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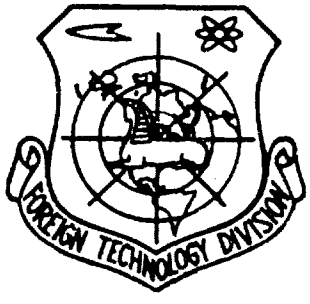
RADIOTOXINS: THEIR NATURE AND ROLE IN THE BIOLOGICAL EFFECT OF HIGH-ENERGY RADIATION

edited by

A. M. Kuzin



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RADIOTOXINS: THEIR NATURE AND ROLE IN THE BIOLOGICAL EFFECT OF HIGH-ENERGY RADIATION

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ABSTRACT The book furnishes theoretical and experimental data on the formation of toxins in irradiated organizms and as compiled from the materials of the First All-Union Conference of Radiobiologists in 1965 dealing with the nature and role of radiotoxins in the biological effects of ionizing radiation. The quantitative relationships for the formation of toxins, their chemical nature, and a wide spectrum of their radiomimetic properties have moved the problem of toxins to the center of attention in the study of initial and activation mechanisms of the biological effects of ionizing radiation. The nature and properties of secondary radiotoxins which are formed with radiation sickness are investigated from the point of view of pathogenesis and radiation-disease therapy. The book is intended for scientific personnel working in radiobiology, graduate students, students in appropriate advanced courses, and for a variety of surgeon-radiologists, and specialists concerned with problems of the use of atomic energy in agriculture.

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A large amount of theoretical and experimental material on questions of the formation of radiotoxins in irradiated organisms, quantitative principles of their development, chemical nature and broad spectrum of the radiomimetic properties of these substances is correlated in the book; these questions place the whole problem of radiotoxins at the center of attention in a consideration of the initial, actuating mechanisms of the biological effect of ionizing radiation.

A number of papers which throw light upon the nature and properties of secondary radiotoxins which develop in the course of radiation sickness are of great interest from the viewpoint of an understanding of the pathogenesis and methods of treating radiation sickness.

The book is intended for scientists working in the field of radiobiology, graduate students and students in the senior courses of the corresponding section, as well as for a wide circle of medical radiologists and specialists interested in questions of the use of atomic energy in agriculture.

FOREWORD

The First All-Union Conference of Radiobiologists on questions of the nature and role of radiotoxins in the biological effect of ionizing radiation took place in 1965 and the material from it is contained in this book.

The concept of radiotoxins as substances forming in the organism from the effect of radiation, which are biologically active and cause radiation effects to one or another degree in the irradiated organism, arose at the very beginning of the development of radiobiology. Thus, D.E. Lee in 1946 in his classical work "The Effect of Radiation on Living Cells" assumed that the products which are formed from the effect of emissions, being cell poisons, even at very low concentrations, can have an injurious effect. However, the absence of real knowledge of the chemical and biochemical changes in the irradiated organism at that time did not permit closer consideration of this hypothesis.

Later, noting the similarity of the effect of ionizing radiation with the effect of bacterial exotoxins or mustard gas and its analogues (radiomimetic substances), many investigators returned again and again to the thought that a number of the aftereffects of the irradiation can be explained by the radiotoxins which are formed.

At the end of the forties and the beginning of the fifties a number of biochemical investigations were conducted on irradiated organisms which as a result showed various changes in the content of different substances in the tissues of irradiated animals.

In 1954, B.N. Tarusov in the monograph "Foundations of the Biological Effect of Radioactive Emissions" indicated the dependence of the radiation effect on the development of physicochemical processes in the irradiated organism which lead to the appearance of "primary toxins." Numerous investigations on the distant effect of radiation, experiments with parabionts, experiments on the radioresistance of cells in the case of their postradiation maintenance in tissue cultures and investigations on the effect of irradiated cytoplasm on nonirradiated cell nuclei pointed to the role of radiotoxins in the development of radiation sickness.

P.D. Gorizontov at the end of the fifties, in analyzing data on the development of radiation lesion in mammals, came to the

conclusion that toxemia occupies an important place in the pathogenesis of radiation sickness.

A.M. Kuzin in 1962 in the monograph "Radiation Biochemistry" gave attention to the fact that the effect of ionizing particles on multiple structures of the cell can change their functions in metabolic processes and lead to the formation in the cell of changed metabolites, whose accumulation and effect on the unique molecules of the cell can cause many of the known radiation effects. The concept "radiotoxin" thus is enlarged. It is not exhausted by any one specific substance. This concept extends to a number of unusual metabolites developing in an irradiated organism and even to ordinary metabolites, but which are formed after irradiation in abnormally high concentration. Radiotoxins which possess the ability to react with the unique molecules of desoxyribonucleic acid (disturbing their structure and information code) and which act on cell membranes with a change in the course of fermentative reactions will have special significance for an understanding of the initial, actuating mechanisms of the effect of ionizing emissions at the cell level. Secondary radiotoxins arising as a result of deeper biochemical changes in the irradiated organism can be of extreme interest for understanding the later stages of the development of radiation lesion in the organism of higher animals and man and, consequently, for rendering them the appropriate assistance.

Classical target theory (or hits on unique cell structures) is capable of explaining only a limited range of phenomena. Many experimental facts require either additional new hypotheses or a radically different interpretation of the initial events in an irradiated cell.

The first conference on radiotoxins had as its task consideration of the problem of radiotoxins as a whole and becoming acquainted with recent investigations in this area.

This book, in correlating the material of the conference, gives an up-to-date presentation of the theory of radiotoxins, substantially supplementing the available information on processes actually arising in the irradiated cell and organism, and promoting the development of a structural-metabolic theory of the biological effect of ionizing radiation.

A.M. Kuzin

RADIOTOXINS. THEIR POSSIBLE NATURE AND ROLE IN THE DEVELOPMENT OF RADIATION LESION

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The distance effect of radiation, shown at the cell level [1-7], in plants [8,9] and in animals [10-13] compels one to assume the possibility of various mechanisms in the direct effect of radiation. Its presence speaks of the formation of substances (radiotoxins) in an irradiated cell or tissue possessing, in virtue of their special chemical structure or as a result of their appearance in abnormal concentrations, the capacity to imitate the effect of radiation. In fact, such phenomena as inhibition of mitosis, delay in division and growth, the appearance of chromosomal aberrations and pycnotic nuclei, the development of sterility, cytolytic cell breakdown, etc., which are characteristic of the direct effect of radiation are observed in the case of the distance effect.

The following processes precede the appearance of the distance effect:

1. *Radiotoxins* [RT] (PT) *must arise from the effect of irradiation in the cells of irradiated tissues.* Having arisen, they will react with characteristic structures of the irradiated cell, causing the corresponding radiation effect. With a small dose of radiation all the RT which have formed react with characteristic structures of the irradiated cell and, consequently, there cannot be an escape of the RT from the cell, that is, the distance effect is not manifested.

2. *The radiotoxins must escape from the irradiated cell into the external environment.* This is possible only when there is a sufficiently high dose of radiation which provides for the formation of an excess of RT which is not absorbed by the characteristic cell structures. Only at a specific concentration of RT in the irradiated tissues is their escape possible from the irradiated cells into the external environment as a result of diffusion or active metabolic transfer.

3. *The radiotoxins must be transfered by the medium flowing around the cells (intercellular liquid, lymph, blood, etc.), to uninjured tissues.* Undoubtedly, during the transfer dilution, non-

specific sorption and, consequently, a decrease in the RT concentration will occur.

4. *The radiotoxin must penetrate into the cells of distantly located tissue in order to manifest its radiomimetic effect.* For this, RT must be present in sufficient concentration in the medium flowing around the cells, in any case, more than that which is formed within unirradiated cells. However, in its turn this minimal RT concentration in the cell must be sufficient for the RT to react with intracellular structures (for example, with the nucleus) and cause the distance effect (for example, a change in the course of mitosis).

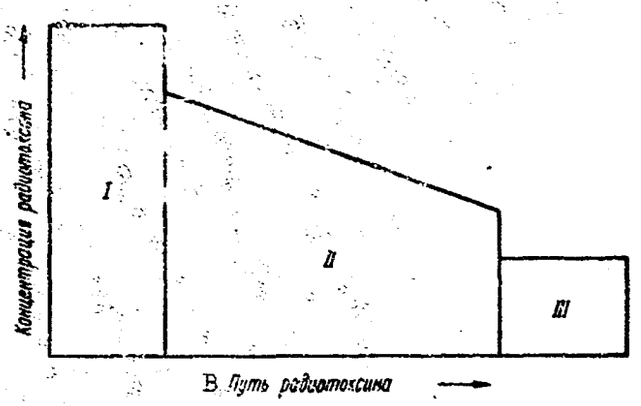

Fig. 1. Diagram of the distance effect. I) Irradiated medium (cell cytoplasm, tissue); II) interstitial medium (cytoplasm, intercellular fluid); III) site of the appearance of the distance effect (cell nucleus, tissue). A) Radiotoxin concentration; B) path of radiotoxin.

Thus, the existence of a distance effect of radiation's action compels one to accept the relative distribution of the RT concentration, represented in Fig. 1.

Such a representation of the various RT concentrations in directly irradiated tissues and tissues in which a distance effect is manifested leads to the following conclusions which are important to the general theory of the biological effect of radiation.

1. All the phenomena observed in distantly located tissues from the effect of the very small RT concentrations which have reached them will be manifested in directly irradiated tissues at much lower doses and to a considerably greater degree.

2. The radiation effects observed in the distance effect of radiation, for example, delay in mitoses, cessation of cell division, inhibition of growth and tissue development, appearance of chromosomal aberrations and pycnoses of the nucleus, inhibition of desoxyribonucleic acid [DNA] (ДНК) synthesis, etc., can also arise in directly irradiated tissue as a result of the formation of RT in it, without necessarily assuming a direct hit of ionizing particles on structures responsible for these effects.

3. The appearance of any radiation effects in cells in the case of direct irradiation which are not manifested in distantly located tissues cannot serve as an argument in favor of the necessity of their development due to a direct hit of ionizing particles. This cannot be asserted if only because the RT concentration in directly irradiated cells and tissues is immeasurably higher than in distantly located cells and tissues, which may be the reason for the appearance of a number of such effects which cannot be caused by a relatively lower RT concentration.

Everything which has been stated above emphasizes the exceptional importance of a quantitative approach in evaluating the significance of RT in the development of radiation lesion. The quantitative approach was widely used in radiobiology, however, the dose-effect curves obtained in experiments were analyzed as a rule from the standpoint of classical target theory. This theory assumes the need for a direct hit of the ionizing particles (one or several) on *unique cell structures* which are the *controlling systems* whose destruction leads in the final analysis to manifestations of various *radiation effects* (to the development of mutations, the cessation of growth, the death of the cell, etc.).

The role of desoxyribonucleic acid in the storage of the cell's hereditary and metabolic information, which has been shown in the last ten years, as well as the determination of the paths of the transmission of this information through informational ribonucleic acid [I-RNA] (И-РНК) to the cytoplasmic ribosomes (which synthesize protein-enzymes determining the direction and intensity of the metabolic processes in the cell) were used in radiobiology for identifying the "unique structures" injured by a direct hit of ionizing particles with structures of the DNA *molecules*.

Thus, according to genetic theory [14], the initial events in an irradiated cell which have a direct relation to the appearance of the final effect arise in the genetically significant DNA macromolecules of the cell nucleus. The primary effect of radiation comes down to a disturbance of the matrix structure of the DNA, as a result of which there is a delay in its reduplication and the appearance of "mistakes" in reading the information code of I-RNA which leads to a decrease in the synthesis of individual enzymes. If these are key enzymes, the death of the cell occurs. A disturbance in the matrix structure of the DNA during its reduplication leads to genetic defects – the appearance of radiation mutants with various deviations from the norm.

The genetic theory of hits on *unique cell structures* (target theory) under discussion leads to the following conclusions:

1. The radiation effect in the cell develops according to the "all or nothing" principle depending on hits or non-hits of ionizing particles on a unique structure.

2. The radiation effect depends only on the entry of ionizing particles into unique structures of the cell nucleus. Irradiation of multiple structures of the cytoplasm is not of decisive importance.

3. The radiation effect is caused by one or several hits of ionizing particles on a unique structure. Hence, according to Poisson's theory it is easy to obtain curves of the dependence of the effect on the dose. Exponential curves indicate that one hit on a sensitive area of the cell is sufficient for the manifestation of the radiation effect. Curves of an S-shaped form speak of the need for two or more hits on a sensitive area of the cell.

H wever, no matter how orderly and conclusive the concept of the direct effect of radiation on unique cell structures may seem, many experimental facts sharply contradict its assumptions and require either additional new hypotheses or a radically new interpretation of the initial events in an irradiated cell.

First of all, at the present time inapplicability of the "all or nothing" principle depending on a hit or a non-hit on a unique structure is well-known: the radiation effect after irradiation with a specific dose can change essentially depending on the cultural conditions of the cells in the post-radiation period. It has also been found that the magnitude of the effect depends on the nature of the metabolic processes in the cell in the post-radiation period [27, 28].

Investigations which showed the effect of irradiated cytoplasm on the functioning of the nucleus placed in doubt the second point of the target theory which is that irradiation of multiple cell structures is not essential for the final radiobiological effect.

The assumption that a hit of an ionizing particle on one or another structure or molecule leads only to its destruction and the loss of its functions (for example, to enzyme inactivation, impossibility of DNA reduplication, etc.), is also erroneous.

Target theory has also ignored another possibility, very likely in an actively metabolizing cell, namely that a hit of an ionizing particle on a biologically active macromolecule or structure can lead to a change in its functions, causing in the post-radiation period the appearance and accumulation in the cell of abnormal metabolites -- radiotoxins.

The obtaining of an exponential curve of the effect-dose dependence is usually cited as abundant proof of the correctness of the target theory, that is, of the possibility of the manifestation of the radiation effect even from one hit on a unique cell structure. It is not difficult to show that the obtaining of an exponential dependence of the radiation effect on the dose equally fits the theory of RT formation.

Let us assume that the radiation effect depends on the hit of ionizing particles on some multiple cell structures, for example, mitochondria. The hit of an ionizing particle on a mitochondrion does not disable it, but disturbs only interlinked processes of electron transport. Let us assume that as a result of this disturbance unusually strongly oxidized compounds begin to form, for example, epoxides of fatty acids or quinones from aromatic amino acids. The striking of only one mitochondrion leads to an infinitesimally

small accumulation of abnormal oxidation products in the cell - radiotoxins. The probability of the manifestation of its effect will be infinitesimally small. However, the greater the irradiation dose, the more altered mitochondria there will be and the more RT will be formed in some specific time interval.

The radiotoxin [RT] concentration will be directly proportional to the number of changed mitochondria [M_1]:

$$[PT] = k[M_n],$$

where k is the coefficient of proportionality. According to Poisson's theory, the number of altered centers in the cells (in the given case mitochondria) will increase depending on the dose D according to the equation

$$[M_n] = [M_0](1 - e^{-aD})$$

under the condition that one hit on an active center is sufficient to cause a change in its functions. Hence, the RT concentration will increase in the irradiated cell depending on the dose according to the equation

$$[PT] = k[M_0](1 - e^{-aD}),$$

that is, exponentially.

It is sufficient to assume that the probability of the manifestation of the radiation effect (for example, the death of the cell or its inability to multiply) within certain dose ranges is directly proportional to the RT concentration:

$$\text{Effect} = K[RT]$$

and consequently,

$$\text{Effect} = Kk[M_0](1 - e^{-aD}) = K'(1 - e^{-aD}),$$

in order to obtain exponential curves of the dose-effect dependence. In our work with Norbayev [15] by the method of electron polarography the dependence of the quinone formation on the radiation dose in plant tissue was obtained in the form of typical exponential curves (Fig. 2).

As was shown on other specimens (plants, yeasts and liver), the accumulation of quinones can occur both according to an exponential curve and according to an S-shaped curve (see this collection, pages 28 and 37), which represents the corresponding dose-effect curves. Thus, the dose-effect curves comply equally well both with the theory of hitting unique structures and with the theory of hitting multiple structures with a change in their functions in the direction of RT formation.

A majority of the radiation effects is manifested only some time after irradiation. A study of the RT concentration in irradiated tissues also showed its increase with the time which had passed after irradiation. This increase clearly is directly connected with the initial change in enzyme activity in irradiated cells shown in a number of papers (see [16-18] and this collection, page 18). If the simplest case of RT formation is assumed (for example, quinone) – through an enzymatic process taking place in one stage – the rate of RT accumulation will be expressed by the following equation:

$$\frac{d[PT]}{dt} = K([A] - [PT]),$$

where [RT] is the radiotoxin concentration;

t is the time after irradiation;

[A] is the initial concentration of the RT precursor.

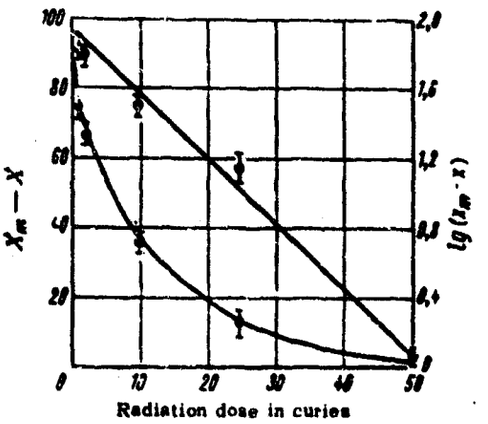

Fig. 2. Dependence of *o*-quinone formation on radiation dose. *X)* Amount of *o*-quinones formed, %; X_m) maximum forming amount of *o*-quinones, %.

The rate of RT formation will decrease in proportion to the increase in its concentration, for example, through the reversibility of the reaction, according to the equation

$$\frac{d[PT]}{dt} = k([A] - [PT]) - k'([B] + [PT]),$$

where [B] is the normal amount of RT in the system. Integration at [RT] = 0 and T = 0 gives

$$[PT] = \frac{k[A] - k'[B]}{k + k'}\left(1 - e^{-(k+k')t}\right).$$

Taking $k/k' = K$, we obtain

$$[PT] = \frac{K[A]-[B]}{1+K}\{1-e^{-(k+k')t}\}.$$

In the equilibrium state $[RT]_r$

$$[PT]_p = \frac{K[A]-[B]}{1+K},$$

$$[PT] = [PT]_p\{1-e^{-(k+k')t}\},$$

i.e., the RT concentration under the assumptions made above will increase exponentially with time. The polarographic study of the increase in quinones in irradiated plant tissue conducted jointly with Norbayev [15] is represented in Fig. 3. As seen from the figure, the amount of quinone increases strictly exponentially in the first 24 hours after irradiation.

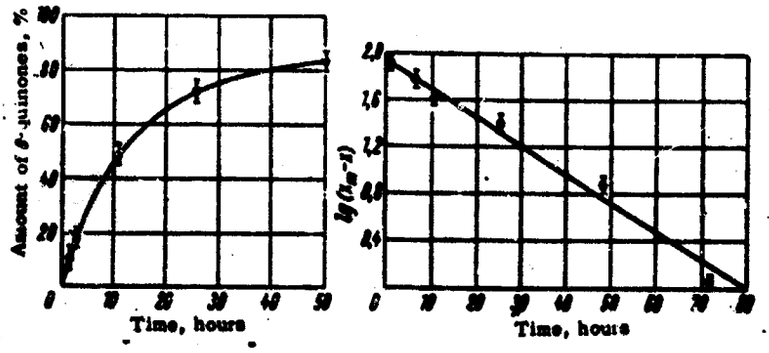

Fig. 3. Dependence of *o*-quinone formation on time after irradiation (X_m is taken as 100%).

The exponential nature of the curve of the increase in the amount of quinones with the time after irradiation leads to two important conclusions.

1. The formation of quinones begins at the moment of irradiation. (This is one of the first processes arising from the effect of radiation on a biological specimen.)

2. The formation of quinones continues after conclusion of the irradiation as a result of the activation of enzymatic systems of phenol oxidation, i.e., there is present here an example of the biochemical intensification of initial processes, whose importance in the theory of the biological effect of radiation was indicated by the author in 1957 [19].

The linking of the radiation-chemical and enzymatic mechanisms of RT formation (semiquinone or quinone) can be represented in the following way.

Normally the following chain of reactions takes place in the cell:

$$\text{Precursor} \xrightarrow{\text{Enzyme A}} \text{Semiquinone} \rightleftarrows \text{Quinone} \xrightarrow{\text{Enzyme B}} \text{Product of condensation or oxidation cycle}$$

Quinones as intermediate substances are found in an infinitesimally small concentration.

During irradiation the radiation-chemical reaction

$$\text{Precursor} \xrightarrow{h\nu} \text{Quinone}$$

develops.

The quinone concentration increases. The quinones react with enzyme B, blocking its active group. The decrease in quinones is slowed down. In virtue of the action of enzyme A the quinones begin to be accumulated in the cells through the enzymatic process.

A different path of linking can be assumed: normally enzyme A is inhibited. The quinones arising as a result of the radiation-chemical reaction bind the inhibitor and from the effect of the liberated enzyme A enzymatic production of quinones develops. A third mechanism also cannot be excluded: the quinones formed as a result of a radiation-chemical reaction react with a repressor which inhibits the synthesis of I-RNA which is responsible for the synthesis of enzyme A. Synthesis of I-RNA takes place and (after its arrival at the ribosomes) regeneration of enzyme A. The increased amount of enzyme A activates enzymatic production of the quinones. The probability of the first method was confirmed by the Kopylov's investigations (see this collection, page 18). Further investigations will show whether other methods of linking occur.

Similar regularities in the increase in the amount of quinones with time were shown in our laboratory in irradiating vegetating corn germinants and seeds and in the irradiation of yeasts (see this collection, page 28), as well as in the liver of irradiated rats (see page 37) and rabbits (see page 201).

It is necessary to particularly emphasize the capacity of quinones arising from irradiation (as well as quinones obtained in model experiments, for example, from the enzymatic oxidation of tyrosine) to inhibit mitoses [20], to cause chromosomal aberrations [21, 22], to stop the growth of tissues [23] and young animals (see this collection, page 90), to cause leucopenia (see page 90), to inhibit and alter DNA synthesis (see page 73), to retard development and to cause the appearance of deformities in acting on developing amphibian eggs (see page 83).

What has been stated above makes it possible to consider the quinones appearing in irradiated tissues as one of the most important examples of RT.

The above-described principles of the formation of RT and

their properties makes it possible to examine many phenomena in radiobiology. The rapid active sorption of RT of quinoid nature by cell nuclei (see this collection, page 60) and their close connection with the proteins and DNA of the nuclear nucleoproteins (see page 53) makes it possible to understand the greater vulnerability of cell nuclei and the change in the transmission of information included in the DNA of the nuclear nucleoproteins from the effect of radiation. The complexing of the quinones with the nitrogenous bases of DNA can cause mistakes in the synthesis of informational RNA and thereby a secondary change in the metabolic processes in the irradiated cell. Similar complexing is especially dangerous during cell division when a break at the site of the blocked base can occur in the complementarily synthesizing DNA which probably leads to chromosome fractures or death of the cell during subsequent mitoses.

The different radiosensitivities of different types of tissues and cells will be determined by conditions which favor or which prevent the accumulation of RT (quinones) in cells and their interaction with structures of the cell nucleus. Polyploids will require 2-3 times greater accumulation of quinones for inactivation of the 2-3 times greater amount of nucleoproteins of the polyploid nuclei.

Albinos (white lines of mice) are more radiosensitive than black mice [24], since enzyme systems are developed in the latter which quickly condense quinones into inert melanines thereby decreasing the concentration of the toxic quinones.

In a comparison of related plants with different radiosensitivities it was shown [25] that from irradiation phenol-oxidizing systems (polyphenoloxidase and peroxidase) are more easily activated in the more radiosensitive plants than in the more radioresistant.

The absence of oxygen at the moment of irradiation sharply retards the radiation-chemical production of quinones which activate enzyme systems in the irradiated tissues which clearly makes its contribution to the mechanism of the manifestation of the universal "oxygen effect."

RT production processes play an important role in the manifestation of the post-radiation recovery effect. The location of irradiated cells under conditions which prevent growth and division, but which provide for the course of normal metabolic reactions considerably decreases the possibility of the manifestation of the radiation effect (see [26-28] and others). These facts sharply contradicted the theory of a direct hit on structures responsible for the manifestation of the observed radiation effect. An additional hypothesis concerning the presence of "hidden" radiation injuries which, depending on metabolic processes in the post-radiation period, can either be manifested or disappear. Since the recovery process takes a long time [for example, for metabolizing yeasts it progresses over the course of 2-3 days [29], and for dry seeds it requires many months (see this collection, page 96)], no real notion of the nature of the hidden injuries which are able to exist for such a long time in a metabolizing

system in the presence of oxygen was suggested.

It seems to us that the problem of post-radiation recovery acquires a different explanation in the light of our data on the formation and role of RT (for example, orthoquinones) in radiation lesion. The radiation effect increase in proportion to the flow of the enzymatic reactions of RT formation, the increase in its concentration, diffusion to nuclear structures and interaction with the latter. If the cells are kept without nutrient media in the post-radiation period (a deficiency of precursors for enzymatic RT production, for example, tyrosine for quinone production) in a large volume of water (which promotes washing out of the RT from the cells) and in the presence of oxygen (which intensifies active excretion of RT through the cell membranes with the consumption of macroergs), the RT concentration inside an irradiated cell will be considerably less, the contribution of the RT to the manifestation of the radiation effect will be absent and a considerably smaller effect will be recorded than under condition favoring production and activity of RT. This can be represented in the form of a diagram (Fig. 4).

The following experimental facts speak for the correctness of the hypothesis concerning the significance of the decrease in RT concentration in the post-radiation decrease in the manifestation of the radiation effect:

1. A specific dilution of cells is required for a decrease in post-radiation lesion: the greater the dilution the stronger the effect. In concentrated suspensions the radiation effect does not decrease. Dilution favors the escape of RT from the cells.

2. It has been established experimentally by the method of electron polarography (see this collection, page 28) that when irradiated yeasts are kept in water for the first and second days, quinones are energetically excreted into the external environment ($E_{\frac{1}{2}}$ = 0.35 v). The excretion proceeds strictly exponentially. The decrease in the radiation effect in yeasts also proceeds exponentially [29].

3. Oxidative reactions which produce energy are required for active RT excretion; the post-radiation decrease in the radiation effect takes place better in an atmosphere of air than in a nitrogen atmosphere [27, 28].

4. Intensive washing of irradiated seeds which promotes washing out of RT decreases the radiation effect (inhibition of growth and germination and number of chromosomal aberrations) (see this collection, page 102).

