation at a dose of 700 R, %	Mice: Δth of muscle tissue (mV) 25-30 min after administration of the preparation	% protection with respect to beta-mercaptoethylamine: Yeast cells, Radiation model* (Concentration of each preparation 0.02 M)	Yeast cells, Radiomimetic model* (Concentration of each preparation 0.02 M)	Erythrocytes, Radiation model* (300 kR), Concentration of preparations 0,01M	Erythrocytes, Radiomimetic model, Concentration of preparations 0,02M	Erythrocytes, Radiomimetic model, Concentration of preparations 0,02M	Chemical model: Oxidation of beta-carotene
Aminothiols	1	$H_2N(CH_2)_2SH$	200	100	—140±19	100 (1,8)	100 (1,7)	100 (3,1±0,1)	60 (2,0±0,2)	100 (5,0±0,3)	100
	2	$H_2N(CH_2)_2S-C(=NH)-NH_2$	250	80	—103±7,0	100	100	100 (3,1±0,1)	—	100 (5,0±0,4)	100
Aminodisulfide	3	$H_2N(CH_2)_2-S$ / $H_2N(CH_2)_2-S$	350	100	—150±11,1	100	100	100 (3,1±0,1)	—	50 (2,5±0,3)	—
Bunte's salts	4	$H_2N(CH_2)_2SSO_3H$	560	98	—149±13,2	30	75	—	40 (1,8±0,1)	2,5 (1,1±0,03)	Preparations insoluble in samples
	5	$(H_3C)_2N(CH_2)_2SSO_3H$	280	74	—90±6,4	35	55	—	80 (2,7±0,2)	Токсичность (0,5±0,13)	
	6	$H_2N(HN=)C-N(CH_2)_2SSO_3H$	—	—	—	—	—	—	80 (2,8±0,1)	30 (2,2±0,44)	
	7	$H_2N-(CH_2)_3SSO_3H$	560	65	—72±7,7	30	75	—	—	—	
	8	$(H_3C)_2N(CH_2)_3SSO_3H$	500	53	—48±4,8	—	—	—	30 (1,6±0,2)	5,0 (1,2±0,05)	0
	9	$(H_3C)_2NCH_2CHSSO_3H$ / CH_3	300	39	—55±4,8	30	45	—	30 (1,6±0,5)	5,0 (1,2±0,1)	0
	10	$H_2NCH(CH_2)_3SSO_3H$ / CH_3	—	—	—	20	15	—	—	—	—

Note. The numbers in parentheses denote the absolute values of the protective coefficient, representing: on erythrocyte models the ratio $\frac{t_{50\%}(n)}{t_{50\%}(k)}$ and on yeast models the ratio $\frac{S_{D50}(n)}{S_{D50}(k)}$, where where t_{50} is the time of 50% hemolysis; S_{D60} is the survival of the colonies in % at the dose D_{50}; n denotes addition of the radioprotector; (k) is the control, i.e., without addition of the radioprotector.

The data cited indicate that the methods used for rapid and preliminary selection of radioprotective properties of the preparations permit an evaluation of the effectiveness of certain aminothiols [5-9]. It is interesting that cystamine, inactive when tested on chemical models, can induce a protective effect upon radiomimetic and radiation models, in addition to lowering the redox potential, which confirms the data known in the literature on the possibility of the conversion of cystamine to an aminothiol in vivo [2].

In view of the above, the problem arose of determining the possibility of using radiation and radiomimetic models to evaluate the effectiveness of potential aminothiols -- the group of preparations known as Bunte's salts. The activity of β-mercaptoethylamine (preparation No. 1), taken in the table as 100%, was selected as the standard activity. As can be seen from this table, aminoalkylthiosulfuric acids exhibit different protective properties. The most active proved to be aminoethylthiosulfuric acid, its N-substituted preparations, and aminopropylthiosulfuric acid (preparations Nos. 4-7). The remaining preparations (Nos. 8-10) did not manifest any protective effect upon any of the models used.

Thus, ineffective or weakly effective radioprotectors cannot be selected for further investigations either on radiomimetic or on radiation models.

In a comparison of the preparations manifesting a radioprotective effect on mice, it is evident that they all possess the ability to sharply lower the potential of the tissues. On cell models, active preparations of Bunte's salts (preparations Nos. 4-7) manifested their activity ambiguously. Aminoalkylthiols protect erythrocytes from radiation and are ineffective on the yeast model. At the same time, these preparations lower the radiomimetic effect on yeast cells and are not active on the erythrocytic radiomimetic model. It is extremely important to note that the duration of the contact of radioprotectors with the cell is not the same on the different models. For example, according to the experimental conditions, under the action of radiation on yeast cells, the protectors were in contact with the cell for 30 min, and with the erythrocytes for two hours.

Under the action of the radiomimetic (oxidized oleic acid), the investigated preparations were in contact with yeast cells for three hours, and with erythrocytes for only 10 min.

A comparison of the effectiveness of the preparations with the duration of their action upon cells permits us to assume that the radiations yeast and radiomimetic erythrocytic models are not sensitive to the action of such potential aminoalkylthiols as Bunte's salts. Two to three-hour exposure of the protector with the cell suspension permitted the detection of a prolonged protective action of Bunte's salts. According to the experimental conditions, this could be done on the yeast radiomimetic and erythrocytic radiation models. The manifestation of the protective activity of aminoalkylthiosulfuric acids evidently depends upon the rate of their penetration into the cell, as well as upon the rapidity of the conversion of the potential

radioprotectors to aminoalkylsulfides.

The available literature data on the absence of a protective effect of Bunte's salts upon suspensions of erythrocytes, hemolyzates, and liver homogenates could also be explained by the brief contact of the preparations with the specimens. According to the experimental conditions, the duration of incubation of the preparations with the specimens was only 10 min [3]. As a result of the potential properties and prolonging action, aminoalkylthiosulfuric acids are less toxic than aminoalkylsulfides.

The toxicity of active preparations of Bunte's salts (Nos. 4-7) is only half as great as that of cysteamine. A 0.02 M preparation of β-mercaptoethylamine, as a result of its toxic properties, lowered the protective effectiveness in comparison with the preparation at a concentration of 0.01 M. From the table it is evident that for a 0.01 M solution, the coefficient of protection is greater (3.5) in comparison with a 0.02 M solution, the coefficient of protection of which is equal to 2.0. The 0.02 molar solutions of Bunte's salts did not manifest toxic properties and exerted an optimal protective effect.

The dependence of the effectiveness of Bunte's salts upon the duration of their action upon the biological specimen is also observed according to the change in the potential of animal tissues with the time after introduction of the preparation (Fig.).

From the figure and table it is evident that the sharpest decrease in the redox potential among the animals is observed after the administration of active radioprophylactic preparations to them.

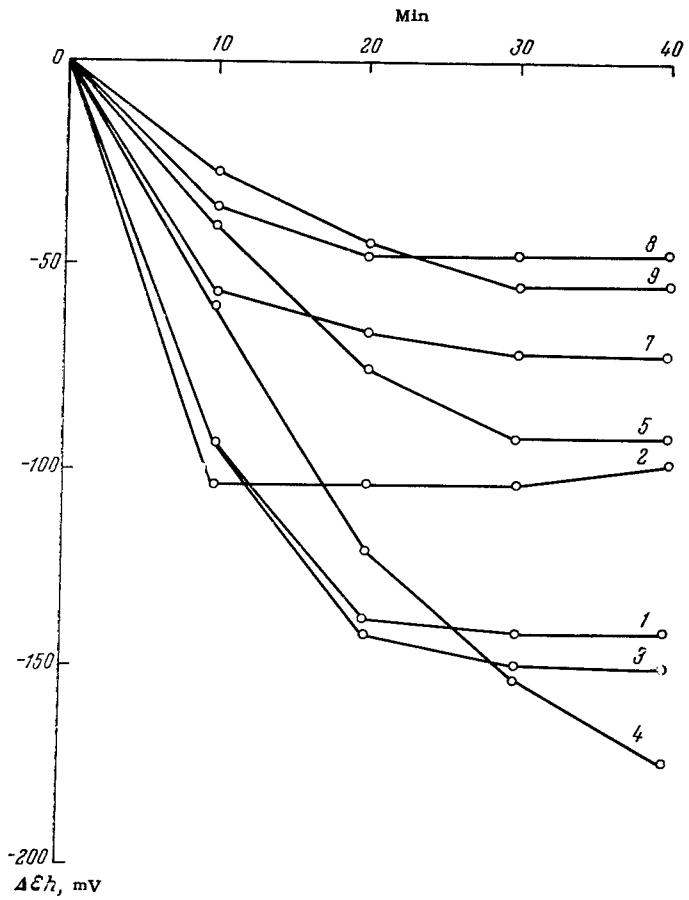
The maximum decrease in the level of the potential is observed 25-30 min after the administration of the preparations to mice for almost all the investigated substances (Fig.). The only exception is aminoethylthiosulfuric acid (Preparation No. 4). The decrease in the level of the redox potential after the injection continues for all 40 min of the experiment.

The data cited confirm the conclusion that the lower the readings of the redox potential of the tissues at the time of irradiation, the stronger the protective effect.

We were unable to evaluate the antioxidant action of all the Bunte's salts on a chemical model in view of the insolubility of many preparations in oleic acid. We were able only to show that inactive preparations (Nos. 8 and 9), soluble in oleic acid, do not change the rate of oxidation of β-carotene, while cysteamine and AET protect β-carotene from oxidation.

The data obtained on mice concerning the high radioprophylactic activity and low toxicity of β-aminoethyl-, γ-aminopropyl-, dimethylaminoethyl-, and β-guanidoethylthiosulfuric acids confirm the conclusions of the protective properties of Bunte's salts [1-3]. Other investigated representatives of aminoalkylthiosulfuric acids proved inactive (or weakly active).

In contrast to the available literature data, we succeeded in detecting a protective effect of the indicated active preparations of



Influence of various protectors on the stationary redox potential of mouse muscles. Along X-axis -- time after introduction of protectors, min; along Y-axis -- redox potential mV; the numbers denote the numbers of the preparations (see table).

Bunte's salts on model specimens as well [3, 4]. By using radiomimetic models and comparing them with radiation models, we succeeded in demonstrating that the protective effectiveness of Bunte's salts on models can be manifested after prolonged exposure of the potential radioprotectors with the cell.

CONCLUSIONS

1. Data on the radioprophylactic effect of certain representatives of Bunte's salts -- potential aminothiols -- were confirmed.

2. It was demonstrated that by using radiomimetic and radiation models for rapid and preliminary selection of radioprotectors, the antiradiation effectiveness of Bunte's salts can be evaluated.

3. The protective effectiveness of Bunte's salts on models can be detected after two to three-hour exposure of the potential radioprotectors with the cell.

4. The drop in the redox potential of the muscles at the time of irradiation of the animals can be used, together with other indices, as a measure of the antiradiation effect of the investigated radioprophylactic substances.

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ON THE INHIBITING EFFECT OF SMALL DOSES OF IONIZING RADIATIONS UPON VEGETATING CELLS

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pp. 278-283

The problem of the influence of a chronically increased radiation background upon living organisms has taken on more and more interest in our time. From experiments on prolonged irradiation of plants on a γ field [1-11], it follows that their radiosensitivity frequently extends into the region of extremely low values of the dose rate. Ehrenberg [2] observed the appearance of a number of morphological changes among the offspring of legumes, which experienced chronic irradiation in the first generation, at a rate of 0.4-6.0 R/day. Miksche [11] noted an appreciable lag in budding in the yew, irradiated for a year with γ rays at a rate of 3.7 R/day. According to the data of Ozol [7], chronic irradiation of wheat by γ rays at 1 R/day increases the number of mutations in comparison with their number under natural conditions. Granhall [12] has found that daily γ irradiation of barley at a dose rate of 0.25-0.58 R/day lowers its fertility. Finally, Neubohm [6] is inclined to believe that for barley, in general, there is scarcely a dose below which irradiation becomes ineffective.

In previous investigations, the authors demonstrated high sensitivity of V. faba L plants to chronic irradiation [13]. The rise in the natural background irradiation to 0.07 R/day, associated with an increase content of U and Ra on the experimental area, led to a substantial disturbance of the growth of V. faba. The data obtained were verified and confirmed in experiments on the influence of chronic irradiation upon wheat and barley.

MATERIALS AND METHODS

Spring wheat of the Garnet variety and spring barley of the Sysol'skii 95 variety were grown from planting until harvesting on field lots with an area of 2 m^2, situated between irradiator boxes, filled with radioactive rock containing $n \cdot 10^{-3}$% uranium and $n \cdot 10^{-7}$% radium. The number of rows in the plot was seven, number of plants to a row 100. On the control area, the plots with plants were situated between partitions that were not filled with radioactive rock. Each experimental and control variation was conducted in four lot repetitions (1961 experiment).

In 1962 the plants were grown in vegetation pots in trenches dug into the same radioactive rock and lined with insulating materials to avoid migration of the radioelements in the plants. The control trenches were dug in normal earth. The number of pots per variation was 45, number of plants per pot -- 50.

According to the readings of the RPP-1 and DP-11B instruments, the average rate of the background radiation on the plots between emitter boxes at a height of 40 cm above the soil varied in γ count from 0.072 R/day (near the wall of the emitter) to 0.005 R/day (in the center of the plot). In 1962 the background on the experimental area was somewhat higher (0.1 R/day), but also more uniform. The fraction of hard β radiation in all cases was below the threshold of sensitivity of the instruments; the influence of α and soft β radiation is excluded on account of the use of insulating materials. The radon content, according to the readings of the SG-11 emanometer, did not exceed the level of the normal frequency of distribution (no more than 0.1 emanation/liter). The rate of background irradiation on the control areas was 15-24 uR/hr.

RESULTS AND THEIR DISCUSSION

The data of two years of investigations of the structure of the wheat and barley crop (Table 1) are evidence of an unfavorable effect of chronic irradiation upon plants. According to most of the characteristics examine (height of the main stem, length of the third internode, air-dried weight of one plant, number of grains in the spike of the main stem), the experimental plants are significantly inferior to the controls. And yet, it was found that under the conditions of irradiation, as a rule, a larger grain is formed in the spike of the main stem of wheat and barley (Table 2). However, since the number of grains in the spike is lowered in the irradiated plants (Table 1), the average yield of grain per spike of the main stem, on the whole, is lower among these plants than among the control plants (Table 2).

Table 1

Characteristics of Plants and Structure of Wheat and Barley Crop Grown Under Conditions of Chronic Irradiation

Indices studied	Wheat, Garnet variety							
	1961				1962			
	Control	Irradiation	Difference $d \pm S_d$	t	Control	Irradiation	Difference $d \pm S_d$	t
Height of plants, cm $(\overline{x} \pm S_{\overline{x}})$	80,8±0,58	67,7±0,88	13,1±1,05	12,5	90,7±0,95	60,3±0,79	30,4±1,23	24,7
» », %	100	83,8	—	—	100	66,5	—	—
Number of plants studied	2197	2089	—	—	87	91	—	—
Length of third internode, cm $(\overline{x} \pm S_{\overline{x}})$	—	—	—	—	20,2±0,53	13,5±0,45	6,7±0,69	9,7
» », %	—	—	—	—	100	66,8	—	—
Number of measurements	—	—	—	—	87	91	—	—
Number of internodes $(\overline{x} \pm S_{\overline{x}})$	—	—	—	—	4,6±0,04	4,4±0,04	0,2±0,05	4,0
Number of plants studied	—	—	—	—	252	252	—	—
Air-dried weight of one plant, g $(\overline{x} \pm S_{\overline{x}})$	2,5±0,07	1,9±0,08	0,6±0,10	6,0	—	—	—	—
Number of measurements	2197	2089	—	—	—	—	—	—
Length of spike, cm $(\overline{x} \pm S_{\overline{x}})$	7,5±0,03	7,1±0,02	0,4±0,03	13,3	6,6±0,08	6,5±0,11	0,1±0,13	0,8
» », %	100	94,7	—	—	100	98,5	—	—
Number of measurements	840	840	—	—	252	252	—	—
Number of spikelets per spike $(\overline{x} \pm S_{\overline{x}})$	13,0±0,07	12,5±0,06	0,5±0,09	5,6	12,0±0,13	12,0±0,12	—	—
» », %	100	91,1	—	—	100	100	—	—
Number of measurements	317	595	—	—	251	251	—	—
Number of grains per spike $(\overline{x} \pm S_{\overline{x}})$	25,5±0,30	24,8±0,22	0,7±0,37	1,9	15,9±0,11	9,8±0,44	6,1±0,45	13,6
» », %	100	97,3	—	—	100	61,6	—	—
Number of measurements	317	595	—	—	76	91	—	—

Table 1 (Continuation)

Indices studied	Barley, Sÿsol'skiĭ 95 variety							
	1961				1962			
	Control	Irradiation	Difference $d \pm S_d$	t	Control	Irradiation	Difference $d \pm S_d$	t
Height of plants, cm $(\bar{x} \pm S_{\bar{x}})$	87,6±0,85	77,2±1,87	10,4±2,05	5,1	99,6±0,80	54,6±1,69	45,0±1,87	24,1
» », %	100	88,0	—	—	100	54,8	—	—
Number of plants studied	1854	1586	—	—	84	42	—	—
Length of third internode, cm $(\bar{x} \pm S_{\bar{x}})$	—	—	—	—	12,5±0,16	8,4±0,23	4,1±0,28	14,6
» » %	—	—	—	—	100	67,2	—	—
Number of measurements	—	—	—	—	83	41	—	—
Number of internodes $(\bar{x} \pm S_{\bar{x}})$	—	—	—	—	5,7±0,05	5,6±0,09	0,1±0,12	0,8
Number of plants studied	—	—	—	—	83	41	—	—
Air-dried weight of one plant, g $(\bar{x} \pm S_{\bar{x}})$	5,5±0,16	4,5±0,18	1,0±0,23	4,3	—	—	—	—
Number of measurements	1854	1586	—	—	—	—	—	—
Length of spike, cm $(\bar{x} \pm S_{\bar{x}})$	9,5±0,04	9,2±0,04	0,3±0,05	6,0	9,3±0,13	7,5±0,23	1,8±0,26	6,9
» », %	100	96,8	—	—	100	80,6	—	—
Number of measurements	840	840	—	—	84	42	—	—
Number of spikelets per spike $(\bar{x} \pm S_{\bar{x}})$	22,1±0,45	20,9±0,54	1,2±0,70	1,7	21,5±0,28	18,3±0,63	3,2±0,69	4,6
» », %	100	90,0	—	—	100	80,9	—	—
Number of measurements	210	210	—	—	84	42	—	—
Number of grains per spike $(\bar{x} \pm S_{\bar{x}})$	21,3±0,50	18,4±0,64	2,9±0,81	3,6	19,9±0,35	15,5±0,73	4,4±0,80	5,5
» », %	100	86,4	—	—	100	77,9	—	—
Number of measurements	210	210	—	—	84	40	—	—

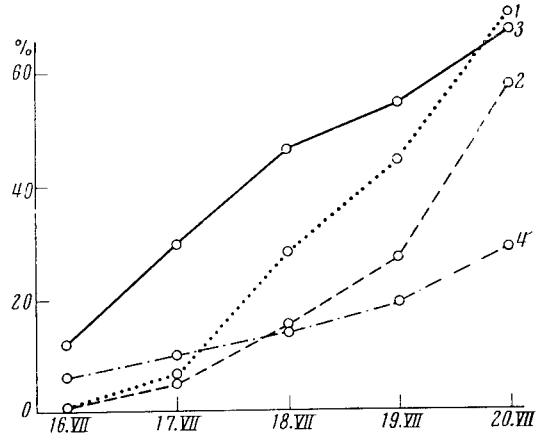

Fig. 1. Periods of heading of wheat and barley under normal conditions and under conditions of chronic γ irradiation. Wheat: 1 -- control; 2 -- chronic irradiation; barley: 3 -- control; 4 -- chronic irradiation. Along X-axis -- observation period; along Y-axis -- number of plants with spikes, %.