The hypothesis concerning the important role of RT (*o*-quinones, peroxides and epoxides) in the mutagenic effect of radiation makes it possible to explain from a single viewpoint a number of biological processes. *o*-quinones are constantly formed in cells. Their amount is small because of the low rate of production and the existence of biochemical processes which neutralize these very active substances (by means of further oxidation, condensation into melanines, binding with proteins and excretion from the cell).

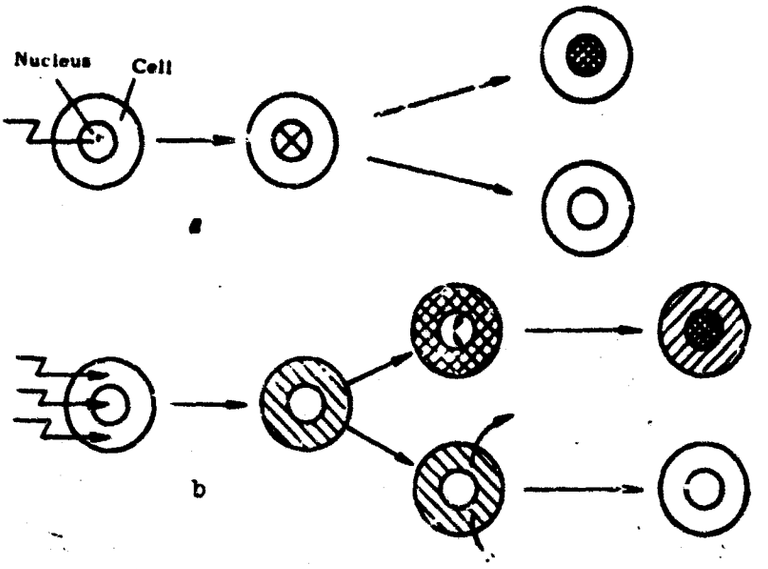

Fig. 4. Diagrams of possible mechanisms of post-radiation recovery. a) Hidden injury theory; b) radiotoxin theory.

Normally these processes are in equilibrium (the *o*-quinone concentration is low). The probability of interaction with DNA of the nucleus is infinitesimally small, but in rare cases is not excluded. Natural mutability (as is known, very low) may be the result of this probability.

As we have shown [30], very low *o*-quinone concentrations stimulate cells to divide, while high concentrations inhibit. Their level in the liver is sufficient for such inhibition and the cells do not divide. In the case of hepatoectomy conditions are created which are favorable for a decrease in their level and intensive division begins. During irradiation enzymes are activated which oxidize phenols to *o*-quinones. It is possible that systems which free the cell from these substances are inhibited (see this collection, page 18). As a result the level of the quinones increases sharply. This leads to inhibition of division and great damage to DNA which is manifested in an increase in the number of chromosomal aberrations and an increase in mutation. From this point of view, natural mutation, mutation from the effect of ionizing radiation and from the action of chemical mutagens is a single process having the same mechanism.

No mutagenic effect of quinones and peroxides has been noted in the literature (see this collection, page 102, and [24, 31].

The set of mutants which develop can, as is known, vary from the use of chemical mutagens of different types. Radiotoxins (for example, *o*-quinones) will be one of the possible chemical mutagens.

In studying the mechanism of the protective effect of sulfhydryl protectors, Bacq [32] found that at the moment of the greatest protective effect (10 min after injection) the mitochondrial structures in the cells of the tissues are sharply changed. Consequently, the protection is connected with inhibition of enzymatic oxida-

tion processes at the time of irradiation which must lead to less production of RT of the quinone, peroxide and epoxide type than, perhaps, is explained by the protection mechanism.

The RT theory recommends new methods of post-radiation protection, namely, as complete as possible washing out of the RT from the organism and the introduction into the organism of substances which actively bind RT.

All the new observations and considerations presented above, it seems to us, speak convincingly of the important role of RT production, and first and foremost, of *o*-quinones in the development and manifestation of many radiation effects. An immediate, direct effect of ionizing particles on unique structures of the cell during its irradiation (DNA molecules and chromosomes) certainly occurs and in the light of numerous data of radiation chemistry and molecular biology can lead to the appearance of localized mutations, chromosome breaks and death of the cell during mitosis. However, in the light of the RT theory all these phenomena can also develop through the secondary action of RT.

On the other hand, RT clearly occupy a leading role in the interphase death of cells which plays an important role in radiation injury of an organism, in inhibition of growth and development and in the appearance of a number of symptoms of radiation sickness.

An understanding of this role is quite necessary for proper treatment of radiation sickness, for a successful search for new prophylactic and post-radiation protective remedies and measures and for further development of methods of radiation therapy of malignant neoplasms.

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Manuscript Page No.	Transliterated Symbols
8	и = i = izmenennyy = changed
10	р = r = ravnovesiye = equilibrium

MECHANISM OF FORMATION AND IDENTIFICATION OF TOXIC SUBSTANCES OF QUINOID NATURE FORMING IN AN IRRADIATED ORGANISM

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While the question of the formation of toxic substances in an irradiated organism is sufficiently clear at the present time, their nature has still been insufficiently studied.

The first assumptions about the nature of the toxic substances were expressed in 1912 by Werner [1]. In observing the formation of choline from irradiation of lecithin with large doses, he suggested the possibility of choline formation from irradiation of the animal organism. And in fact, it was shown considerably later [2] that choline is formed in an irradiated organism. When choline was injected into animals, a number of symptoms characteristic of radiation lesion was noted. Caspari [3] believed that the toxic substances might be cell-decomposition products, which he called necrohormones. Of the substances which may play a specific role in the etiology of the radiation syndrome, imidazone derivatives, which Menkin [4] found in an irradiated organism, are of interest. Lewis [5] expressed the hypothesis that the toxic substance formed in an irradiated organism may be histamine. This hypothesis became known as the "histamine theory" after the work of Ellinger [6]. Krichevskaya [7] expanded the notion of histamine's role in the development of radiation sickness and expressed a number of new hypotheses.

In 1963, Mano [8] reported isolating in crystalline form from the serum of irradiated rats the substance $C_6H_{15}N_2O_3$, which at a dilution of 1:3000 caused leucopenia. In recent years in the laboratory of B.N. Tarusov, Yu.B. Kudryashov and colleagues have been intensively studying the nature of a toxic substance which they isolated, now conditionally called "cytotoxic factor" [9, 10]. Budnitskaya [11] believes that some of the toxic substances formed in an irradiated plant may be unsaturated higher fatty acids. The question of the formation of organic peroxides in the irradiated organism was introduced by Horgan and Philpot [12]; however, there is still no clear proof of this in spite of the large number of studies which have been carried out. A.M. Kuzin, based on the structural-metabolic theory which he developed, ascribes a major role in the development of radiation lesion to toxic substances formed in the irradiated organism and suggests that *o*-quinones in semiquinone form are one of the primary toxic compounds [13, 14].

This is a far from complete list of those toxic substances which are formed in an irradiated organism. However, these data are sufficient to suggest that irradiation causes the formation of a whole series of toxic substances, the combination of which can create the qualitative peculiarity of ionizing radiation's specific effect on the organism. Therefore, the notion, which has existed for a long time, of the toxic substance radiotoxin (RT) as some unique compound which is capable by its presence of causing all the symptoms of radiation sickness, seems incorrect to us. The idea of a unique RT was engendered when there were still no clear notions of the nature of primary disturbances as a result of the effect of ionizing radiation and of those biochemical changes which occur in this case.

The question of which toxic substances are formed directly as a result of irradiation (primary RT) and which are the result of previously formed toxic substances is appropriate at the level of present-day knowledge. It is important to establish which metabolic links are disturbed from the effect of one or another toxic substance and to isolate those which, in acting on the cell nucleus, cause such characteristic symptoms of the radiation reaction as cessation of cell division, growth inhibition, leucopenia, suppression of DNA synthesis, development of chromosome fractures, etc.

The experiments of A.M. Kuzin and L.M. Kryukova have played a major role in the investigation of such RT. From irradiating a leaf of the *Vicia faba* plant with complete screening of all of the rest of the plant, the authors noted inhibition of mitotic division at the growth points, and removal of the irradiated leaf in the 4 hours immediately after irradiation led to elimination of the noted effect [15]. It turned out that substances which are formed in an irradiated leaf are easily extracted and if plant seeds are moistened in such extracts, inhibition of mitotic division is also noted [16]. It was established that along with inhibition of mitotic division, a disturbance in the chromosome apparatus occurs [17].

After the introduction of such extracts into animals there occurs a drop in weight with subsequent inhibition of growth (see [18] and this collection, page 90), a sharp decrease in the number of leucocytes in the first hours after injection (see page 90) and a change in the weight of some organs, as is found in radiation sickness. The fact that, as Kryukova, et al, [19] showed, these extracts selectively act more actively on malignant tumor tissues was also interesting. The fact that extracts obtained from irradiated plants contain 1.5-2 times more *o*-dioxyphenols than extracts from nonirradiated plants draws attention.

It is known that a whole series of phenols have an expressed toxic effect. Some phenols at concentrations of $1 \cdot 10^{-4}$-$1 \cdot 10^{-6}$ *M* inhibit mitotic division, disturb the chromosome apparatus and have an effect on a whole series of enzyme systems [20, 21]. The toxic properties of the phenols are determined by their structure, the arrangement of the hydroxyl groups and the nature of the substituent. One important property, from our point of view, is that the toxicity of the phenols is determined by their capacity for the formation of free radical forms [22].

Kuzin, et al, [23] showed that in potato tubers irradiated with a dose of 15 curies, the amount of such *o*-phenols as chlorogenic and caffeic acids increases considerably. In joint work with T.V. Volkova, we noted an increase in 3,4-dioxyphenylalanine in *Vicia faba* leaves. Chlorogenic and caffeic acids can have an effect on a whole series of metabolic links and on growth processes. However, as Sokolova showed [24], for the development of the toxicity of chlorogenic acid, concentrations far exceeding the limits of the physiological norms are needed, whereas in the irradiation of potato tubers the concentration of chlorogenic acid increases only by 70-80%.

Consequently, the toxic effect which is noted in extracts of irradiated plants is connected not only with substances of phenolic nature, but also with some other substances. The assumption that these can be substances of peroxide nature (for example, lipid peroxides) is unlikely since the separation method which was used excluded their presence in large amounts. But even if it is assumed that part of them is extracted, the concentration of these substances will clearly be so insignificant that it is difficult to expect any effect.

An examination of the toxicity of solutions of hydrogen peroxide on plant seeds (on which the toxicity of extracts obtained from irradiated plants was determined) showed that only a concentration of 10^{-2} M indisputably inhibits germination of the seeds, whereas a concentration of 10^{-3} M hardly inhibited growth. Siegal [25], in studying the toxicity of some peroxides, showed that linoleic acid in a concentration of $2 \cdot 10^{-4}$ M when oxidized with lipoxidase for 20 hours inhibits seed germination in comparison with linoleic acid only by 12%.

In order to determine the nature of the toxic substances formed in irradiated plants, potato tubers of the "Lorkh" variety were irradiated in a GUPOS-2 cesium apparatus with γ-rays at a dose rate of 700 r/min in a dose of 15 curies. Twenty-four hours after irradiation the potato tubers (control and experimental) were chilled to 0° to prevent oxidative processes during separation. The surface layer (3-4 mm in thickness) was removed and the middle part was homogenized in 96% ethyl alcohol chilled to −20°. The extraction lasted 60 minutes with mechanical stirring. After precipitation of the stroma with alcohol the extract was concentrated under vacuum at 28-30°. The sediment which forms as a result of the concentration was removed and the clear light yellow solution was acidified with hydrochloric acid to pH 2.0 and extracted with ethyl acetate. The ethyl acetate layer obtained (to which, according to the data in the literature, the quinones must have moved) was applied to a paper chromatogram (20 × 20 cm) and fractionated in two solvents, namely, in 2% acetic and in a benzene-acetic acid-water mixture (in a ratio of 2:4:1). The results of the chromatographic separation are shown in Fig. 1.

In examining the chromatograms in ultraviolet light, several fluorescing spots (1-5) are found and a substance *X* is contained in the irradiated tubes in contrast to the control. Substance *X* fluoresces with a blue light which in a few seconds changes to dark blue, and it also gives a positive reaction with aniline

reagent which indicates the quinoid nature of this substance. A special reagent for quinones, consisting of sulfuric acid, zinc powder and a basic thiazone dye also gave a positive reaction [26]. Kuzin and Norbayev [27] found by polarography that water extracts of irradiated potato tubers give a half-wave at −0.35 v typical of quinones. Spots 1 and 2 give a positive reaction for *o*-dioxyphenols (Arnow reaction) [28].

Quinones are very reactive compounds. They can be compared with α, β-unsaturated ketones, but they are considerably more active. The high reaction capacity of the quinones is connected with the nature of their structure which is characterized by relatively little additional stabilization through resonance energy. Quinones are compounds which are widely distributed in nature, whose great significance in biochemical processes is becoming more and more evident. However, this pertains primarily to the *p*-quinones; it is usually not possible to isolate *o*-quinones from normally metabolizing cells. *o*-quinones have higher potentials than the corresponding *p*-quinones. *o*-quinones are actually the strongest oxidizers of all natural organic compounds. In connection with this, it was important to establish to which quinone series the quinones found in the irradiated samples belong. For this purpose, $1 \cdot 10^{-4}$ M ascorbic acid was added to the test solution, which, as is known, quantitativlly reduces quinones to the corresponding phenols. After separation on a two-dimensional paper chromatogram under the same conditions as described above, the chromatograms shown in Fig. 2 were obtained.

As seen from Fig. 2, the spot (see *X* in Fig. 1) belonging to quinone disappeared, whereas the intensity of Spots 1 and 2 increased sharply. As was shown above, Spots 1 and 2 contain substances of a phenolic nature with *ortho*-located hydroxyl groups. Hence it follows that the addition of ascorbic acid to the test solution leads to the formation of *o*-dioxyphenols, which speaks of the *ortho*position of the carbonyls in substance *X*. Substance *X* was eluted from the chromatograms with alcohol and the absorption spectrum in the ultraviolet region was recorded on a self-recording spectrophotometer. The absorption spectrum of substance *X* in the ultraviolet region is given in Fig. 3.

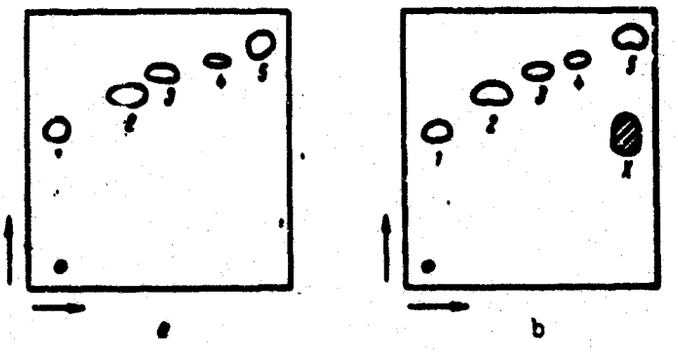


Fig. 1. Chromatogram of ethyl acetate fraction separated from nonirradiated and irradiated (in a dose of 15 curies) potato tubers (middle part). a) Control; b) after irradiation. Along ordinate – mixture of benzene, acetic acid and water (2:4:1); along abscissa – 2% acetic acid. Numbers 1-5 – number of spots.

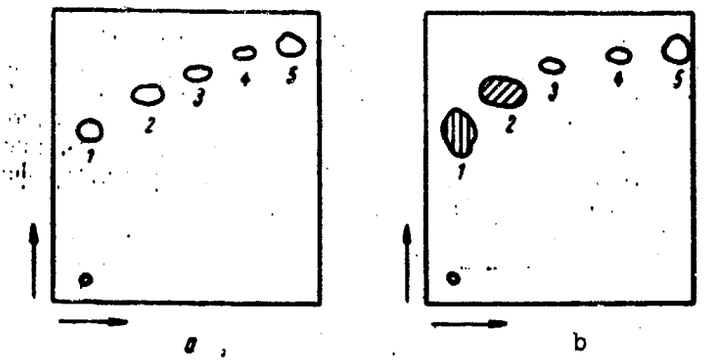


Fig. 2. Same as in Fig. 1, but with the addition of ascorbic acid in a concentration of $1 \cdot 10^{-4}$ M.

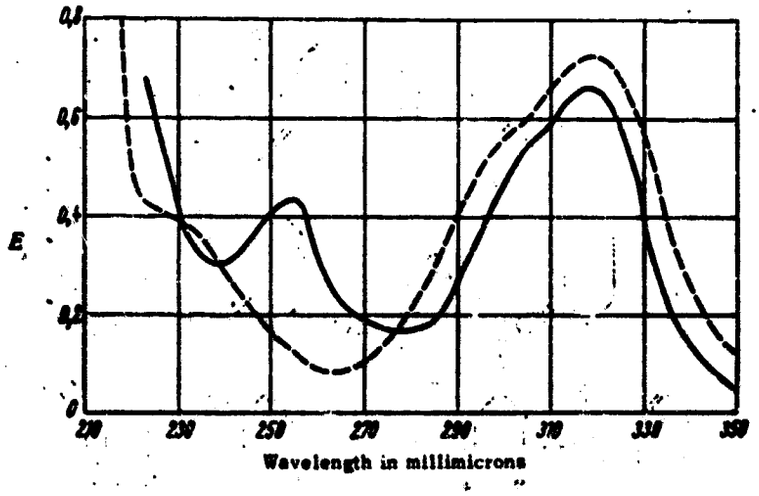
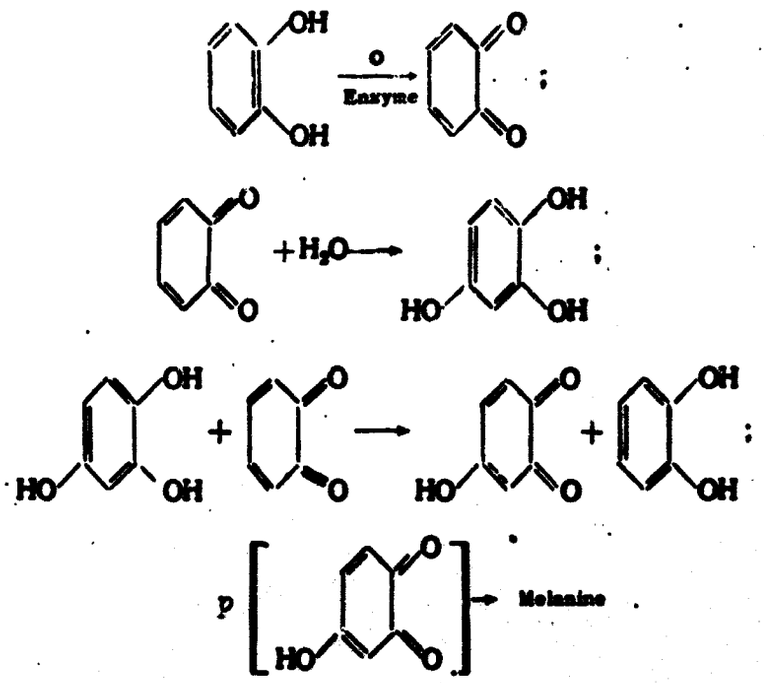


Fig. 3. Spectrum of substance *X* in ultraviolet region, taken on a self-recording spectrophotometer. Unbroken curve – substance *X*; broken curve – chlorogenic acid.

As follows from the curves obtained, substance *X* has two clear maxima – at 255 and 322-324 mμ. The absorption spectrum of chlorogenic acid was taken for comparison. In the long wave region substance *X* has a spectrum characteristic of cinnamic acid derivatives, in the short wave region (250-255 mμ), it is closer to substances of quinoid nature. Thus, extracts of irradiated plants which have toxic properties contain *o*-quinones in addition to phenols.

An increase in quinones after irradiation occurs not only in plants where, as is known, there is a large number of substances of phenolic nature, but also in animal organisms. The results have been published earlier [29], here we should only like to emphasize that later analogous data were obtained by Plyshevskaya et al. (see this collection, pages 37 and 60) and Ivanitska and Kuzin [30]. One of the possible reasons for the appearance of *o*-quinones in an irradiated organism may be a disturbance in the linkage of enzyme

systems responsible for the oxidation of polyphenols. Earlier it was believed that polyphenoloxidase is radioresistant. Thus, according to Sussman's data [31] a dose of 300 curies is insufficient to inactivate this enzyme. However, in 1958 Schwimmer [32], in 1959 Rubin and Mikheyev [33] and in 1960 Kuzin and Kopylov [34] reported that after irradiating plants with a dose of 10-15 curies, a disturbance in the activity of polyphenoloxidase occurs. The principal path of the oxidation of polyphenols by polyphenoloxidase has been suggested by Dawson [35] and consists in the following:



As seen from this diagram, for oxidation of pyrocatechol by polyphenoloxidase the participation of the enzyme is necessary only in the first stage, that is, the oxidation of pyrocatechol to *o*-benzoquinone. If ascorbic acid is added to the reaction medium, the whole further process of oxidation, according to this scheme, must be interrupted. It is possible to judge the rate of the enzymatic process at this stage of oxidation from the amount of ascorbic acid which went into the reduction of quinone. To determine the polyphenoloxidase activity one can use Povolotska and Sedenko's method [36], based on this principle. The results of measurements made by this method of the activity of polyphenoloxidase isolated 24 hours after irradiation of potato tubers in a dose of 15 curies are given in the table.

It is seen from these data that the polyphenoloxidase activity at the given stage of oxidation is not disturbed during irradiation. We have developed another method of determining polyphenoloxidase activity which consists of recording the reaction at the stage of the formation of the penultimate reaction product – oxyquinone.

Among the products of the enzymatic oxidation of pyrocatechol only oxyquinone has an absorption maximum at 255 mμ [37]. By measuring its amount on special ultraviolet equipment capable of absorbing only in the region of 255 mμ, it is possible to determine the rate of the enzymatic process.

Potato tubers	Ascorbic acid oxidized (enzyme activity), mg			
	Expt. I	Expt. I	Expt. III	Averages
Control	120,1	103,7	101,5	108
Irradiated	107,5	115,7	118,4	114

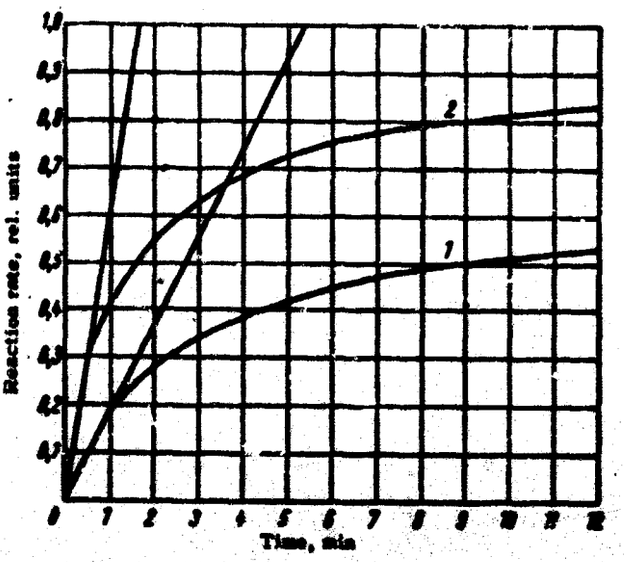


Fig. 4. Rate of polyphenoloxidase reaction determined from absorption at 255 mμ. Reaction conditions: pH 6.8; $T = 27 \pm 0.1°$; phosphate buffer; pyrocatechol substrate ($1 \cdot 10^{-4}$ M). 1) Normal; 2) Irradiation.

The kinetics of the enzymatic process were continuously recorded on a type EPP-92 potentiometer. The average results of three experiments are presented in Fig. 4. The initial pyrocatechol concentration was $1 \cdot 10^{-4}$ M; an acetone preparation of the enzyme isolated from irradiated and nonirradiated tubers was taken in an amount of 5.8 mg of protein in 1 ml both in the control and in the experiment (24 hours after irradiation of potato tubers in a dose of 15 curies).

As seen from Fig. 4, the activity of polyphenoloxidase isolated 24 hours after irradiation proved to be more than 2 times higher than in the control, with the selected method of determination. Consequently, while in the first stage of oxidation (see data in table) as a result of irradiation a disturbance in enzyme activity does not occur, in subsequent stages a sharp increase in the amount of oxyquinones is noted.

According to the scheme suggested by Dawson [35], oxidation of

pyrocatechol after the formation of *o*-quinones occurs without enzyme participation. However, if it is assumed that oxidation of pyrocatechol by polyphenoloxidase is carried out by the enzyme not only in the first stage of oxidation, but, possibly (by analogy with the oxidation of dioxyphenylalanine by tyrosinase, according to Reper and Masson) also at other stages, it is easy to understand the results obtained in this work. As a result of irradiation inhibition of one of the enzymes of the polyphenoloxidase complex which oxidizes oxyquinones occurs; as a result they are accumulated as intermediate products which normally are oxidized further thanks to the orderliness of this entire scheme.

This can be compared with the specific effect of a poison on a polyenzyme system, where normally it is not possible to isolate intermediate products of the reaction, while from the action of the poison on one of the enzymes accumulation of intermediate compounds sufficient for quantitative determination occurs.

Consequently, the noted increase in oxyquinones in irradiated samples rather is the result of the inhibition of one of the enzymes of the polyphenoloxidase complex than activation of another.

The data obtained by Anbar [38] are interesting in this connection. He notes that molecules carrying a copper ion have an advantage in radiolytic separation over the other organic compounds dissolved in the system. This effect increases with an increase in oxygen concentration. Many enzymes of the polyphenoloxidase complex contain copper, which perhaps causes the observed inactivation.

Thus, the toxicity of extracts obtained from irradiated plants can be connected with the accumulation of *o*-quinones which, as is known, possess the clearly expressed ability to inhibit cell division and to cause chromosomal aberrations [39, 40].

Clearly, the toxicity of the *o*-phenols is also determined by their ability to be easily oxidized to quinones in the organism. As Reed showed [41], quinones accumulated from the oxidation of *o*-dioxyphenols by polyphenoloxidase inhibit mitotic division at the prophase stage. The addition of cysteine removes this effect. The formation of quinones in an irradiated organism possibly is the result of a disturbance in the activity not only of polyphenoloxidase, but also of peroxidase. The activity of the latter increases considerably in irradiated organisms. Shifting of the pH to the alkaline side in irradiated plants and animals is one of the possible causes of the auto-oxidation of polyphenols to quinones. It is interesting that the products of the auto-oxidation of polyphenols are considerably more toxic than the products of enzymatic oxidation. For example, if the toxicity of chlorogenic acid is taken as one, the products of its enzymatic oxidation will be three times more toxic, and the products of auto-oxidation - 10 times [42]. It is also necessary to take into account that during irradiation the radiation-chemical oxidation of *o*-phenols to quinones will also occur. The quinones which are produced in catalytic amounts can have an effect on the activity of individual enzymes of the polyphenoloxidase complex, which will lead to subsequent, post-radiation enzymatic accumulation of quinones.

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QUANTITATIVE PRINCIPLES OF THE APPEARANCE OF QUINONES IN IRRADIATED PLANT TISSUES AND YEAST CELLS

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(Institute of Biophysics USSR Academy of Sciences)

In order to understand the possible role of quinones which appear in irradiated tissue and cells [1-6], it is necessary to know the quantitative principles of their formation in relation to dose, time after irradiation and dose rate of the irradiation. The method of electron polarography was used for this purpose: the height of the polarographic wave was measured at $E_{\frac{1}{2}} = -0.35$ v in equipment of the PA-2 type.

It was shown earlier [6] that after irradiation of potato tubers the amount of quinones increases strictly exponentially in relation to the dose. The increase in the amount of quinones continues for 3 days after irradiation (in an irradiated tuber at room temperature) and the curve of this increase corresponds to the curve of the formation of a product of a reversible first order reaction.

It seemed of interest to follow the regularity of the increase in amount of quinones under the more complex conditions of actively metabolizing tissue. For this purpose the formation of quinones and their excretion through the roots of irradiated corn germinants was investigated.

Seven-day corn germinants (of the "Sterling" variety) were irradiated in a GUPOS-2 cesium apparatus with γ-rays with a dose rate of 700 r/min in increasing doses. 15 minutes, 6, 24, 48 and 72 hours after irradiation, 7 g of tissue was taken, pulverized with 20 ml of 96% alcohol and centrifuged at 8000 rpm. All procedures were carried out at a temperature of 0°C. The oxygen was displaced from the solution obtained at this temperature by purified nitrogen and recording of the polarographic wave was conducted in the presence of a base electrolyte (phosphate buffer, pH 6.8) with an equipment sensitivity of 1.0.

The average data from six series of experiments are presented in Fig. 1.

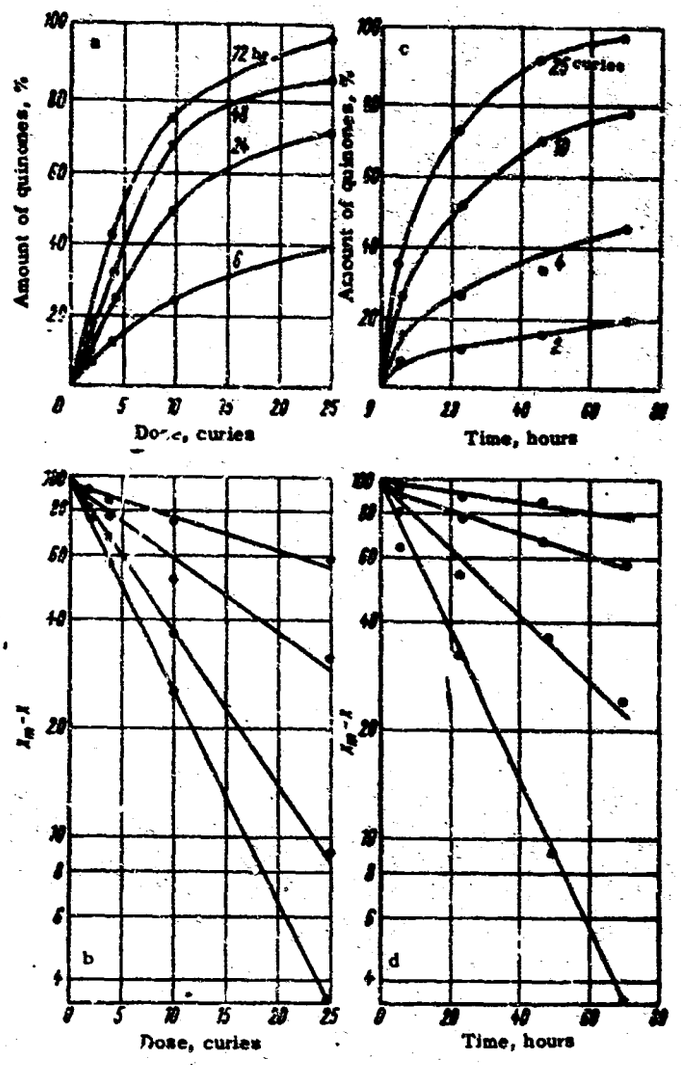

Fig. 1. Quinone content of corn germinants depending on dose and time after irradiation (X_m is taken as 100%): a,b) Dependence on dose at different times after irradiation (time indicated on curves); c,d) dependence on time with different doses (doses indicated on curves).

As seen from Fig. 1, the amount of quinones formed in the irradiated germinants increases strictly exponentially depending on the dose and time in the post-radiation period. The pattern of quinone formation in actively metabolizing tissues of corn germinants is close to the earlier described patterns of their formation in irradiated potato tubers [6].

It can be assumed from the rapid increase in the amount of quinones in irradiated tissue that in the case of the toxic action of the quinones the plant will attempt to excrete them from the tissue through the root system. For a quantitative study of this phenomenon, 40 normally growing corn plants were selected

on the seventh day after sprouting of the germinants and irradiated in the GUPOS-2 with γ-rays with a dose rate of 700 r/min in doses of 2, 4, 10 and 30 curies. After irradiation the roots of these germinants were placed in 75 ml of distilled water. After 24 hours the water in which the roots were kept was removed and concentrated in a vacuum to 5 ml. The oxygen was driven off with pure nitrogen and the polarographic wave was determined at $E_{\frac{1}{2}}$ = −0.35 v with an equipment sensitivity of 0.1. Distilled water was used in order to promote escape of the compounds which interest us through the hypotonic conditions. In these experiments nonirradiated plants also excreted in 24 hours a small amount of substances giving a polarographic wave at $E_{\frac{1}{2}}$ = −0.35 v. The change in the excretion of quinones from the effect of irradiation with different doses (average data of six experiments) is presented in Table 1.

TABLE 1

Excretion of Quinones by the Roots of Corn Germinants 24 Hours After Irradiation

Radiation dose curies	Height of polarographic wave (average of six experiments) in mm	Radiation dose curies	Height of polarographic wave (average of six experiments) in mm
Control	27,0±1,4	10	56,0±4,2
2	21,0±2,9	30	75,0±1,3
4	16,0±2,5		

Two details from these data are of interest: 1) at doses below 5 curies noticeably fewer quinones are excreted than in the control, and 2) with a further increase in the irradiation dose progressive excretion of the quinones is observed which reflects the pattern of their accumulation in tissues shown above. The amount of quinones formed in plant tissues at 10 curies in 24 hours is approximately 300 times more than the quinones excreted in 24 hours by the same plant.

The question of the formation of quinones in seeds after their irradiation is of great interest. The work of Berezina and Yazykova [7], Fonshteyn [8] and others showed that removal of the embryo from irradiated endosperm and corymb makes it possible to decrease considerably radiation lesions arising in the embryo. It is likely that the RT which are formed in the seed on entering the embryo play an important role in its radiation injury. If you consider that quinones appear as a result of a disturbance in oxidative processes which take place particularly intensively in the corymb of the seed during its germination, it can be assumed that particularly intensive quinone formation takes place in the corymb. In order to verify this assumption, corn seeds were soaked for 24 hours, then irradiated with γ-rays in doses of 2, 4, 10 and 40 curies, immediately after irradiation the embryos were removed and the seeds (40 specimens) placed in 25 ml of sterile distilled water. After 24 hours in water the amount of substances giving a polarographic wave at $E_{\frac{1}{2}}$ = −0.35 v was determined at equipment sensitivity of 0.1. Nonirradiated seeds, also with the embryo removed, served as a control. The results are presented in Fig. 2.

It is seen from these data that after irradiation intensive formation of quinones begins in the seed (its endosperm and corymb) and that their level, determined after 24 hours, increases, depending on the dose, strictly exponentially. It is interesting to note that when the embryo is removed quinones are excreted into the external medium and, consequently, in a whole seed they will go from the corymb to the embryo tissue, causing radiation effects in the latter.

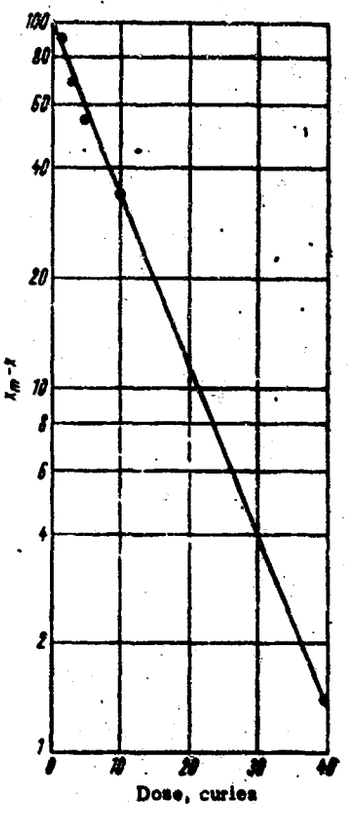

Fig. 2. Excretion of quinones by irradiated corn seeds in the 24 hours after removal of embryo depending on the irradiation dose.

The increase in the amount of quinones in irradiated tissue depending on the dose and time which was shown on potato tubers as well as on corn germinants and seeds stimulated studies of other specimens to confirm the universality of this process.

Diploid wine yeasts, *Saccharomyces vini*, isolated from the vinous material "Bayanshirin" (grown in grape must of the "Riesling" variety: saccharinity 16.8%, specific gravity 1.071, pH 3.36, total acidity 6.1) were used in the studies. The must was sterilized for 20 minutes at 0.5 atm in 1 liter Ehrlenmeyer flasks. 2 vol. % of a rejuvenated 2-day culture was added to 200 ml of the must. Cultivation was carried out under aerobic conditions (on a shaker at 180-200 rpm) for 2 days at 27-28°.

To separate the cells, the cell suspension in the must was filtered on the second day and washed several times with sterile distilled water and suspended in 12 ml of sterile distilled water (the concentration of the suspension was approximately 83 million/ml). The suspension was irradiated in cesium equipment with γ-rays with a dose rate of 700 r/min in doses of 50, 100, 300 and 700 curies. After 24 hours of storage at 27°, the yeasts were removed by filtration, the transparent solution saturated with nitrogen and the height of the polarographic waves was determined at $E_{\frac{1}{2}}$ = −0.35 v at an equipment sensitivity of 0.04. The average results obtained from three series of experiments are presented in Fig. 3. As seen from the data obtained, quinone excretion proceeds according to an S-shaped curve, which evidently reflects the nature of its formation in irradiated yeast cells.

The dynamics of quinone excretion in the time after irradiation was investigated in a special series of experiments. In these experiments the yeast suspension was irradiated in a dose of 100 curies and kept for 24 hours in water. After this the cells were removed by filtration, washed and transferred to pure sterile water. The procedure of filtration, washing and transfer was carried

out every day for 5 days. The amount of quinones excreted on the 1st, 2nd, 3rd, 4th and 5th days was determined by the above-described method. The data obtained are presented in Fig. 4.

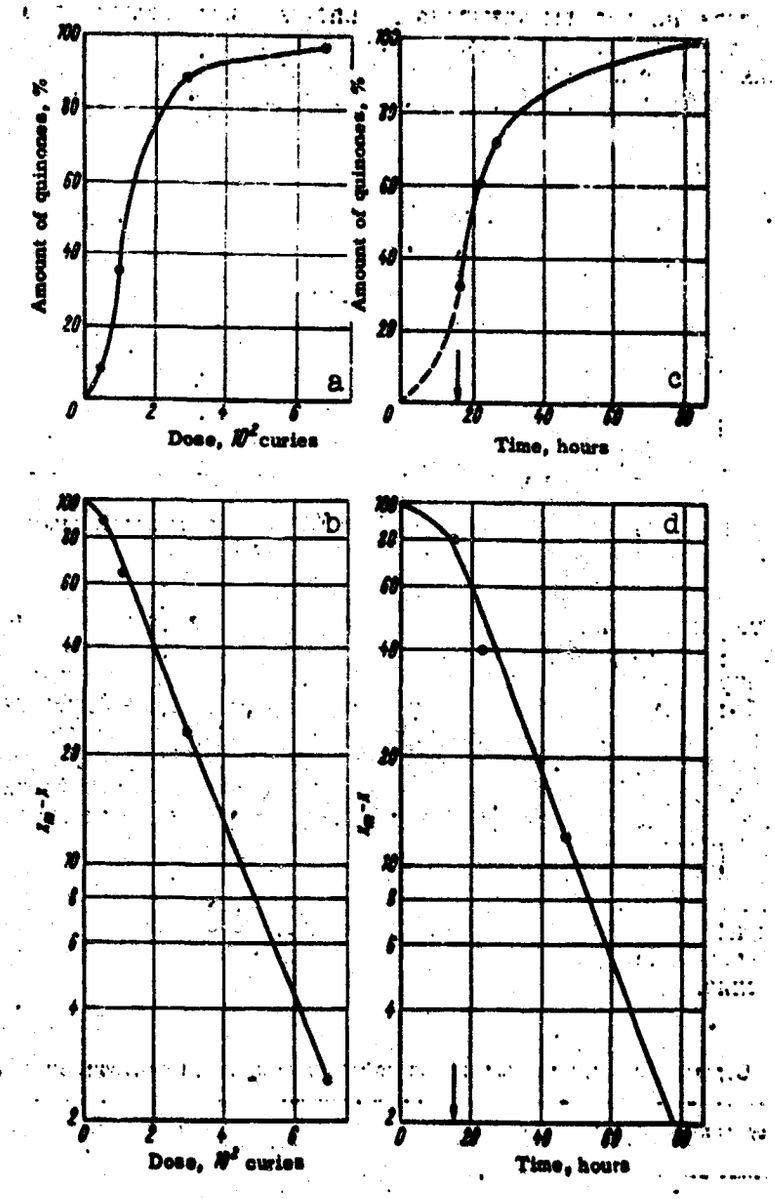

Fig. 3. Excretion of quinones by irradiated yeast cells of *Saccharomyces vini* depending on dose and time after irradiation (X_m taken as 100%). a,b) Dependence on dose (24 hours after irradiation); c,d) dependence on time after irradiation (in a dose of 700 curies); the arrow indicates duration of irradiation.

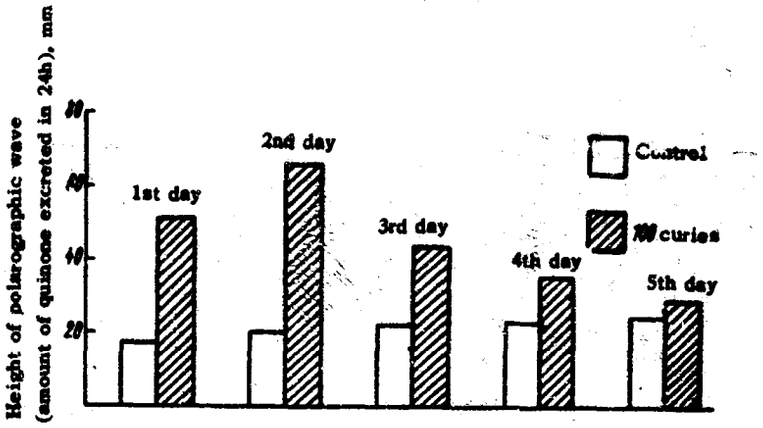

Fig. 4. Excretion of quinones by irradiated (in a dose of 100 curies) yeast cells of *Saccharomyces vini*.

As seen from these experiments, most intensive quinone excretion occurred on the 1st and 2nd day after irradiation. Then it fell sharply, hardly exceeding the control, by the 4th day.

The increase in the quinones which was found in the time after irradiation made the enzymatic nature of their formation through oxidation of phenol likely (see this collection, page 44). In the literature, the hypothesis has been stated many times that the activation of the enzymes is the result of a radiation-chemical change in the physical properties of intracellular structures [9], their breakdown [10] or damage to subcellular membranes [11]. If this is correct, then by increasing the dose rate of the irradiation, greater effectiveness of this initial disturbance of the elementary structures and thereby increased quinone formation might be expected. To verify the hypothesis, irradiation of potato tubers with γ-rays of Co^{60} (at the Institute of Organic Chemistry) with a dose rate of irradiation of 300 r/sec was carried out, at the same time as another batch of the tubers was simultaneously irradiated in Co^{60} equipment (Institute of Biophysics) with a dose rate of 1.6 r/sec.

The kinetics of the quinone formation in the time after a total irradiation dose of 20 curies is presented in Table 2 (sensitivity of the equipment equals 2). The data obtained clearly shows an increase in quinone accumulation with an increase in the dose rate of irradiation.

A quantitative investigation of quinone formation in irradiated tissues of young germinants and in yeast cells showed that, as in potato tubers, after irradiation of actively metabolizing cells oxidative processes arise which lead to a rapid increase in the appearance of biologically highly-active quinones. The obtaining of similar results on four different specimens (potato tubers, corn germinants, corn seeds and one-celled organisms – the yeast *Saccharomyces vini*) indicates that quinone formation is a common response reaction to irradiation for plant tissue.

TABLE 2

Quinone Formation in Potato Tubers Depending on Dose Rate

Time of measurement after irradiation, days	Radiation doserate, r/sec	Height of polarographic wave, mm
1st	Control	55,0±2,9
	1,6	91,0±6,1
	Control	54,0±2,7
	300	113,0±8,3
2nd	Control	57,0±3,7
	1,6	102,0±12,0
	Control	58,0±3,1
	300	132,0±8,1
3rd	Control	59,0±3,6
	1,6	128,0±7,3
	Control	56,0±2,8
	300	152,0±6,3
4th	Control	58,0±3,4
	1,6	139,0±7,4
	Control	59,0±1,8
	300	168,0±4,8

The dose curves (exponential or S-shaped) examined above completely imitate dose curves obtained according to other criteria and interpreted according to target theory as one- and multiple-hit curves. However, having shown that the accumulation of toxic quinones is expressed by the same dose curve, we can easily explain other radiation effects as the result of the action of increasing amounts of quinone in place of the hypothesis of one- and multiple hits of an ionizing particle on unique structures. The exponential nature of the curves describing the increase in quinones with the time after irradiation is of special interest. It indicates that the reaction of quinone production is one of the first reactions of the effect of radiation, which arises directly during the irradiation and at the same time having the characteristic that having begun, it continues after irradiation, which clearly indicates its enzymatic nature. The assumption concerning enzyme activation as its basis is in accordance with the data on the dependence of quinone yield on the dose rate of the irradiation.

The active excretion by irradiated tissues and cells (germinant rootlets and yeast cells) of the radiotoxins – quinones – which are formed in them which was found is of extremely great interest. Processes of the yeasts' recovery from radiation lesion during their storage in tap water which have been described by a number of investigators [12-14] finds a natural explanation in the elimination of RT, and does not require any hypothesis of "hidden" radiation injuries (see this collection, page 4).

It is interesting to compare the unique character of the dose curve of quinone excretion by the roots of irradiated plants with concepts of the mechanisms of the stimulatory and inhibitory effect of radiation developed by Kuzin, et al. [15]. If their point

of view that the same RT in small doses stimulates plant development and in large doses suppresses it is correct, the data in Table 1 have a simple explanation: at low stimulatory radiation doses, the quinones which have this stimulatory effect are retained by the tissues, and only at large doses, toxic for the plants, are they excreted from it via the root system.

As is known from the literature, a number of changes observed in the germinants of irradiated seeds increased exponentially depending on the dose (for example, the number of chromosomal aberrations). The exponential increase shown in the present work in the quinones which enter the embryo from the irradiated seed makes it possible to see the true cause of the exponential increase in the lesions in the embryo in the regularities of the entry of RT into embryos in a supplement to the hypothesis of a direct hit on the structures which are injured. Such a point of view agrees well with the results of a number of studies which showed the origin of radiation injuries, including chromosomal aberrations in the action of RT [16, 17].

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QUINONE FORMATION IN THE LIVER OF IRRADIATED ANIMALS

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It has been shown in a number of investigations [1-3] that the amount of *o*-quinones in plant tissues increases after their irradiation. The radiomimetic properties of these compounds have also been demonstrated and the hypothesis has been stated that *o*-quinones are the principal component of plant radiotoxins (RT) [4-11].

The question has arisen of whether *o*-quinones also appear in the animal organism when it is irradiated.

There is data in the literature on an increase in the amount of tyrosine, which can be a precursor of *o*-quinones, in the tissues and blood of irradiated animals [12-14]. Tyrosinase activation has been shown in the skin of rats soon after irradiation [15].

The appearance of quinones in the liver of irradiated rats was found in a reaction with aniline [16]. However, the low specificity of this reaction did not give an unequivocal answer. In the same work there are also observations of an increase in the absorption peak in ultraviolet at 255 mμ of extracts from the liver of irradiated animals.

It was the purpose of the present work to study the quantitative principles of the initial quinone accumulation in the liver of irradiated animals depending on the dose and time after irradiation.

Unbred white rats weighing from 80 to 100 g were the subject of the study. Irradiation was carried out in a cesium apparatus with γ-rays with a dose rate of 700 r/min.

The liver was removed at different times after irradiation. The livers (10 g) of three rats were homogenized at 0° and extracted with a five-fold volume of 96% alcohol for 2 hours at room temperature. Then the homogenate was centrifuged at 8000 rpm for 30 minutes, the extract was decanted and the alcohol driven off in vacuo. The residue in a volume of 2-3 ml was neutralized with 0.1 N NaOH and brought to 5 ml with pH 7.4 phosphate buffer.

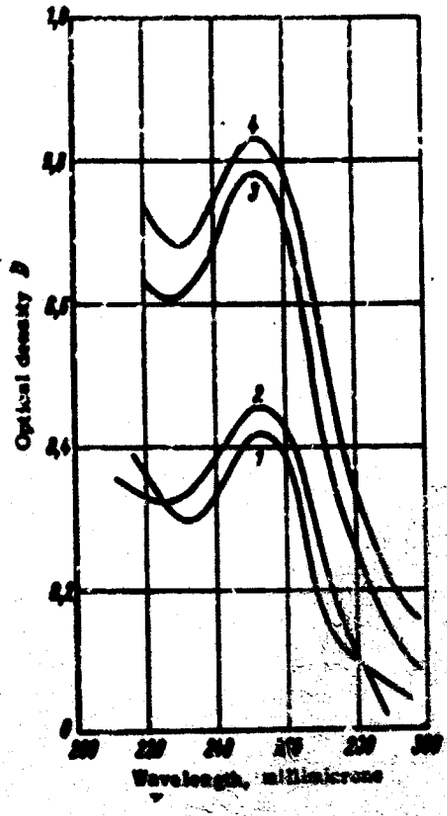

Fig. 1. Ultraviolet absorption spectra of alcoholic extracts of the liver of rats irradiated with a dose of 2 curies, for different times after irradiation. 1) Unirradiated; 2) after 15 minutes; 3) after 4 hours; 4) after 24 hours.

After a short stay in the refrigerator, the extract was again centrifuged to remove the finely dispersed sediment which precipitates out. The extracts obtained were studied at a 40-fold dilution on a self-recording spectrophotometer, manufactured on the basis of the SF-4 spectrophotometer. As a result of all the operations, the substances extracted from 10 g of liver were contained in 200 ml of solution.

The absorption spectra of the extracts under investigation which were extracted 15 minutes, 4 and 24 hours after irradiation with a dose of 2 curies are presented in Fig. 1.

As seen from this figure, extracts of the livers of unirradiated rats have an absorption spectrum typical of *o*-quinones with a maximum of 255-256 mμ [17-19]. The spectra obtained clearly indicate (from the increase in absorption in ultraviolet at 255 mμ) an increase in the *o*-quinone content of the liver tissues 15 minutes after irradiation (in comparison with the control), an increase in 4 hours and some increase 24 hours after irradiation.

The increase in the *o*-quinone content of the livers of irradiated rats which was found stimulated the authors to investigate the dose dependence and dynamics of their accumulation in time.

TABLE 1

o-Quinone Accumulation in the Liver of Irradiated Rats, Obtained Spectrophotometrically at the Maximum (255 mμ) Depending on Time After Irradiation and Dose (D – Optical Density; % – Percent of Control)

Time elapsed after irradiation	Orthoquinone accumulation at various radiation doses							
	0.5 cu		1 cu		2 cu		4 cu	
	D	%	D	%	D	%	D	%
10–15 min	—	—	0.503±0.017	111	—	—	0.718±0.018	159
4 hr	0.649±0.025	143	0.774±0.016	171	0.880±0.027	190	0.973±0.036	215
24 hr	—	—	0.765±0.023	169	—	—	1.017±0.036	225
48 hr	—	—	1.121±0.037	248	—	—	1.266±0.043	280

Note. 78 rats in control; average of 30 experiments 0.452 ± 0.006*D*.

TABLE 2

o-Quinone Accumulation in Liver of Irradiated Rats, Obtained Polarographically, Depending on Time After Irradiation and Dose (Relative Units – Height of Polarographic Wave in Millimeters; % – Percent of Control)

Time elapsed after irradiation	Orthoquinone accumulation at various radiation doses									
	0.2 cu		0.5 cu		1 cu		2 cu		4 cu	
	rel. units	%	rel. units	%	rel. units	%	rel. units	%	rel. units	%
10–15 min	—	—	—	—	43.0±2.6	122	—	—	58.3±4.9	180
4 hr	43.7±2.6	134	49.0±2.4	150	59.0±1.5	181	66.0±1.6	202	73.3±4.1	224
24 hr	—	—	—	—	60.3±4.8	185	—	—	77.0±6.1	238
48 hr	—	—	—	—	77.0±6.5	237	—	—	98.0±6.1	301

Note. 81 rats in control; average of 33 experiments 32.4 ± 0.8 rel. units

The *o*-quinone content of the liver of the control rats is relatively constant (Table 1). In irradiating rats (each result is the average value of 3-4 independent experiments) with a dose of 1 curie, the *o*-quinone content 15 minutes after irradiation reliably increases in comparison with the control. Four hours after irradiation this increase reaches 171%, and after 48 hours a second rise is noted – to 248% of the control.

After irradiation with a dose of 4 curies, the same pattern is observed, but the level of *o*-quinones accumulation is considerably higher and 15 minutes after irradiation reaches 159% of the control, while after 48 hours it is 280%. Four hours after irradiation the level of *o*-quinone accumulation reaches the first plateau, at which it remained until 24 hours after irradiation. This time was used for finding the dose dependence. The data obtained are presented in the same table.