Table 2

Absolute Weight of Seeds and Yield of Wheat and Barley

Plants	Years of investigation	Weight of 100 seeds, g						Yield of grain per spike, g	
		Control		Chronic irradiation		Difference between control and experimental data $d \pm S_d$	Index of significance of difference t	Control	Chronic irradiation
		No of determinations	$\bar{x} \pm S_{\bar{x}}$	No of determinations	$\bar{x} \pm S_{\bar{x}}$				
Wheat	1961	20	12,1±0,41	20	16,3∓0,41	4,2∓0,58	7,2	—	—
	1962	4	8,2∓0,42	3	10,8∓0,19	2,6∓0,43	6,0	0,13	0,11
Barley	1961	20	38,0∓0,54	20	40,7∓0,50	2,7∓0,73	3,7	—	—
	1962	3	29,4∓1,97	3	25,8∓0,42	—3,6∓2,02	1,8	0,59	0,40

Phenological observations indicated that chronic irradiation had no significant effect upon the rate of appearance of wheat sprouts, while causing only a negligible lag in the appearance of sprouts in barley. Among these same plants, some retardation of the onset of the phase of tillering was observed. Data on measurements of the plants at the root in the phase of bolting, obtained for a number of years (Table 3), show that the differences between the control and irradiated plants are indefinite at the early phases of growth. Only in the case of barley (1963 experiment) was the appearance of an inhibiting effect

Table 3

Height of Main Stem of Wheat and Barley from Control and Experimental Areas
(Phase of Bolting), cm

Experimental plants	Years of investigation	Periods of observation	No of plants measured	Control	Chronic irradiation	$d \mp S_d$	t
Wheat	1961	3.VII	392	25,5∓0,90	26,7∓0,40	1,2∓0,98	1,2
	1962	4.VII	1125	24,0∓0,19	26,0∓0,20	2,0∓0,27	7,4
Barley	1961	3.VII	784	29,0∓0,80	27,2∓1,20	1,8∓1,37	1,3
	1963	5.VIII	146	28,1∓0,60	23,8∓0,45	4,3∓0,74	5,8
	1963	12.VIII	152	30,0∓0,46	27,4∓0,24	2,9∓0,57	5,1

Fig. 2. Inhibition of growth of wheat and barley under conditions of chronic γ irradiation. a -- Wheat; b -- barley; 1 -- control; 2 -- irradiated plants.

of chronic irradiation upon the growth of the main stem in height noted.

In the phase of heading of wheat and barley, the differences between the control and irradiated plants are already distinctly manifested. The inhibiting effect of chronic irradiation is exerted both on the periods of heading of the plants (Fig. 1) and on a number of morphological characteristics (Fig. 2).

The results outlined are again evidence of an unusually high effectiveness of the action of small doses of ionizing radiations upon vegetating plants. In our opinion, a substantial fraction of this effect should be ascribed to the unique geometry of the uranium-radium radiation source that we used, which may be characterized as voluminous representing the sum of small point sources in the volume of the rock. The apparatus available to us does not yet permit an exhaustive description of the radiation environment in the experiment. However, using the plants themselves as an "instrument," we were able to ascertain that different degrees of "immersion" of the plants in the same radiation field (compare the 1961 and 1962 experiments, Table 1) can be used to achieve different effectiveness of the action of this field upon the plants.

CONCLUSIONS

Chronic influence of small doses of ionizing radiation (0.005-0.1 R/day) upon vegetating plants of spring wheat and spring barley appreciably inhibit their growth and development, leading to a substantial decrease in the biological productivity of the plants.

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ON THE PROTECTIVE EFFECT OF CALCIUM AGAINST DEPOSITS OF RADIOSTRONTIUM IN THE ORGANISM

Z. V. Dubrovina, P. M. Malkin

pp. 284-287

The protective effect of calcium against radiostrontium has been the subject of a substantial number of studies published in the literature. However, since in each individual investigation, no more than two to three different concentrations of calcium were used for comparison, it did not seem possible to give a quantitative evaluation of the dependence of the deposition of strontium upon the calcium content in the diet. And yet, such a quantitative evaluation is vital for predicting the magnitude of the effect from an increase in the calcium intake.

To obtain information on this problem, in this work we used the materials of 11 experiments on adult rats weighing from 150 to 250 g. Before the beginning of the experiments, all the animals received normal calcium nutrition. During the experiment, calcium was taken in either contained in food products (in two experiments) or in the form of a feed additive. When calcium was added to the diet, in most cases its chloride was used.

Strontium-90 was also taken in in the form of the chloride in eight experiments, while in three experiments it was ingested in the composition of meat or milk. In the overwhelming majority of cases, the radioisotope was introduced mixed with the feed, and hence was consumed by the animals uniformly over a period of 24 hours; only in one experiment was strontium introduced into the stomach once through a probe.

At the same time, in five chronic experiments we determined the absorption of calcium from the gastrointestinal tract according to the method described in [1-3]. The amount of calcium-45 absorbed from the gastrointestinal tract was calculated according to the difference between the activity taken in with the diet and excreted with the feces, considering endogenous calcium. In accord with this method, the entire mass of calcium passing from the gastrointestinal tract into the blood is taken as the amount of absorbed calcium; moreover, part of this absorbed calcium is subsequently returned to the intestines as endogenous

calcium. The duration of the experiments was varied from eight hours to 24 days.

The experimental conditions, as is evident from the above, varied greatly, which increased the dispersion of the results, but made it possible to draw broader generalizations.

Experiments with 58 groups of animals, four to ten rats in each with 29 different calcium concentrations in the diet, varying from 2 to 150 mg/day, were subjected to statistical treatment according to the method of least squares. The results obtained are presented in Fig. 1.

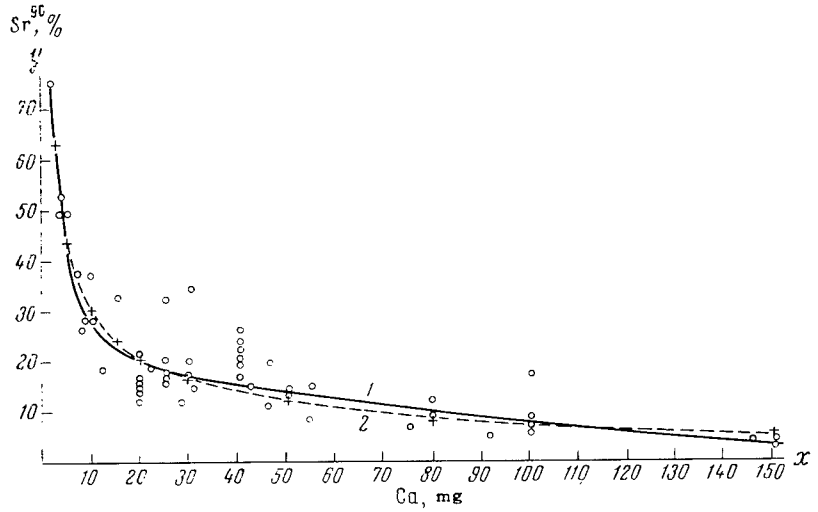

Fig. 1. Dependence of the deposition of strontium-90 in the skeleton upon the calcium content in the diet (from 2 to 150 mg). 1 -- Curve according to equation (1); 2 -- according to equation (2); the circles denote experimental points, the crosses -- calculated points according to a step model.

As was found, the dependence of the deposition of radiostrontium in the organism upon the calcium content in the diet is satisfactorily described by the sum of two exponential functions or by one power function with high correlation ($\rho = -0.75 \pm 0.06$) according to the equations:

$$y = 78\, e^{-0,24x} + 22\, e^{-0,008x} \qquad (1)$$

or

$$y = 96\, x^{-0,51}, \qquad (2)$$

where y is the deposition of Sr^{90} in the organism in percent of that

taken in with the diet; x is the calcium content in the diet, mg/day.

The power or biexponential character of the dependence of the retention of strontium upon the calcium content in the diet is correct only within the limits of the concentrations studied, that is from 2 to 150 mg per day per animal. It should be mentioned that for small intervals between concentrations, this dependence can be described by a linear function.

As can be seen, the effect from an increase in the calcium intake drops sharply as the level of the normal physiological requirement is approached. For animals of a given age, 40-60 mg/day can be taken as the normal level of calcium nutrition [3].

When the calcium intake is increased three to four times above the normal, the retention of radiostrontium decreases by 1.5-2-fold, while the addition of calcium to a diet sharply deficient in it causes an almost proportional effect.

Figure 2 presents the retention of Sr^{90} in the presence of various amounts of calcium absorbed from the gastrointestinal tract. The results were treated analogously to the preceding. From the figure it is evident that the regression line constructed according to exponential or power functions satisfactorily describes all the results obtained.

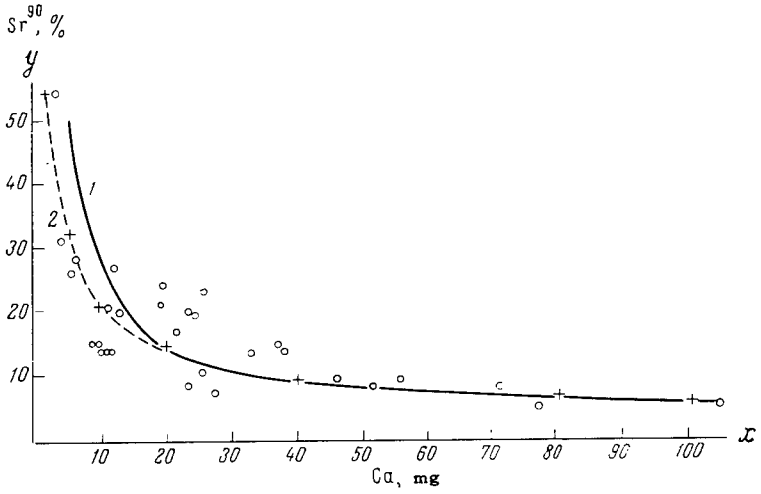

Fig. 2. Dependence of the deposition of strontium-90 in the skeleton upon the amount of absorbed calcium (from 3 to 150 mg). 1 -- Curve according to equation (3); 2 -- equation (4); the circles denote the experimental points.

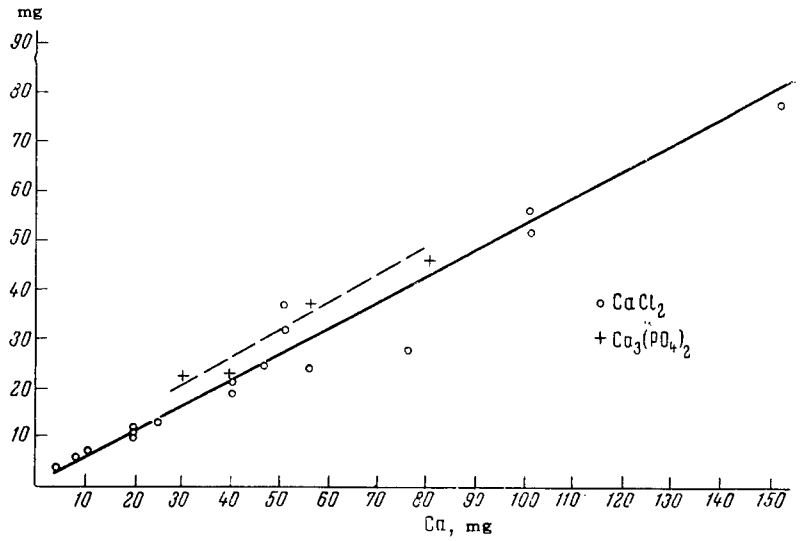

Fig. 3. Dependence of the amount of absorbed calcium on the calcium content in the diet. Along Y-axis -- assimilated calcium, mg; along X-axis -- calcium of the diet, mg.

The regression equation in this case took the form:

$$y = 86\, e^{-0,17x} + 14\, e^{-0,007x} \tag{3}$$

and

$$y = 80\, x^{-0,56}, \tag{4}$$

where x is the amount of calcium absorbed from the gastrointestinal tract, mg/day.

Noteworthy is the similarity of the last equations to those cited above. This means that a direct proportional dependence should exist between the calcium concentration in the diet and the amount of absorbed calcium.

Figure 3 presents the dependence of the amount of assimilated calcium upon its content in the diet.

As can be seen, for the same salt the amount of absorbed calcium is proportional to its content in the diet. This conclusion contradicts the data of [4]. However, in it actually not the absorption of calcium from the gastrointestinal tract, but its disappearance from the duodenum in a definite period of time was determined. In this case, the endogenous discharge of calcium into the contents of the duodenum during this period was not considered at all.

From Fig. 3 it is also evident that the absorption of calcium from various salts is not the same. Two experiments on rats that received the same amount of calcium, but in the form of 10 different compounds, both organic and inorganic, indicated that the protective effect among calcium salts differs, and it is well correlated with the amount of calcium absorbed from a given compound: the more calcium was absorbed, the better this salt protected [5-6].

All the aforementioned permits us to conclude that only absorbed calcium exerts a protective effect against the deposition of radiostrontium in the case of calcium-deficient diets: the more calcium is assimilated from the gastrointestinal tract, the less radiostrontium should be assimilated. In connection with this, it is interesting to draw an analogy with the nature of the protective action of calcium of the soil against the intake of radiostrontium into plants: according to the data of [7], only the exchange fraction of calcium in the soil exerts a protective effect.

The exponential nature of the dependence of the deposition of radiostrontium in the organism upon the calcium of the diet, obtained on rats, evidently will also be observed among other individuals, including man; however, the degree of this dependence should be different since the calcium requirements of rats are considerably higher than those of man (200-300 mg/kg of weight and 7-10 mg/kg of weight, respectively). For this reason, we might expect a greater sensitivity of rats to calcium deficiency in comparison with man. Further investigations are needed on animals closer to man in level of calcium requirement.

CONCLUSIONS

1. The effect from the addition of calcium to the diet is sharply reduced when the level of normal physiological requirement of calcium is approached.

2. At a calcium content in the daily diet of rats from 2 to 150 mg, the dependence of the deposition in the organism of radiostrontium, administered orally, upon the calcium content is satisfactorily described by the sum of two exponential functions with exponents -0.24 and -0.008 or by a power function with exponent -0.52.

3. The protective effect of calcium in the case of oral intake of strontium-90 is determined by the amount of calcium absorbed.

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INFLUENCE OF γ IRRADIATION OF RYE SEEDS ON THE DYNAMICS OF THE ACCUMULATION OF CHLOROPHYLL AND CAROTENE

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pp. 288-291

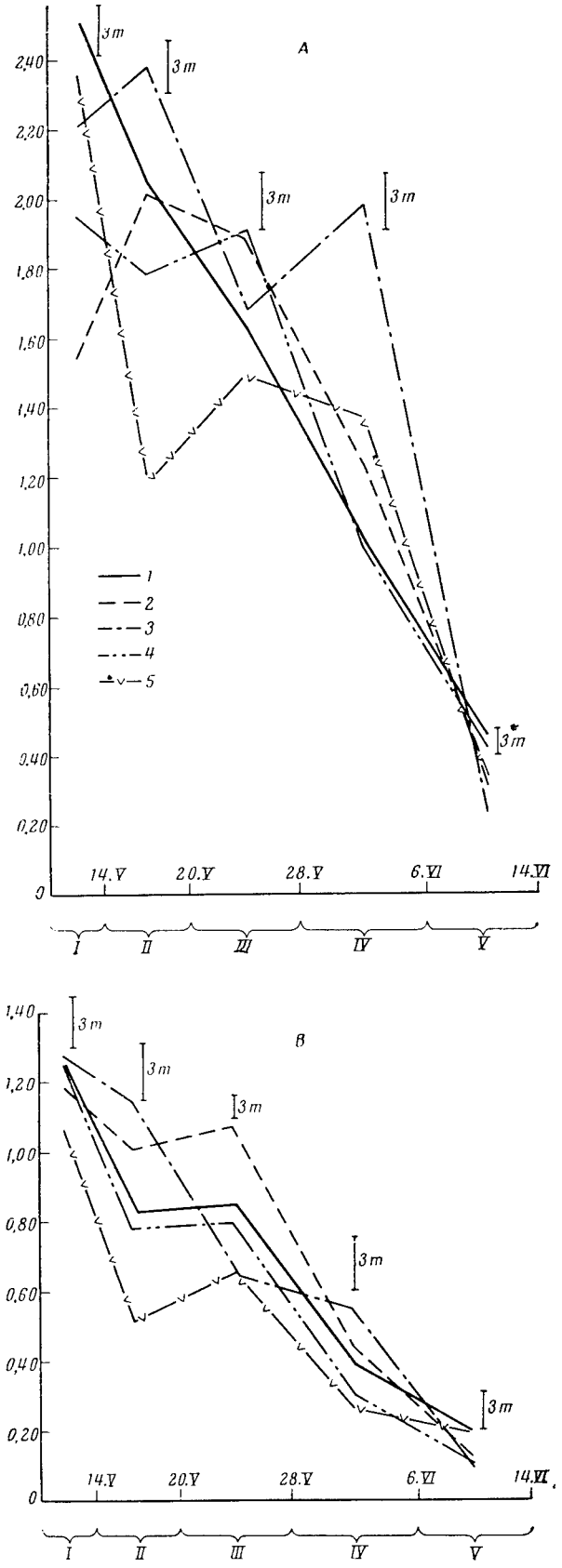
The positive influence of ionizing radiation upon the chlorophyll content in the leaves of spring rye has been noted by Breslavets [1]. Moreover, the literature contains only brief information on the increased chlorophyll and carotene content in one of the leaves of corn, grown from seeds subjected to γ irradiation [2-4].

The purpose of this work was to investigate the influence of γ irradiation of dry seeds of spring rye on the dynamics of the accumulation of chlorophyll and carotene in the plant.

The irradiation of seeds of the Petkus variety at doses of 0.5, 1, 2.5, and 5 kR was performed on the γ apparatus of the Institute of Biophysics, Academy of Sciences USSR. Ten days after irradiation, the seeds were planted in soil without the introduction of fertilizers. The field repetition was eight times for the control, twice for the experimental. Samples were collected to determine dry weight, chlorophyll and carotene content at definite phases of development of the plants. The pigments were determined colorimetrically [5]. The data obtained, presented in Tables 1 and 2, permit us to conclude that the absolute amount of the two pigments increases with development, both among the control plants and in the experimental.

In this case, all the experimental plants contain an increased amount of pigments at one phase of development or another. Evidently γ irradiation of seeds has a substantial influence upon the accumulation of pigments in the chloroplasts. However, the absolute amount of these substances in the plant cannot serve as a criterion for judging the dynamics of their accumulation in the plant.

The amount of the pigment accumulated by a unit mass of the plant per unit time was referred to as the "intensity of accumulation" (IA) of chlorophyll or carotene at one phase of development of the plant or another. We calculated this value for each phase of development on the basis of the experimental data of Tables 1 and 2, dividing



Influence of γ irradiation on the intensity of the accumulation of pigments by the above-ground organs of spring rye. A -- Chlorophyll; b -- carotene. Along X-axis -- phases of development: I -- opening of first leaf; II - opening of second and third leaves; III -- tillering; IV -- bolting; V -- heading; along Y-axis -- increase in pigment in mg per g of dry matter per day; 1 -- control; 2 -- 0.5 kR; 3 -- 1 kR; 4 -- 2.5 kR; 5 -- 5 kR.

Table 1

Influence of γ Irradiation on the Chlorophyll and Carotene Contents in the Above-Ground Organs of Spring Rye (in mg per Plant)

Irradiation dose, kR	Development of 1st leaf, 14 May	Development of 3d leaf, 20 May	Tillering, 28 May	Bolting, 6 June	Heading, 14 June
			Chlorophyll		
Control	0,082 ±0,004	0,40 ±0,01	2,10 ±0,05	6,40 ±0,25	14,30 ±0,70
0,5	0,053 ±0,005	0,35 ±0,004	2,00 ±0,08	6,80 ±0,03	11,70 ±0,60
1,0	0,070 ±0,003	0,49 ±0,005	2,20 ±0,01	10,80 ±0,08	14,80 ±0,70
2,5	0,070 ±0,0	0,36 ±0,04	2,80 ±0,12	6,80 ±0,50	13,70 ±0,50
5,0	0,090 ±0,002	0,27 ±0,0	1,30 ±0,01	5,30 ±0,15	10,90 ±1,10
			Carotene		
Control	0,040 ±0,002	0,170 ±0,004	1,06 ±0,04	2,74 ±0,018	6,08 ±0,40
0,5	0,037 ±0,001	0,186 ±0,002	0,97 ±0,06	2,86 ±0,24	4,85 ±0,20
1,0	0,040 ±0,004	0,251 ±0,005	0,92 ±0,04	3,27 ±0,08	4,83 ±0,20
2,5	0,044 ±0,002	0,172 ±0,002	1,20 ±0,06	2,48 ±0,01	4,86 ±0,28
5,0	0,040 ±0,001	0,119 ±0,007	0,57 ±0,02	1,37 ±0,03	4,56 ±0,20

Table 2

Influence of γ Irradiation on the Dry Weight of the Above-Ground Portions of Spring Rye Plants (in g per Plant)

Irradiation dose, kR	Coleoptiles, 10 May	Development of 1st leaf, 14 May	Development of 3d leaf, 20 May	Tillering, 28 May	Bolting, 6 June	Heading, 14 June
Control	0,0029 ±0,0001	0,0123 ±0,0010	0,0402 ±0,0040	0,2183 ±0,0250	0,7372 ±0,0400	3,4900 ±0,0800
0,5	0,0032 ±0,0001	0,0115 ±0,0010	0,0380 ±0,0030	0,1805 ±0,0200	0,6817 ±0,0800	2,9030 ±0,1000
1,0	0,0025 ±0,0001	0,0132 ±0,0011	0,0462 ±0,0045	0,2085 ±0,0100	0,7567 ±0,1800	3,3870 ±0,1400
2,5	0,0036 ±0,0001	0,0138 ±0,0010	0,0409 ±0,0040	0,2817 ±0,0100	0,6222 ±0,0300	3,2510 ±0,1900
5,0*	0,0044 ±0,0001	0,0145 ±0,0010	0,0362 ±0,0070	0,1369 ±0,0001	0,4950 ±0,0800	3,6580 ±0,0700

*Beginning with the phase of development of the first leaf, many of the plants of this variation began to die.

the increase in the pigment (in mg) in each phase by the dry weight of the above-ground portion of the plant (in g), the average during this time, and by the duration of the phase (in days). The results of the calculation are given in Fig. 1, A and B.