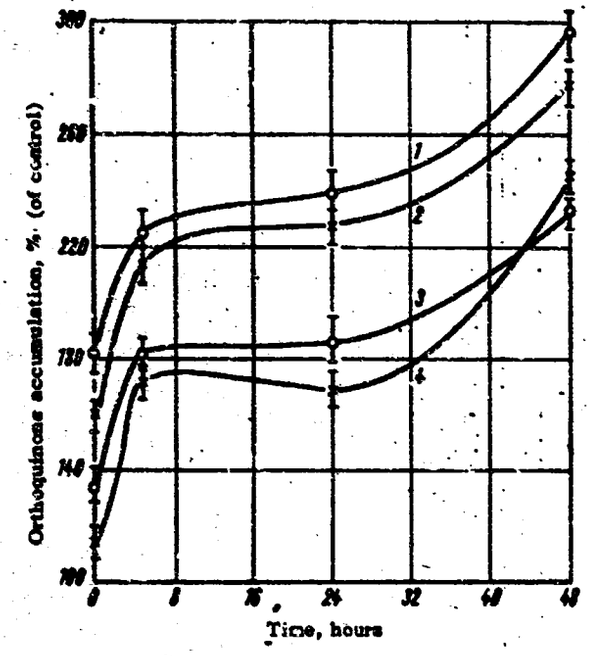

Fig. 2. *o*-quinone accumulation in liver of irradiated rats depending on the time after irradiation. 1,3) Polarographic method; 2,4) spectrophotometric method; 1,2) irradiation with a dose of 4 curies; 3,4) irradiation with a dose of 1 curie.

Analogous studies were conducted in parallel by the method of electron polarography. The *o*-quinone content was simultaneously determined in the same extracts by the polarographic and spectrophotometric methods.

The polarographic method is set forth in detail in this collection (see page 28). The amount of the substances under investigation was determined from the height of the polarographic wave at a half-wave potential of $E_{\frac{1}{2}}$ = 0.35 v, which corresponds to the quinones. The original extract (4 ml) was placed in the polarographic cell, to which 4 ml of base electrolyte was added. Phosphate buffer at

pH 6.8 served as the polarographic background for these compounds. The determination was carried out in a nitrogen atmosphere.

The *o*-quinone content in liver extracts obtained by the polarographic method is presented in Table 2. As in the case of the spectrophotometric method, the time (at doses of 1 and 4 curies) and dose (4 hours after irradiation) dependences of the *o*-quinone accumulation were determined.

The data obtained by the methods of electron polarography and ultraviolet absorption are compared in Fig. 2. As already noted above, at a dose of 1 curie, the *o*-quinone content increases sharply, and at 4 hours after irradiation reaches a plateau, remains at approximately the same level until 24 hours, after which a second sharp rise follows. At a dose of 4 curies, this pattern has a similar character, but at a somewhat higher level.

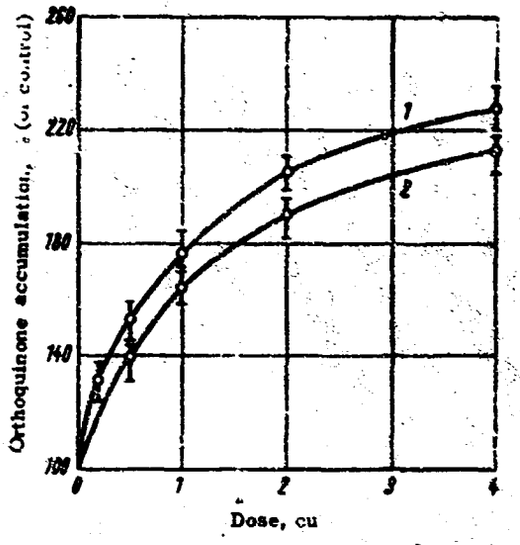

Fig. 3. *o*-quinone accumulation in liver of irradiated rats (4 hours after irradiation) depending on the radiation dose. 1) Polarographic method; 2) spectrophotometric method.

The curves of the dose dependence of *o*-quinone accumulation in the liver of irradiated rats in per cent of the control are presented in Fig. 3. As seen from this figure, the curves have an exponential character which is confirmed by the straight-line relation on a semilogarithmic scale (Fig. 4).

As seen from Fig. 4, for both methods the points lie on one straight line and, consequently, there is good agreement of data obtained by the two different methods.

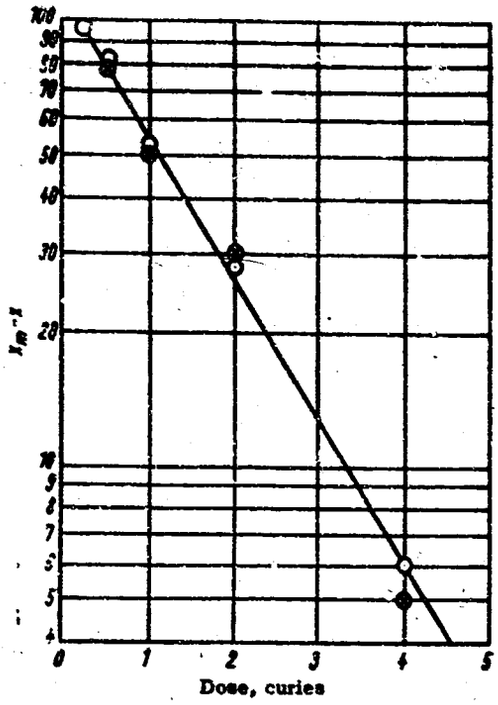

Fig. 4. The same as in Fig. 3, but on a semilogarithmic scale. X is the amount of *o*-quinones formed in per cent of the control; X_m is the maximum amount of quinones forming; the open circle is the polarographic method; circle with cross is spectrophotometric method.

Thus, the data obtained by two independent methods – polarographic and spectrophotometric – make it possible to establish the following:

1. In the liver of irradiated rats, as in the case of plant specimens, there is an increase in the *o*-quinone content in comparison with the control animals,

2. The dose dependence of *o*-quinone accumulation in the liver of irradiated rats is of an exponential character. This factor makes it possible to believe that *o*-quinone accumulation in the liver of irradiated rats reflects primary reactions of the interaction of the ionizing radiation with specific cell components.

3. The continuing increase in the quinones with time after irradiation indicates a linking of the primary radiation-chemical processes arising in the cells with subsequent enzymatic ozidative processes leading to quinone formation.

4. The regular and considerable increase in the quinones in the first 4 hours after irradiation makes probable the hypothesis concerning their role as RT in the further development of the radiation lesion of the animal organism.

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RADIATION ACTIVATION OF ENZYMATIC OXIDATION AND THE POSSIBLE ROLE OF THIS PROCESS IN THE FORMATION OF RADIOTOXINS OF QUINOID NATURE

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As seen from previous work [1-4], the *o*-phenol and *o*-quinone concentration increases in tissues of irradiated animals and plants and phenolase activation occurs. It can be assumed that the *o*-quinones or their semiquinone form, which have strong radiomimetic properties, play an essential role in the development of the radiation effect (see this collection, page 18). For an understanding of the mechanisms of formation of the *o*-quinones it is interesting to study the *in vitro* effect of radiation on the enzymatic oxidation of tyrosine by tyrosinase which leads, as is known [5,6], to the formation of the compounds which interest us.

Numerous investigations have been devoted to questions of the effect of radiation on enzymes [7]. Some have concerned the effects of substrate (DNA, proteins and starch) irradiation on the rate of subsequent enzymatic splitting [8]. And only in the work of Schachinger and Chug [9] were dehydrogenases irradiated with X-rays with a dose rate of 15 curies/minute at the moment of the reaction. Here interesting shifts in the direction of inhibition of oxidation reactions and intensification of reduction reactions were found.

It was assumed that it is possible to explain, by means of a radiation change in substrate, enzyme or intermediate products of the reaction, changes in the course of the enzymatic oxidation of tyrosine at the time of irradiation, which can also occur from irradiation of living, metabolizing systems.

Commercial crystalline tyrosinase in a concentration of 2 micrograms/ml and a 10^{-2} *M* solution of tyrosine were used in the experiments. The reaction was carried out in 0.15 M phosphate buffer at pH 7.4. The rate of the initial stage of the multistage reaction of tyrosine oxidation was followed by determining *o*-phenols and *o*-quinones by Utevskiy and Butom's method [10].

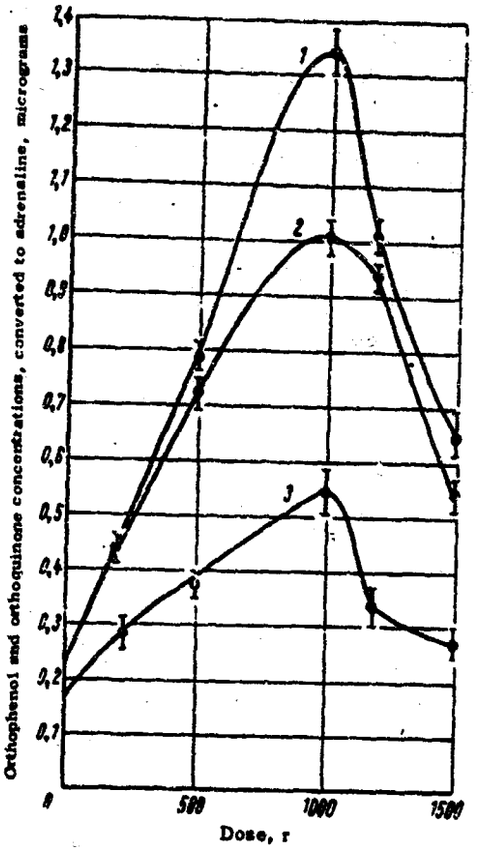

Fig. 1. Change in the concentration of total *o*-quinones and *o*-phenols during a 10-min reaction in a tyrosine-tyrosinase mixture and tyrosine solution from irradiation. 1) Irradiation of tyrosine-tyrosinase mixture; 2) irradiation of tyrosine solution with subsequent incubation for 10 minutes with unirradiated tyrosinase; 3) irradiation of tyrosine solution.

In the first series of experiments the rate of *o*-phenol and *o*-quinone formation was determined at 37.00 ± 0.01° in the first 10 minutes of the enzymatic reaction. The reaction took place during irradiation in RUP-3 equipment (180 kv, 15 ma, without a filter) with dose rates of irradiation from 20 to 150 r/min which in 10 minutes produced a dose from 200 to 1500 r respectively. The results are presented in Fig. 1 (Curve 1).

As seen from the data obtained, at a radiation dose of 200 r acceleration of the enzymatic formation of *o*-quinones and *o*-phenols from tyrosine takes place. This acceleration increases up to a dose of 1000 r. At this dose (dose rate of irradiation 100 r/min) the reaction was accelerated more than six times. A further increase in the dose rate of irradiation and, consequently, of the dose leads to some decrease in the effect. It was of interest to explain the reason for such strong acceleration of the reaction of tyrosine oxidation by tyrosinase during irradiation. Irradiation of the enzyme alone under the same conditions with subsequent addition of tyrosine did not change the rate of the enzymatic reaction.

The assumption developed that the radiation-chemical oxidation of tyrosine leads to the appearance in the system of *o*-phenols and *o*- quinones which, as is known, accelerate the enzymatic process of tyrosine oxidation by tyrosinase.

The following series of experiments was set up for the purpose of explaining the role of the *o*-phenols and *o*-quinones forming during the radiation-chemical oxidation of tyrosine in the increase in the rate of the enzymatic process. A solution of tyrosine was irradiated for 10 minutes at 37° with different dose rates. Curve 3 in Fig. 1 corresponds to the amount of *o*-phenols and *o*-quinones which form in this case through radiation-chemical oxidation. In the next series of experiments radiation-chemically formed tyrosine was added to an unirradiated enzyme and incubated for 10 minutes at 37°. If the acceleration in the first series of experiments was caused by the products of the radiation-chemical oxidation of tyrosine which gradually appear in 10 minutes, from the addition of maximally oxidized (in 10 minutes) tyrosine one could expect an even greater acceleration of the reaction. However, as seen from Curve 2 of Fig. 1, although the addition of irradiated tyrosine led to acceleration of the enzymatic reaction, the effect was less than from irradiating the system at the moment when the enzymatic reaction takes place (compare Curves 1 and 2). This indicates that along with radiation-chemical oxidation of tyrosine, other factors participate in the acceleration of the enzymatic reaction (probably, a reversible change in the enzyme's structure, inclusion of water in the chain of radiolytic products and others).

With the method of experiment selected several dose rates of irradiation were used. At higher dose rates the decrease in the effect was evidently the result of a decrease in the radiation chemical yield of *o*-phenols and *o*-quinones. In fact, specially set up experiments on the irradiation of a tyrosine solution with a dose of 1500 r at dose rates of 150 and 50 r/min gave 0.35 ± 0.10 and 0.80 ± ± 0.17 microgram of oxidation products.

All these data indicate that the appearance of radiation-chemical reaction products plays an essential role in the radiation acceleration of the enzymatic process.

However, the question of whether it is possible to reduce the entire mechanism of the acceleration merely to elimination of the induction period of the reaction was raised. In order to answer it, one further series of experiments was set up: the products of the radiation-chemical oxidation of tyrosine obtained at 37°, with one dose rate of irradiation (56 r/min) and different doses (see dose curve in Fig. 2) were added to unirradiated tyrosinase and the effect observed after 10 minutes of incubation at 37°.

The results of these experiments are presented in Fig. 3 (Curve 1) which also contains Curve 2 calculated by adding the amounts of added products of the radiation-chemical oxidation of tyrosine to the amount found in the experiment of *o*-phenols and *o*-quinones which form in 10 minutes at a constant rate of the enzymatic reaction (0.15 micrograms in recalculation to adrenalin in 10 minutes in the period between the 10th and 20th minutes of the reaction under the conditions indicated above).

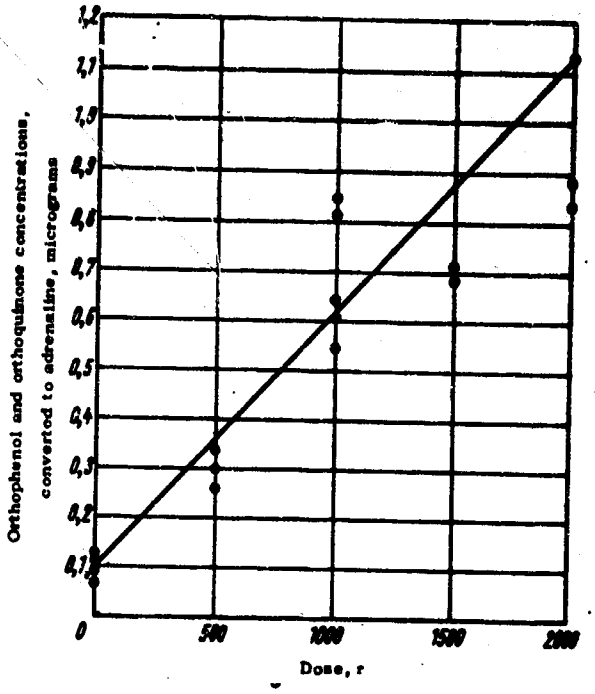

Fig. 2. Dependence of *o*-phenol and *o*-quinone concentration in tyrosine solution on radiation dose.

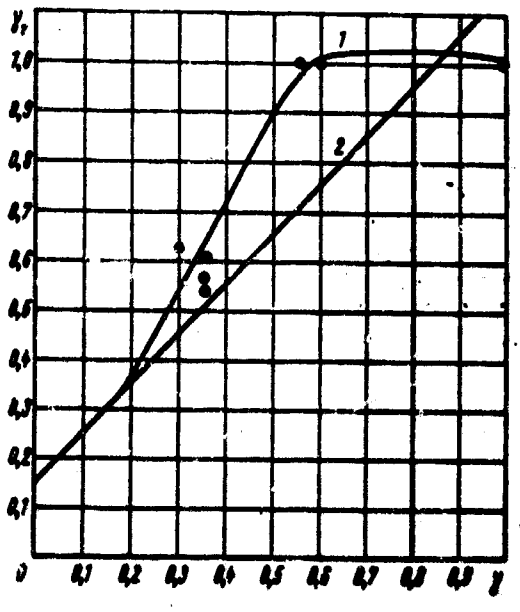

Fig. 3. Activation of tyrosinase depending on the concentration of the added *o*-quinones and *o*-phenols. 1) Concentration of *o*-phenols and *o*-quinones in tyrosine-tyrosinase mixture after incubation with different initial concentrations of *o*-phenols and *o*-quinones; 2) theoretical curve. (Along abscissa – initial concentration of *o*-phenols and *o*-quinones in recalculation to adrenalin in micrograms; along ordinate – the same, but after incubation with tyrosine).

As seen from Fig. 3, Curve 1 does not coincide with the theoretical curve (Curve 2). This indicates that the products of the radiation-chemical oxidation of tyrosine not only eliminate the induction period, but also have an effect directly on the rate of the enzymatic reaction of tyrosine oxidation.

It seems possible that the phenomenon of sharp acceleration of the enzymatic reaction of tyrosine oxidation at the moment of irradiation which was found in the experiments plays an essential role in the appearance of *o*-phenols and *o*-quinones after the irradiation of plant and animal tissues.

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INHIBITION OF DNA SYNTHESIS AFTER LOCAL IRRADIATION OF CYTOPLASM AND NUCLEUS

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Studies on the local irradiation of the cell are greatly furthering an explanation of the role of toxic products in the development of radiation sickness [1]. By using this method, an attempt was made in the present work to explain the role of radiotoxins [RT] (PT) forming after the irradiation of cytoplasm in the development of injury to the nucleus. In the investigation a study was made of the dose dependence of the inhibition of desoxyribonucleic acid [DNA] (ДНК) synthesis after the irradiation of the cytoplasm and nucleus of HeLa cells. Selection of the process of DNA synthesis for study was determined by its high radiosensitivity, connected with the ease of injuring the DNA molecules themselves [2] or the enzyme systems of the phosphorylation of the precursors, also located in the nucleus [3].

The HeLa cells intended for irradiation were cultivated on mica sheets 3-4 μ thick on medium No. 199 containing 20% bovine serum and 100 units of penicillin and streptomycin per ml. The duration of individual phases of the mitotic cycle in the given cell line was determined on the basis of a curve of labelled mitoses, after brief incubation of the cells with thymidine-H^3 (TH^3) [4] and averaged (in hours): T = 28, G_1 = 8.5, S = 10.5 and G_2 = 8. The average dimensions of the nucleus were 8.7 × 11.0 μ.

Local irradiation of the cells was carried out at room temperature with the help of a special attachment to the MBI-3 microscope [5]. A Po^{210} preparation of the RDS-10 type (10 millicuries, diameter of the active surface about 1.3 mm, product of the Amersham Radiochemical Center) served as the source of the α-particles. A narrow beam of α-particles was isolated with bronze diaphragms 0.1 mm thick with a central opening 1-2 μ in diameter "drilled" on an ionic etching unit [6]. A collimating system made it possible to obtain beams of α-particles with diameters down to 4 μ: beams of a diameter of 8-8.5 μ were used in this work. The dose absorbed was calculated on the basis of the number of α-particles in the microbeam, their energy distribution and the thickness of an "average" cell (8 μ in the region of the nucleus and 4.5 μ in the region of the cytoplasm). The number of α-particles in the beam passing through mica plates of different thicknesses was de-

termined with the help of a nuclear photoemulsion.

The rate of DNA synthesis was measured autoradiographically after brief (20 min) TH^3 labeling 1 hour after irradiation. The method of evaluating the effect of irradiation on DNA synthesis was similar to that described earlier [7] and consisted in comparing the synthesis rates of irradiated and control cells growing on one mica plate. At each dose of local irradiation the rate of synthesis was measured on 60-80 cells; here 3-10 irradiated cells were found in one preparation.

In local-irradiation experiments, we used cell cultures synchronized in *S*-period by the addition at 21 hours of (G_1 + *M* + G_2)--thymidine to a final concentration of $4 \cdot 10^{-3}$ M [8,9]. The excess thymidine was removed 3 hours before the beginning of irradiation. Preliminary experiments showed that the radiosensitivity of DNA synthesis in synchronous cells is barely more than or equal to the sensitivity of this process in normal cells. The principal advantage of the use of a synchronized culture in experiments on local irradiation was that at the moment of irradiation almost all the cells were in the same *S* phase; the number of synthesizing cells was increased to 70-80% against 25% in a normal culture and the more compact distribution of the cells according to rate of synthesis made it possible to deal with a smaller number of cells in order to obtain reliable results. The results obtained are presented graphically in the figure.

As seen from the data obtained, after local irradiation of the nucleus the dose curve of the inhibition of DNA synthesis is clearly a two-component curve (Curve 3), which indicates the presence of no fewer than two mechanisms of radiation inhibition of DNA synthesis. It is interesting that even with comparatively large doses of local irradiation of the nucleus, DNA synthesis is inhibited no more than 40%.

From local irradiation of the cytoplasm (about half of its volume) and with complete exclusion of α-particles hitting the cell nucleus (irradiated area of the cytoplasm was 2-3 μ from the nucleus) considerable inhibition of DNA synthesis is observed 1 hour after irradiation (Curve 1). At 6.1 krad DNA synthesis is 18% inhibited. At an equal dose of irradiation of the nucleus DNA synthesis is 34% inhibited. Three hours after irradiation of the cytoplasm the inhibition of DNA synthesis is noticeably increased, and 18% inhibition is observed at a dose of 2 krad (Curve 2). It is likely that the slowing down of DNA synthesis in the nucleus after local irradiation of the cytoplasm may be the result of the formation of RT in it. The effect of irradiated cytoplasm on the unirradiated nucleus which was noted earlier [1, 10-13] also can be explained in the same way. In connection with this it is interesting to note that proteins and nucleic acids of the nucleoproteins of rat thymus nuclei rapidly and firmly bind *o*-quinones and *o*-phenols which comprise an important part of the RT [14] *in vitro* (see this collection, page 53).

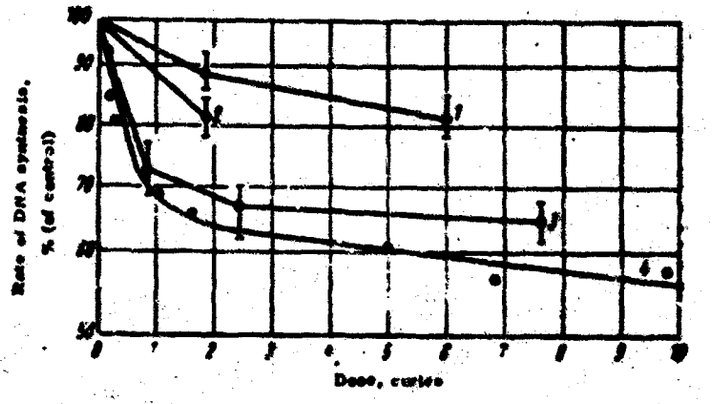

Fig. 50. Inhibition of DNA synthesis under different conditions of α-particle irradiation depending on the dose. 1) Local irradiation of cytoplasm 1 hour after irradiation; 2) the same, but 3 hours after irradiation; 3) local irradiation of the nucleus; 4) total irradiation of the cell.

It is very likely that after total irradiation of the cell (Curve 4) the formation of toxic products in the cytoplasm will be greater than that demonstrated in the experiments under consideration because in the latter there always remained an unirradiated zone 2-3 μ wide between the irradiated part of the cytoplasm and the nucleus which could absorb considerable amounts of toxic products as a result of the great reactive capacity of the latter.

The results of the experiments which have been conducted show that RT formation has great significance in inhibition of DNA synthesis. The mechanism of action of these compounds may consist both in direct damaging of the desoxyribonucleoproteins and in decreasing the activity of DNA-polymerase and enzymes participating in the production of DNA precursors.

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THE INTERACTION OF QUINONES WITH CELL NUCLEI

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The high radiosensitivity of the cell nucleus which usually shows up in inhibition of mitoses, disturbances in the mitotic cycle, chromosomal aberrations, the appearance of mutations and the frequently observed death of the cell during post-radiation division has led to concepts of the leading importance of the direct effect of radiation on unique structures of the nucleus – the chromosomes or their components – deoxyribonucleoproteins [DNP] (ДНП). On the other hand, the existence of radiation's distance effect and many facts presented in other articles of this collection speak of activation in cells during irradiation of oxidation reactions and the appearance of strongly oxidized compounds, among which a leading role in the manifestation of a number of radiation effects is ascribed to the *o*-quinones. The apparent contradictions between the theory of a hit on unique cell structures and the theory of the primary formation of radiotoxins [RT] (РТ) are easily eliminated by assuming a rapid reaction of RT with the chromosomes of the nucleus which causes subsequent visible changes in nuclear structures and functions.

The purpose of the present investigation is to show on model systems the existence of such reactions between *o*-quinones and substances of the cell nuclei. As is known [1], the enzymatic oxidation of natural phenols with the formation of active *o*-quinones is activated by the effect of irradiation. On this basis, products of the enzymatic oxidation of tyrosine – which is one of the possible precursors of active *o*-quinones – were used as a model for study. It is known from the literature that *o*-phenols and *o*-quinones which later condense into melanines begin to be accumulated from the effect of tyrosinase in a tyrosine solution. It was shown earlier that at the moment of the greatest accumulation of *o*-quinones the tyrosine-tyrosinase system displays radiomimetic properties, inhibiting the mitoses of plant cells [2] and the division of *E. coli* [3]. Preliminary experiments on incubation of a tyrosine solution (0.05% in 0.04% $NaHCO_3$) with an equal volume of a tyrosinase solution (0.1 unit per ml) at 30° showed a maximum accumulation of *o*-quinones, determined with α-naphthol [4] and by Osinskaya's method [5], 60 minutes after the beginning of the experiment. At this time the absorption spectrum of the solution in the ultraviolet region clearly changed. As is seen from the experimental data presented in Fig. 1, new absorption

spectra appear in the regions of 303-310 and 450 mμ. According to the literature data, tyrosine is oxidized to dioxyphenylalanine [DOPA] (ДОПА) and changes into DOPA-quinone and DOPA-chrome. These two latter compounds also produce an absorption maximum in the ultraviolet region at 305 mμ (pH 6.8-8.4) [6].

The reaction products of 30-60 minute enzymatic oxidation of tyrosine with native thymus nuclei were investigated in the present work. As is clear from the preceding and from data in the literature, *o*-quinones with their accompanying *o*-phenols predominate among the oxidation products of tyrosine at this time. Since these compounds easily change into each other and since the chemical reactivity of the *o*-quinones is especially great, in the future we will be talking about *o*-quinones with allowance for possible admixtures of other oxidation products.

Native nuclei of rat thymus cells were isolated by Downs' method (see [7]). Microscopic examination of the suspension of nuclei obtained after two washings did not show impurities. A suspension of cell nuclei in isotonic sucrose solution (10^8 nuclei per 1 ml) was added at a temperature of 30° to a tyrosine-tyrosinase system, which was first incubated at 30° for 0.5 hours in the presence of isotonic phosphate buffer (pH 7.4). After different times of incubation (5, 15, 30, 90 and 150 min) the nuclei were separated by centrifugation and the amount of absorption at 303 mμ was determined in the supernatant liquid. The decrease in *o*-quinones depending on the length of incubation is presented in Fig. 2.

As seen from the results obtained, native nuclei actively absorb *o*-quinones. In 5 minutes almost 50%, in 15 minutes about 50% and in 30 minutes about 70% of the total amount was bound by the nuclei. A similar effect was obtained from the addition of a suspension of nuclei whose structure was first destroyed by freezing and thawing. This brought on the thought that *o*-quinones are directly bound by the DNP of the nuclei. In order to study in more detail the interaction of *o*-quinones with the DNP of the nuclei, the method of radioactive indicators was used.

Tyrosine uniformly labeled with C^{14} with a specific activity of 12 millicuries/millimole was used for the experiment. Under the conditions described above the labeled tyrosine was oxidized in 30 minutes by tyrosinase at a temperature of 30° which led to the formation of C^{14} labeled *o*-quinones. Since at the time of the addition of the cell nuclei about 80% unchanged tyrosine remained in the solution, its inclusion in the proteins of the cell nuclei might have interfered with determination of the absorption of the oxidation products (*o*-quinones and *o*-phenols). In order to avoid this difficulty, puromycin in a concentration of 20 micrograms/ml or chloramphenicol in a concentration of 2 mg/ml was added to the nuclei suspension. According to the data of Mirsky and Ris [8] at such inhibitor concentrations the inclusion of amino acids in thymus nuclei decreases by 70 and 90%, respectively.

Nuclei in the presence of protein synthesis inhibitors were added to the initial labeled tyrosine solution (control) and to the same solution after 30-minute tyrosinase action, that is, to one which contains, besides tyrosine, its oxidation products, *o*-quinones and *o*-phenols.

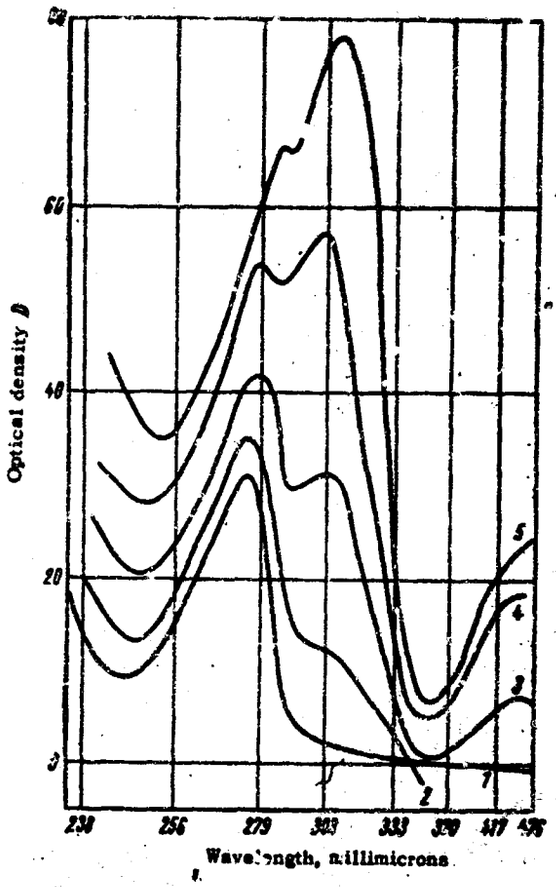

Fig. 1. Absorption spectra of a tyrosine-tyrosinase system. 1) Initial mixture; 2,3,4 and 5) after 10, 15, 30 and 60 minutes of reaction at a temperature of 30°, respectively.

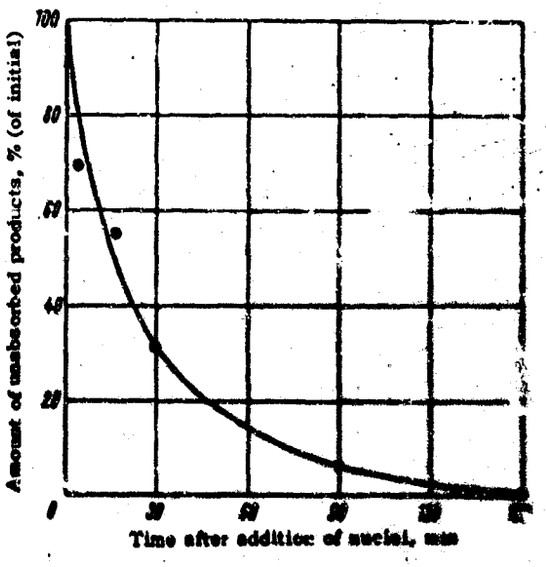

Fig. 2. Absorption of tyrosine oxidation products from a tyrosine-tyrosinase system by nuclei of thymus cells.

TABLE 1

Radioactivity of Cell Nuclei

Control, pulses in 5 min	Experiment	
	pulses in 5 min	% (of control)
900	6000	666
580	4360	750
575	6967	1200

TABLE 2

Radioactivity of Individual Fractions of Thymus Nuclei

Fraction	Radioactivity, pulses in 5 min			Percentage of total radioactivity represented by activity of fraction
	Expt. 1	Expt. 2	Expt. 3	
Fraction soluble in 0.14M NaCl (RNP proteins)	6 360	6 990	6 800	25,5
Fraction soluble in 1M NaCl (DNP)	28 000	12 000	18 518	69,3
Insoluble residue (acid proteins and membrane proteins)	2 000	970	—	5,2

TABLE 3

Radioactivity of Proteins and Nucleic Acids of Thymus Nucleus Nucleoproteins

Fraction	Activity, pulses in 5 min	Ratio of nucleic acid activity to protein activity
DNP $\left(\frac{\text{DNA}}{\text{protein}}\right)$	$\frac{1959}{7300}$	0,26
RNP $\left(\frac{\text{RNA}}{\text{protein}}\right)$	$\frac{600}{2800}$	0,21

After 30 minutes of incubation under the above-described conditions, the nuclei were removed by centrifugation at 4000 rpm and washed three times with pH 7.4 phosphate buffer. In order to determine the amount of radioactive compounds absorbed by the nuclei, the nuclei were precipitated on a cellulose membrane filter with a pore diameter of 1.2 μ. The nuclei on the filter were washed three more times with phosphate buffer, then with a 10% solution of trichloracetic acid, and then successively with 70 and 96% alcohol. The activity of the nuclei on the filters was determined with a "Tritiomatic-100" type of liquid scintillation counter, using a nonpolar scintillator.

The results of three experiments are presented in Table 1.

The first two experiments were without the addition of antibiotics, the antibiotic puromycin was used in the last experiment.

As seen from the data obtained, the oxidation products of tyrosine are actively absorbed by thymus cell nuclei which is in complete accord with data from the investigation of ultraviolet spectra. In connection with the fact that the nuclei in the experiment were in a medium containing tyrosinase which is absent from the control for 30 minutes, it was necessary to determine whether the observed effect is a result of an increase in the absorption of tyrosine by the nuclei from the action of tyrosinase on them.

The following experiment was set up for this purpose. The nuclei of rat thymuses were divided into two portions, one of which was incubated for 30 minutes with phosphate buffer, the other – with phosphate buffer in the presence of tyrosinase. After washing nuclei of both portions in the presence of chloramphenicol (2 mg/ml) were placed for 30 minutes in a medium with C^{14} tyrosine. Then the nuclei were washed three times with buffer, precipitated on cellulose filters where they were washed as described above. The count intensity in the control and experimental samples was 2.13 and 2.70 imp per microgram of protein in 5 minutes, respectively.

The results of this experiment lead to the conclusion that incubation of the nuclei with tyrosinase in itself does not increase the absorption of tyrosine. Thus, the sharp increase in the radioactivity of the nuclei which was obtained in the presence of oxidized tyrosine indicates the active absorption of the oxidation products.

It seemed of interest to investigate which components of the nucleus are responsible for the observed absorption. For this purpose, the cell nuclei after 30-minute incubation with the products of enzymatic tyrosine oxidation were removed by centrifugation, washed and lysed with distilled water.

The lysates were treated with 0.14 M NaCl (1:10). The residue was removed by centrifugation and washed 3-4 times for as complete as possible extraction of the ribonucleotides, enzymes and globular proteins. The part which was insoluble in 0.14 M NaCl was extracted three times with 1 M NaCl for separation of the DNP. According to Mirsky and Osawa's data [9], it is possible to isolate 80-90% of the DNP of the nuclei by this means. The part which

remained represented acid proteins, part of the ribonucleoprotein (RNP) and insoluble proteins of the nuclear membranes. Determination of the radioactivity of each fraction was carried out in the following way: Trichloroacetic acid was added to the 0.14 M NaCl solution to a final concentration of 10%. All of the precipitated residue was applied to several filters with pores of a diameter of 0.3-0.5 μ in such a way that there was 3-4 mg of the substance on one filter. Six volumes of water was added to the 1 M solution. The DNP which precipitated out in this case was transferred with a glass rod to 1-2 ml of a 1 M NaCl solution, redissolved and the whole solution applied to several filter paper circles which were dried under an infrared lamp. The insoluble residue was also transfered with a glass rod to a membrane filter. All the filters were washed with a 10% trichloroacetic acid solution and successively with 70 and 96% alcohol.

The total radioactivity of all the filters belonging to a given fraction is presented in Table 2.

As seen from the results obtained, the bulk of the tyrosine oxidation products is bound with the DNP of the nucleus (69.3%).

The labeled DNP which was isolated was separated by Sevag's method, in which their solution in 1 M NaCl was shaken for 1 hour with a mixture of chloroform and isoamyl alcohol (9:1). After removal of the protein from the DNA the total activity of each fraction was determined; the fraction soluble in 0.14 M NaCl [RNP] (РНП) was studied in the same way. The separation was carried out in the presence of 0.1% sodium dodecylsulfate. The results obtained are presented in Table 3.

An analysis of the DNP and the desoxyribonucleic acid under investigation showed protein contents of 15% and 6%, respectively (protein determination according to Lowry, and DNA according to Spirin [10]) which could not explain the radioactivity of the DNA only by protein contamination. The decrease of activity in DNA in comparison with DNP is 5-fold, while the decrease in the amount of protein is 25-fold.

Thus the investigation which was conducted showed that native nuclei isolated from rat thymus cells actively absorb products of enzymatic tyrosine oxidation (*o*-quinones). The bulk of the *o*-quinones are bound by the nuclear nucleoproteins (69.3%). In the nucleoproteins the *o*-quinones are mainly associated with histones (80%), however, the remaining part (20%) is firmly bound by DNA.

The combining of the *o*-quinones with the nucleoproteins of the nucleus – the principal nuclear macromolecules which store genetic and metabolic information – which has been shown explains well the radiomimetic effect of the *o*-quinones.

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RADIOMIMETIC EFFECT OF ORTHO-QUINONES ON THE SORPTION PROPERTIES OF CELL NUCLEI

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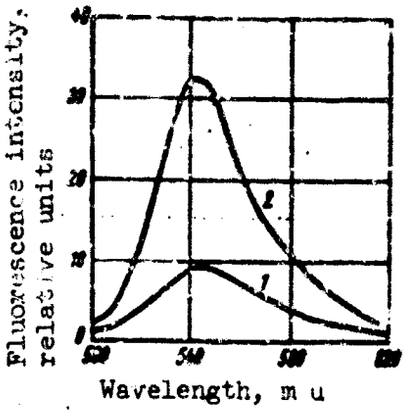
A change in the sorption of acridine orange [AO] (AO) by the nuclei of rat thymus cells 15 minutes after irradiation of the animals with a dose of 1 curie was established earlier [1].

This phenomenon consists in the fact that thymus nuclei isolated 10-15 minutes after irradiation of rats, from a measurement of the fluorescence excited by ultraviolet radiation, showed an increase in fluorescence in the presence of an 0.002% AO solution of 30-40% in comparison with the nuclei of unirradiated rats. The fact that similar irradiation of isolated nuclei (in a sucrose-buffer solution) did not change their fluorescence in the presence of AO brings on the thought that the effect is caused by the interaction of the nuclei with substances formed in the irradiated cytoplasm. On the basis of Kuzin's hypothesis (see this collection, page 4) that these substances are *o*- quinones, a series of experiments on the effect of the latter on cell nuclei was conducted.

Isolated thymus nuclei were selected as the subject of the investigations. The cell nuclei were isolated from the thymuses of unbred white male rats weighing from 80 to 100 g by a modified Downs method (see [2]). The concentration of the nuclei was determined in a Goryayev chamber. The suspension of nuclei was diluted with isotonic sucrose-phosphate buffer (0.25 M sucrose solution prepared in 1/15 M phosphate buffer at pH 7.4) so that 1 ml contained $1 \cdot 10^8$ nuclei. The AO concentration was always 0.02 mg/ml of the suspension. In 1-2 minutes after the addition of AO the suspension was centrifuged, the supernatant fluid was poured off, the nuclei again suspended in the same volume of sucrose-phosphate solution and the fluorescent spectrum recorded. Irradiation of rats and potato tubers was carried out with γ-rays of Cs^{137} (dose rate of 700 r/min). Alcoholic extracts of the irradiated potato tubers were obtained by the same method as from the liver of irradiated rats which was described in detail in this collection (see page 37).

Measurement of the fluorescent spectra was carried out in an ISP-51 spectrograph with an FEP-1 photoelectric unit with recording on an automatic self-recording ПС1-02 potentiometer. A PRK-2

mercury-quartz lamp served as the excitation source. Excitation was carried out with the 366 mμ line which was cut out with special light filters.



Fluorescent spectrum of nuclei in the presence of AO. 1) AO (0.02 mg/ml); 2) nuclei (10^8 nuclei/ml) + AO (0.02 mg/ml).

The fluorescent spectra of AO and of a suspension of nuclei with AO are presented in the figure. The fluorescent maximum of AO lies in the region of 540 mμ which is in good agreement with the literature data. The addition of AO to a suspension of normal nuclei evokes bright green fluorescence of the latter, and in this case a considerable increase is observed in the intensity of the fluorescence at the maximum without a noticeable change in its position in the spectrum in comparison with AO.

Later the change in intensity of the fluorescence of the nuclei in the presence of AO served as a test for detecting the effect of various factors (*in vivo* and *in vitro*) on cell nuclei.

Model experiments [3], conducted with products of enzymatic tyrosine oxidation, clearly showed that whereas tyrosine itself and the products of its final oxidation, melanines, did not possess antimitotic properties, intermediate products of the enzymatic oxidation, as is well-known, containing quinoid and semiquinoid forms, possessed the ability to sharply inhibit cell division.

In connection with this, it was interesting to study the effect of the intermediate products of enzymatic tyrosine oxidation on the fluorescence of nuclei *in vitro*. For this purpose a tyrosine solution (concentration 0.5 mg/ml) was incubated in the presence of tyrosinase (activity 1 unit/ml) and sucrose-phosphate buffer for 1 hour at a temperature of 30°. In this time the solution acquired a bright rose color and the quinone concentration reached a maximum.

$5 \cdot 10^6$ nuclei were added to 10 ml of this solution and incubated 15 minutes more at the same temperature. Then the nuclei were removed by centrifugation, again suspended in sucrose-phosphate solution and after the addition of AO their fluorescence was measured by the usual method. The data obtained are presented in Table 1.

TABLE 1

The Effect of Products of the Enzymatic Oxidation of Tyrosine by Tyrosinase (15 min After Action) on Fluorescence of Nuclei in the Presence of AO (on Maximum at 540 mμ)

Exp. No.	Control rel. units	Experimental	
		Rel. units	% (of control)
1	35	56	160,0
2	31	53	170,5
3	33	51	154,5
4	34	48	141,2
5	32	57	178,1
6	32	60	187,5
7	39	59	151,3
8	39	56	143,6
9	32	58	181,3
Average	—	—	162,5±5,2

Note. Reliability of the difference $p < 0.001$.

In control experiments with nuclei incubated with oxidized tyrosine, but without the addition of AO, fluorescence at 540 mμ was not found.

As seen from the table, fluorescence of the nuclei in the presence of AO from the effect of the intermediate products of tyrosine oxidation increases 62.5%.

It is seen from the experiments presented that the change in the fluorescence of the nuclei in the presence of AO, caused by total irradiation of the animal, is completely imitated by the products of enzymatic tyrosine oxidation.

In accord with earlier observations concerning the formation of toxic metabolites in irradiated plant tissue [4-7], the effect of substances extracted by 96% alcohol (1:5) from irradiated potato tubers on the fluorescence of nuclei in the presence of AO was investigated. 50 g of irradiated (in a dose of 50 curies) and unirradiated potato tubers of the Lorkh variety was homogenized at a temperature of 0° and extracted with a five-fold volume of 96% alcohol for 2 hours at room temperature. Then after centrifugation at 8000 rpm for 30 minutes and removal of the alcohol in a vacuum the extract was diluted in sucrose-buffer solution. The substances extracted from 50 g of tissue were contained in the 10 ml of the original extracts which were obtained. The effect of these substances on thymus nuclei was studied.

The original extracts were diluted 40 times and incubated with isolated thymus nuclei for 30 minutes at 30° (10 ml of diluted extract with $5 \cdot 10^6$ nuclei).

As seen from the data obtained (Table 2), at large dilutions the extracts from the control tubers do not have an effect on the fluorescence of the nuclei in the presence of AO, whereas extracts of irradiated tubers imitate the picture of radiation lesion of the nuclei.

TABLE 2

Increase in Fluorescence of Nuclei From the Effect of Extracts of Potato Tubers Irradiated With a Dose of 50 Curies in the Presence of AO

Extract	Number of Experiments	Increase in fluorescence % (of control)	Reliability of difference, r
From irradiated potato tubers	22	141,3±3,1	<0,001
From unirradiated potato tubers	22	100,0±2,5	<0,001
From an irradiated potato treated with ascorbic acid (1 mg ascorbic acid per ml of original extract)	5	118,7±5,2	<0,01

The preliminary addition of ascorbic acid to the extracts (the reduction of *o*-quinones to *o*-phenols) considerably decreases the fluorescence, which confirms the dependence of the observed effect on *o*-quinones or semiquinones.

An attempt was made to increase the content of *o*-quinones in the organism by injecting massive doses of adrenaline into the rats. As is known, when adrenaline is injected into the organism it is rapidly broken down with the formation of products of quinoid nature, in particular, the *o*-quinone of adrenochrome [8,9]. 100 micrograms of adrenaline per 100 g of animal weight was injected into rats intra-abdominally. This amount exceeds by many hundreds of times the physiological discharge of adrenaline from the adrenal depots during stress reactions [10, 11]. The nuclei were removed 10-15 minutes after the effect. As seen from Table 3, such an effect leads to an analogous reaction of the thymus nuclei, which serves as further proof of the correctness of the indicated assumptions.

An increase in *o*-quinones in the liver of irradiated rats in comparison with the control was established earlier (see this collection, page 37). It is interesting to verify whether the investigated extracts of the liver of irradiated rats, like extracts of irradiated potato tubers, possess radiomimetic properties. For this, a rat was irradiated with a dose of 2 curies (the *o*-quinone content of rat liver at this dose 4 hours after irradiation increases almost two-fold in comparison with the control; see this collection, page 37).

Extracts of the liver were obtained 15 minutes, 4 and 24 hours after irradiation. Then they were incubated at a 20-fold dilution

under the same conditions as the extracts of potato tubers with isolated nuclei.

The data on fluorescence are presented in Table 4.

TABLE 3

Increase in Fluorescence of Nuclei After *In Vivo* Injection of Adrenaline in a Dose of 100 Micrograms per 100 g of Animal Weight

Exp. No.	Control, rel. units	Experimental	
		Rel. units	% (of control)
1	30	37	123,3
2	30	38	126,7
3	29	37	127,6
4	31	38	122,6
5	36	46	127,8
Average	—	—	125,6±1,2

Note. Reliability of difference $p < 0.01$.

TABLE 4

Increase in Fluorescence of Nuclei From Effect of Extracts of the Liver of Rats Irradiated With a Dose of 2 Curies in the Presence of AO

Extract	Number of experiments	Increase in fluorescence (of control)	Reliability of difference, r
Of liver of unirradiated rats (control)	9	100,0±2,2	<0,001
From liver of rats after irradiation			
After 15 min	9	119,0±[illegible]	<0,03
After 4 hours	9	148,6±5,4	<0,001
After 24 hours	3	170,0±4,3	<0,01

These results clearly indicate the radiomimetic effect of extracts from the liver of irradiated rats.

The studies which were carried out show that the change in the fluorescence of thymus cell nuclei in the presence of AO which develops rapidly after total irradiation of the rats can be fully explained by the formation of radiotoxins of quinoid nature in the irradiated cells.

The data obtained are in full accord with the results of experiments (see this collection, page 53) which showed rapid sorption of *o*-quinones by native nuclei in *in vitro* experiments.

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THE FORMATION OF COMPLEXES WITH THE TRANSFER OF A CHARGE BETWEEN NUCLEOTIDE BASES AND TETRACHLORO-*p*-BENZOQUINONE

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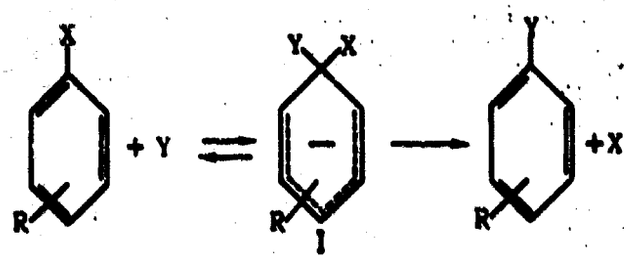
The detected and studied (see this collection, Pages 4, 18 and 28) participation of oxidation products of phenolic substances – the quinones – in the radiobiological effect has a clearly universal character since it has been demonstrated by irradiating plants, animals and micro-organisms. In the light of this work, an investigation of the mechanism of interaction of the quinones with biologically important compounds which regulate and direct biological processes seems of interest. The range of substances which can enter into an interaction with quinones, as well as the nature and conditions of this interaction can be outlined in an examination of the structure and reaction capacity of the quinones.

In the quinone molecule the oxygen and carbon atoms are joined by a π bond, which is partially polarized, that is, the electrons forming it are shifted in the direction of the more negatively-charged oxygen atoms, and the electron densities of the conjugated system of π-electrons of the aromatic ring are shifted in the direction of the oxygen atoms. Nucleophilic substitution reactions are, as is well-known, a common property of compounds containing electron acceptor groups. In an examination of nucleophilic substitution at unsaturated centers [1], it has been shown that it differs radically from nucleophilic substitutions at saturated centers in the existence of an intermediate complex and can be described by the following reactions (cited from Bannet [1] in simplified form):



where X is the substituted group, Y is the substituent group and I is the intermediate complex.

"Any degree of binding of a carbon atom (substituting group – our note) must be accompanied by a rupture either of the bond with the replaceable group or of the π bond of the unsaturated system" [1]. In the latter case the negative charge of the nucleophile is placed somewhere within the limits of the unsaturated(acceptor) π-system, but "not at the reaction center" [1]. Some expenditure of energy is necessary in order to displace the charge, however, it is decreased if substituents which draw off the electrons are present, which, in the case of quinones, are carbonyl groups. In accordance with the concept developed by Bannet [1], the structure of the intermediate complex can be depicted in two ways: 1) the bonding of the nucleophile with the unsaturated system of π-electrons is carried out locally somewhere close to the reaction center (Fig. 1a); 2) the bonding of the nucleophile is carried out directly with the unsaturated system of π-electrons (Fig. 1b).

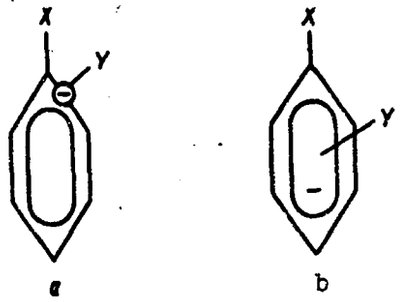


Fig. 1. Structure of intermediate complexes during nucleophilic substitution.

Thus, the intermediate complex I is clearly a molecular π-complex of the original substance and the nucleophile which is formed through overlapping of the nucleophile's donor orbit and the replaceable compound's acceptor orbit. Consequently, in the nucleophilic substitution of quinones the intermediate stage can be molecular complexes with the transfer of a charge, which, possibly, will be stable, particularly when the quinones interact with aromatic molecules possessing a donor system of π-electrons, namely, with aromatic amines, phenols and others [2,3]. Sjent-Georgi [4] indicates the biological importance of reactions of this type.

During the formation of the complex a partial transfer of one of the donor's electrons to the acceptor's orbit takes place. In the case in which the donor has a sufficiently low ionization potential and that of the acceptor is very high, complete transfer of the electron with the formation of two ion-radicals can occur. The degree of electron transfer depends on the polarity of the medium: more polar conditions promote strong electron transfer with subsequent division into ion-radicals [4]. Proteins, as is known, contain nonpolar side chains, in individual cases up to 49% of the total number of side groups (35% on the average) [5]. As a result of this, the protein environment becomes sufficiently hydrophobic which, in particular, was shown by spectral methods [6,7]. Under cellular conditions the interaction of quinones with various compounds takes place in a protein environment, therefore reactions inherent to nonpolar conditions can predominate in the interaction of quinones with cellular components, particularly with nucleic acids which are present in cells mainly in the form of nucleoproteins.

The possibility of the reaction of quinones with nucleotide bases is most interesting. The interaction of tetrachloro-*p*-benzoquinone with guanine, guanosine, cytidine and xanthine at various polarities of the medium was studied as a model system in the present work.

In the first series of experiments the absorption spectra of 10^{-3}-10^{-4} M solutions of tetrachloro-*p*-benzoquinone (chloranil) and nucleotides in 50% ethyl alcohol with 1/15 M phosphate buffer (pH 7.0) were recorded in the course of heating the solutions at 50°. The measurements were made on an SF-10 spectrophotometer.

In the second series of experiments chloranil and guanine were incubated at 38° for 10-12 hours in different solvents: sample No. 1 in a mixture of methyl alcohol and phosphate buffer (pH 7.0, 1/15 M) (1:1); No. 2 – in a mixture of ethyl alcohol and phosphate buffer (1:1); No. 3 – in *n*-amyl alcohol; No. 4 in a mixture of *n*-butyl alcohol and phosphate buffer (1:3); No. 5 in chloroform; No. 6 in carbon tetrachloride; No. 8 in *n*-propyl alcohol and sample No. 9 in a mixture of acetone and phosphate buffer (1:1). All the incubations were carried out in an excess of the solid phases of the reagents. The product which interested us was carefully washed from the excess of unreacted substances on a No. 4 glass filter with the appropriate solvents. In a number of cases after washing the visible and ultraviolet spectra of the solvent was recorded in order to make sure the unreacted reagents were removed.