Among the control plants, the curve of the "intensity of accumulation" of chlorophyll represents a straight line with maximum in the first phase of development. Among the plants irradiated at a dose of 500 R, the IA of chlorophyll at the very beginning of development is very low (38% below the control), but then it increases and subsequently does not differ from the control. Plants that received a dose of 1 kR exhibit an extremely substantial increase in the IA of chlorophyll (by 16%) during the period of development of the third leaf and during bolting (by 97%); in the remaining phases of development, they accumulate chlorophyll with the same intensity as the control plants. Among plants grown from seeds irradiated at 2.5 kR, we observed a more intensive accumulation of chlorophyll only at the beginning of tillering (by 17%); in all the other phases of development they accumulate chlorophyll considerably less intensively (from 1% to 26%) than the control plants. In the variation with a dose of 5 kR, the experimental plants in all phases of development exhibit a less intensive accumulation of chlorophyll (from 9 to 28%), except for the phase of bolting (by 36%).

Analyzing the curves of the intensity of accumulation of carotene we see that among the plants irradiated at a dose of 500 R, pigment accumulation is more intensive than on the control plants (from 16 to 24%) in all phases of development except for the first and last. Irradiation at a dose of 1 kR causes a more intensive accumulation of carotene in the phase of development of the third leaf (by 39%) and before bolting (by 29%); in all the other phases the intensity of this process is equal to the control or lower. Irradiation at a dose of 2.5 kR has no effect upon the IA of carotene, while a dose of 5 kR sharply reduces the intensity of this process (by 20-40%) in all phases of development except for the last, when it is comparable with the control.

Thus, the results of our experiments show that γ irradiation of seeds in small doses stimulates the formation and accumulation of chloroplast pigments to one degree or another. A dose of 1 kR is the most effective. Stimulation is not observed immediately after germination of the seeds, but only in later phases of development (development of the third leaf, tillering, bolting). With the onset of heading, the stimulating effect disappears.

We should like to take this opportunity to express our deep gratitude to Professor L. P. Breslavets for his aid and consultation in the work.

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REDISTRIBUTION OF FREE SUGARS IN PLANTS AFTER γ IRRADIATION

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pp. 292-293

Individual metabolic reactions in plants possess different radiosensitivity [1]. The degree of their radiosensitivity can be judged according to the disproportionate change in the concentration of biologically important substances in irradiated plants. However, a summary evaluation does not give exhaustive information on the processes that occur during irradiation within a concrete metabolic reaction. If is of interest to determine how the carbohydrate balance varies among irradiated plants in the course of their metabolic transformations.

In the experiments we used seven-day pea sprouts of the Pobeditel 5-288 variety, which were subjected to γ irradiation by Co^{60} at a dose of 50 kR and a dose rate of 45 R/min. Six hours after irradiation, part of the sprouts were placed for four hours in a chamber with $C^{14}O_2$ according to the procedure described earlier [2]. Another portion of the sprouts was grown for nine days after irradiation under greenhouse conditions, after which the plants were labeled in an atmosphere of $C^{14}O_2$, just as in the first lot. The controls were nonirradiated plants. The radioactivity of the leaf homogenates and fractions was calculated per unit dry weight. The summary activity of free sugars and other alcohol-soluble substances was determined in the alcohol extract. The total sugars was determined in the alcohol extract, deionized on ion exchange resins. The amounts of free sugars -- glucose, fructose, and sucrose -- were determined separately by a chromatographic method [3]. The experimental results are presented in the table and in Figs. 1 and 2.

From the table data it is evident that the photosynthetic intensity decreases somewhat (by 14%) in the first hours after irradiation and remains at the same level up to nine days. However, the relative amount of alcohol-soluble substances increases somewhat in comparison with that which was observed in the first period after irradiation.

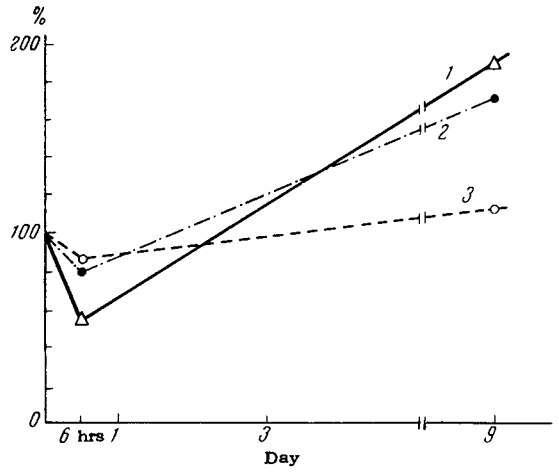

Fig. 1. Phase character of the changes in the free sugars after irradiation. Along X-axis -- time after irradiation; along Y-axis -- radioactivity of monosugars and sucrose, % of control; 1 -- sucrose; 2 -- fructose; 3 -- glucose.

Distribution of C^{14} in Plant Material After Irradiation at a dose of 50 kR (Counts/Min per 10 mg of Dry Weight)*

Object of investigation	Control	Six hours after irradiation	% of control	Control	Nine days after irradiation	% of control	Activity after nine days: Control	Activity after nine days: Irradiation	Activity after nine days: % of control	Difference between original activity: Control	Difference between original activity: Irradiation	Difference between original activity: % of control
Leaf homogenate	13 500	11 593	85,9	19 459	16 252	83,5	5993	7793	130,0	7507	3800	50,6
Alcohol extract	9 333	7 627	81,7	12 987	11 282	86,9	—	—	—	—	—	—
Sugars	7 170	6 231	86,9	7 313	7 574	103,6	—	—	—	—	—	—

*The error in the analysis for radioactivity was 6%.

The total amount of soluble carbohydrates nine days after irradiation (in percent of control) is 17% increased with respect to the initial period. But this is most distinctly detected in a radiochromatographic investigation of the free sugars (Fig. 1). The concentration of individual carbohydrates with respect to the control was sharply reduced, and then increased disproportionately with respect to the first period after irradiation (sucrose by 134%, fructose by 71%, glucose by 23%), which indicated a different sensitivity of the elementary

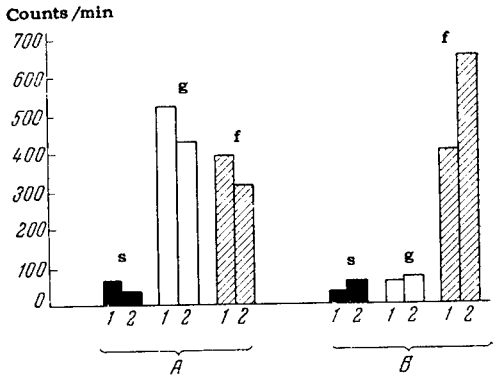

Fig. 2. Redistribution of free sugars after irradiation. A -- Six hours after irradiation; B -- nine days after irradiation; 1 -- control; 2 -- irradiation at a dose of 50 kR; s -- sucrose; g -- glucose; f -- fructose.

reactions of carbohydrate metabolism.* There was also a redistribution of the amount of free sugars (Fig. 2). Such a state evidently is not associated with any intensification of the synthesis of these substances, since we observed no increase in the photosynthesis, but rather with a slowdown of their further conversion during the process of respiration.

In order to compare the representation of the magnitude of the decrease in the respiration intensity during irradiation, part of the plants, which originally received the C^{14}-label, were left under greenhouse conditions for nine days. The respiration rate was followed according to the loss of the radioactive label (see table). The respiration rate of the sprouts was decreased by an average of 50% as a result of irradiation.

The process of assimilation of C^{14} and distribution of the label in the plant material, which we followed quantitatively, varies with time. The phase character of the dynamics of the assimilation of C^{14} was manifested in the alcohol fraction, in the summary sugar fraction, and especially distinctly in an investigation of glucose, fructose, and sucrose. No phase character was observed in plant homogenates. The data obtained agree with the phase character of the change in respiration after irradiation of plants [4-6], since the investigated carbohydrates are the basic respiratory substrates.

*In our experiments the fraction of the investigated carbohydrates comprised only part of the sum of all the carbohydrates. In the first period after irradiation, two spots with Rf = 0.44 and 0.53 appeared on the chromatograms. After nine days a substance with Rf = 0.32 appeared.

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INFLUENCE OF THE CONDITIONS OF CULTURING SEED MATERIAL ON THE MANIFESTATION OF RADIOBIOLOGICAL EFFECTS DURING THE SUBSEQUENT IRRADIATION OF SEEDS BY FAST NEUTRONS

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pp. 294-297

The effects of neutrons on biological specimens have been little studied in comparison with those for X rays and γ rays. It is believed that the injuries induced by neutron irradiation are practically independent of the physiological state of the specimen [1, 2].

The data obtained in this article contradict this hypothesis.

MATERIALS AND METHODS

Barley seeds of the Krasnoyarskiĭ 1 variety, bred against a background of low soil fertility, were used as the objects of investigation. In 1961, part of the seeds were planted on fallow black soil (high soil fertility), while another portion was planted in a fall-plowed field after wheat (low soil fertility). In 1962, N^{40}, P^{40}, and K^{20} were applied to the area with high soil fertility in doses of 100, 120, and 100 kg/hectare, respectively. Moreover, in 1963 part of the seeds of the crop from the high soil fertility area were planted in a fall plowed field after wheat (low soil fertility), part after peas (high soil fertility). The barley was sown on medium-strength high-alkali chernozem. The yield from the low soil fertility background in 1962 was 16.6 centners/hectare and in 1963 11.5 centners/hectare, while from the area with high soil fertility, the values were 32.6 centners/hectare and 41.8 centners/hectare, respectively. Large, equalized seeds were used for the neutron treatment. The weight of 1000 grains from the high-soil-fertility background in 1962 was 56.7 g and in 1963 -- 57.5 g, and from the low background -- 47.2 g and 54.0 g, respectively.

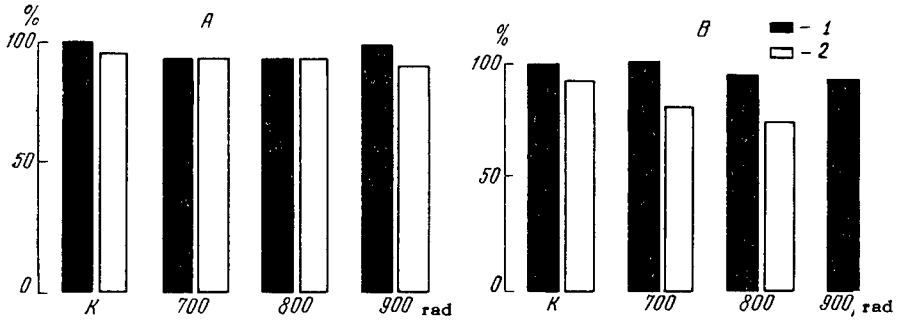

Fig. 1. Germination of seeds in pots (A) and in open soil (B). K -- Control; 1 -- plants from high soil fertility background; 2 -- plants from low soil fertility background.

The seeds were treated in the IRT-1000 nuclear reactor with fast neutrons in the doses: in 1962 -- 700, 800, and 900 rad, in 1963 -- 950 and 1100 rad. The accompanying γ admixture was 942, 1080, 1215, 665, and 775 rad, respectively. The admixture of thermal and slow neutrons was negligible. Considering the negligible effect of γ radiation at the total dose obtained in treatment of the seeds in a nuclear reactor [3], we shall henceforth mention only the fast neutron doses.

Irradiated and nonirradiated seeds of the 1962 and 1963 crops were germinated in Petri dishes on filter paper at room temperature. A week after the beginning of germination, the sprouts were transferred to tap water and placed in the light. After another week, the length of the roots and sprouts was measured, and the dry weight of the roots was determined. Part of the seeds of the 1962 crop were planted in a hothouse and under field conditions. For the hothouse experiments we used meadow soil with the introduction of mineral fertilizers into the pot. The soil moisture content in the pots was maintained at a level of 70% of its total moisture capacity. A field experiment was conducted in 1963. The soil moisture content at a depth of 5-20 cm varied from 24.0 to 39.6% during the first two months, with a minimum on the first days after planting.

EXPERIMENTAL SECTION

Treatment of seeds of the 1962 crop from backgrounds of low and high soil fertility with doses of up to 900 rad had no appreciable effect upon the germination of the seeds in Petri dishes; nor were any significant differences in the sensitivity of the seeds from the two backgrounds to neutron treatment according to germination rate detected in the pots (Fig. 1). In open soil, among seeds from a background of

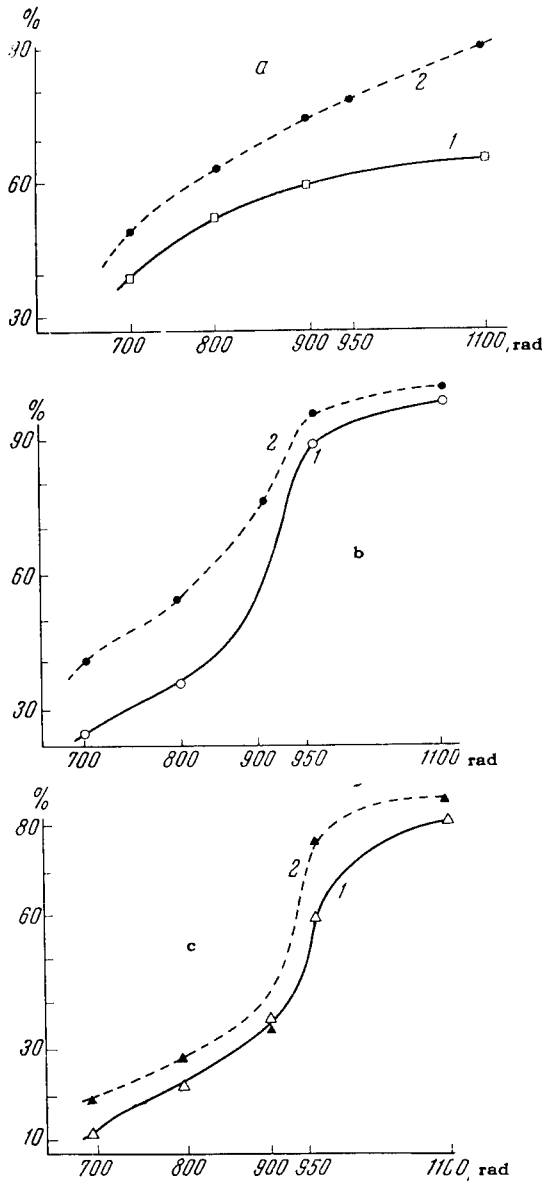

Fig. 2. Influence of neutron irradiation on the germination of seeds from different backgrounds of soil fertility. a -- Inhibition of growth of roots in length; b -- inhibition of elongation of sprouts; c -- inhibition of accumulation of dry mass by roots; 1 -- for seeds from high soil fertility background; 2 -- for seeds from low soil fertility background.

high soil fertility, treatment at a dose of 900 rad lowered the germination rate by only 6.5%, while for seeds from a background of low soil fertility, this dose proved lethal. The effect of smaller doses on the germination of the seeds was considerably weaker. Comparing the germination rates of seeds in pots and under field conditions, we may assume that the basic cause of the difference of the biological effect of the same irradiation doses lies in a different water system.

The results of experiments conducted in Petri dishes on seeds of the 1962 and 1963 crops, expressed in percent inhibition of growth, are presented in Fig. 2. Even under favorable conditions of germination substantially greater inhibition of the growth processes is manifested among the irradiated seeds. The elongation of the roots is most substantially inhibited, especially among the seeds of a background of low soil fertility at a dose of 1100 rad, although they were obtained after one year of growth under such conditions.

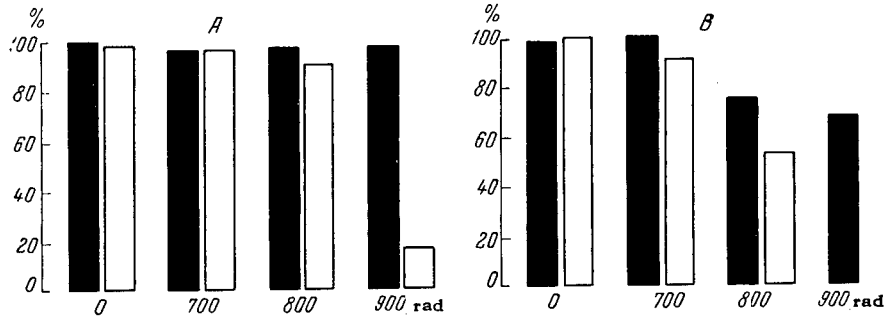

Fig. 3. Survival rates in pots and in open soil. Notations the same as in Fig. 1.

The appreciably greater sensitivity to neutron irradiation of seeds from a background of low soil fertility, manifested during germination, was revealed in a weakening of the recovery processes and a very high death rate of the plants. In spite of the fact that the most favorable conditions for growth and development were created in the pots, the survival rate in the variations of seeds from a background of low soil fertility proved significantly lower than in the variations of seeds from a high soil fertility background (Fig. 3). Especially significant differences were manifested at a dose of 900 rad. Although this dose chiefly affected vegetation for the variation of seeds from a background of high soil fertility, in the variation of seeds from a fall-plowed field, there was excessive thinning of the plants. It is interesting to note that even under favorable conditions of growth in pots, the survival rate in the 800-rad variation of seeds from a background of low soil fertility proved lower than in the 900-rad variation of seeds from a background of high soil fertility. The difference in survival rates was even more substantial under field conditions (Fig. 3).

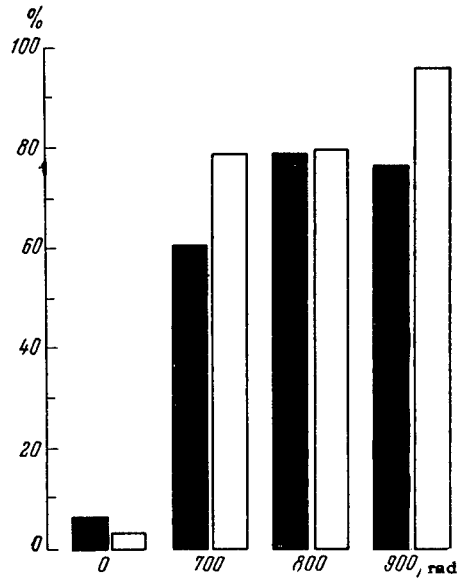

Fig. 4. Sterility of the main generative shoot. Notations the same as in Fig. 1.

Ultimately the different sensitivities of the two lots of seeds to neutron treatment was expressed in sterility of the first generation in the case of growth in pots. Sterility was calculated according to the main spike and was expressed in percent of spikelets unfilled with grain among the total number of spikelets per spike. The data are presented in Fig. 4. In this case also, seeds from a low background of soil fertility after neutron treatment showed substantially greater sterility of the plants grown from them.

The different sensitivities of seeds to neutron treatment noted can be combined on the basis of injuries to vitally important compounds of the type of respiratory enzymes and storage compounds with high-energy bonds. It is quite possible that the difference in the nitrogen and water contents of the medium might lead to a difference in the seeds at the level of the respiratory enzymes or concentration of compounds with high-energy bonds. It is precisely seeds from a background of low soil fertility that may contain relatively more of the above mentioned compounds, damage of which by neutrons leads to the detected effects.

CONCLUSIONS

Under conditions of insufficient nutrition, seed material is formed which is substantially more sensitive to the effects of fast neutrons than under conditions of normal formation of the crops.

In conclusion, the author would like to express deep gratitude to V. V. Khvostova and Yu. I. Bregadze, who participated in the treatment of the seeds with fast neutrons.

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DEPTH DISTRIBUTION OF THE ABSORBED ENERGY IN LOW-Z MATERIALS, IRRADIATED BY ACCELERATED ELECTRONS WITH ENERGIES 0.4-1.2 MeV

G. V. Radzievskiĭ, D. P. Osanov

pp. 298-307

In conducting research in the field of radiobiology, radiation chemistry, medicine, physics, etc., irradiation by accelerated monoenergetic electrons with energy 0.5-5 MeV is frequently used. The main advantages of such irradiation in comparison with X ray and γ irradiation lies in the possibility of obtaining very high dose rates, of the order of 10^5-10^7 rad/sec, as well as in the comparatively simple and accurate determination of the integral dose in the specimen, entirely absorbed by the electron beam. The integral dose in this case is equal to $IE_0t(1 - p)$, where I is the current strength, E_0 is the energy of electrons incident on the specimen, t is the time of irradiation, and p is the so-called energy coefficient of backscattering, equal to the ratio of the summary energy of electrons reflected from the sample to the energy of electrons incident upon the sample. The values of I and t are easily determined; E_0 is known for a given accelerator, while the value of p can be estimated according to the literature data for most cases of practical importance, as will be shown below. It is important that for low-Z materials and the case of normal incidence of the beam, $p \ll 1$, and no great accuracy in the determination of this value is required.

In addition to the integral dose, in the case of irradiation it is also necessary to know the dose distribution in the material. In the case of a comparatively low energy of the electrons, it is usually necessary to find the unidimensional dose distribution for the geometry of an "infinitely broad" electron beam. Such distributions can be calculated approximately by solving the kinetic equation for electrons with certain simplifications [1-3]; however, it is simpler to measure them. Individual results of such measurements for a number of materials and initial electron energies E_0 are cited in the literature [4-10]. Frequently the "reversibility of the dimensions" of the radiation beam and detector are used in dose measurements in broad-beam geometry, and a narrow (collimated) beam of electrons and an "infinitely" broad flat detector, for example, a slit ionization chamber, are utilized.

The dose distributions from electrons in low-Z materials (water, plastics, paraffin, etc.) for the broad-beam case, measured by various authors, differ rather greatly. In all cases, the dose curves cited in the literature possess the characteristic dome shape, with maxima situated at a depth of approximately one third the range of the electrons. However, the relative heights of these maxima, estimated as the ratio of the maximum dose P_{max} to the dose at the surface P(0), according to the results of various authors, differ greatly. Thus, for example, it was found in [4] that $P_{max}/P(0)$ within the energy range 2-5 MeV increases with increasing E_0, and at E_0 = 2 MeV comprises about 1.3. In [10] the dose curves are cited for water or tissue, showing that $P_{max}/P(0)$ increases with increasing E_0 all the way up to 9 MeV, and when E_0 = 1 MeV, it is about 1.1. In earlier studies [6-8], no increase in $P_{max}/P(0)$ with increasing E_0 was detected, and the value of the ratio $P_{max}/P(0)$ for water and the energies 0.5-1.5 MeV is equal to about 1.5. From the results of [5, 9], the opposite tendency is evident -- with increasing E_0 (in the intervals 3-15 and 6-21 MeV), $P_{max}/P(0)$ decreases monotonically; moreover, extrapolation of the dependences $P_{max}/P(0)(E_0)$ at E_0 = 1 MeV leads to a value of about 1.4-1.5.

All the dose distributions cited in the literature correspond to the case of normal incidence of the beam on the phantom. Although the case of normal incidence is encountered most often in irradiation practice, finding the dose distributions for an oblique incidence of the beam is also of undoubted interest.

The purpose of this work was to measure the relative dose distributions in low-Z materials from electrons with energies in the range 0.4-1.2 MeV at various angles of incidence of the beam upon the sample. Moreover, the work discusses methods of standardizing the relative dose curves, i.e., methods of determining the absolute values of the dose rate, corresponding to a set value of the electron current on the sample.

2. The radiation source in this work was an apparatus with a valve-cascade voltage multiplier, calculated for an energy of up to 1.2 MeV at a beam current of up to 2-3 mA. Certain methodological questions of the work on this apparatus were discussed in [11, 12]. Calibration of the apparatus according to electron energies was performed with the aid of a total absorption calorimeter, simultaneously serving as a Faraday cylinder [13].

The measurement of the relative dose distributions was performed with the aid of a broad plane-parallel ionization chamber, depicted in Fig. 1. Here the vertical arrows denote the electron beam, emerging from the window of the accelerator tube and passing through the collimator 1, 2. A plane-parallel ionization chamber -- monitor 6 was constructed in the collimator for continous monitoring of the emergent electron beam. The beam, emerging from tube 2 of the collimator, was incident upon a flat sample 3 of the test material, under which was situated the measuring chamber 4, 5. The working volume of the chamber was a flat slit, bounded from above by a terylene film about 10 μ thick, and below by a disk 4 of a material close to the material

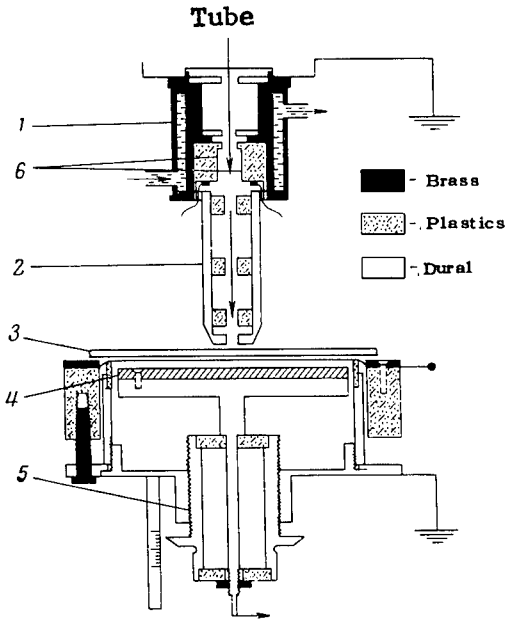

Fig. 1. Scheme of plane-parallel ionization chamber.

of the sample 3. The film was tightly stretched over the framework of the chamber, while the screw 5 with dial and scale served to measure the height of the working volume of the chamber. The position of the chamber and sample in Fig. 1 corresponds to the case of normal incidence of the beam. For measurements at other angles of incidence, the chamber with sample was set up at an angle to the beam.

Determinations of the relative depth dose distributions reduced to measurements of the ionization current in the chamber; the true ionic current was determined as half the sum of the currents of the chamber in the case of opposite polarities of its electrodes. The height of the working volume of the chamber was usually established at 0.5 mm, and in all the cases in the measurements the presence of proportionality between the ionic current and height of the gap was verified.

The diameter of the working volume of the chamber was about 80 mm, and as was shown by special experiments, it was sufficient for the chamber to be considered an "infinitely wide" detector at angles of incidence of the beam all the way up to 60°.

In measurements with an ionization chamber, generally speaking, not the distribution of the absorbed dose, but the distribution of the ionization density according to the depth of the phantom was determined. The absorbed dose rate and ionization density were related by the well known Bragg-Gray function. In the case of a small difference in the atomic numbers of the wall and gas of the chamber and in the case of a narrow ionization gap, the gas of the cavity does not disturb the flow

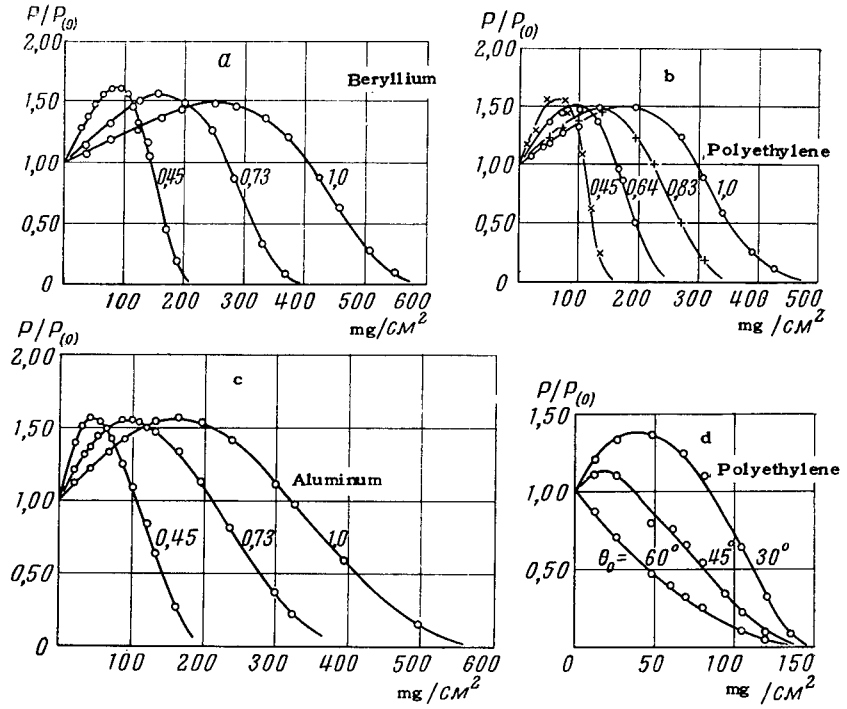

Fig. 2. Relative dose distributions obtained with the aid of a plane-parallel ionization chamber for various materials. Along Y-axis -- relative dose rate; along X-axis -- thickness of material.

of ionizing particles in the wall. The average work of formation of an ion pair can be considered independent of the electron energy with sufficient accuracy. At the same time, the relative retarding powers of low-Z materials depend very weakly upon the electron energy (within the energy range 10 keV to 1 MeV, they are constant with an accuracy of ±3%). Hence, the coefficient of proportionality between the ionization density and the dose rate is practically the same for various depths under the surface of the phantom, and the distribution of ionization densities will be proportional to the depth doses.

Examples of relative dose distributions obtained are presented in Fig. 2. Graphs a-c correspond to normal incidence of the beam; d corresponds to an ablique incidence of the beam at E_0 = 0.45 MeV.

The curves for normal incidence possess pronounced maxima with relative height (P_{max}/P_0) about 1.5, which agrees with the data of [6-8] and differs from the results cited in [4, 10]. The depth of the maxima for the same E_0 differ among materials with different Z. In accord with the assumption that the main cause of the formation of a maximum is elastic scattering of electrons, in materials with greater Z, the maximum is situated closer to the surface. This is especially

noticeable in a comparison of the curves for beryllium and aluminum -- two materials with comparatively greatly differing Z and approximately the same mass stopping powers for electrons. On the curves for oblique incidence (Fig. 2, d), a gradual disappearance of the maximum with increasing angle of incidence θ_0 of the beam upon the sample is noticeable. When $\theta \approx 60^0$, the maxima on the dose curve disappear entirely for all the energy values used in the work.

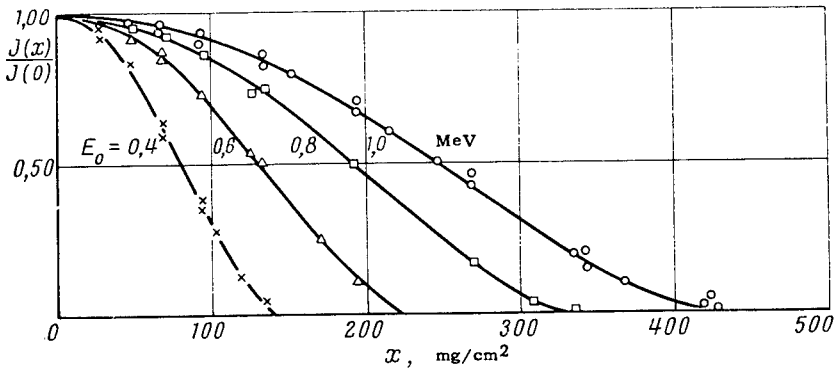

Fig. 3. Relative depth distributions of the electron current in a polyethylene phantom for normal incidence.

In addition to the relative dose distributions, in this work we also measured the depth distributions of the electron current reaching a set depth. For the measurements we used an electron collector, close in design to that described in [11], upon which flat samples of the test materials were placed from above. In certain cases the "current" distributions were measured with the aid of a camera (Fig. 1), and in this case the value of the electron current absorbed in the lower electrode was determined as the half difference of the currents corresponding to different electrode polarities. Examples of relative "current" distributions, presented in Fig. 3, correspond to the case of normal incidence of the beam on a polyethylene phantom.

3. The relative depth distributions of the electron current possess an interesting property, first established in [14]. It was found that if the thickness of the material, expressed in fractions of the total range R, is plotted along the X-axes of the graphs of Fig. 3, then the curves for different initial energies E_0 coincide. Subsequently [15-19], this result was confirmed for many materials and extended to a broad range of energies, from several keV to approximately 1 MeV. It was found that with an accuracy of about 10%, the "current" distributions for the same material and different energies are described by a "single" (universal) function, depending on the ratio $x/R(E_0)$. This function can be approximated [18, 19] by exponential functions (at large Z) or by positive branches of a Gauss curve (small Z).

The depth distributions of the electron current, measured in this work, are also universal in form; moreover, it was found that this property is possessed both by curves for normal incidence of the beam (Fig. 3) and by curves for oblique incidence. The "current" curves for polyethylene at normal pressure and at $s/R \leqslant 0.8$ are satisfactorily described by the expression

$$\exp\left[-\left(\frac{1{,}5\,x}{R}\right)^{2.5}\right],$$

the curves for $\theta_0 = 45^\circ$ by the expression

$$\exp\left[-\left(\frac{1{,}9\,x}{R}\right)^{2}\right].$$

The form of the depth distributions of the electron current and the shape of the depth dose curves are determined essentially by the same processes: inhibition and multiple scattering of electrons in the irradiated material. Hence, the universality of the shape of the relative "current" curves is evidence in favor of the possibility of "universalization" of the dose distributions as well. This hypothesis is confirmed, and the dose curves that we measured both for the case of normal incidence of the beam and for the case of oblique incidence proved to be very close in shape for different energies E_0, with all other conditions equal (material, angle of incidence θ_0). When we convert to relative thicknesses x/R, the dose curves for different E_0 approximately coincide. The maximum error of such "universalization" for the energy range 0.4-1.2 MeV and normal incidence of the beam does not exceed 10-15%, while when $\theta_0 = 45^\circ$ it does not exceed 20%. Comparing the dose distributions that we measured with the results cited in the literature [6, 8, 20-24], we can conclude that the universal shape of the dose curves, at least for the case of normal incidence of the beam, is observed within a rather broad range of energies -- approximately from hundreds of keV to 5-6 MeV. The universality of the shape of the dose curves for different initial electron energies permits comparatively simple estimates of the dose distributions from electron fluxes with arbitrary energy spectrum, since the calculation in this case can be reduced to a single integration of a known function [25], describing the "universal" dose distribution.

4. Let us determine the factors leading to the disappearance of maxima on the dose curves with increasing angle of incidence of the beam upon the sample. In Fig. 4a, the vertical arrow depicts a narrow electron beam, incident upon the surface of a phantom I (in the case of normal incidence) or II (oblique incidence). The originally narrow electron beam gradually expands beneath the surface of the phantom, which is schematically depicted in Fig. 4a by a diverging beam of dotted lines. We shall determine the distribution of the absorbed energy over the depth of the phantom and assume that under the plane I or II are

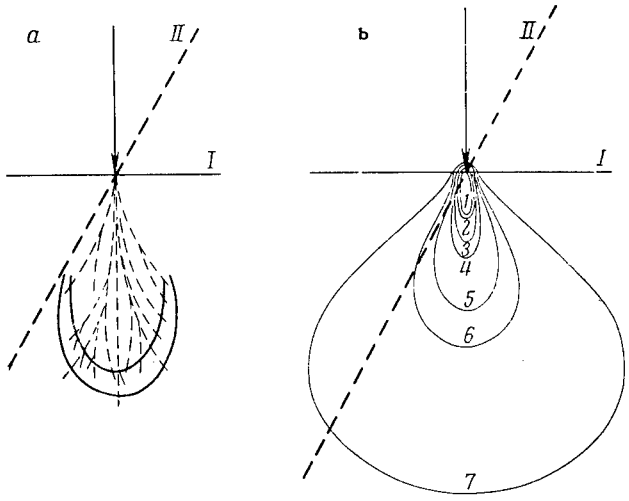

Fig. 4. Explanations in text.

arranged successively thin layers of material of the same thickness, parallel to the surface I or II. The energy of the radiation absorbed in 1 sec in each of such infinitely broad layers will correspond to the dose rate P(x) from an infinitely broad beam of electrons (x is the depth of the layer beneath the surface I or II).

The value of P(x) depends upon: 1) the total number of electron tracks intersecting a given layer; 2) the distribution of these tracks according to angles of inclination to the plane of the layer, and 3) the energy distribution of the electrons intersecting the layer. Instead of integrating the distribution functions, we can use the average values and write that $P(x) = N(x)\overline{l}(x)\overline{\frac{dE}{dx}}$, where N(x) is the number of electrons intersecting the layer in 1 sec, $\overline{l}(x)$ is the average length of the track in the layer, and $\overline{\frac{dE}{dx}}$ is some average value of the stopping power for the electrons intersecting the layer. Let us express N(x) in terms of I(x) -- the value of the resulting current, flowing in the direction along a normal from the surface of the phantom. I(x) is the current whose depth distribution was measured in our experiments by the electron collector (section 2). Let us introduce the functions $I_+(x)$ and $I_-(x)$, denoting the current flowing in the direction of increase in x and the current in the opposite direction, respectively. $I(x) = I_+(x) - I_-(x)$ and $N(x) = I_+(x) + I_-(x)$. For each depth, X, $I_-(x)/I_+(x) = \varepsilon(x)$, where $\varepsilon(x)$ is the average value of the backscattering coefficient according to number of particles for the depth X. From the functions written, it follows that:

$$N(x) = I(x)\frac{1+\bar{\varepsilon}(x)}{1-\bar{\varepsilon}(x)} \text{ and } P(x) = I(x)\frac{1+\bar{\varepsilon}(x)}{1-\bar{\varepsilon}(x)}\left(\frac{\overline{dE}}{dx}\right)\bar{l}(x).$$

Having determined the nature of the depth variation of each of the four functions determining P(x), we determine what conditions will be the most favorable for the formation of maxima on the dose curves.

I(x) for all angles of incidence θ_0 decreases monotonically to zero with increasing x. In the second cofactor $\bar{\varepsilon}(x) < 1$, and the expression $\frac{1+\bar{\varepsilon}(x)}{1-\bar{\varepsilon}(x)}$ itself increases with increasing $\bar{\varepsilon}(x)$. The backscattering coefficient ε increases with decreasing energy of the incident electrons and with increasing angle of incidence; moreover, the energy dependence is comparatively weak, while the monotonic dependence upon the angle of incidence is strongly manifested. For simplicity, we shall consider that ε does not depend upon the energy, and evaluate the quantity $\frac{1+\bar{\varepsilon}(x)}{1-\bar{\varepsilon}(x)}$ for small and for large depths x according to the literature data.

Experiments in which the scattering of electrons during passage through layers of variable thickness was studied [26] indicated that at first, at small depths beneath the surface of the sample, electrons are scattered for the small angles symmetrically with respect to the axis of the beam, and they thus still "remember" the direction of their original motion. Subsequently, with the development of the process of multiple scattering, the electrons gradually "forget" their original direction, and their motion more and more resembles diffusion with an almost equiprobable angular distribution of the velocities. The region close to the ends of the tracks, in which there is a diffusion of electrons, is depicted in Fig. 4, a in the form of a plateau between two solid curves.

At great depths, in the diffusion region, the coefficient $\bar{\varepsilon}$ for plastics and water will evidently take a value of about 0.2-0.3, as for a "diffuse" beam and materials with $Z \sim 6$-7 [27, 28]. Thus, at great depths $\frac{1+\bar{\varepsilon}(x)}{1-\bar{\varepsilon}(x)} \simeq 1.5$-1.9, independent of the angle of incidence of the beam upon the phantom. As for small depths, here the value of the expression $\frac{1+\bar{\varepsilon}(x)}{1-\bar{\varepsilon}(x)}$ depends upon the angle of incidence; moreover, it may be either smaller than 1.5-1.9 (small angles of incidence) or larger than it (incidence at large angles). The cofactor $\frac{1+\bar{\varepsilon}(x)}{1-\bar{\varepsilon}(x)}$ will thus increase with the depth in the case of small angles of incidence and decrease with the depth in the case of large angles of incidence.

Let us consider the third cofactor -- the quantity $\bar{l}(x)$. In the case of small depths, $\bar{l}(x) \simeq 1/\cos\theta_0$. It can be shown that at large depths x, when the "indicator" layer falls in the diffusion region, $\bar{l} = 2$; moreover, this value of $\bar{l}$ is also reached independent of the angle

of incidence of the beam upon the phantom. The fourth cofactor in the formula for P(x) -- the quantity $\frac{\overline{dE}}{dx}$ increases monotonically with the depth as a result of the gradual stopping of the electrons, but still remains limited.

From what was said on the nature of the change in the four cofactors determining P(x), it becomes evident that the most favorable conditions for the formation of maxima of the dose curves exist in the case of normal incidence of the beam, while with increasing θ_0, the relative height of the maxima should decrease. When $\theta_0 = 60^\circ$, both $\bar{\zeta}(x)$, and $\frac{1+\bar{\varepsilon}}{1-\bar{\varepsilon}}$ practically do not change with the depth, and only the increase in $\frac{\overline{dE}}{dx}$ remains. In Fig. 2,d, we can follow the disappearance of the maxima on the curves when $\theta_0 \to 60^\circ$, and this agrees with the hypotheses formulated above on the mechanism of the formation of maxima on the dose curves.