In order to obtain sample No. 7 xanthine and chloranil were incubated in a mixture of ethyl alcohol and phosphate buffer (1:1) for 12 hours, after which the solution was separated. The residue which precipitated upon cooling to room temperature was separated from the solution and examined.

Infrared [IR] (ИК) spectra were recorded on a UR-10 apparatus in liquid petrolatum; electron paramagnetic resonance [EPR] (ЭПР) spectra were recorded on an EPR-spectrometer at the temperature of liquid nitrogen without evacuation.

In the first series of experiments the interaction of chloranil with guanosine and cytidine was studied. After heating the chloranil with the nucleotide at a temperature of 50° for 20 minutes or more the appearance of new absorption bands with maxima at 540 and 550 mμ for guanosine and cytidine, respectively was found. An absorption band in this region also appeared from heating a solution of chloranil alone for a longer time(more than 1 hour) and, in our opinion, corresonded to a hydrolysis product of chloranil - chloranilic acid (2,5-dihydroxy-3,6-dichloro-*p*-benzoquinone or 3,6-dihydroxy-2,5-dichloro-*p*-benzoquinone) which absorbs in this region (maximum 536 mμ) [8]. It is possible that the observed spectra belong not to chloranilic acid itself, but to its derivatives replaced at some position by the nucleotide. It has not been excluded that in the latter case the formation of a molecular complex between the nucleotides and chloranil as an intermediate product might occur. This complex possibly is dissociated into ion-radicals as a result of high polarity of the medium with a relatively larger yield than in nonpolar solvents. This assumption is confirmed by the fact that under such conditions in the reaction of chloranil with nucleotide bases

EPR-signals were found [9]. In this work the observed appearance of absorption bands in approximately the same region as found by the authors of the present article is explained by the formation of a molecular complex between chloranil and nucleotide bases. However, on the basis of the aforesaid, it is possible to interpret this phenomenon as a result of a far reaching chemical reaction with the formation of chloranilic acid and its complexes.

In the second series of experiments the interaction of chloranil with guanine was studied at 38° for 10-12 hours in different solvents in an excess of the undissolved phase of both components. The product obtained was carefully washed off from the unreacted original substances and studied by methods of EPR and IR spectroscopy. The appearance of an EPR-signal clearly indicates the formation in this system of complexes with the transfer of a charge.

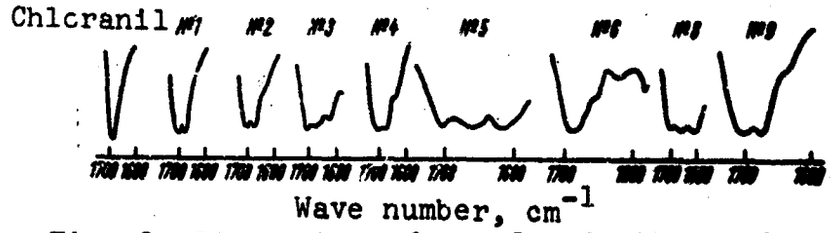


Fig. 2. IR-spectra of samples in the region of absorption of carbonyl groups.

The IR-spectra of chloranil and the product obtained in the absorption region of carbonyl groups is shown in Fig. 2. As is known, the position of the absorption band of carbonyl groups depends on the polarity of the C = O bond; with a decrease in polarity the frequency of the absorption decreases [10]. This follows, in particular, from work [11] in which it was shown that the frequency of the absorption band of the C = O groups of quinones decreases with an increase in the effective electronegativity of the carbonyl C-atoms. As seen in Fig. 2, on interaction with guanine, splitting of the absorption bands of chloranil's carbonyl groups occurs; a majority of the newly formed lines is shifted to a lower frequency region in comparison with chloranil. This is characteristic of samples obtained from nonpolar or low polar solvents (Samples No. 3, 5, 6 and 8). It is interesting to note that sample No. 4, obtained from a mixture of *n*-butyl alcohol and phosphate buffer (1:3) did not show splitting. It is assumed that the changes in the IR-spectra of the samples obtained which are observed in this work in the region of absorption of carbonyl groups are caused by the partial location of the electron transferred with the donor (guanine) in the collectivized orbit of the π-electrons of chloranil in positions which have a different effect on the polarity of the C = O-bonds; this causes splitting of the absorption band, in which lines with a higher frequency correspond to a location which causes a small decrease in the polarity of the bonds, and lines with a lower frequency to a location which strongly decreases the polarity of the bond. With a location in the region of an oxygen atom, an increase in the bond's polarity could occur with a corresponding increase in the frequency of absorption. This is

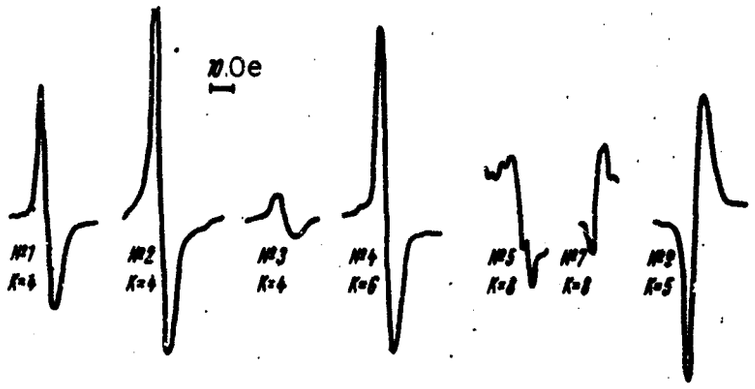


Fig. 3. EPR-spectra of samples. K – index of intensification (each degree of intensification increases the sensitivity two-fold). Samples No. 1, 2, 3, 4, 5, 7 and 9 had the following weights, respectively: 24.9, 37.8, 41.1, 16.7, 20.1, 0.9 and 37.8 mg.

somewhat reminiscent of the splitting of the absorption frequency of the carbonyl band of chloroacetone during the formation of rotational isomers which received a similar explanation [12].

In polar solvents (samples No. 1, 2, 4 and 9) the formation of a complex could be accompanied by strong electron transfer, possibly even with the formation of ion-radicals of guanine and chloranil. Data from a study of the EPR-spectra (Fig. 3) confirm this assumption, since in samples obtained from polar solvents the number of unpaired electrons was higher (of the order of 10^{19}-10^{20} per g) than in samples from nonpolar solvents. As seen from Fig. 3, the signals are asymmetric (half width of approximately 7-8 Oe, which indicates the possible presence of unresolved fine structure. It should be noted that the EPR-spectra of complexes with charge transfer known from the literature [13] also have the form of asymmetric singlet bands sometimes containing against the background of a large singlet a narrower (less than 1 Oe) small signal shifted in the direction of large values of the g-factor. Samples No. 6 and 8 produced very weak signals on the borderline of the equipment's sensitivity with a number of radicals less than 10^{14} per g.

Guanine is the best donor of the nucleotide bases, due to which it, more than the other bases, tends toward interaction with quinones; xanthine is the worst donor among the nucleotide bases. It seemed of interest to verify the possibility of the formation of a complex of xanthine with chloranil. As seen from Fig. 3 (sample No. 7), the product of their interaction also gives an EPR-signal, that is, xanthine reacts with chloranil, similarly to guanine, with the formation of a molecular complex, but, probably, with a smaller yield (which is indicated by the lower intensity of the EPR-signal) in comparison with the guanine complex obtained under the same conditions.

The ability of the quinones to interact with nucleotide bases

can have a great effect on a number of cellular processes. Those changes which may be caused by this interaction in the synthesis and metabolism of the nucleotide bases, nucleotides and nucleotide phosphates will not be considered here, but only certain results of this reaction of the quinones with nucleoide bases which are part of the nucleic acids which deal with the realization of genetic information will be examined.

A chemical change in the matrix of desoxyribonucleic acid [DNA] (ДНК) (the inclusion of analogues of the bases, nucleophlic substitution with alkylating agents, the effect of free radicals, etc.) can have one of two consequences – either the DNA molecule can lose the capacity for reduplication or this change may only disturb the correct reading of the sequence of nucleotide bases from DNA to informational ribonucleic acid [I-RNA] (И-РНК). The first possibility is achieved evidently from the effect on DNA of bifunctional alkylating agents which form, as is believed [14], cross bonds – cross linkings of neighboring DNA molecules. Some quinones can also have such an effect on DNA at a sufficiently high concentration in virtue of their similarity to polyfunctional alkylating agents. This type of effect leads to death of the cells during the mitotic process [15]. In this case, the appearance of mutations should not be expected since the cells in which DNA was subjected to such a strong effect do not produce progeny. An intermediate stage in the alkylating effect of quinones on DNA – the formation of a molecular complex with nucleotide bases (shown in the present work) – is accomplished through the transfer of one electron without the formation of a chemical bond in the commonly accepted sense. Since only one donor molecule (a nucleotide base) participates in the formation of the molecular complex, the DNA molecule can retain its matrix properties. However, a change in the electron configuration of the nucleotide base during the formation of the complex can be the cause of a disturbance in the reading process. This disturbance can consist in improper (erroneous) reading at the site of the complex formation or in blocking further reading in this segment. In the first case the appearance of point mutations can be expected; in the second, the result of not reading whole segments of DNA molecules is genomic mutations. In evaluating the relative importance of these two processes – the formation of a molecular complex and nucleophilic substitution – it must be considered that DNA molecules are bound in the cell with proteins which evidently take upon themselves the "first blow" of the quinones, entering into interaction with them. Such a protective effect from natural alkylating compounds penetrating into the cells may be one of the functions of the nuclear proteins. Only after penetration through the protein defenses [omission in original] by the interaction of the quinones with the nucleotide bases of DNA.

Thus, the mechanism of the mutagenic, fungicidal and antiviral action of the quinones [16-18] can be explained by the interaction of these substances, in particular, the quinones, with the nucleotide bases of the DNA molecule.

During the formation of complexes of the quinones with nucleotide bases of I-RNA mistakes can occur in the assemblage of proteins from amino acids which, of course, are not of a genetic character, but affect cell metabolism and lead to the synthesis of enzymes with

altered activity or specificity or devoid of a control mechanism.

The formation of molecular complexes between the nucleotide bases and tetrachloro-*p*-benzoquinone shown in the present work can also have general significance for the quinones which form during the irradiation of organisms, which makes the continuation of similar studies, but with natural analogues of the *o*-quinones, extremely interesting.

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THE EFFECT OF RADIOTOXINS ON DESOXYRIBONUCLEIC ACID SYNTHESIS IN PLANTS

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A change in the native state of desoxyribonucleic acid [DNA] (ДНК) is the principal cause of the development of genetic and somatic radiation lesions of cells [1,3].

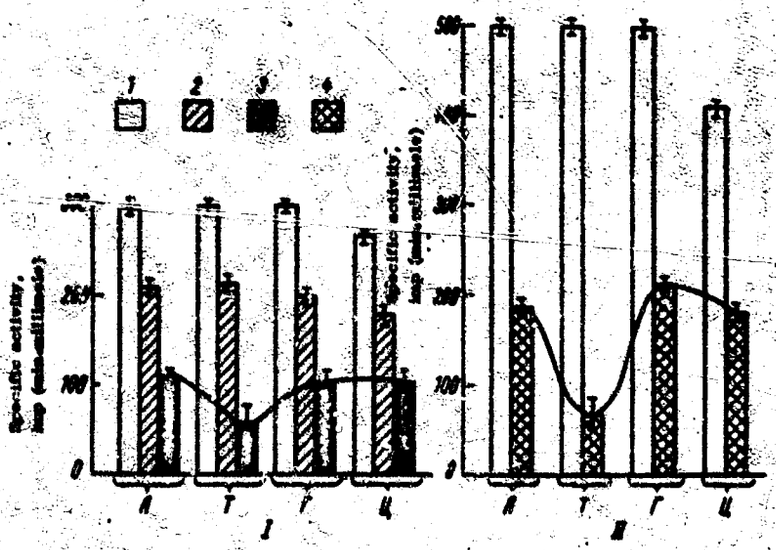
In spite of Li's hypothesis [4] that these changes in DNA are caused only by a direct hit of ionizing particles on DNA molecules, at the present time more and more data are being accumulated which speak of the possibility of the development of analogous changes in DNA by a second means – from the effect of changed metabolites appearing in a cell or tissues of an irradiated organism [5-7].

It has been shown in previous investigations [8-11] that in an irradiated plant organism not only is DNA synthesis inhibited, but also during residual DNA synthesis the inclusion of thymine is clearly decreased and DNA is synthesized with a changed ratio of specific activities of adenine and thymine (A/T). It appears that it is possible to explain this phenomenon both by the direct action of radiation on the DNA-matrix and by the effect of the radiotoxins [RT] (РТ) which form on DNA synthesis. To resolve this question, it is of interest to determine whether DNA synthesis will change in plants from the effect of isolated RT just as from the effect of direct irradiation. The present study has been devoted to this.

Radiotoxins were extracted from irradiated potato tubers of the "Lorkh" variety which were first subjected to γ-irradiation from a Cs^{137} source in a dose of 15 curies at a dose rate of the irradiation of 700 r/min. 24 hours after irradiation the tubers were chilled and the skin removed (only the middle part of the tuber was used in the experiment). The potato pulp was extracted with ethyl alcohol (1:3) in the cold. The extract was concentrated under vacuum at a temperature of 30° until the alcohol was completely removed. Simultaneously, a similar preparation was made from unirradiated tubers (control II, see below). To determine the RT activity in the obtained extracts a series of dilutions of the original extracts was prepared and pea seeds of the "Pobeditel" variety were moistened in them. Extracts in a dilution of 1:5 which in the experimental samples retarded pea growth by 50% and in the controls hardly had an inhibitory effect were used in the experiments.

Three-day germinants of a radiosensitive pea variety ("Pobeditel'") were used to determine the effect of RT on DNA synthesis. The germinants were divided into three parts (150 specimens each). The first part was placed with the roots in tap water (Control I), the second in an extract of unirradiated tubers (Control II) and the third in an extract of irradiated tubers (experimental).

After the plants were kept under the indicated conditions for 5 hours, the germinants were transferred to tap water and after 12 hours were placed in an illuminated chamber with radioactive $C^{14}O_2$ for labeling of newly synthesized DNA under conditions described earlier [13]. After 24 hours in an atmosphere with radioactive carbon dioxide, the plants were extracted, homogenized and after determination of the total radioactivity of the homogenate the DNA was extracted from it by the Schmidt and Tanhauser method. The specific activity of the extracted DNA was determined before its hydrolysis with hydrochloric acid by Wyatt's [14] method and the bases obtained were separated quantitatively by paper chromatography, as described previously [9]. The adenine, thymine, guanine and cytosine spots were eluted, the amount of the bases was determined from the ultraviolet absorption on an SF-4 apparatus and their specific activity on a B-2 unit.



Change in DNA synthesis in pea germinants. I) From the effect of extracts of unirradiated tubers; II) after direct irradiation with γ-rays in a dose of 10 curies. 1) Control, A/T = 1.0; 2) extract of unirradiated tubers, A/T = 1.0; 3) extract of irradiated tubers, A/T = 1.8; 4) γ-rays in a dose of 10 curies, A/T = 2.7.

TABLE

Effect of Extracts of Irradiated Potato Tubers on DNA in Pea Germinants

1 Вариант[1]	2 Выделение СО₂ в растительной ткани, мкл/(мкг·час)	3 Выход ДНК из 100 г ткани, мг	4 Удельная активность ДНК		7 Удельная активность ферментов окисления ДНК, имп/(имп·мин)				Отношение удельных активностей	
			5 имп/(имп·час)	6 % к контролю	8 аденин (А)	9 тимин (Т)	10 гуанин (Г)	11 цитозин (Ц)	12 А/Т	13 Г/Ц
14 *Опыт 1*										
15 Контроль I	764±35	7,7	287	100	340	334	300	225	1,05	1,33
15 Контроль II	672±30	5,5	233	83	213	215	205	185	0,99	1,10
16 Опыт	466±48	4,0	105	37	120	62	104	102	1,93	1,01
14 *Опыт 2*										
15 Контроль I	800±64	9,4	300	100	292	305	320	241	0,96	1,32
15 Контроль II	684±53	6,5	256	85	170	156	185	175	1,08	1,06
16 Опыт	430±20	5,1	145	48	176	86	204	160	2,04	1,27
Опыт 3										
15 Контроль I	480±30	8,5	270	100	200	185	206	138	1,08	1,50
15 Контроль II	290±35	6,0	220	81	237	255	233	178	0,93	1,31
16 Опыт	203±24	4,8	96	35	171	111	180	151	1,54	1,19

[1]Control I – normal; Control II – extract of unirradiated tubers.
[key on next page]

1) Variants[1]
2) C^{14} inclusion in plant tissue, imp/(min·mg)
3) Yield of DNA per 100 g of tissue, mg
4) Specific activity of DNA
5) imp/(min·mg)
6) % (of control)
7) Specific activity of nitrogenous bases of DNA, imp/(min·millimole)
8) Adenine [A] (А)
9) Thymine [T] (Т)
10) Guanine [G] (Г)
11) Cytosine [C] (Ц)
12) Ratio of specific activities
13) G/C (Г/Ц)
14) Experiment
15) Control
16) Experimental

The experiments were repeated three times with the same results, which are presented in the figure (average data) and in the table.

As was shown earlier [15], the RT under investigation, similarly to direct irradiation, sharply inhibited plant growth. The inclusion of CO_2 during photosynthesis which as is well-known, is relatively resistant to the effect of radiation hardly changed under the influence of RT (as is seen from the second column of the table). However, during the general inclusion of C^{14} carbon in the plant material, the inclusion of C^{14} in DNA was inhibited approximately 50% from the effect of RT in comparison with Control II, which, as is known, is also characteristic of the direct effect of radiation.

It is interesting to note the considerably smaller yields of DNA from plants subjected to the action of RT. This is evidently connected with its partial depolymerization, which is always observed in working with directly irradiated plants.

However, the change in the ratio of the specific activities of adenine and thymine described by the authors is the most specific for the effect of radiation. It is seen from the data in the table that an analogous change is observed from the effect of RT. It is necessary to especially emphasize that extracts of normal potato tubers, which also somewhat inhibit plant growth and DNA synthesis, do not have any effect on thymine inclusion, as is seen from the table.

Thus, the investigation which has been conducted showed that RT which are formed in irradiated plant tissue not only cause, similarly to direct γ-irradiation, inhibition of DNA synthesis by approximately 50% of the control, but also have an effect on the composition of newly synthesized DNA, decreasing its thymine content (and accordingly increasing the A/T coefficient).

A comparison of the results obtained from literature data on the inhibition of DNA synthesis in the presence of irradiated cytoplasm (see [16,17] and this collection, page 49) leads to the conclusion that such a process, characteristic of radiation lesion,

as inhibition and distortion of DNA synthesis is explained to a considerable measure by the appearance of RT in irradiated cells.

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THE EFFECT OF RADIOTOXINS FROM IRRADIATED PLANTS ON ANIMAL ASCITES CANCER CELLS

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The use of various chemical anticancer preparations is due to their action primarily on tumor cells with a relatively smaller toxic effect on cells of normal tissues. Therefore, in the investigation of such preparations chief attention is paid to the difference in their effect on cancer and normal cells.

On the other hand, it is well-known that tumor tissue is more sensitive to the action of ionizing radiation than normal tissues of the organism. Modern radiotherapy of malignant neoplasms is based on this property. Based on concepts developed above (see this collection, page 4) of the role of radiotoxins [RT] (PT) in the manifestation of the radiation effect, it is natural to assume that perhaps RT isolated from irradiated tissues will, like direct irradiation, preferentially inhibit the growth of tumor cells.

There is little information in the literature at present on the effect of RT on malignant tumor cells. For example, inhibition of the growth of Yoshida's sarcoma tumor tissue by certain fractions of extracts from the liver of irradiated animals [1] and inhibition of the growth and formation of necroses of a Brown-Pearce tumor in rabbits from the effect of toxins which develop as a result of high doses of ionizing radiation [2] has been reported. Of the biological properties of RT forming in plant tissue after its irradiation with a dose of 25 curies, special attention was paid to their effect on malignant tumor cells [3]. In a study of RT isolated from irradiated leaves of *Vicia faba* plants it was shown in tissue cultures that at an appropriate dilution they completely inhibit the growth of tumor tissue – Geren's carcinoma, in a somewhat higher concentration, leading to its destruction, whereas cells of normal uterus tissue only decreased the growth coefficient 20-30%. It has been shown by the tissue-culture method [4] that whereas at a suitable dilution RT did not decrease the amounts of growing heart, spleen, lung and adrenal explantates and only somewhat decreased (20-40%) the growth of liver, lymph node, kidney and uterus explantates, these RT decreased the growth of a Geren's malignant tumor by 86%.

The strong effect of plant RT on tumor cells has been shown [5] in a study of the survival of a suspension of tumor tissue after preliminary action of RT on it. Survival with respect to the control (taken as 100%) fell to 0% for sarcoma-45, was 16.4% for sarcoma-M-1, 25% for Geren's sarcoma and 51% for Jensen's sarcoma.

The results of a study of the effect of RT from irradiated plants on ascites tumor strains are presented in this article.

The test extracts were prepared from various plants: bean (*Vicia faba*), mint (*Mentha piperitae*) and potato tubers. The plant tissue was irradiated in a GUPOS-2 cesium apparatus with γ-rays in a dose of 25 curies at a dose rate of 700 r/min. 24 hours after irradiation extracts of these plants were prepared. An extract of unirradiated plants was similarly prepared (control). The extraction was carried out with 96% ethyl alcohol (1:5). After extraction the alcohol was removed in a vacuum at 35°, and the aqueous extract which remained was brought with physiological solution to 1/10 of the initial volume.

Ascitic strains of rat ovary tumors – OT (ОЯ) and Ehrlich's mouse ascites were the subjects of the study.

A study of the effect of extracts on ascites strain cancer cells was carried out both *in vitro* and *in vivo*. The effect of the extracts on cancer cells was examined by the following method. The test extract was added to a suspension of ascites cells in a proportion of 1:1, the suspension was incubated at 37° for 2 hours with periodic mixing. After incubation the preparations were treated with congo red. In this case, only dead and injured cells were stained. A count of the living and dead cells was made in a Goryayev chamber.

For a study of the effect of the extracts on the mitotic activity of ascites cells the extracts were injected into the tumorous animals intraperitoneally (1 ml of extract once for rats) or subcutaneously (0.5 ml daily for 4-6 days). Preparations for counting dividing cells were made by fixing the smear in methyl alcohol and subsequent staining with methylene blue. A count of dividing cells was made on 100 cells.

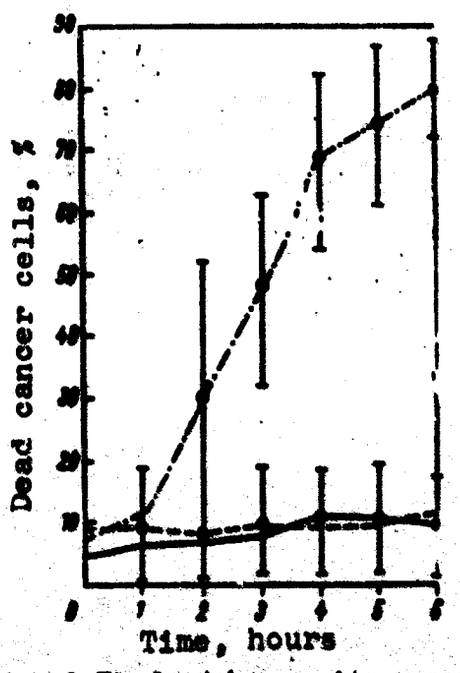
The effect of extracts of irradiated plants on ascites cells *in vitro* was studied on rat ovary tumor cells – OT and on Ehrlich's mouse carcinomas. From contact action of the extracts on the tumor cells it was established that most of the cells die within the first hour. The results of the studies are presented in Table 1 and in the figure.

As seen from the figure, death of the cells gradually increases in proportion to an increase in the time that the cancer cells are in direct contact with extracts of irradiated plants.

TABLE 1

Death of Cancer Cells From the Effect of Extracts of Irradiated Plants (After 2 Hours Incubation)

Plant	Strain	Dead cancer cells,%		
		In control	From the effect of plant extracts	
			Normal	Irradiated
Mint (leaves)	Ehrlich's carcinoma Rat ovary tumor – OT.	5,4 5,1	27,2 15,8	75,8 81,0
Potato (tubers)	Ehrlich's carcinoma	4,8	7,8	40,0
Beans (leaves)	» »	7,7	17,4	67,6



Death of Ehrlich's ascites carcinoma cells from the effect of extracts of potato tubers. ——) Control; – – –) normal; -.-.-) irradiation.

TABLE 2

Effect of Extracts of Irradiated Mint Plants on Mitotic Activity 24 Hours After Direct Contact With Ascites Cells (Intraperitoneal Injection)

Strain	Mitotic index		
	Control	Normal	Irradiated
Ehrlich's ascites	1,20±0,05	1,1±0,1	0,40±0,05
OT ascites	2,6	—	0,5

TABLE 3

Effect of Extracts of Irradiated Bean Plants (*Vicia faba*) on Ehrlich's Ascites Cells (Six Injections)

Variant	Amount of ascites, ml	Number of mitoses, %	Number of ascites cells in 1 ml
Unbred white mice			
Control	10,0	2,3±0,4	1 461 100
Normal	10,3	2,0±0,6	840 100
Irradiated	7,5	0,5±0,1	365 100
Mice CC_{57} Bl			
Control	—	1,9±0,2	206 000
Irradiated	—	1,0±0,2	160 000

TABLE 4

Effect of Extracts of Irradiated Mint Plants (*Mentha piperitas*) on Ascites Cells (Four Injections)

Strain	Variant	Amount of ascites, ml	Number of mitoses, %	Number of ascites cells in 1 ml
OT ascites	Control	49,5	0,70±0,05	5 [illegible]
	Normal	48,1	0,40±0,03	6 [illegible]
	Irradiated	13,3	0,20±0,05	5 700
Ehrlich's ascites	Control	9,2	1,6±0,4	150 000
	Normal	11,1	1,5±0,2	170 000
	Irradiated	6,1	0,70±0,05	82 700

The effect of the extracts on the mitotic activity of cancer cells was studied *in vivo:* the extracts were injected subcutaneously or intraperitoneally into animals infected with ascites. In the latter case, the effect of the extracts was examined 24 hours after direct contact with the ascites cells. The results obtained from intraperitoneal injection are presented in Table 2. As seen from this table, extracts of irradiated plants in 24 hours have an inhibitory effect on cell division (data presented in Table 2 are the results of three experiments).

It was established from subcutaneous injection of the extracts that after 4-6 injections not only is cell division inhibited, but the total amount of ascites is decreased and the concentration of cells in 1 ml of ascites fluid is decreased. The results presented in Tables 3 and 4 are the averages of three experiments (there were 10 animals in each experiment).