The same results can also be obtained in a different way. Figure 4, b, depicts the isodoses in air from a narrow monodirected beam of electrons ("source -- electron irradiation unit") with $E_0 = 0.4$ MeV, constructed according to the results of [29]. Lines 1, 2, 3, etc., represent the cross-sections of the isodose surfaces, corresponding to doses of 100, 50, 10, 5, etc. units. The portions of the isodose curves close to the point of entrance of the beam into the phantom are depicted approximately, by extrapolation. Let us imagine a thin layer of material parallel to surface I or II of the phantom and situated at some depth x beneath it. The intersection of the isodose surfaces with the plane of the layer forms a system of closed curves on the latter (in the case of normal incidence, these curves will be concentric circles). The radiation energy absorbed in the layer can be expressed in the form of a sum of products of the areas between neighboring close curves (the areas of "rings") of the corresponding average dose values for these areas. If the layer is displaced into the phantom parallel to plane I (normal incidence), then the area of the "rings" in the case of small depths beneath the surface will increase on account of the pear shape form of the isodose surfaces, which also leads to the formation of a layer parallel to the plane II (oblique incidence at a large angle); such growth of the areas between neighboring isodose lines will not occur, and there will be no maximum on the dose curve. The calculation in [29] was made for an unbounded medium. The influence of the surface of the phantom can consist only of the fact that part of the electrons will be dispersed "upward." In this case, the pea shape of the isodose surfaces becomes more pronounced, and our conclusions are qualitatively unchanged.

5. Since in this work we measured only the relative dose distributions, the problem arises of determining the absolute doses, corresponding to a set (measured) electron flux on the sample. For standardization of the relative dose curves, we can determine the surface dose rate $P(0,E_0,\theta_0)$ from the condition of conservation of the

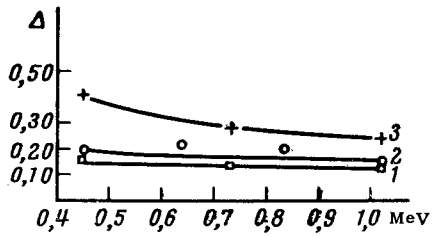

Fig. 5. Dose coefficients of backscattering for Be (1), $(CH_2)_n$ (2), and Al (3).

energy of the beam incident on the sample. In this case we obtain the function: $P(0,E_0,\theta_0)\int_0^\infty f(x,E_0,\theta_0)dx = E_0\cos\theta_0(1-p)$. The quantity $P(0,E_0,\theta_0)$ here corresponds to a single electron flux, incident upon the surface of the phantom at an angle θ_0; $f(x, E_0,\theta_0) = P(x,E_0,\theta_0)/P(0,E_0,\theta_0)$ is the relative dose distribution. The surface dose rate can be evaluated, and the relative dose distribution $f(x,E_0,\theta_0)$ thereby normalized in a different way. The quantity $P(0,E_0,\theta_0)$ is equal to $\frac{dE}{dx}(E_0)[1+\Delta(E_0,\theta_0)]$, where we call the coefficient Δ the dose coefficient of backscattering. It is equal to the relative increase in the dose rate in a thin surface layer of the irradiated material on account of the contribution to the dose of the electrons reflected from a thick sample. Eliminating $P(0,E_0,\theta_0)$ from the last expressions, we obtain a relationship between the backscattering coefficients:

$$(1+\Delta)\frac{dE}{dx}(E_0)\int_0^\infty f(x, E_0, \theta_0)\,dx = E_0\cos\theta_0(1-p)$$

This formula can be used to find the dose coefficient Δ when p and f are known. The value of p can be measured [30] or estimated according to the results of experiments in which the energy distribution of the backscattered electrons was investigated [26, 31-33]. The total energy distributions are available only for normal incidence of the beam (the case of azimuthal symmetry), and here for different materials and energies E_0, we can estimate the ratio $\bar{E}/E_0$ according to the literature data, where $\bar{E}$ is the average energy of the electrons reflected from the sample. It can be shown that the energy coefficient of backscattering $p \simeq \varepsilon\bar{E}/E_0$, where ε is the numerical coefficient of backscattering.

Figure 5 depicts the values of the dose coefficients of backscattering for three materials and energies E_0 within the range 0.45-1 MeV. These values were calculated according to the experimental dose distributions [numerical integration of functions $f(x,E_0,0)$] and the energy coefficients of backscattering, estimated according to the literature data. In this case, the values of ε were taken from [27, 28],

while the ratios $\bar{E}/E_0$ were taken from [26, 33]. The coefficients p for beryllium and polyethylene are about 0.01 and 0.02, respectively, and for aluminum -- about 0.04, and in the range of E_0 from 0.4 to 1 MeV are almost independent of E_0.

The dose coefficients of backscattering Δ can also be estimated independent of the dose distributions $f(x,E_0,\theta_0)$ and the energy coefficients p. According to the results of [26], it is evident that the angular distribution of electrons reflected from the sample (in the case of saturation of backscattering) is rather close to cos θ. Moreover, for the dose coefficient of backscattering Δ we obtain an estimate: $\Delta \simeq 2\varepsilon\frac{dE}{dx}(\bar{E})/\frac{dE}{dx}(E_0)$. Again using the literature data for the values of ε and $\bar{E}/E_0$, we obtain the following values of Δ:

Energy	0.45 MeV	1.0 MeV
$\Delta_{beryllium}$	0.10	0.08
$\Delta_{aluminum}$	0.43	0.33
$\Delta_{plexiglas,\ celluloid}$	0.19	0.15

As can be seen, these values are rather close to the results presented in Fig. 5.

CONCLUSIONS

In connection with the problems of dosimetry of accelerated electrons, in this work we determined the depth distributions of the absorbed energy in low-Z materials for the geometry of an "infinitely broad" electron beam.

1. Measurements were made of the relative dose distributions in a number of materials in the case of normal incidence of the electron beam with energies in the range 0.4-1.2 MeV. The partially contradictory data cited in the literature concerning the dose distributions in the case of normal incidence were refined.

2. The relative dose distributions in the case of oblique incidence of the beam were measured for certain materials within the range of angles of incidence θ_0 up to 60°. The disappearance of maxima on the dose curves when θ_0 is increased to $\sim 60^\circ$ was detected, and an explanation was proposed for this phenomenon.

3. The question of normalization of the relative dose distributions, i.e., of the determination of the absolute doses corresponding to a set magnitude of the electron beam on the sample, was considered. The proposed methods of normalization require a knowledge of the dose (Δ) or energy (p) coefficients of backscattering of the electrons. The coefficients Δ were estimated for certain low-Z materials and energies E_0 in the range 0.4-1.0 MeV.

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DISTRIBUTION OF THE ABSORBED DOSE OF FAST NEUTRONS IN A HETEROGENEOUS PHANTOM

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pp. 308-311

In conducting radiobiological experiments with fast neutrons, it is necessary to consider the heterogeneity of the irradiated specimens. As is well known, in the irradiation of biological specimens by fluxes of fast neutrons, the values of the absorbed dose are determined basically by the hydrogen content in the specimen. Recoil protons, arising in the interaction of a flux of fast neutrons with hydrogen atoms, contained, for example, in muscle tissue, are responsible for the absorption of 85-90% of the energy, and only 10-15% of the absorbed dose is due to heavy recoil nuclei.

Thus, for the same neutron flux, the absorbed dose will differ for bone, muscle, or adipose tissue, the chemical compositions of which differ.

Data on the elementary chemical composition of various normal tissues, cited in the literature, are contradictory. This pertains especially to the composition of bone tissue. According to the data of Randolph [1], for example, the hydrogen content in bone tissue comprises 4% by weight. Bewley [2] cites a value of 8.3%. This is evidently due to structural peculiarities of different parts of the skeleton, the density of impregnations of soft formations, etc. The chemical compositions of the mammal tissues that we used in the calculations are cited in the table.

While the topography of the dose field has been investigated in detail for X rays and γ rays, considering the heterogeneous structure of real biological specimens [4], the so-called transitional effects at the boundary of muscle and bone tissue and the topography of the dose field considering the structure of bone tissue [5] for neutron fluxes have not been thus investigated.

In most studies with neutrons, in the best case the so-called average tissue dose for muscle tissue of standard composition, containing 10-10.5% by weight hydrogen, is calculated. The average tissue

Elementary Chemical Composition of Various Mammal Tissues, % by Weight

Tissue	H	O	C	N	Remaining elements	Density g/cm^3
Muscle*	10,2	72,9	12,3	3,5	1,1	1,0
Bone*	6,4	41,0	27,8	2,7	22,1	1,85
Adipose [2]	12,5	12,5	75,0	—	—	0,91

*Data of the International Commission on Radiological Units (ICRU) [3].

dose is determined on the basis of measurements of the distribution of the tissue dose in a homogeneous phantom of a tissue equivalent substance and does not reflect the actual distribution of the absorbed dose in the irradiated animal.

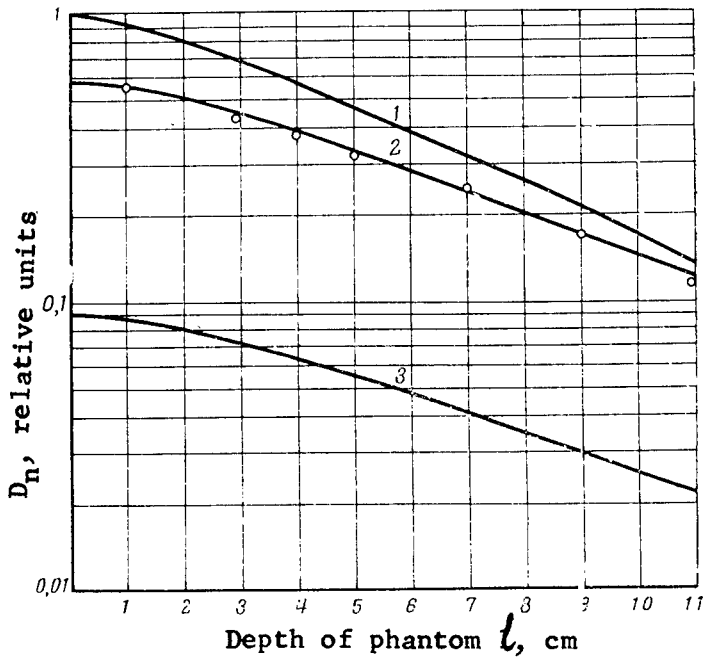

Fig. 1. Variation of absorbed neutron doses along the depth of phantoms of various materials. 1 -- Polyethylene; 2 -- polystyrene (solid curve calculated according to formula (2), points -- experimental); 3 -- graphite.

In this work we determined the variation of the absorbed dose over the depth of a heterogeneous phantom during its irradiation by a flux of fast neutrons in the horizontal channel of the IRT-1000 reactor, adapted for radiobiological research [6]. We preliminarily

determined the variation of the absorbed dose over the depth of homogeneous phantoms of materials with various chemical compositions. The measurements were performed with two homogeneous ionization chambers of polyethylene, filled with ethylene, and of graphite, filled with carbon dioxide, which permitted separate determination of the absorbed dose of fast neutrons and of γ radiation accompanying the neutron flux. The method of measurement and calculation of the absorbed doses in materials with various chemical compositions was described in our earlier studies [7, 8]. Polyethylene (14.3% H and 85.7% C by weight), polystyrene (7.7% H and 92.3% C), and graphite were selected as the phantom materials. A cylindrical phantom 80 mm in diameter and 110 mm thick consisted of individual disks. The measurements of absorbed doses of fast neutrons D_n in the indicated materials were conducted at points along the axis of the phantom, set up in a biological channel 100 mm in diameter, at various distances l from the end surface, turned toward the neutron flux.

Knowing the relative variation of the absorbed dose of fast neutrons along the depth of a graphite phantom $D^C_{n,l}/D^C_{n0} = f_C(l)$ and the variation of the absorved dose in a polyethylene phantom $f_{p\text{-}et}(l)$, we can calculate the function $f_m(l)$ for any material m, representing a compound of C and H. If ρ is the density of the substance of the phantom, while p^H and p^C are the weight contents of hydrogen and carbon in it, then it can be shown that

$$f_m(l) = \frac{f_C\left(\frac{\rho_m}{\rho_C} p_m^C l\right) f_{p\text{-}et}\left(\frac{\rho_m}{\rho_{p\text{-}et}} \frac{p_m^H}{p_{p\text{-}et}^H} l\right)}{f_C\left(\frac{\rho_m}{\rho_C} p_{p\text{-}et}^C \frac{p_m^H}{p_{p\text{-}et}^H} l\right)}. \quad (1)$$

Figure 1 depicts the variation of the absorbed doses of fast neutrons over a depth of the phantoms. With the aid of the results obtained on polyethylene and graphite phantoms, we calculated the variation of the absorbed dose of fast neutrons along the axis of a polystyrene phantom.

When $\rho_C = 1.85$, $\rho_{p\text{-}st} = 1.05$ and $\rho_{p\text{-}et} = 0.915$

$$f_{p\text{-}st}(l) = \frac{f_C(0{,}52\, l)\, f_{p\text{-}et}(0{,}62\, l)}{f_C(0{,}26\, l)}. \quad (2)$$

The experimental points fit well on the theoretical curve described by function (2). Since the cross-sections of the interactions of fast neutrons with carbon and oxygen are approximately the same within a broad range of energies, excluding the comparatively narrow regions of resonances, function (1) can be used to calculate the distribution of the absorbed dose in any irradiated specimen, if its density and hydrogen contents are known. It is also possible to calculate the dose field in a heterogeneous phantom. As an example, we calculated the deep absorbed doses in a heterogeneous phantom of

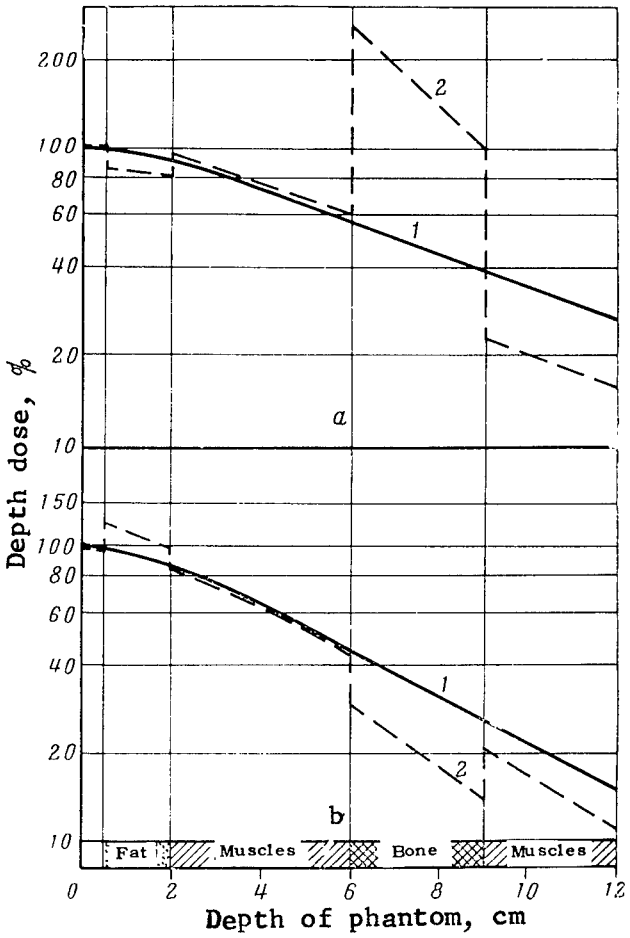

Fig. 2. Distribution of % depth dose in a homogeneous phantom of muscle tissue (1) and a heterogeneous phantom consisting of layers of adipose, muscle, and bone tissues (2). a -- X rays with $d_{1/2}$ = 1.5 mm Cu; distance from the source 150 cm; irradiation field 100 cm^2 [4]; b -- fast neutrons of reactor.

muscle, adipose, and bone tissues, considered in [4]. Figure 2 illustrates the variation of the absorbed dose of fast neutrons in a homogeneous phantom of muscle tissue and the curve obtained as a result of consideration of the heterogeneity of the specimen. This curve is illustrative in character. In each concrete case, one must consider the geometry of irradiation and perform the calculation according to the scheme cited above for the concrete specimen. For comparison, Fig. 2 presents the depth doses of X ray radiation, taken from [4]. When the effective atomic number of the substance Z_{eff} is increased, the absorption of X ray radiation increases, since the process of photo-

electric absorption, manifesting a strong dependence upon Z, plays a substantial role in the interaction of soft X ray radiation with the substance. Hence, the large content of heavy elements, especially calcium (14-15%), in bone tissue leads to an increase in the absorbed X ray energy. At the same time, the lower hydrogen content in the bone tissue results in a decrease in the absorbed dose of fast neutrons in comparison with muscle tissue.

In Fig. 2 the change in the dose in passage from one tissue to another is depicted in the form of a jump. In a real case, the jump at the interface occupies a finite region, the dimensions of which do not exceed the range of secondary particles with maximum energy. In the case of irradiation by fast neutrons, the thickness of the transition layer is several tens of microns.

We must also take into consideration the structural heterogeneity of the bone tissue, i.e., the presence of soft impregnations. If the dimensions of these impregnations are comparable with the range of the recoil protons, then calculation of the absorbed dose is a rather complex problem. For X rays and γ radiation, such a calculation was performed in [5]; however, no analogous investigations have been conducted for fast neutrons.

CONCLUSIONS

1. In evaluating the influence of fast neutrons upon biological specimens, it is insufficient to determine the average tissue dose. The absorbed doses in muscle, bone, and adipose tissues of mammals vary substantially as a result of the difference in the chemical compositions of these tissues.

2. The depth doses of fast neutrons in homogeneous phantoms of various chemical compositions, obtained in the work, permit a determination of the topography of the dose field in heterogeneous specimens.

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BRIEF COMMUNICATIONS

INTENSIFICATION OF THE ANTITUMORAL EFFECT OF X RAYS WITH THE AID OF A WEAK DIRECT ELECTRIC CURRENT

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pp. 312-315

An attempt to increase the sensitivity of tumoral tissue to the action of ionizing radiation, i.e., to increase the difference between the sensitivities of tumoral and normal tissue, has proved justified in a number of studies published in recent years [1-3]. Among the various factors characterizing the peculiarities of cell growth, and, consequently, the sensitivity of cells to the actions of external agents, a special place is occupied by the state of the electric charge of the cell. A number of investigations have shown that the density of the surface charge of tumoral cells is substantially higher than among normal cells of the same type, and that the increase in the degree of malignancy of the tumor is paralleled by an increase in the charge density [4-6].

Individual communications have appeared in the literature, indicating that under the direct influence of weak electric current, the growth of certain tumors is retarded [7, 8]. However, there are rather few such studies.

EXPERIMENTAL SECTION

The experiments were conducted on 620 white rats. Transplanted tumors were used: Walker's carcinosarcoma, sarcoma-45, transplanted alveolar cancer of the rat liver RS-1, and Pliss' lymphosarcoma.

The influence of a weak direct electric current upon the tumor was imposed by an apparatus for galvanization with a current strength of 4 mA, voltage 6-7 V, duration 40 min and two hours. The region of localization of the tumor was X rayed (50 kV, 5 mA, filter 2.5 mm Al)

daily in amounts of 240 R up to summary doses: 3500 R (in 14 sessions) for Pliss' lymphosarcoma, 4500 R for Walker's lymphosarcoma (in 18 sessions), 5000 R for sarcoma-45 (in 18-20 sessions), 3900 and 6000 R for transplanted alveolar cancer of the rat liver RS-1 (in 3 and 22 sessions, respectively).

The experiments were conducted during the period when the tumors had reached the size of an average of 2x2 cm, and were continued for three to four weeks. The percent inhibition of tumor growth was calculated according to the formula:

$$\frac{B_к - B_0}{B_к} \times 100,$$

where B_0 is the weight of the tumor in animals of the experimental group; $B_к$ is the weight of the tumor in the animals of the control group.

The experimental results are presented in Table 1. As can be seen from the table, a weak direct electric current exhibits an antitumoral activity. In experiments with sarcoma-45, the weight of a tumor for the experimental animals was only a third as great as in the control at the end of the experiment, while with Walker's carcinosarcoma, it was two-and-a-half times smaller than the control.

In all the experimental series, the average weight of the tumor at the end of the experiment in the group with pure X ray irradiation was greater than in the group where the animals were subjected to a local combined action of weak direct electric current and X rays. Moreover, the symmetrical action of the two factors (electric current and X ray irradiation) is accompanied by a more rapid decrease in tumor size than in the case of a single X ray irradiation. The dynamics of the variation of the average tumor size are presented in the figure.

In the experimental series cited, a weak direct electric current in the case of prolonged influence, causes an appreciable inhibition of tumor growth. And therefore, the intensification of the antitumoral influence of X rays under conditions of their combination with direct electric current may be considered as a result of addition of the antitumoral effects of the two factors. However, the data obtained in experiments with the strains RS-1 and Pliss' lymphosarcoma to some degree contradict this hypothesis; the antitumoral effect of the combined action of electric current and irradiation is only slightly intensified in comparison with the action of X ray irradiation alone.