It follows from the tables that an extract of irradiated plants inhibits cell division, whereas an extract of unirradiated plants differs little from the control. It was observed from *in vivo* use of the extracts that at the concentrations used they are not toxic for the animals: the mice did not lose weight in comparison with the control. The experiments which were carried out show that RT from irradiated plants inhibit the division of cancer cells and lead to their death both *in vitro* and *in vivo* which can be of interest to medicine.

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THE COMPARATIVE EFFECT OF γ-RAYS AND RADIOTOXINS OF QUINOID NATURE OF THE GROWTH OF LOACH ROE

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Developing egg cells of various organisms are classical subjects for investigating the role of the nucleus and cytoplasm in general radiation lesion. The eggs of the sea urchin, fruit fly, and ichneumon fly *Habrobracon*, frog ova, loach roe, silkworm eggs – this is a far from complete list of convenient subjects which are widely used for these research purposes [1,2].

Irradiation of a developing egg cell leads to such radiation effects as death, cessation of growth and the appearance of deformaties and anomalies in the development of the embryo. A sharp change in radiosensitivity at different stages of development is also characteristic of a developing egg cell.

At the present time it can be considered as established that all these phenomena are very closely connected with a disturbance in the normal entry into the cell of the information coded in the desoxyribonucleic acid of the nucleoproteins and with a change in the metabolic interrelations between cellular structures – interrelations supporting the self-regulation of cellular processes [3]. The initial mechanism of the damage to unique cell structures remains unclear.

The structural-metabolic theory of the biological effect of radiation [3] ascribes an essential role in the radiation injury of a cell to radiotoxins [RT] (PT) which are formed directly in the irradiated cell and secondarily affect unique structures of the nucleus.

Whereas classical target theory (the strike theory) allows injury of unique nuclear structures only through a direct hit by ionizing particles [4], the structural-metabolic theory of the biological effect of radiation, along with a direct effect, allows an essential role in radiation injury to RT which also form in the cell through primary processes and secondarily act on unique structures of the nucleus [5]. To confirm these assumptions it seemed of interest to determine on developing egg cells whether radiotoxins of quinoid nature isolated from an irradiated organism

imitate direct γ-irradiation not only in a qualitative, but also in a quantitative respect.

Loach roe, *Misgurnus fossilis L.*, which have been well studied in a radiobiological respect, were used as the subject of investigation [6]. To avoid genetic heterogeneity of the spawners, the ovum for each series of experiments was taken from one pair of loaches. On the night before the experiments in order to mature the sexual products the females were injected with the gonadotropic hormone preparation, choriogonin, and incubated in an ultraincubator at a temperature of 17-18°. Then the ovum was fertilized with the male's testicular fluid. Incubation of the ovum was carried out in Petri dishes at a temperature of 20-21°. Under these conditions the development of the ovum took place in the following way (we shall note only certain time stages to which we will refer below): 0 hours – fertilization, 1.5 hours – 2 blastomeres, 2.5 hours – 8 blastomeres, 6-9 hours – blastula, 10-19 hours – gastrula, 43 hours – tail still does not reach head. It is most convenient (see [6] and others) to characterize the development of the loach ovum in hours; therefore the names of the individual stages will not be given below, but the hours of development corresponding to these stages.

The unfertilized ova were not taken into account in the experiments, but were removed in the blastulation period. Irradiation of the ova was carried out in small thin-walled cups 3 cm in diameter in the GUPOS and RUP apparatus.

The irradiation conditions were: GUPOS apparatus with a Cs^{137} emission source and a dose rate of 700 r/min; RUP apparatus with a voltage of 210 v and a current strength of 15 ma without a filter with a dose rate of 3020 r/min at a distance of 9 cm.

The radiotoxins (quinones) were obtained from potato tubers irradiated to a dose of 15 curies (see this collection, page 73). At the same time an analogous preparation was obtained from control, unirradiated tubers. Both preparations were diluted to concentrations at which the control solution did not have an effect on the object under investigation; thus, all the cited effects pertain only to substances formed as a result of irradiation, that is, to RT of quinoid nature (see this collection, page 18). The preparations were stored at a temperature of 0°.

Dose curves. In the first series of experiments the nature of the dose curves was studied in relation to death of the embryos. The ovum was irradiated in different doses or treated with various RT concentrations in the blastulation period (8 blastomere stage).

A total of four series of experiments were set up. The nature of the curves from γ-irradiation and the effect of RT were monotypic in each series. The results obtained from one such series of experiments are represented graphically in Fig. 1. Up to 150-300 roe were taken at each point. The dose of γ-irradiation varied from 25 to 2000 r. The radiotoxin was used at different dilutions, namely: 1, 0.5 and 0.25 ml in 10 ml of water. The roe were kept in these dilutions from 15 minutes to 5 hours, after which they were washed and placed in Petri dishes with water for further development.

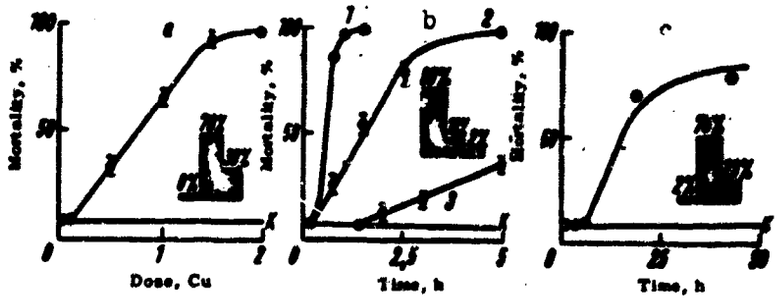

Fig. 1. Dose (a) and concentration (b and c) curves of ovum death. b) RT concentration 1, 0.5 and 0.25 ml in 10 ml of water (Curves 1, 2 and 3, respectively); c) chronic effect of toxin at a concentration of 0.1 ml in 10 ml of water; K) control.

The effect was evaluated from the death of the roe, which is easily fixed from their blanching. Experiments on the chronic effect of small concentrations of RT (0.1 ml of toxin in 10 ml of water) were set up in parallel. Death at low RT concentrations was determined after 19 and 43 hours of chronic action.

As seen from the data obtained (see Fig. 1a) death of the embryos from γ-radiation in relation to the dose of irradiation occurs according to an S-shaped curve. Similar curves were obtained from brief action of RT. The slope of the curve depended heavily on the concentration. At high concentrations (Fig. 1b, Curve 1) rapid death of all the roe set in, by decreasing the dose curves were obtained which imitate well the shape of curves from direct γ-irradiation (see Fig. 1b, Curves 2 and 3). Curves close to those of γ-radiation were obtained from the chronic effect of small RT doses (Fig. 1c).

The nature of the death of the roe by days of development shown in Fig. 1 by the black columns was different in experiments with the brief action of high RT doses [the maximum came on the first day of development (see Fig. 1b)] and with γ-irradiation [the maximum came on the second day of development (see Fig. 1a)]. However, from the chronic action with small RT doses, as is seen from Fig. 1c, good agreement with the effect of γ-radiation was also obtained according to this characteristic (compare with Fig. 1a).

Sensitivity of roe at different stages of embryonic development to γ-irradiation and effect of RT. It is known from the literature [7] that the roe's sensitivity to radiation decreases with an increase in age. In the present work the sensitivity of loach roe at the developmental stages of 2.5, 19 and 43 hours to the effect of γ-irradiation and RT was studied. The results of the experiments are presented in Fig. 2.

As seen from the data obtained (see Fig. 2a) radiosensitivity drops sharply at 19 hours of development and then decreases somewhat more at 43 hours. In experiments with the brief effect of

large RT doses (1:10) (see Fig. 2b, Curves 1 and 2) it is not possible to detect a difference in sensitivity at the developmental stages of 2.5 and 19 hours, but at 43 hours the sensitivity to RT falls sharply, similarly to experiments with γ-irradiation.

With a decrease in RT concentration (Curves 3-5 in Fig. 2b; concentration of 0.5:10) a decrease in sensitivity is clearly observed at 19 hours and further at 43 hours of development. A sharp decrease in sensitivity to RT was observed at 19 hours from the chronic action of small RT doses (0.1:10) (see Fig. 1c). It is seen from the data of the experiments which were conducted that the decrease in radiosensitivity at different developmental stages of loach roe fully correlates with the decrease in sensitivity to RT.

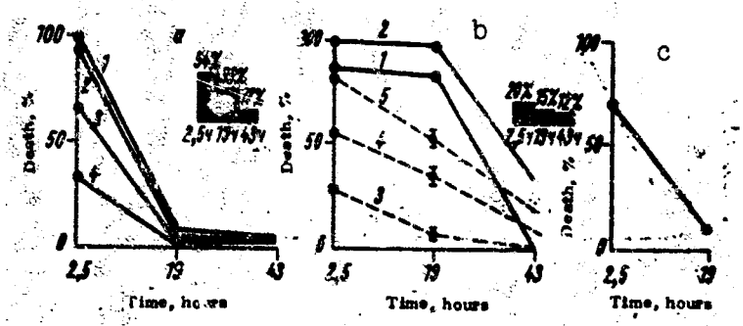

Fig. 2. Sensitivity of roe to γ-irradiation (a) and effect of RT (b and c) at different stages of development. a) Death of roe for doses of 2, 1.5, 1 and 0.5 curies (Curves 1, 2, 3 and 4, respectively); b) RT concentrations of 1:10 (Curves 1 and 2), 0.5:10 (Curves 3-5); c) chronic action of RT at a concentration of 0.1:10. Black columns - percentage of deformities from irradiation to doses of 0.5 curie (a) and from effect of RT in a concentration of 0.5:10 (b) according to time of development. Ч = hours.

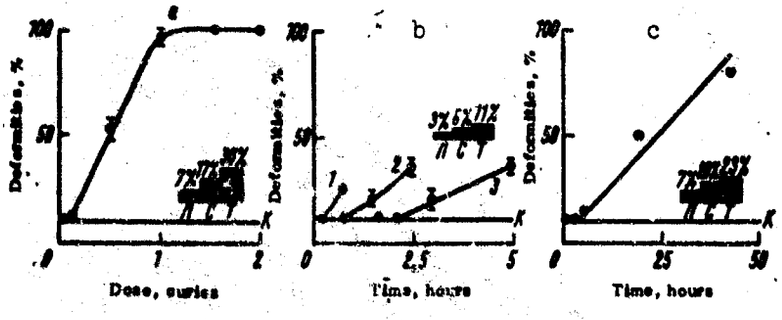

Fig. 3. Dose (a) and concentration (b and c) curves of roe deformities. b) RT concentrations in dilutions of 1:10, 0.5:10 and 0.25:10 (Curves 1, 2 and 3, respectively); c) chronic action of RT at concentration of 0.1:10; K) control; black columns) nature of distribution of deformed larvae for 0.5 curie (a), for RT at a concentration of 0.5:10 (b) and from chronic RT action at a concentration of 0.1:10. (Л - slight; С - average; Т - severe).

The formation of developmental deformities from the effect of γ-irradiation and RT. Developmental deformities as a rule which arise from the effect of γ-irradiation undoubtedly are connected with a change in the unique molecules of desoxyribonucleic acid in the cell nuclei and, consequently, with a disturbance in the entry of deformation for normal development. Therefore, a study of the question of whether RT can also imitate the direct effect of radiation in this characteristic was of special interest.

In this series of experiments the roe were irradiated or subjected to the action of RT at the 8-blastomer stage (2.5 hours). After hatching of the larvae, all visible anomalies were counted and their percentage expressed with respect to all larvae which survived.

All the visible anomalies were conditionally divided into three groups:

1) slight (slight curvatures of the spine; the larvae actively move around the aquarium);

2) average (sharper curvatures of the spine in dorsal and lateral directions; the larvae either move in a curve or perform rotary movements);

3) severe (severe deformations of the head, sharp reduction in the body, twins, etc.; the larvae perform only convulsive, paralytic movements).

As seen from the data of the experiment presented in Fig. 3a from the effect of γ-irradiation the total number of deformities increases with the dose according to an S-shaped curve and at 1 curie reaches 100%. From the effect of higher RT concentrations after brief action (see Fig. 3b) the formation of deformities was also observed; however, their number did not exceed 30-35% of the surviving larvae.

A different type of curve was obtained from the chronic action of small RT doses (concentration 0.1:10). As seen from Fig. 3c, the curve imitated well the dose curve obtained from the direct effect of γ-radiation (see Fig. 3a).

It is interesting to note that the nature of the distribution of the anomalies, as seen from Fig. 3 (black columns) and the table, is similar to the direct effect of γ-radiation. Under these conditions, just as from irradiation in high doses, the hatching time is delayed at exposures of 43 hours.

Of course, the external effect of RT, even if prolonged, does not completely simulate all processes developing in an irradiated egg cell. Thus, in the action of RT it was not possible to observe the characteristic picture of large doses of γ-radiation (20 curies) given at the 0-6 hour stages of development, when division continues up to the late blastula stage and the egg cell dies only in changing to the gastrula stage [6]. From the effect of large RT doses death occurred earlier, and at low doses development continued further.

TABLE

Distribution of Deformed Larvae From the Effect of γ-Irradiation and RT

Group of anomalies	Distribution of deformed larvae, %					
	At a dose of γ-irradiation of		From RT action of different durations			
			Experiment 1		Experiment 2	
	0,5 Curies	1 Curies	19Hours	43Hours	19Hours	43hours
Slight . . .	7,50±1,11	—	7	15	8	18
Average . .	17,20±1,24	27,0±3,5	20	26	24	28
Severe . . .	30,50±1,65	72,0±3,1	23	50	24	50

In this case other processes which are not imitated by the quinones under the conditions of the experiments which were conducted are probably involved in the irradiated cell. It may be that the kinetics of their development in the irradiated object is different than in the external action of a constant RT concentration. It is also likely that the effect of direct injury of the cell structures during irradiation is added to the effect of the RT.

The investigation which has been carried out clearly showed that RT can sufficiently fully reproduce in egg cells such characteristic radition effects as 1) curves of death in relation to the dose; 2) times of death; 3) different radiosensitivities at different stages of development; 4) basic patterns of the development of deformities; 5) a delay in hatching from an increase in the dose.

Therefore, all the effects listed cannot unconditionally be considered as the result only of a direct hit of ionizing radiation on nuclear structures. It is more likely that RT forming from the effect of radiation make their own important contribution to the development and manifestation of all the indicated consequences of irradiation.

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THE EFFECT OF PLANT RADIOTOXINS ON THE ANIMAL ORGANISM

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Anomalous metabolites of phenolic and quinoid nature are formed in plant tissue from the effect of γ-irradiation [1-3]. Substances of phenolic and quinoid nature are extremely active: their bacteriostatic effect is well-known [4,5]; many quinones possess fungicidal and insecticidal properties [6,7] and antimitotic [8] and antitumor [9] activities. The ability to inhibit certain enzymes [10, 11], particularly enzymatic oxidation [12-14], is characteristic of a number of phenolic and quinoid substances, for example, some benzoquinones are capable of inhibiting oxidation and phosphorylation in the mitochondria of the ox heart [15], as well as succinoxidase in the respiratory chain [16].

In a number of papers, Emanuel' et al. [17, 18] found that extremely active intermediate oxidation forms (semiquinones) are produced during the oxidation of inhibitors of free radical reactions (phenols). Selective suppression of the activity of oxidation-reduction enzymes in tumor cells [19], inhibition of lactate dehydrogenase [20] and suppression of the biosynthesis of cellular proteins of Yoshida's ascitic hepatoma [21] is noted in the activity of inhibitors of chain processes (propylgallate). Emanuel' and Lipchina [22] in experiments on mice with an acute transplanted leucosis established the antileukemic activity of substances of this type.

Substances forming in plant tissue from the effect of γ-irradiation are also capable of retarding mitosis [24], inhibiting plant development [3, 25] and causing chromosomal aberrations [26].

However, data on the effect of these substances on the whole animal organism are almost absent from the literature. Kryukova (see this collection, page 78) in investigating the effect of comparatively dilute extracts of irradiated *Vicia faba* plants on tumorour rats and mice did not observe a drop in the animals' weight.

In the present work the effect of large doses of rather concentrated radiotoxins [RT] (PT) which form in potato tubers after irradiation was studied on normal animals. Plant RT were obtained in the following way: potato tubers were irradiated with Cs^{137} γ-rays at a dose rate of 700 r/min. The irradiation dose was 15 curies.

The irradiated tubers together with control unirradiated tubers were kept for 24 hours at room temperature. Further treatment was carried out at a temperature of 0-2°. The outer layer of a thickness of 3-4 mm was removed from the potato tubers and the tissue which remained was homogenized in a three-fold volume of 96% ethyl alcohol. Extraction was carried out for 1 hour with continual mixing, the stroma was separated and the alcohol was removed under vacuum at a temperature of 28-33°. The concentrated extract was diluted with distilled water. From 100 g of the original tissue 35 ml of final extract was obtained.

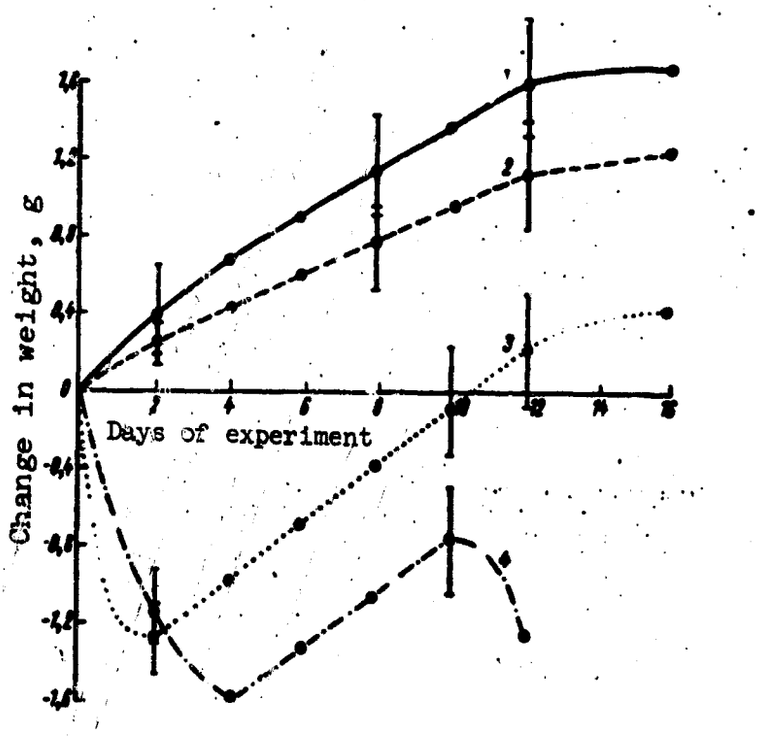

Fig. 1. Change in weight of mice from RT injection and direct γ-irradiation. 1) Control I; 2) control II; 3) injection of extract of potato tubers irradiated in a dose of 15 curies; 4) control III.

In the first series of experiments the effect of such extracts on a change in weight of mice after one extract injection was investigated. The experiment was carried out on white unbred male mice weighing 21-28 g. 0.2 ml of extract was injected subcutaneously into each animal. After the injection the animals were weighed daily for several days. The experiment had three controls: control I – uninjected animals; control II – injection of 0.2 ml of extract obtained from unirradiated potato tubers; control III – irradiation of the animals with Cs^{137} γ-rays in a dose of 700 r. There were 15 animals in each group. A total of three series of experiments (180 mice) was carried out. The average data are presented in Fig. 1.

As seen from Fig. 1 (Curve 3), a sharp drop in the animals' weight takes place in the first two days after the injection under the influence of the plant RT's; in 10 days the animals do not even

reach the original weight and in 16 days a considerable lag in the growth of these animals behind the controls is still observed.

The curves of the change in the body weight of mice from the effect of extracts of irradiated potato tubers and of mice directly irradiated in a dose of 700 r are very similar for 9 days (compare Curves 3 and 4).

A drop in weight does not occur from the effect of extracts of unirradiated potato tubers (Curve 2), and this curve almost coincides with Control I (Curve 1).

20 days after the injection the mice (weight 26-30 g) were killed and some internal organs weighed: liver, testicles, kidneys and spleen. As seen from the data presented in Table 1, the weight of the liver and testicles decreased 17 and 18%, respectively from the effect of plant RT, the weight of the kidneys did not change and the weight of the spleen increased 33%.

TABLE 1

Weight of Internal Organs of Mice 20 Days After Injection of Extracts of Irradiated (With a Dose of 15 curies) and Control Tubers

Organ	Extract of irradiated potato tubers		Extract of control potato tubers		Weight in irradiated samples in comparison with control,%	Reliability of difference, p
	Number of animals	Weight, mg	Number of animals	Weight, mg		
Liver	11	1230±60	11	1480±50	83	<0,01
Testicles	10	230±10	11	280±13	82	<0,02
Kidneys	5	427±20	4	446±23	—	—
Spleen	10	320±16	10	240±20	133	<0,01

In preliminary experiments mice were subcutaneously injected with increasing amounts of extracts (0.3, 0.5 and 0.75 ml) of the surface layer of potato tubers obtained according a method described earlier [3]. In this case an increase in death of the animals was observed: 20, 50 and 75%, respectively. Extracts of control tissue caused death of 20-25% only from the injection of 0.75 ml.

The change in the number of leucocytes in the peripheral blood of rats after one subcutaneous injection of 1 ml of extracts obtained according to the above-described method was also investigated. For this, white unbred male rats weight 160-230 g were used. Blood was taken from the tail vein and the number of leucocytes counted in a Goryayev chamber. As seen from the data presented in Table 2, 24 hours after the injection the number of leucocytes decreased 42% from the effect of plant RT. A decrease in the number of leucocytes from the effect of extracts of unirradiated tubers is not observed (in some cases a slight temporary leucocytosis occurs). The dynamics of the change in the number of leucocytes is presented in Fig. 2. The sharpest drop is noted 6 hours after injection, on the fifth day the number of leucocytes still remained decreased.

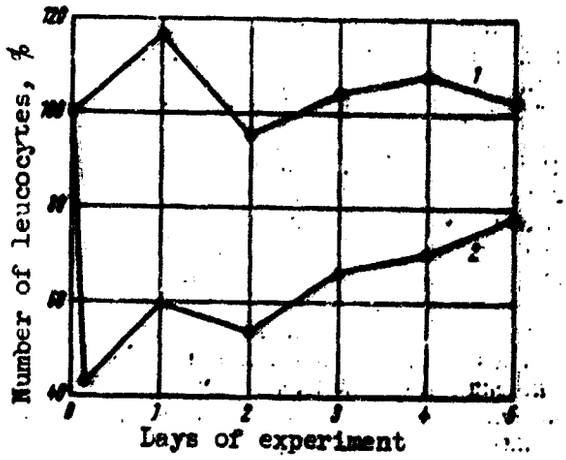

Fig. 2. Change in number of leucocytes in peripheral blood of rats after one RT injection. 1) Control (injection of extract of unirradiated potato tubers); 2) injection of extract of potato tubers irradiated in a dose of 15 curies (RT).

TABLE 2

Number of Leucocytes in 1 mm³ of Blood in Rats 24 Hours After Injection of Extracts of Irradiated (Dose of 15 curies) and Control Tubers

Exp. No.	Extract of irradiated potato tubers		Extract of control potato tubers	
	Before injection	After injection	Before injection	After injection
1	9040	4000	6000	7000
2	5100	3000	4400	4100
3	7200	4000	6000	7000
4	5100	3000	4000	8000
5	5000	4000	8000	10000
6	6400	3100	8000	6000
7	7400	4000	7400	7000
8	5100	3000		
Average	6000±300	3600±200	6000±500	6000±600

Note: Reliability of difference $p < 0.01$.

Discussion of the results. The curves of the change in body weight of mice from the effect of one subcutaneous injection of plant RT and after total γ-irradiation in a dose of 700 r are very close in the first 9 days. As from the effect of radiation, a drop in the animals' weight accompanied by a further lag in growth is noted from the effect of RT. High RT concentrations caused death of the animals. The decrease in the weight of the testicles 20 days after one injection is analogous to the effect of γ-irradiation in small doses [27].

Total X-ray irradiation in a dose of 600 r causes a drop in the weight of the liver of the mice, and on the 21st day after irradiation, the weight of the organ still remains reduced [28]. In experiments which were carried out 20 days after the injection the weight of the liver was 17% lower than the control.

The spleen is unusually sensitive to the injurious effect of ionizing emissions. Initially from total X-ray irradiation with a dose of 600 r a sharp decrease is noted in the organ's weight which is accompanied after 14 and 21 days by the beginning of hypercompensation of the organ [28]. The clear hypercompensation reaction caused by plant RT also emphasizes the similarity of their effect to direct irradiation. The observed decrease in leucocytes indicates injury of the hemogenetic system which is extremely sensitive to the effect of radiation.

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THE RECOVERY OF SEEDS FROM RADIATION INJURIES IN THE LIGHT OF THE TOXIN PRODUCTION THEORY

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If it is considered that radiotoxins [RT] (PT) which are accumulated in irradiated tissue in the post-radiation period play an essential role in radiation injury, then, as was stated in one of the preceding articles (see page 4), the so-called post-radiation recovery from radiation injuries may be due in considerable measure to a decrease in the entry of RT into structures responsible for manifestation of the radiation effect.

Irradiated seeds are interesting subjects for verification of this hypothesis. There is very little data in the literature on the question of the recovery of irradiated seeds from radiation injuries during storage, and they are extremely contradictory [1-4].

It was shown earlier [5] that if the germs of corn seeds irradiated with a lethal dose of 40 curies are separated from the endosperm at the very beginning of the moistening and the former are grown on an artificial medium, the injurious effect of the irradiation can be eliminated to a considerable degree.

The conclusion follows from these experiments that not so much the direct irradiation of the cells as the postradiation influx, from the irradiated endosperm and corymb, of the RT formed in them from the effect of irradiation plays a decisive role in the death of the germs. Actually, as has been shown (see this collection, page 28), if the germs are removed from germinating irradiated seeds, quinones, whose concentration increases with an increase in irradiation dose, will be excreted from the endosperm and corymb into the external medium.

Active toxin production begins from the moment of swelling of the irradiated seeds - from the moment of the beginning of active metabolism. Before moistening, dry irradiated seeds evidently store absorbed energy in the form of free radicals of macromolecules, whose prolonged existence was shown by direct determinations by the method of electron paramagnetic resonance [6, 7].