At the same time, electric current itself, in influence on the indicated strains, retards tumor growth. In view of this, we conducted experiments in which X ray irradiation was conducted according to the method of coarse fractionation (Table 2). As can be seen from the table weak direct electric current (4 mA, 6-7 V) with two-hour exposure in the case of three influences had practically no effect upon tumor growth of the strain RS-1. At the same time, its combination with three irradiations by massive doses of X rays (1300 R daily, rate 300-320 R/min, total 3900 R) gave a sharp intensification of the therapeutic effect.

Table 1

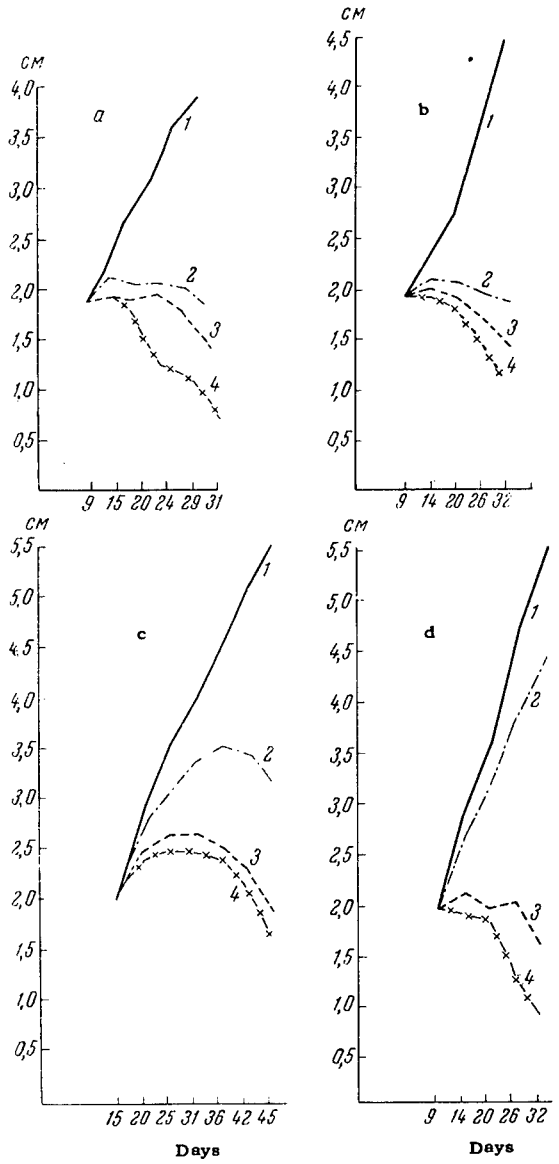
Influence of Weak Electric Current and its Combination with Radiation Upon Transplanted Tumors

Strains	No of animals	Weight of tumor at end of experiment, g		% inhibition of tumor from electric current	p	Weight of tumor at end of experiment, g		% of increase in effect of irradiation in conjunction with electric current	p
		Control $M \pm m$	Electric current, $M \pm m$			Irradiation, $M \pm m$	Electric current plus irradiation, $M \pm m$		
RS-1	84	85,0±10,8	49,3±4,9	42,0	<0,001	27,2±3,8	25,0±3,9	7,9	<0,5
Sarcoma-45	188	33,5±4,1	11,3±2,2	66,3	<0,001	4,7±1,4	2,5±0,7	46,9	<0,1
Walker's carcinosarcoma	128	56,4±2,9	22,7±3,3	59,8	<0,001	10,8±1,8	7,9±1,5	35,2	<0,001
Pliss' lymphosarcoma	66	39,4±3,5	26,3±3,5	33,2	<0,001	23,1±3,0	22,8±2,6	1,3	<0,5

Table 2

Influence of the Joint Action of Weak Electric Current with a Massive Dose of Radiation Upon Growth of Strain RS-1 Tumor

No of rats	Weight of tumor at end of expt, g		% inhibition from electric current	p	Weight of tumor at end of expt, g		% of intensification of effect of irradiation in conjunction with electric current	p
	Control $M \pm m$	Electric current, $M \pm m$			Irradiation, $M \pm m$	Electric current + irradiation, $M \pm m$		
68	92,0±3,0	79,0±3,3	14,1	<0,001	14,7±1,5	6,1±0,9	58,5	<0,001



Influence of weak direct electric current, X ray irradiation, and their joint action on the growth of transplanted tumors. According to the method of fine fractionation of the summary dose: a -- sarcoma-45; b -- Walker's carcinosarcoma; c -- strain RS-1; d -- according to the method of coarse fractionation, strain RS-1; 1 -- control; 2 -- electric current; 3 -- X ray irradiation; 4 -- electric current in conjunction with irradiation.

The average weight of the tumors in the X ray irradiation group at the end of the experiment was 6.2 times lower than in the control, while when radiation was combined with electric current, it was 15 times lower. The fact that electric current separately did not appreciably affect tumor growth, but caused a sharp intensification of the effect of radiation in the case of combined action is evidence that a weak direct electric current exhibits a radiosensitizing effect. The dynamics of the variation of tumor size are presented in the figure, d.

CONCLUSIONS

1. A weak direct electric current, exerting no harmful influence upon the animal organism, causes an inhibition of growth of the transplanted tumor strains that we tested in rats.

2. A weak electric current, in conjunction with X rays, intensifies the antitumoral effect of the radiation, evidently as a result of summation of the effects of the two factors, as well as on account of the manifestation of a radiosensitizing influence of the electric current.

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PHASE CHANGES IN THE THERMOREGULATION IN RABBITS AFTER TOTAL X RAY IRRADIATION (THERMOGRAPHIC OBSERVATION)

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pp. 315-318

In recent years, the dynamics of the changes in various functions of the organism during an ionizing influence have been attracting more and more attention [1, 2]. It has been established, for example, that the direct action of ionizing radiation upon the organism causes profound hypotension [3, 4], a change in the bioelectric activity of the cerebral cortex [5], and physicochemical changes in its tissue [6]. In the course of irradiation, a change arises in the composition of the peripheral blood and hematogenic organs [7], as well as a disturbance of the unconditioned reflex responses [8]. In the case of irradiation among rats, the oxygen consumption is increased; section of the spinal cord, on the other hand, prevents the development of such a metabolic reaction [9]. It might be assumed that a study of such a complex function of the intact organism as thermoregulation under conditions of irradiation would permit a better understanding of the nature of the changes in the energy processes in the organism and an estimate of the possible role of these disorders in the outcome of radiation injury.

PROCEDURE

The investigation was conducted on 39 rabbits, each weighing more than 3 kg. Before, during total irradiation, and in the post-radiation period, a simultaneous remote recording of the temperature of the skin (left side of the chest cavity), striated muscles (longest muscles of the back), rectal and liver temperatures was conducted with a thermograph according to the method developed previously [10]. During the experiment, the animals remained in the natural position, in a state of relative physiological rest, which was ensured by placing the rabbits

in latticed wooden cages, limiting movement of the animals. Before irradiation, control experiments were conducted to observe the dynamics of the temperature quantities studied with multi-hour recording. The animals were irradiated on the RUM-11 apparatus under standard conditions: voltage 200 kV, current strength 20 mA, filter 0.5 mm Cu, skin-focal length 70 cm, dose rate 15-24.1 R/min. The total dose was varied from 221 to 10,000 R.

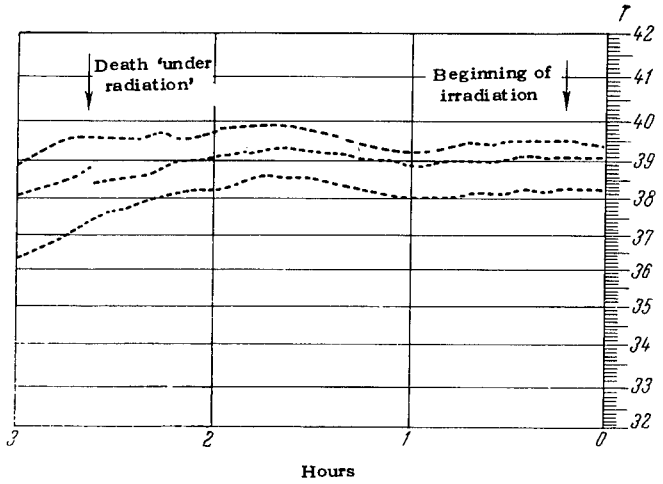

Fig. 1. Variations of temperature of liver (upper curve), rectal temperature (middle curve), and skin temperature (lower curve) in total irradiation of rabbit No. 34. Death "under radiation," total dose 2042 R, dose rate 15.8 R/min. The thermogram should be read from right to left.

RESULTS OF THE INVESTIGATIONS

A total of four series of experiments were conducted, directed toward determining the nature of the temperature variations "during irradiation."

In the first series, the animals were subjected to prolonged total X ray irradiation. Under the radiation influence, unidirected three-phase changes in the temperature of the liver, muscles, rectum, and skin temperature arose among all the rabbits, although the time of their appearance and degree of expression differed for each of these organs.

The first phase (hypothermal), arising in a number of cases at the first 2-8 min of irradiation, was characterized by a negligible decrease in the temperature of the liver (up to 0.5°), muscles (up to 0.5°), rectum (up to 0.6°), and skin (up to 0.7°). This phase usually lasted for 20-53 min, reaching a maximum at total irradiation doses from 270 to 1200 R.

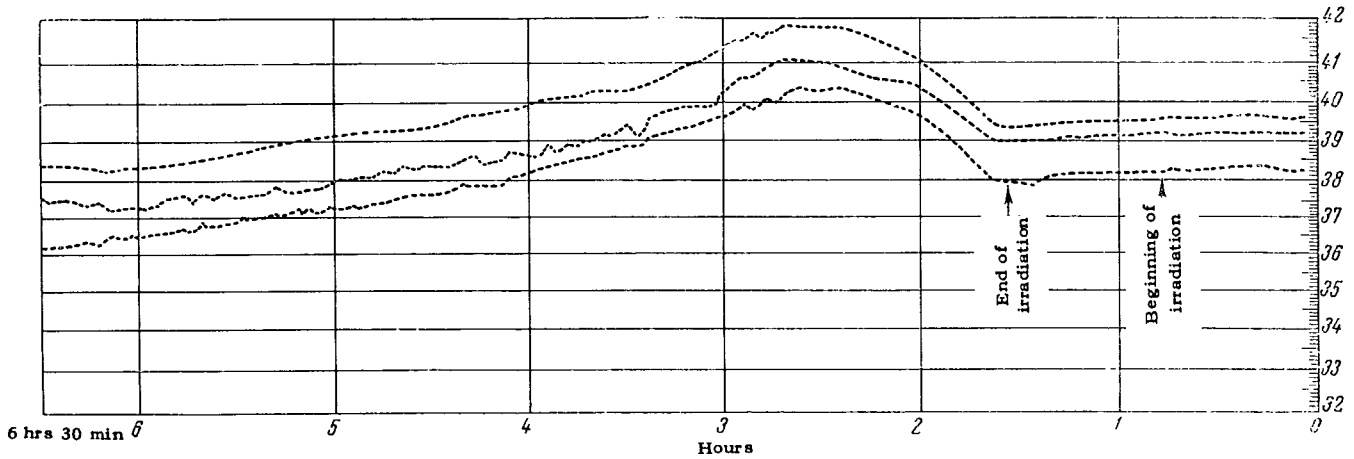

Fig. 2. Variations of liver temperature (upper curve), rectal temperature (middle curve), and skin temperature (lower curve) in the case of total irradiation of rabbit No 10. Irradiation was stopped at the beginning of the development of the second phase of temperature changes (742.6 R). Dose rate 15.8 R/min. The thermogram should be read from right to left.

The second phase -- the phase of relative hyperthermia, developing immediately after the first during irradiation -- was characterized by an increase in the temperature of the liver, muscles, rectum, and skin. This increase was either slight, when the temperature increased but did not reach the original levels recorded before irradiation, or the temperature of the liver, muscles, rectum, and skin rose 0.9--1.2° above the background values. The maximum expression of the phase was observed at total irradiation doses from 948 to 2042 R.

The third phase, arising during continuing irradiation immediately after the second, was characterized by a substantial (up to 3.0-3.5°) drop in the temperature of the liver, muscles, rectum, and skin. The decrease continued to definite, individually differing temperature values, upon the reaching of which relative stabilization of the temperature set in. It was found that among those rabbits that died "during irradiation" (1555-2169 R), the second phase -- increase in the temperature -- was especially pronounced (Fig. 1). In the same experiments, when the second phase was distinguished by a slight, only relative increase in the temperature, the rabbits survived the period of irradiation (3-10 kR) and died during various postradiation periods.

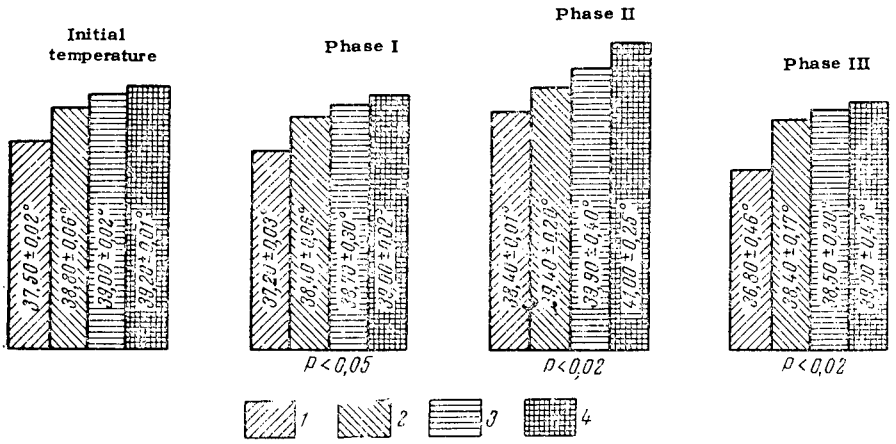

Fig. 3. Temperature of various organs before, during total irradiation of rabbits (first and second phases) and immediately after irradiation (third phase). 1 -- Skin temperature; 2 -- muscle temperature; 3 -- rectal temperature; 4 -- liver temperature.

Subsequently it was of interest to determine the possible role of an increase in the temperature in the rapid death of the rabbits "during irradiation." For this purpose we conducted experiments (second series) on six rabbits subjected to irradiation in a state of fever. It was induced by intravenous injection (0.4-0.6 ml/kg) of boiled defatted milk, which led to an increase in the liver temperature of $1.2\pm0.3^{\circ}$, in the rectal temperature $1.2\pm0.2^{\circ}$, and in the skin temperature of $1.1\pm0.2^{\circ}$. Against a background of maximum increase in the temperature, the rabbits were X rayed at a rate of 15.4 R/min. It was established that among the feverish animals, ionizing radiation also induces changes in the thermoregulation and death "during irradiation," from comparatively small doses (1386-2294 R). The animals that had survived the period of irradiation (3 kR) died in the following 12 hours. Consequently, the radiosensitivity of the feverish rabbits proved higher than that of the intact rabbits. However, although experimental fever increases radiosensitivity, it does not do so to such a degree as might explain the death "during irradiation" of the intact rabbits in which the hyperthermic phase was well expressed.

Then it seemed vital to determine the degree to which temperature changes "during irradiation" were due to continuing total irradiation of the animals. It was found that cessation of irradiation at the very beginning of the development of the phase of relative hyperthermia (third series of experiments) not only does not prevent the increase in the temperature of the liver, muscles, rectum, and skin, but, on the contrary, in all cases leads to a more intensive and prolonged increase in the temperature than was established in the case of continuing

irradiation (Fig. 2). Cessation of irradiation at the very beginning of development of the third (hypothermic) phase of the temperature changes (fourth series of experiments) did not prevent its development.

On the basis of the experimental data, it seems to us that the first (hypothermic) phase, which develops rapidly during irradiation, is due to a decrease in the heat formation, with a simultaneous limitation of heat removal. In this case, the exothermic reactions in the liver and muscles are inhibited, which also results in a decrease in the general body temperature (rectal) and partially in the skin temperature. The subsequent development of the phase of relative hyperthermia arises on account of intensification of the energy processes in the irradiated organism, since together with the increase in the liver and muscle temperature, the rectal temperature and skin temperature also rose. This phase of intensification of energy processes, accompanied by an increase in the heat production, was especially graphically manifested in the case when irradiation was stopped at the very beginning of the second (hyperthermic) phase (Fig. 3).

CONCLUSIONS

1. Total X ray irradiation induces disturbances of the thermoregulation and heat production, which occur in three phases: a phase of hypothermia with reduced heat production in the liver, striated muscles, a drop in the rectal and skin temperatures; a phase of relative hyperthermia, characterized by a relative increase in the heat production in the liver, striated muscles, and an increase in the rectal and skin temperatures; a phase of hypothermia, with a drop in the heat production in the liver, striated muscles, and a decrease in the rectal and skin temperatures.

2. Cessation of total X ray irradiation at the beginning of the second (hyperthermic) phase not only does not prevent its development, but, on the contrary, leads to an even more intensive and prolonged increase in the temperature than in the case of a continuous radiation influence. Cessation of X ray irradiation at the very beginning of the third (hypothermic) phase also does not prevent its development.

3. X Ray irradiation of rabbits existing in a state of fever revealed their greater radiosensitivity in comparison with intact animals.

4. The three-phase character of the temperature vatiations arising under the action of X rays should be taken into consideration in a study of other phenomena, in particular, biochemical reactions directly or indirectly associated with the energetics of the organism.

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INFLUENCE OF SPECIFIC POLYSACCHARIDES FROM TYPHOID FEVER BACTERIA AND PROTEUS WITH A STRUCTURE PARTIALLY MODIFIED BY ACID HYDROLYSIS, ACETYLATION, OR OXIDATION UPON THE RADIORESISTANCE OF MICE

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pp. 318-321

The protective properties of corpuscular vaccines, total antigens, and lipopolysaccharides, administered before irradiation, are evidently associated with stimulation of the protective forces of the organism, its cellular and humoral immunity, and are probably due to the presence of specific polysaccharides [1-3]. It was of interest to determine whether fractionation of the polysaccharides will lead to the isolation of fractions exhibiting a stronger protective effect, as well as to study how changes in the basic chemical structure of the polysaccharides influence their protective properties. The prerequisite was the literature data indicating that the antigenic and a number of other properties of preparations of endotoxins are almost entirely broken down after weak hydrolysis [4-7]; when the molecular weight of dextrans is changed, their radioprotective properties are changed [8]; acetylation of endotoxins reduces the toxic and pyrogenic properties while retaining increased resistance to infection [9]; oxidation of polysaccharides by iodic acid leads to a change in the content of aldehyde groups, in the structural bonds, and may change the qualitative and quantitative ratios of the monosaccharides [10], etc.

MATERIALS AND METHODS

We selected the specific polysaccharides from total antigens of S ty2-4446 and Proteus vulgaris. The former is complex in monosaccharide composition; the latter contains chiefly only galactose and glucose. Culturing and isolation of specific polysaccharides were described in [2].

The specific polysaccharides were fractionated by precipitation with alcohol at various concentrations. Originally, after solution of the specific polysaccharides, the zero fraction was removed by centrifuging (8000 rpm, 50 min). The first fraction was precipitated at an alcohol concentration up to 45%, the second up to 86%. Each fraction was reprecipitated four to five times and washed with alcohol. The following were obtained from the specific polysaccharides of typhoid fever bacteria: fraction 0 -- practically absent; fraction 1 -- 10-20%; fraction 2 -- 70-80%. From the specific polysaccharides of Proteus: fraction 0 -- 25-30%; fraction 1 -- 20-30%; fraction 2 -- 25-30%. The dependence of the radioprotective effect upon the influence of various factors was tested with two fractions. The cleavage of the specific polysaccharides by stepwise hydrolysis was performed in a medium of 1N CH_3COOH at 100°, with collection of samples two, four, and six hours after the beginning of the process [11].

Table 1

Chemical Composition of Polysaccharide Preparations

Polysaccharides	Carbohydrates	Reducing substances	N	P	Protein	Nucleic acids	Pentoses	Monosaccharides
	(in % of air-dried substance)							
From typhoid fever bacteria								
Initial	82	78	0,9	0,9	Traces	1,6	0,6	Ga, Gl, Ma, Ra, T(U)(Hs)
Fraction 1	92	59	0,6	0,8	3,8	3,6	None	Ga, Gl, Ma, Ra, T(U)(Hs)
Fraction 2	100	73	0,0	0,5	0,0	0,7	None	Ga, Gl, Ma, Ra, T(U)(Hs)
Hydrolyzate 1	100	65	0,0	0,4	0,0	0,4	None	Ga, Gl, Ma, Ra, (T)U(Hs)
Hydrolyzate 2	100	70	0,0	0,2	0,0	0,6	None	Ga, Gl, Ma, Ra, (U)(Hs)
Hydrolyzate 3	100	67	0,0	0,1	0,0	0,5	None	Ga, Gl, Ma, Ra, (U)(Hs)(0)
Acetylated	—	—	0,0	0,06	0,3	0,5	—	
Oxidized	90	—	0,0	0,4	—	2,1	None	
From Proteus								
Initial	90	67	0,4	2,6	1,5	7,3	6,7	Ga, Gl, U, (Hs)(Ri)(Ra)(X)
Fraction 0	90	74	2,1	0,7	1,4	3,5	1,4	Ga, Gl, U, (Hs)(Ri)(Ra)(X)
Fraction 1	77	73	1,9	0,4	0,6	3,3	2,4	Ga, Gl, U, Hs, (Ri)(X)
Fraction 2	97	76	2,7	0,3	0,4	1,3	1,6	Ga, Gl, U, Hs, (Ri)(X)
Hydrolyzate 1	95	72	2,1	0,0	0,5	0,5	—	Ga, Gl, U, (Hs)(Ri)(X)(0)
Hydrolyzate 2	97	72	1,5	0,0	Traces	0,4	—	Ga, Gl, U, O(Hs)(X)
Hydrolyzate 3	99	71	1,3	0,0	0,3	0,3	—	Ga, Gl, U, O(Hs)(X)
Acetylated	—	—	1,1	0,2	—	—	—	
Oxidized	99	—	—	0,3	—	1,6	—	

Arbitrary notations: Ga -- galactose; Hs -- hexosamine; Gl -- glucose; Ma -- mannose; Ra -- rhamnose; Ri -- ribose; T -- tivelose; U -- uronic acids; X -- xylose; O -- oligosugars (trace amounts indicated in parentheses).

Table 2

Survival of Mice Irradiated at a Dose of 750 R, Protected with Preparations of Typhoid Fever Bacteria and Proteus

Preparations	No of animals	Survival rate in %	p	Average lifetime, days
Specific polysaccharide from S. ty$_2$-4446	100	31,7±4,6	0,001	15,5
Fraction 1	55	25,3±5,8	0,002	17,4
Fraction 2	100	24±3,5	0,05	15,1
Hydrolyzate 1	70	1,3±1,3	—	9,7
Hydrolizate 2	45	4±2,9	—	12,2
Hydrolyzate 3	100	21±4,0	0,001	15,7
Oxidized	75	28±5,1	0,001	12,6
Acetylated	75	13,6±3,9	0,1	12,9
Control	100	5±2,1		11,6
Specific polysaccharide from Proteus vulgaris	85	31,4±5,1	0,001	16,2
Fraction O	85	27,7±4,8	0,001	15,5
Fraction 1	85	32,5±5,0	0,001	17,3
Fraction 2	85	22,2±4,5	0,002	14,5
Hydrolyzate 1	85	8,7±3,0	0,5	10,9
Hydrolyzate 2	45	23,5±6,5	0,01	16,9
Hydrolyzate 3	85	5,5±2,4	0,5	10,3
Oxidized	45	25±6,4	0,01	16,4
Acetylated	20	0	—	4,3
Control	85	5±2,3		10,3

Note: dose -- 100 γ per mouse, method of administration intraperitoneal; the table summarizes the data of four experiments; in each group of preparations, 20-30 animals per preparation; p -- significance of the difference.

The oxidation of the specific polysaccharides was performed with 1N iodic acid for 15 days at 0° in darkness [10]. The specific polysaccharides were acetylated with acetic anhydride in the presence of sodium acetate for 3.5 hours at 100° [9, 12]. The methods of analysis were described earlier [2].

RESULTS AND DISCUSSION

Eight preparations were obtained from typhoid fever bacteria and nine from Proteus vulgaris. Table 1 presents their basic chemical composition. All the fractions of specific polysaccharides contain from 80 to 100% carbohydrates, 60-75% reducing sugars. Preparations from typhoid fever bacteria contain from 0.5 to 3% nucleic acids (their number decreases with purification), and the preparations from Proteus from 0.5 to 7.3%. These data are confirmed by the results of a determination of phosphorus, nitrogen, and pentoses, and by paper chromatography of the monosugars. All the preparations were practically protein-free.

Alcohol fractionation introduced no changes into the qualitative

composition of the monosugars, just like 2-, 4-, and 6-hour acid hydrolysis.

Table 2 presents average data on survival and average lifetime of mice protected by preparations from typhoid fever bacteria and Proteus vulgaris. As can be seen, the specific polysaccharides from the typhoid fever bacteria always exerted a protective effect in the irradiation of mice at a dose of 750 R. On the average, when specific polysaccharides were administered, 31% of the mice survived at a dose of 100 γ (from 12 to 50% in different experiments), as against almost total death in the control (average survival of the unprotected animals 5% in 30 days of observation). Similar results were obtained with preparations from Proteus; the administration of specific polysaccharides protected an average of 31.5% (from 15 to 40% in different experiments). Fractionation of the specific polysaccharides with alcohol slightly reduced the protective properties of the preparations. The protective properties were sharply reduced in acid hydrolysis; moreover, the first and second hydrolyzates of preparations from typhoid fever bacteria and the first and third hydrolyzates from Proteus were practically inactive. Unexpected results, repeated in a number of experiments, were obtained with a preparation from typhoid fever bacteria extracted after six-hour hydrolysis, as well as from a preparation from Proteus, obtained after four-hour hydrolysis. They possessed some radioprotective activity. The data obtained will require a more detailed study.

The least influence was exerted by oxidation with iodic acid. The protective effect of the preparations was almost the same as among the original specific polysaccharides, or slightly inferior. Acetylation caused a practically total loss of radioprotective properties. The acetylated fractions of specific polysaccharides from typhoid fever bacteria were substantially less effective, while those from Proteus entirely lost their ability to protect animals from radiation.

Thus, the data that we cited indicated that a partial change in the structure of specific polysaccharides from typhoid fever bacteria and Proteus leads to a decrease in the radioprotective properties.

CONCLUSIONS

1. The specific polysaccharides from typhoid fever bacteria and Proteus, when administered 24 hours before irradiation (intraperitoneal) protect about 31% of the mice from γ irradiation at a sublethal dose.

2. Alcohol fractionation of the specific polysaccharides does not lead to the isolation of fractions more active in protective properties.

3. Hydrolysis of the specific polysaccharides with 1N acetic acid sharply reduces the radioprotective properties.

4. Oxidation of the specific polysaccharides with iodic acid does not cause any changes in their protective properties.

5. Acetylation of the specific polysaccharides leads to a sharp reduction of their radioprotective properties.

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ON THE RATE OF DEVELOPMENT OF SALMON (SALMO SALAR L.) ROE UNDER CONDITIONS OF RADIOACTIVE POLLUTION OF THE HYDROSPHERE WITH $Sr^{90}+Y^{90}$

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pp. 321-323

During the 1963-1964 period (October-March), we conducted an experiment on the development of salmon roe (Salmo salar L.) in aquaria polluted with radioactive isotopes $Sr^{90}+Y^{90}$, existing in radioactive equilibrium. The following radioactivity levels of the water in the aquaria were used: 10^{-6} curie/liter, 10^{-8} curie/liter, and 10^{-10} curie/liter. The salmon roe developed under conditions of radioactive pollution of the hydrosphere several hours after fertilization, up to the end of the experiment. Aquaria without radioactive pollution were used as controls. In each aquarium we placed approximately 1000 eggs. The eggs were incubated under the conditions provided for by the instructions for culturing of salmon roe [1].

After one to three days, seven to ten eggs were collected from all the aquaria, and then fixed in Bouin's solution. The membranes were removed from the fixed eggs. The stages of development were studied under a binocular microscope. Photomicrographs of various stages of development of the roe were taken simultaneously.

Figure 1 presents a photomicrograph of an egg at the stage of cleavage (eight blastomeres). This stage was noted among the eggs taken from the control and active aquaria, a day after fertilization.

Figure 2 presents photomicrographs of the stages of eggs collected on the same day from active and control aquaria.

The table presents the data on the duration of the development of roe at individual stages in an aquarium with radioactive pollution of the water with 10^{-10} curie/liter and in the control aquarium. The results are presented in days and in degree days. The table data show that the development of salmon roe in both aquaria was the same up to the stage of one third envelopment of the yolk sac. However, subsequently, the development of the roe in the aquarium with Sr^{90} and Y^{90} was more rapid than on the control, with the exception of the period from the appearance

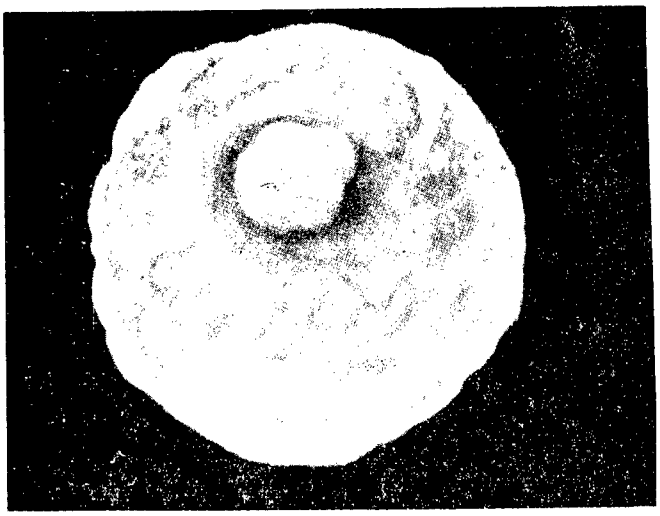

Fig. 1. Roe at the stage of cleavage, eight blastomeres.

Duration of Individual Stages of Development of Salmon Roe and Larvae, Days and Degree Days

Stages of development	Control aquarium		Aquarium with $Sr^{90}+Y^{90}$	
	No of days	No of degree days	No of days	No of degree days
From fertilization to beginning of cleavage	1	7	1	7
Cleavage to beginning of envelopment of yolk sac	4	26	4	26
From beginning of envelopment to envelopment of yolk sac by 1/3	10	51,5	10	51,5
From 1/3 envelopment of yolk sac to tail bud	7	32	4	19
From tail bud to rudiments of pectoral fins	28	55,5	15	38,5
From rudiments of pectoral fins to beginning of pigmentation	17	17,1	22	37
From beginning of pigmentation to beginning of hatching	80	101,9	60	75,6

of pectoral fins to the beginning of pigmentation. In this case, hatching of the larvae in the aquarium with the radioactive isotope also began earlier. On the whole, the period of incubation of the roe from the beginning of fertilization to the beginning of hatching was 147 days and 291 degree days in the control aquarium. In the aquarium with the radioactive isotope $Sr^{90}+Y^{90}$, in days 116, and in degree days 255.

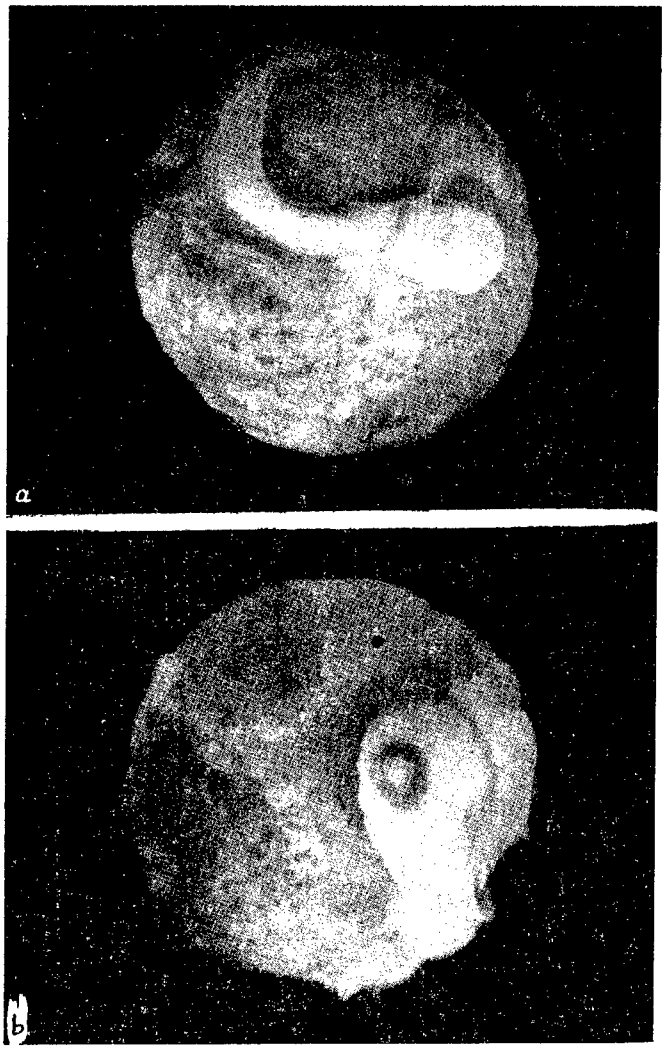

Fig. 2. Eggs at the stage of formation of rudiments of the pectoral fins (control aquarium) (a); egg at the stage of pronounced pigmentation of the "eye" (active aquarium) (b).

Thus, the rate of development of salmon roe in an aquarium with radioactive pollution of the water proved higher than in the control aquarium. Moreover, the percentage of death of the roe during incubation and the number of deformities in the polluted aquarium (10^{-10} curie/liter) did not exceed the same indices in the roe and larvae from the control aquarium.

The rate of development of the roe and hatching of larvae from aquaria with radioactive pollution $Sr^{90}+Y^{90}$ in concentrations of 10^{-6}

and 10^{-8} curie/liter was the same in individual stages as in the aquarium with radioactive pollution 10^{-10} curie/liter. However, the percentage of lethality and number of deformities was higher in the aquaria with higher level of radioactivity.

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INFLUENCE OF IRRADIATION ON THE BIOLOGICAL PROPERTIES OF THE CAUSATIVE AGENT OF BACTERIAL CANCER OF TEA BUSHES

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pp. 323-325

Extensive experimental material is now available on the action of irradiation upon seeds and plants. However, studies devoted to the action of radioactive isotopes upon phytopathogenic bacteria are very few.

In this work we studied the effects of various doses of a sulfur isotope upon certain biological properties of the agent causing bacterial cancer of the tea bush Xanthomonos Gorlencovianum Dan et Zil. The bacteria were cultured in test tubes on solid nutrient media, containing various quantities of radioactive sulfur S^{35} in theform of a solution of Na_2SO_4. The Burkholder solid nutrient media had the following activities: 50.0, 25.0, 12.5, 6.25, 3.12, 1.56, and 0.78 microcuries/ml. Five-day cultures were reinoculated onto normal medium in Petri dishes and cultured at 25°. The size of the colonies was determined after four, seven, 15, 24, and 30 days. The results are presented in Fig. 1. The dimensions of the colonies obtained on radioactive medium with high specific activity, from 50.0 to 1.56 microcuries/ml, were less than the controls; the bacteria from the medium with activity 0.78 microcurie/ml grew somewhat better than the controls.

Thus, high doses retard the growth of the causative agent of bacterial cancer. The same action is exerted by the sulfur isotope both upon the agent causing a bacterial disease of the silkworm Ps. mori and on that of tobacco wildfire Ps. tabaci [1, 2].

To establish the moment of maximum accumulation of the isotope in the bacteria, we used Burkholder's liquid medium, containing S^{35}. After two, five, 15, 30, and 50 days of growth, the activity in bacteria washed free of medium by repeated centrifuging with distilled water was measured on the DP-100. As can be seen from Fig. 2, the maximum amount of radioactive sulfur is contained by five-day cultures, which coincides with the data that we obtained earlier for Ps. mori and Ps. tabaci [1, 2].

In the following series of experiments, five-day cultures grown on radioactive nutrient media were reinoculated onto special media to study the morphological, culture, and biochemical properties. The medium where the strains containing the isotope were grown lost its ability to fluoresce. This was especially noticeable on strains grown on media with specific activities 50.0 and 25.0 microcuries/ml. They did not liquefy gelatin; they decomposed starch weakly.

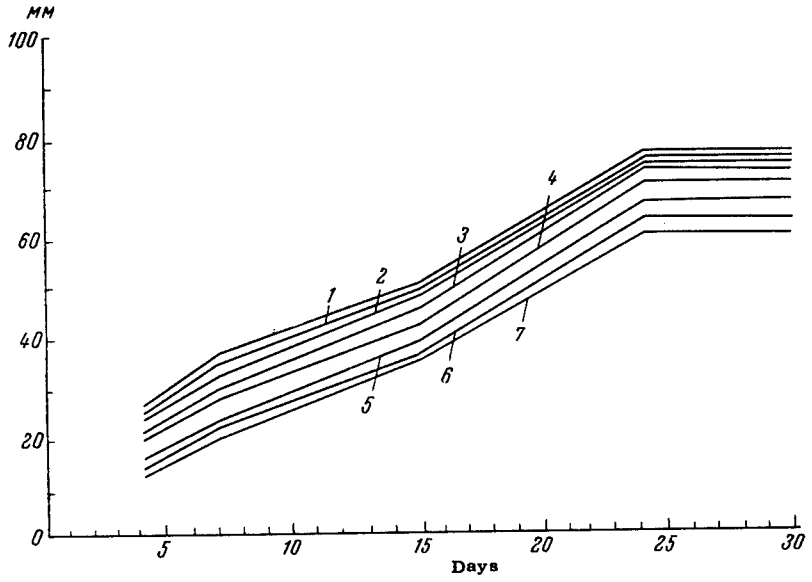

Fig. 1. Dependence of the linear dimensions of the colonies on the time of culturing on radioactive media. Along Y-axis -- size of colonies, mm; 1 -- control. Isotope content in the medium, microcuries/ml; vertical line -- 0.078; 2 -- 1.56; 3 -- 3.12; 4 -- 6.25; 5 -- 12.5; 6 -- 25.0; 7 -- 50.0.

The disease took a different course in infection of tea leaves with the radioactive and control strains. For the control bacteria and the bacteria cultured on a medium with specific activity 0.78 microcuries/ml, the incubation period lasted 25 days, and infection was strong; for bacteria cultured on media with a higher specific activity, the incubation period was lengthened in proportion to the content of the isotopes; the injury was weak. The same results were obtained in the case of Ps. mori and Ps. tabaci [1, 2].

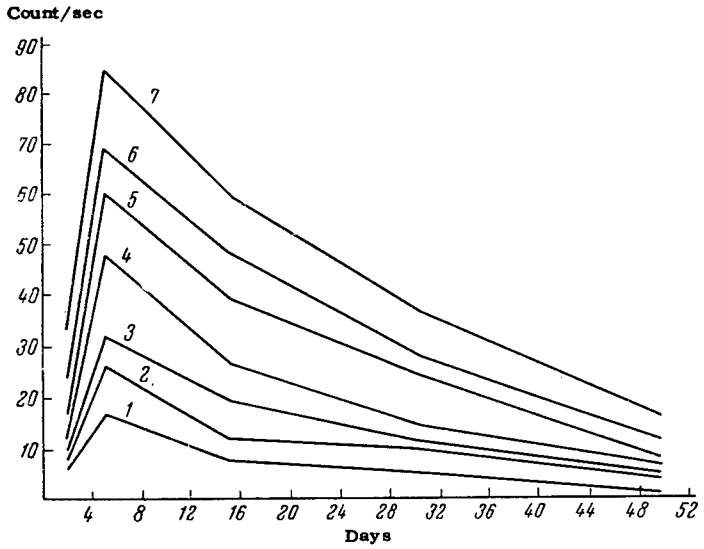

Fig. 2. Accumulation of the isotope as a function of time of culturing on radioactive media. Notations the same as in Fig. 1.

CONCLUSIONS

1. Growth of X. Gorlencovianum on nutrient medium with high specific activity is retarded in comparison with the control or with growth on a medium with low specific activity.

2. The maximum amount of the isotope is contained by five-day cultures.

3. A medium on which five-day radioactive cultures were grown lost its ability to fluoresce. The radioactive cultures weakly decompose starch; they do not liquify gelatin.

4. Bacteria grown on nutrient medium with high specific activity exhibit weakened virulence. Bacteria cultured on a medium with activity 0.78 microcurie/ml exhibit stronger virulence than the control. The incubation period in the case of development of the former on the tea leaf is longer than for the controls.

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ON THE METHOD OF PREPARING TISSUE SAMPLES FOR RADIOMETRY

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pp. 325-326

Radiometry of β emitters in biological specimens is most often conducted by a preparation of homogeneous crude samples of the investigated tissue. Bones, however, are difficult to obtain in such form. Hence, the method of sample preparation usually consists of the following. The extracted bone is calcined in a muffle furnace at a temperature of 800° for six hours. The remaining mineral ash is dissolved in 5N hydrochloric acid. The solution is applied to a target, evaporated until a thin layer of precipitate is obtained, and measured under a counter.

This laborious method, requiring special equipment, can be replaced by a more convenient one. The crude purified bone is immersed in concentrated nitric acid in amounts of 50 mg per ml and left overnight until it dissolves completely. The solution obtained is diluted 1:2 with distilled water so as to avoid the influence of nitric acid vapors upon the mica window of the counter, and applied to a target. The measurement can be conducted in a thick layer, considering self-absorption, or the sample can be evaporated to a thin layer of precipitate.

It should be indicated that foil targets are not suitable for the method described as a result of the strong acidity of the specimen. We used glass targets 1.5 cm in diameter and 0.7 cm high. After washing, they can be used in further work. When it is desirable to use foil targets, the original solution should be diluted not two-fold, but 10-fold.

The proposed method is acquiring great importance in cases of measurement of radioisotopes that are capable of giving volatile compounds under the influence of high temperature on bone. They include, for example, sulfur-35, which is selectively accumulated in the skeletal cartilage. In the case of ashing in a muffle furnace, the sulfur that is contained in the bones can form gases oxides $S^{35}O_2$ and $S^{35}O_3$, which are volatilized, and the subsequent measurement does not reflect the

Measurement of Activity in Bone Samples, Counts/Min

Ca^{45}			S^{35}			Ca^{45}			S^{35}		
Animal No	Ashed	Nonashed	Animal No	Ashed	Nonashed	Animal No	Ashed	Nonashed	Animal No	Ashed	Nonashed
1	405	449	1 *	21	169	6	440	424	6	228	270
2	410	468	2 *	73	176	7	536	521	7	164	265
3	320	355	3 *	10	55	8	514	551	8	206	259
4	464	446	4	245	262	9	630	615	9	211	192
5	340	318	5	172	247				10	148	199
									11	192	166

*These animals received a smaller dose.

true content of the isotope. Dissolving the bone in nitric acid eliminates the possibility of such an error.

As a check on the proposed method, we conducted investigations with the isotopes C^{45} and S^{35}. Both radioactive substances, in the form of $Ca^{45}Cl_2$ and $Na_2S^{35}O_4$, were administered interally to male rats in doses: Ca^{45} -- 0.6 microcuries/g, S^{35} -- 3 microcuries/g.

All the animals were killed simultaneously. Paired long cylindrical bones were extracted. One of the paired bones from each rat was treated with ashing in a muffle furnace, the other by simple dissolving. Measurements were conducted in a thick layer, considering self-absorption. The data of the measurements are cited in the table.

The comparatively small decrease in the count after ashing of bones containing radioactive sulfur should evidently be explained by the fact that not all the organic sulfur is burned out at this temperature to $S^{35}O_2$ and $S^{35}O_3$.

A statistical investigation of the data obtained shows unreliability of the difference between the two methods ($t = 1.01$) in the case of Ca^{45}, i.e., identity of the results of the measurements. In the case of sulfur-35, the difference between measurements of ashed and nonashed bones was significant at $p = 0.01$ ($t = 3.26$).

Thus, the proposed method is the most correct for determining sulfur-35 in bones.

UDC 621.039.55

ON EVALUATING ABSORBED DOSES FROM RADIOPHOSPHORUS INCORPORATED INTO TISSUES

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pp. 326-327

According to the literature data [1-4], as well as the results of our own experiments, it follows that radiophosphorus is nonuniformly accumulated by various tissues in the case of chronic penetration into the animal organism. However, for purposes of approximate dose calculations, in a number of cases the differences in the levels of incorporation of P^{32} by most of the soft tissues can be neglected. This permits a substantial simplification of the solution of the problem of evaluating absorbed doses from radiophosphorus incorporated into tissues.

Below are discussed the possibilities and limits of applicability of such a simplification. We have carried out an experiment in which noninbred white rats (60 females with an average weight of 140±15 g) inhaled radiophosphorus in the form of a $Na_2HP^{32}O_4$ solution in doses of 2 microcuries/day per animal. At various periods after administration (up to 100 days), the animals were killed, and the activity in the tissues was determined by direct radiometry of crude samples by the method of relative measurements.

In the table the tissues of the organism are divided into groups according to their specific activity. Noteworthy is the fact that the equilibrium concentrations of radiophosphorus in the soft tissues are quantities of approximately the same order of magnitude, while the specific activity of bone tissue on account of P^{32} is an order of magnitude higher. On the other hand, among the soft tissues, the brain, blood, and skin tissue are isolated (second group); in them the equilibrium concentration (C_p) is appreciably lower than in the tissues of the first group of organs.

Calculation shows that the combination of 10 vitally important organs into one group with average equilibrium concentration of P^{32} in this group 913±55 microcuries/g leads to an overestimation of the true value by no more than 33% (uterus) and an underestimation of no more than

Equilibrium Concentrations and Time of Establishment of Equilibrium in the Soft Tissues and Bones of Rats After Daily Inhalation of 2 Microcuries P^{32}

Organs and tissues	Equilibrium concentration $(C_p) \cdot 10^{-4}$ microcuries/g	Relative deviation of difference of C_p from $\overline{C}_p$ in %, $\frac{C_p - \overline{C}_p}{\overline{C}_p} \cdot 100\%$	Время наступления равновесия, сутки
Group 1			
Heart	692	—14	20—40
Liver	1103	+36	18—30
Lungs	690	—14	18—30
Adrenals	887	+ 9	18—30
Kidneys	990	+22	20—35
Spleen	801	— 1	20—30
Intestines	1041	+28	20—30
Ovaries	701	—14	16—25
Uterus	545	—33	25—35
Muscles	600	—26	25—40
Hypophysis	662	—19	16—30
	$\overline{C}_p$ 813±55		
Group 2			
Blood	157	—40	18—25
Brain	312	+30	30—50
Skin	305	+18	25—35
	$\overline{C}_p$ 258±23		
Group 3			
Bone	5374		35—60

36% (liver).

Since the dose rate and absorbed dose are linear functions of the equilibrium concentration of activity in the tissue, with all other conditions equal, the accuracy of the dose calculations will be determined unambiguously by the accuracy in the determination of the equilibrium concentration. Obviously if we assume that P^{32} is distributed uniformly in the tissues of the 10 enumerated organs of the animal, then this kind of assumption leads to an error in the calculation of the absorbed dose of no more than 36%, which fully satisfies the purposes of dosimetry. Such an assumption is supported by the fact that equilibrium in the soft tissues sets in approximately simultaneously. Consequently, in the preequilibrium state of accumulation of activity also, the differences in the dose loads will be small.

Thus, we can draw the following conclusion. In the case of chronic penetration of radiophosphorus into the animal organism (by inhalation), in the tissues of the heart, liver, lungs, adrenals, kidneys, spleen, intestines, ovaries, uterus, muscles, and hypophysis,

P^{32} is distributed uniformly at equilibrium. These ratios can be used quite well in evaluating the absorbed doses, and the error in this case will not exceed 36%.

The specific activity of the blood, skin, and brain, is two to three times lower, while that of the bone tissue is about 10 times higher, which prevents averaging of the data obtained in a calculation of absorbed doses.

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LETTERS TO THE EDITOR

UDC 535.31:577.15

INFLUENCE OF PRELIMINARY TREATMENT WITH THE SUBSTRATE ON THE THERMAL AFTEREFFECT IN UV-IRRADIATED TRYPSIN

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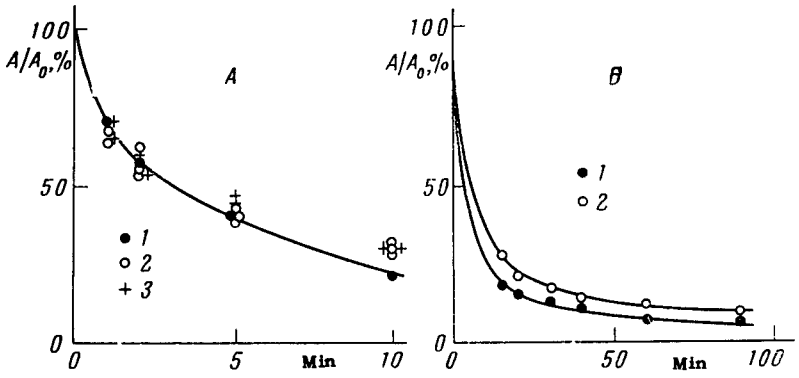
pp. 328-329

It has been hypothesized [1, 2] that treatment of irradiated enzyme solutions with the substrate will reduce the effect of their thermal inactivation. However, the experimental data cited have given no answer to the question of the cause of the protective action of the substrate. In the indicated studies, the operations of decomposition of the substrate by the enzyme and thermal inactivation of the latter were not distinguished in time. Hence, the protective effect could be explained either by the formation of an enzyme-substrate complex stable to heating or by conversion of the latently damaged enzyme molecules to the native form as a result of reaction with the substrate. In this work, the substrate was entirely decomposed with an irradiated trypsin solution before heating of the latter. Possible participation of intermolecular reactions in the process of the thermal aftereffect could be neglected.

Immediately after irradiation (40% inactivation "under the beam") five volumes of a solution of the ethyl ester of N-benzoyl-L-arginine (c = 0.86 mg/ml) in 0.05 M phosphate buffer, pH 8 were added in small portions in the cold to one volume of the trypsin solution (c = 2.0-2.4 mg/ml). The final concentrations in the mixture were $2.2 \cdot 10^{-3}$ M and $1.7 \cdot 10^{-5}$ M, respectively. Another portion of the irradiated solution was mixed in a 1:5 ratio with a solution of the ester preliminarily hydrolyzed at 96.8°. The completeness of hydrolysis in phosphate buffer (pH 8) was verified according to the optical density at 253 mμ. The value of D_{253}, corresponding to total cleavage of the substrate, could be obtained in enzymatic hydrolysis, which occurs very rapidly. After decomposition of the irradiated trypsin solution, its inactivation at 44.5° was followed. The figure compares the aftereffect for trypsin solutions in phosphate buffer (pH 8, dilution with irradiated solution in a 1:5 ratio) and for a solution preliminarily treated with the



Influence of preliminary treatment with substrate on the kinetics of the thermal aftereffect in trypsin solutions irradiated by UV light (phosphate buffer pH 8, temperature 44.5°). 1 -- Inactivation of irradiated trypsin in phosphate buffer; 2 -- substrate was added to irradiated trypsin before heating; 3 -- hydrolyzed substrate was added to irradiated enzyme.

substrate. In the latter case a very small protective effect was observed, probably due to the presence of hydrolysis products of the ester in the heated solution, since such products gave exactly the same effect.

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RADIOPROTECTIVE EFFECT OF BONE MARROW TRANSPLANTED BEFORE IRRADIATION

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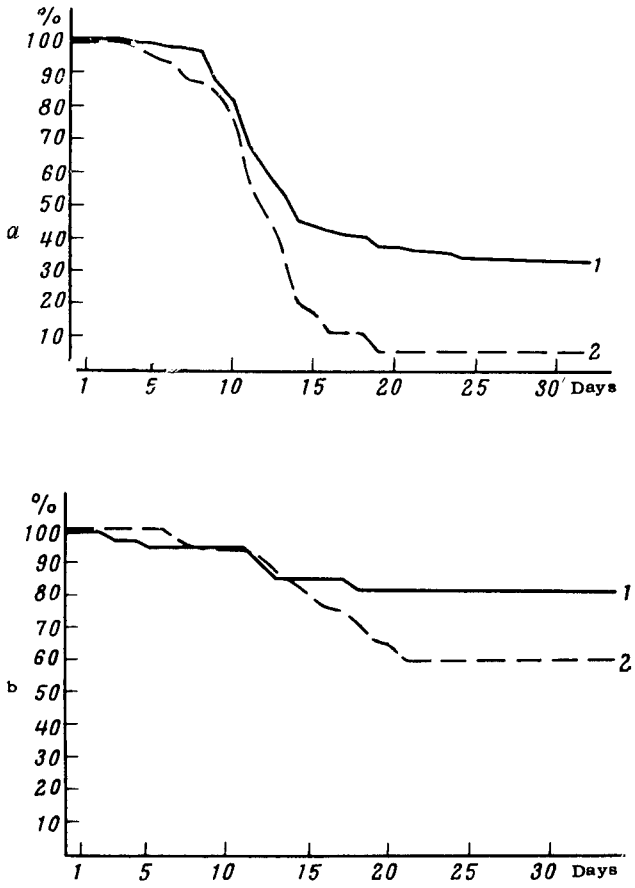
pp. 329-330

In recent years a large number of communications devoted to the investigation of the radioprotective effectiveness of transplantation of hematogenic tissue soon after irradiation have appeared in the literature [1, 2]. There have been no studies of the prophylactic effect of injections of hematogenic cells. And yet, this question is of substantial theoretical interest.

We investigated the influence of isotransplantation of bone marrow cells, performed one to three hours before γ irradiation of mice of the C3H line. The animals were irradiated on the EGO-2 apparatus at doses of 700 and 650 R (dose rate 200-208 R/min).

A suspension of bone marrow cells was prepared by washing out the bone marrow from femurs with medium 199, followed by repeated passage through a steel sieve mounted in a syringe to separate the cellular conglomerates. In the evaluation of cell viability in the suspension with a 0.1% solution of Trypan Blue it was found that this method of preparation ensures the presence of 85-90% viable cells. The suspension was injected intravenously in amounts of $2.3\text{-}2.8 \cdot 10^{-7}$ viable nucleus-containing cells in a volume of 0.5-1.0 ml. The animals were kept in special cages with automatic water and feed dispensers, five mice to a cage. The experiment was conducted in six repetitions. The injection of 23-28 million isologous bone marrow cells before irradiation ensures a greater survival rate of the animals than without injection. In six experiments we irradiated 158 mice, including 82 mice with preliminary injection of bone marrow cells and 76 without injection. From four experiments with irradiation at a dose of 700 R, it is evident that 5% of the animals survive without injection of bone marrow cells, while 32% of the protected animals survived (figure, a).

In the experiments with irradiation at a dose of 650 R, we used 78 mice, 39 of which had preliminary injections of bone marrow cells, while 39 did not. In this case the survival rate of the animals that were only irradiated was 61%, while that of the protected mice was 82% (figure, b).



Influence of prophylactic injection of bone marrow cells upon the survival of mice of the C3H line, irradiated at doses of 700 R (a) and 650 R (b). Along Y-axis -- percent of surviving animals; along X-axis -- time after irradiation, days. 1 -- Mice with injection of bone marrow cells before irradiation, 2 -- irradiated mice without preliminary injection of bone marrow cells.

The effect obtained permits the following explanations. Transplanted cells, found in the blood stream or sites of their primary settling soon after intravenous injection, are distinguished by reduced radiosensitivity or greater ability for postradiation recovery.

Hematogenic tissues, including the bone marrow, contain some hormonal protective factor.

Transplantation before irradiation of a sufficiently large number of viable hematogenic cells creates a different quantitative background of hematogenic (radiosensitive) tissue of the irradiated organism from the normal.

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PECULIARITIES OF THE CLINICAL COURSE OF RADIATION SICKNESS DEPENDING UPON THE SPATIAL DISTRIBUTION OF ABSORBED ENERGY

N. Ya. Savchenko

pp. 330-331

In the study of radiobiological effects after the influence of strongly absorbed ionizing radiations, it is especially important to investigate the distribution in space of the deep dose [1-3]. In our experiment we compared the biological effects of X rays with energies of 80 and 180 kV, giving various dose distributions. In the experiment we used 15 dogs weighing 8-10 kg. The animals were irradiated on a 12-tube X ray apparatus, ensuring a uniform multidirectional influence [4]. At a voltage of 180 kV, current strength 24 mA, focal length 60 cm, the exposure dose rate was 37 R/min, and the original half value layer 0.75 mm Cu. At a voltage of 80 kV, current strength 56 mA, no filtration was used. The half-value layer was 1.1 mm Al. The distribution of the absorbed dose was evaluated on a tissue equivalent heterogeneous phantom with the aid of a condenser roentgenometer of the Krasnogvardeets type and a ferrosulfate dosimeter.

The exposure dose (550 R) was selected as the basis for comparison. Clinical observations of the animals after the influence revealed substantial differences in the course of radiation sickness. In the case of uniform distribution of the absorbed dose, the dogs developed the classical picture of radiation sickness, with death of all the animals on the 12th to 18th day. In the case of irradiation with a drop in the absorbed dose, all the dogs remained alive. Symptoms of acute radiation injury of the skin and mucosa, expressed in the development of inflammation of the conjunctiva of the eyes, glossitis, gingivitis, as well as edema and acute painfulness of the skin predominated in the clinical picture of these animals. From the 14th to 20th days, foci of epilation began to appear. Hemograms of these two groups of animals also exhibited substantial differences. Among the animals that died, the number of leukocytes reached the lowest value on the 10th to 14th day (two to three days before death) and practically approached zero. The change in the number of red blood cells and amount of hemoglobin was less pronounced. The erythrocyte sedimentation rate was accelerated from the first to third day on, reaching a maximum also two to three

days before death. Among the animals that survived after irradiation at an energy of 80 kV, the total number of leukocytes was at the level of the greatest inhibition on the 10th to 20th days, but no lower than 50% of the original value. Although the drop in their number was gradual, no return to the initial level was noted during the period of time studied. As for the red blood cells, the erythrocyte sedimentation rate, and hemoglobin level, no significant changes could be determined among these animals.

CONCLUSIONS

A comparison of the biological effects of radiations with various models of dose distribution revealed substantial differences both in the death rate and in the peculiarities of the clinical manifestation of radiation injury.

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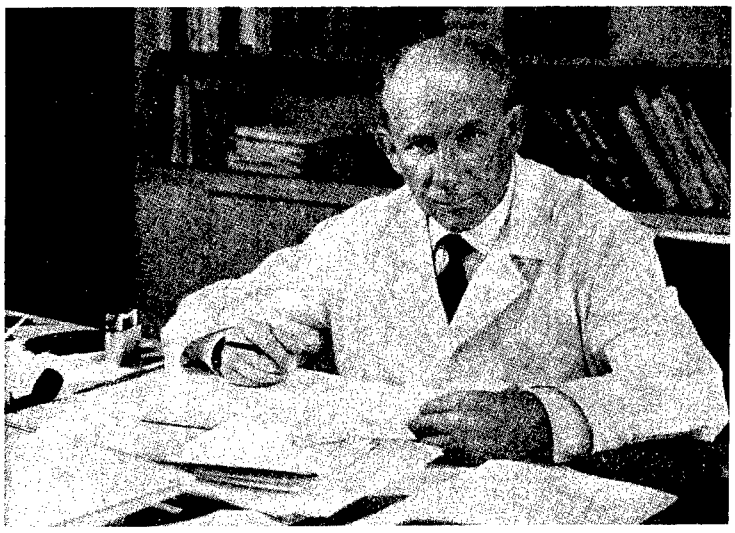
Ferdinand Gerchik*

p. 332

On 20 January 1966, a great radiobiologist, director of the Institute of Biophysics of the Czechoslovakian Academy of Sciences, Professor Ferdinand Gerchik, Doctor of Biological Sciences, Corresponding Member of the Czechoslovakian Academy of Sciences and Member of the Scientific Council of the University imeni J. E. Purkinje, died in Brno after a brief but severe illness.

Professor F. Gerchik was one of the leading scientist of the world in the field of biology. He devoted his entire life to scientific research. He worked in many scientific laboratories of a number of countries. He published more than 140 scientific works, both in Czechoslovakia and abroad.



F. Gerchik made a great contribution to the development of biophysics. Back in 1934, his monograph "Surface Tension in Biology and Medicine" was published in Dresden. One of the first among the

*[Gerchik is Russian transliteration of Hercik.]

Czechoslovakian scientists, he began to take up the study of the influence of radiation upon cells and the organism as a whole. His studies in this field were the most fruitful. Together with experimental scientific works in the field of radiobiology, his original scientific investigations found reflection in books: "From Atom to Life" and "Introduction to Quantum Biology." His name is also closely linked with the development of electron microscopy in biology. In this field he was occupied mainly with the study of bacteriophage formation.

For his book "The Problem of the Bacteriophage," he was awarded the state prize in 1954. His further researchers found reflection in the book, "Biophysics of the Bacteriophage," published in 1959 in Berlin.

Professor F. Gerchik devoted much time and attention to pedogogical activy at the Medical and Natural Faculties of the university; he trained a whole series of young scientists. Since 1955 he had headed the Institute of Biophysics at Brno and had been elected Corresponding Member of the Czechoslovakian Academy of Sciences. In May 1965, he was awarded the Order of Labor in connection with his sixtieth birthday.

A great merit of Professor F. Gerchik was his work in international organizations. As a representative of the Czechoslovakian Soviet Republic he was a member, and then in 1961/62 Chairman of the Scientific Committee of the United Nations Organization for the Study of the Effects of Atomic Radiation Upon Man, as well as a member of a number of other international scientific organizations.

F. Gerchik is also known as an outstanding popularizer of scientific work. He wrote the books: "Young Biologists," "Radiation and Life," "At the Boundaries of Life," as well as a whole series of articles published in various journals. F. Gerchik was Editor in Chief of the Czechoslovakian journal "Science and Life."

In the person of Professor Ferdinand Gerchik, Czechoslovakian and world science has lost one of its outstanding representatives.