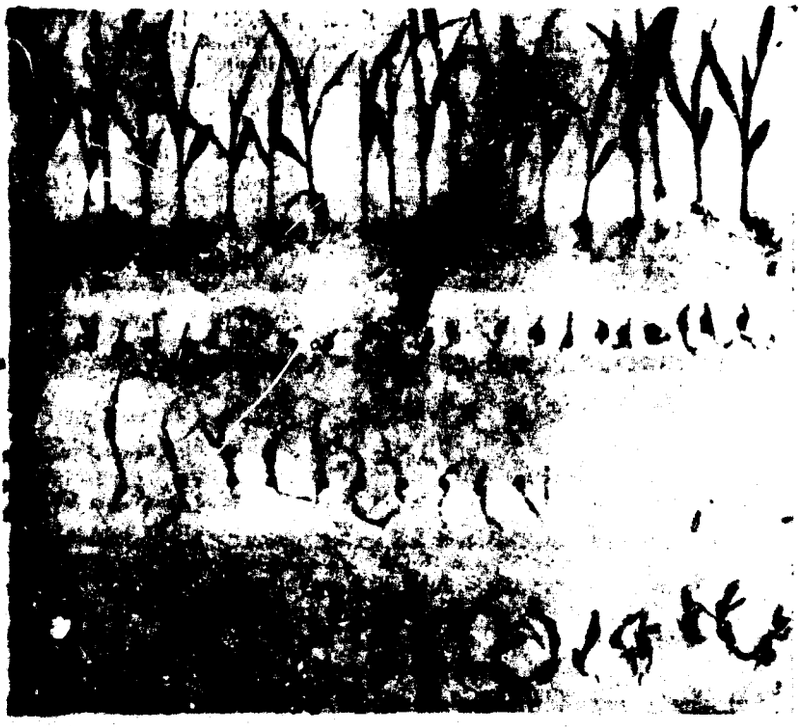

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Fig. 1. 13-day germinants of freshly irradiated corn seeds with different storage periods after irradiation in a dose of 40 curies. a) Control (without irradiation); b) 1 day; c) 6 months; d) 2 years.

The active substances which develop initially by means of radiation-chemical processes, upon prolonged storage of the air-dried seeds, will gradually break down which decreases the possibility of their effect on metabolism upon subsequent moistening of the seeds and the probability of RT formation will also decrease, which in turn leads to the absence of a radiation effect (to recovery).

To verify these assumptions, experiments on prolonged storage of irradiated seeds were set up. All the experiments were carried out on air-dried corn seeds of the "Sterling" variety with 8-10% moisture. One batch of seeds was irradiated with Cs^{137} γ-rays of a dose of 40 curies at a dose rate of 700 r/min. Some of the seeds were sown 24 hours after irradiation, others after 6 months and a third group after 2 years of storage at room temperature. The results obtained are presented in Fig. 1.

As the results of the experiment showed, from irradiation in a dose of 40 curies all the germinants of corn seeds sowed 1 day after irradiation died on the 13th day of vegetation (Fig. 1b). After storage of these seeds for 6 months after irradiation, individual germinants developed for 25-30 days and produced 2-3 real leaves (Fig. 1c), but then died. Seeds stored for 2 years after irradiation recovered their vital capacity. When these seeds were sown, normal germinants developed which vegetated un-

der laboratory conditions in Knopp's nutrient solution up to 45 days (Fig. 1d). In vigor of development these germinants only lagged a little behind germinants of control plants grown from unirradiated seeds (Fig. 1a).

When the experiment was repeated with simultaneous sowing of seeds stored after irradiation for 2, 3 and 4 years and freshly irradiated seeds the same pattern was invariably repeated: after prolonged storage of seeds irradiated in a dose of 40 curies, recovery of their capacity for growth and development was observed (Fig. 2).

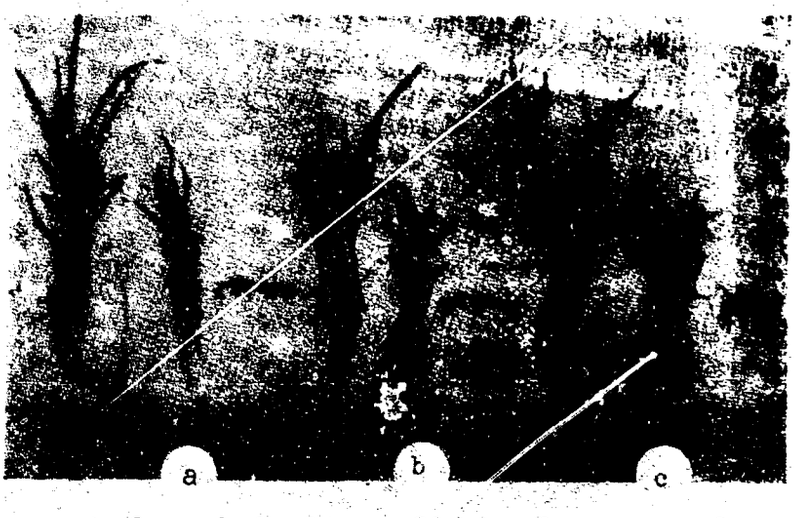

Fig. 2. Recovery from injurious effect of γ-rays of corn seeds irradiated in a dose of 40 curies at different times of storage: a) 4 years; b) 3 years; c) 2 years; 1) control (without irradiation); 2) germinants of seeds stored for the indicated time (a, b, c); 3) germinants of freshly irradiated seeds.

A sharp change was observed in the activity of some oxidizing enzymes in germinants of corn seeds irradiated with a lethal dose. In the first days of germination the activity of these enzymes decreased, comprising only 43% of the activity of the control, and on the 6th, 8 and 13th day of development (to the time of the germinants' death) it increased sharply by 300-400% in comparison with the control.

The maximum change in the activity of these enzymes was observed in the seed corymbs which during germination deliver macroergs and nutrients to the embryo.

The increase in the activity of oxidizing enzymes from the effect of radiation disturbs the usual course of metabolic processes in the germinating seeds and leads to increased production of quinones – which are evidently the principal components of plant RT (see this collection, page 18).

Excess entry of *o*-quinones into the embryo is the cause of the inhibition of their growth and death

In considering the observed phenomenon of the recovery of growth and development of germinants irradiated with a lethal dose from the viewpoint of the RT theory, it can be assumed that during prolonged storage of irradiated seeds as the result of the disappearance of the initial free radicals, initial changes in the activity of oxidizing processes will not arise and the cause of postradiation accumulation during germination of the irradiated seeds will thereby be eliminated. In fact, a study of the activity of two oxidative enzymes – peroxidase and lipooxidase – completely confirmed this hypothesis.

The results of a determination of peroxidase activity on different days of the germination of freshly irradiated seeds (Curve 1) and seeds stored for 2 years (Curve 2) are presented in Fig. 3. As seen from the figure, a sharp deviation from normal is observed in germinants of freshly irradiated seeds (dose of 40 curies), whereas after long storage the activity of peroxidase from germinants of irradiated seeds is almost equal to the activity in the control (unirradiated) plants.

The determination of the lipooxidase activity in the germinant corymbs (by the spectrophotometric method) is presented in Fig. 4.

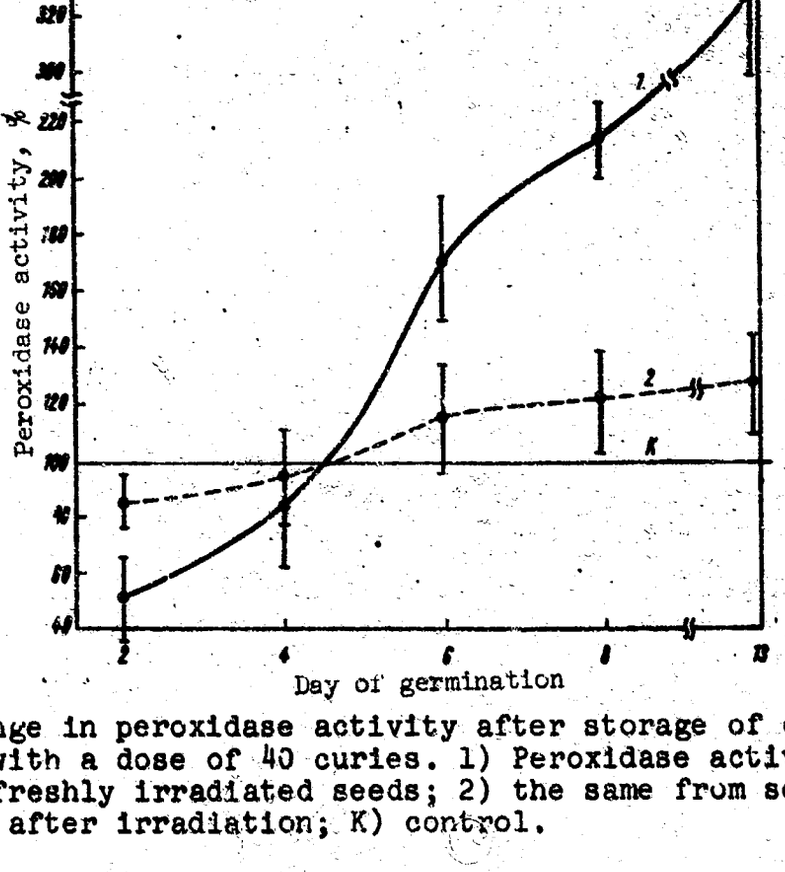

Fig. 3. Change in peroxidase activity after storage of corn seeds irradiated with a dose of 40 curies. 1) Peroxidase activity in germinants of freshly irradiated seeds; 2) the same from seeds stored for 2 years after irradiation; K) control.

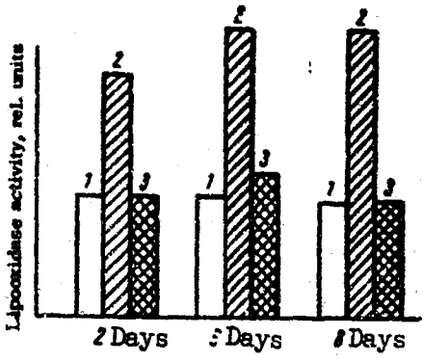

Fig. 4. Change in lipooxidase activity during storage of irradiated seeds (corn). 1) In corymbs of germinating unirradiated seeds; 2) in freshly irradiated seeds (40 curies); 3) in seeds stored 2 years after irradiation (40 curies).

It is seen from this figure that in the first days of germination in freshly irradiated seeds (with a dose of 40 curies) the activity of this enzyme exceeds its activity in the control by more than double. In seeds irradiated but then stored for 2 years, the lipooxidase activity does not differ from the control.

It was interesting to determine whether the production and entry into the embryo of toxic quinones forming in the corymb and endosperm of irradiated seeds changes during storage. For this purpose, both freshly irradiated seeds and seeds stored for 2 years were moistened for 24 hours, after which the embryo was removed. The seeds which were operated on (40 specimens) were placed in containers with 25 ml of distilled water. After 24 hours in the water, the quinones were determined polarographically at $E_{\frac{1}{2}} = -0.35$ v (see this collection, page 28).

The data obtained are presented in the table.

TABLE

Amount of Quinones Excreted by Seeds Irradiated With a Dose of 40 curies After Removal of the Embryo

Seeds	Height of polarographic wave, mm				Amount of quinones in irradiated seeds with respect to the control, %
	Exp. 1	Exp. 2	Exp. 3	Average	
Control	39	30	35	35	100
Freshly irradiated	106	79	94	93	265
Stored for 2 years after irradiation	48	42	55	48	137

As seen from this table, there was a sharp decrease during germination in quinone production in seeds stored for 2 years after irradiation in comparison with freshly irradiated seeds. The principal cause of inhibition of embryo development was thereby eliminated.

The investigation which was carried out showed clearly that after prolonged storage of seeds irradiated with a lethal dose the causes of the activation of oxidative processes and the production of RT of quinoid nature during germiantion of the seeds are eliminated. The absence of RT makes it possible for the embryos to develop normally.

Thus, postradiation recovery processes which are clearly observed after prolonged storage of irradiated seeds are associated not with the recovery of cytogenetic nuclear structures of the embryo damaged by the direct effect of radiation, but with the elimination of the inflow into the embryo of the RT which are the principal cause of the cessation of development and the death of irradiated seeds.

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RECOVERY OF γ-IRRADIATED SEEDS FROM RADIATION INJURIES

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It has been shown in a number of pages that toxic substances produced in irradiated plant tissues can to a considerable degree cause such a manifestation of radiation injury as a delay in cell division and inhibition of plant growth and development (see, for example, [1-4]). The toxic substances are easily extracted with ethyl alcohol and water. It is known that alcoholic or aqueous extracts of normal (unirradiated) plants also have the ability to inhibit plant growth [5]. Attempts have been made more than once to identify the substances responsible for this effect, however, up to the present time there is no single point of view on the nature of these substances. A number of authors believe that such compounds may be substances of phenolic nature [6, 7]. In this connection, it is interesting to note that a considerable increase in the concentration of polyphenols is observed in plants in a quiescent stage, and, as a rule, at the sites of intensive growth [8]. The viewpoint that polyphenols possibly are natural inhibitors and regulators of the rate of cell division is also tempting. At least in the auxin-auxinoxidase system the regulatory role of the polyphenols is becoming more and more evident [9-11]. Aqueous extracts of normal plants have an effect not only on growth processes but also on radiosensitivity and certain biochemical processes. For example, after moistening radiosensitive *Brassicanigra* seeds in extracts obtained from radioresistant *Pinus sylvestris* seeds, the radioresistance of the former is increased, and vice versa (12].

It was shown earlier [13] that a sharp increase in substances of phenolic nature occurs as a result of irradiation of plants and, as the authors believe, growth inhibition by aqueous and alcoholic extracts of irradiated plants is connected with an increase in the concentration of polyphenols and, in particular, with their oxidized form — *o*-quinones (see this collection, page 18). Consequently, if polyphenols in fact normally play the role of inhibitors and regulators of growth processes, it can be said that irradiation causes a disturbance in this system. Normally natural inhibitors of phenolic nature found in seeds can be removed by simple washing out with water. If these starting points are true, then by washing irradiated seeds with water the polyphenols produced as a result of irradiation will be removed and their growth will be improved in comparison with the control.

ried out until gathering of the harvest. The data obtained are presented in Tables 2 and 3 and in Fig. 2.

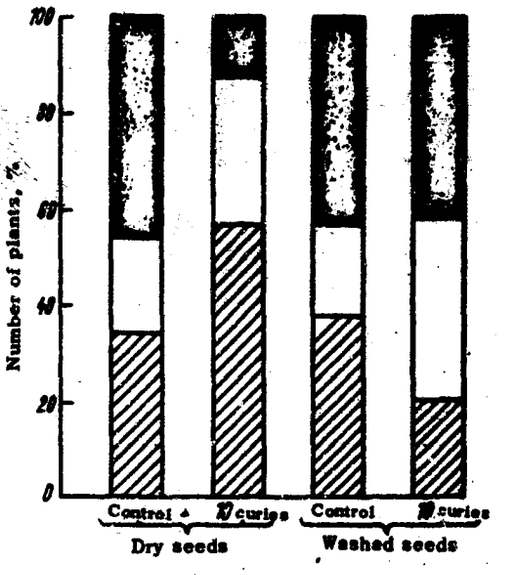

Fig. 2. Result of determination of efflorescence on 31 August (experiment with seed washing). Average data from 100 plants. White column – with panicle; cross hatched column – without panicle; black column – flowering panicle.

TABLE 2

Height of Corn Plants Grown From Seeds Washed After Irradiation With a Dose of 10 Curies (averages from 20 plants, three repetitions)

Variant of experiment	Height of plant					
	8 June		26 July		20 August	
	cm	%		%	cm	%
Dry control	19,0±0,6	100	60,0±4,6	100	137,0±1,0	100
Irradiated with a dose of 10 curies dry seeds	16,0±0,5	84	48,0±3,6	80	117,0±8,4	85
Washed control	21,0±0,9	100	61,0±3,9	100	136,0±5,9	100
Irradiated with a dose of 10 curies washed seeds	18,0±0,6	86	57,0±2,8	93	126,0±3,0	92

In the present work this hypothesis is examined experimentally. Corn seeds of the "Sterling" variety harvested in 1963 were used. Air-dried seeds with 10-11% moisture were irradiated with Cs^{137} γ-rays in GUPOS equipment (dose rate of irradiation 700 r/min) in doses causing strong growth inhibition (10-20 curies). After irradiation some of the seeds were placed in a container with running water at a temperature of 4-6°. They were washed for 2 days at this temperature. Other irradiated seeds were kept at the same temperature in a small amount of non-running water. Unirradiated plants were also divided into two parts, one of which was washed under the same conditions as the irradiated plants. After conclusion of the washing all the seeds were grown under standard conditions.

Data from measurement of the length of the coleoptile on the seventh day of growth are presented in Table 1.

TABLE 1

Length of Coleoptile on Seventh Day of Growth

Variant of experiment	Length, cm		Dry seeds with respect to control, %	Washed seeds with respect to control, %
	Dry seeds	Washed seeds		
Control	11.7±0.7	12.4±0.6	100	100
Irradiation with a dose of 10 curies	7.1±0.4	11.9±0.4	60.5	96
Irradiation with a dose of 20 curies	5.3±0.5	10.9±0.5	45	88

A photograph of one of the experiments is given in Fig. 1.

As seen from the results obtained, washing of the seeds at a low temperature almost completely eliminates radiation inhibition in the first days of development. It is interesting to emphasize that keeping them at a low temperature alone not only does not produce recovery, but even somewhat intensifies the injurious effect of the radiation.

In the second series of experiments, similarly treated seeds were sown under field conditions in experimental plots with threefold alternating repetition. Observations of the plants were car-

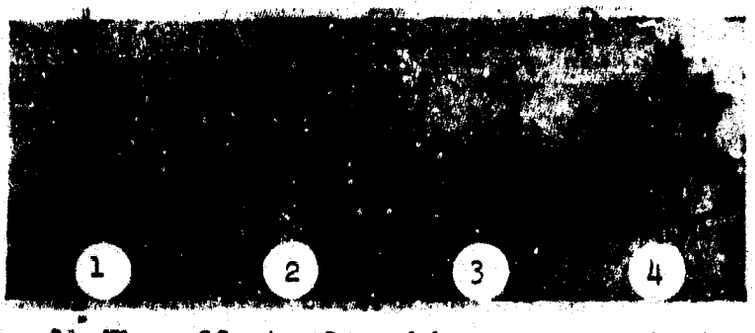

Fig. 1. The effect of washing corn seeds irradiated with a dose of 20 curies on their growth rate.

TABLE 3

Results of Corn Harvest in Experiment With Washed Irradiated Seeds (1963)

Variant of experiment	Height, cm	Raw weight, g	Number of cobs	Weight of cobs, g	
				from one plant	from one cob
Dry control	205	820	1,1	233	210
Dry seeds irradiated with a dose of 10 curies	181	618	0,9	149	165
Per cent of control	88	75	82	64	79
Washed control	207	878	1,1	238	216
Washed seeds irradiated with a dose of 10 curies	196	792	1,2	224	193
Per cent of control	94	90	109	94	89
Ratio of percentages of irradiated washed to irradiated dry seeds	1,07	1,20	1,33	1,47	1,13

It is seen from the data presented that postradiation washing of the seeds almost completely restores such remote effects of irradiation as a decrease in the weight of the cobs, a decrease in their number and a lag in moving from one developmental stage to another (the formation of panicles and efflorescence).

However, it is doubtful whether it is possible to explain recovery from radiation injuries by a simple washing out of growth inhibitors of polyphenolic nature. As seen from Fig. 2, washed seeds (unirradiated) have the same rate of development by phases as unwashed unirradiated seeds, whereas the percentage of plants with a panicle with relative decrease in their flowering, increases considerably in irradiated washed seeds. Here there is a qualitative redistribution by phases of development through washing. According to unpublished data of N. Norbayev, the wash waters from irradiated plants contain a large amount of quinones which are directly related to the irradiation dose. On the other hand, it is noted that the amount of substances of phenolic nature in plants grown from irradiated seeds is also definitely related to the irradiation dose and it should be emphasized that with an increase in the irradiation dose, a regular increase in substances of radical nature – melanines – occurs in the root system [14].

Thus, if an increase in natural inhibitors of polyphenolic nature occurs as a result of irradiation of seeds, it is clearly by quite a different means than takes place normally. Accumulation of substances of quinoid nature which, in condensing, form melanines occurs as a result of such a disturbance.

As indicated above, natural inhibitors and quinones which possess clear toxic properties are removed by washing irradiated seeds. However, it is doubtful whether it is possible to remove them completely; the small concentration which remains possibly has a stimulatory effect on some metabolic links, as a result of which there is noted the increase in plants with a panicle in the present work. On the other hand, evidently even an infinitesimal concentration

can be toxic for the flowering phase (see Fig. 2).

As is known, in discussing the causes of the recovery processes a hypothesis was expressed concerning the important role in them of hidden injuries of macromolecules which become evident in the admission of oxygen and under the influence of heat.

Under the conditions of the present work, washing of the seeds was carried out with water saturated with oxygen, which, it would seem, could not prevent the appearance of an oxygen effect. The low temperature used for delaying the start of metabolic processes in seeds could, of course, delay the appearance of postradiation inactivation; however, this inactivation should be fully manifested when the seeds are sown under germination conditions.

Thus, the theory of the reversal of hidden injuries does not have any advantages over the theory of the washing out of toxic substances. If it is considered that it is possible to completely reproduce the radiation effect of inhibition of seed development by action on the seeds of toxins extracted from irradiated plant tissues, as well as the absence of a recovery effect from keeping the seeds in nonrunning cold water, the explanation of the observed facts suggested here has, it seems to us, a clear advantage over the others.

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THE EFFECT OF PROTECTIVE SUBSTANCES ON THE YIELD OF SOME TOXIC PRODUCTS OF AMINO ACID RADIOLYSIS

N.A. Duzhenkova and A.V. Savich

It is indicated in the work of A.M. Kuzin and colleagues, V. Deyl and others that some products of amino acid radiolysis are cell poisons. Particularly great importance is attached to products of tyrosine breakdown [quinones, 3,4-dioxyphenylalanine (3,4-DOPA) and others] [1]. There is also information on the toxic effect of ammonia [2, 3].

The radiation-chemical conversion of aqueous amino acid solutions has been investigated by many authors. However, it is difficult to compare the results obtained because of the different conditions of conducting the experiments. Some processes of the radiolysis of glycine, tryptophan and tyrosine have already been studied [4-6]. The data obtained are presented in the table.

Amino Acid Radiolysis

1 Аминокислота	2 Выход NH_3 на 100 эв, число молекул			3 Превращение кольца на 100 эв (водный раствор), число молекул	4 Выход разложения на 100 эв (водный раствор), число молекул
	pH 3	pH 7	pH 11		
5 Глицин	2.0	1.6	1.9	—	3.0
6 Триптофан	0.7	0.58	0.9	2.5	5.3
7 Тирозин	0.37	0.28	0.65	0.75	2.1

1) Amino acid; 2) yield of NH_3 per 100 ev, number of molecules; 3) ring conversion per 100 ev (aqueous solution), number of molecules; 4) yield of decomposition per 100 ev (aqueous solution), number of molecules; 5) glycine; 6) tryptophan; 7) tyrosine.

An analysis of the data presented in the table shows that the yields of products and the total decomposition of amino acids do not exceed the yield of free radicals which are produced in the radiolysis of water. The yields are approximately of the same order for aliphatic and cyclic compounds. Only the effect of the radiation deamination of cyclic amino acids is less than for glycine since in the case of their radiolysis the effect of the radia-

tion is directed not only toward the side chain, but also toward the ring structure. The yield of products having a toxic effect and connected with hydroxylation of the phenol ring of tyrosine (3,4-DOPA) is 0.75 molecule per 100 ev and is approximately half of the yield of total amino acid decomposition.

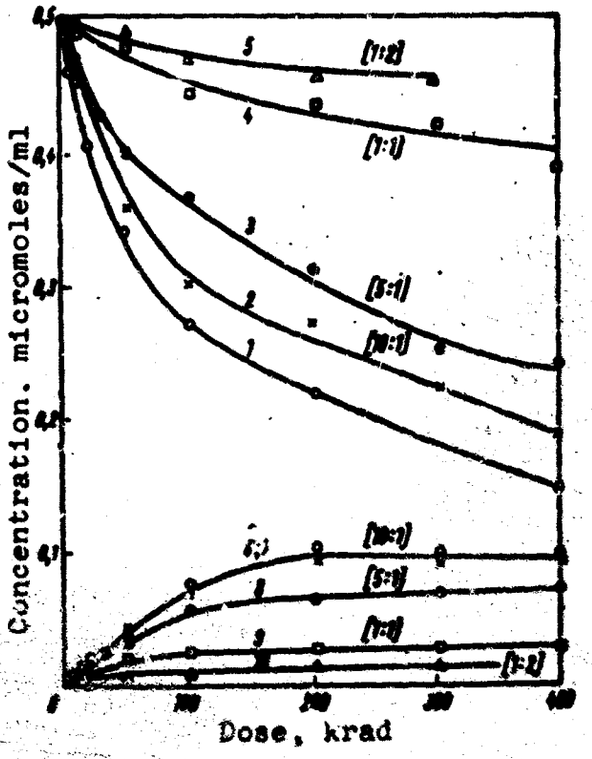

Fig. 1. Dependence of the decomposition of $5 \cdot 10^{-4}$ M aqueous solution of tyrosine and the accumulation of 3,4-DOPA on the dose of γ-radiation. 1) Tyrosine without addition; 2-5) tyrosine + cysteine hydrochloride; 6) accumulation of 3,4-DOPA; 7-10) accumulation of 3,4-DOPA in tyrosine + cysteine hydrochloride system. The proportion of molar concentrations of tyrosine and cysteine hydrochloride is given in square brackets directly on the curve.

The effect of inhibitors of free radical processes – cysteine hydrochloride and propylgallate – on radiation deamination of glycine and tryptophan at different pH values of the medium was studied earlier [4-6]. It was shown that cysteine's protective effect is connected with competition primarily for OH radicals forming during the radiolysis of water and is most clearly expressed in acid medium. The protective effect of propylgallate is due mainly to competition for H radicals and is displayed most clearly in alkaline medium.

At the present time investigations are being conducted on the effect of a number of protective substances on the radiation-chemical conversion of tyrosine. Data obtained in experiments with cyst-

eine hydrochloride are presented in Fig. 1. Curves 1 and 6 depict respectively the dependence of tyrosine decomposition and 3,4-DOPA accumulation on the dose during Co^{60} γ-irradiation (dose rate 150 rad/sec) of a 5×10^{-4} M aqueous solution of the amino acid. The analysis of the solutions obtained was carried out by Arnow's method [7]. The protective effect was calculated from the decrease in tyrosine decomposition and from the decrease in 3,4-DOPA accumulation at a dose of 37% amino acid preservation, equal to 200 krad. Both tyrosine decomposition and 3,4-DOPA accumulation are of an exponential nature. The yield of tyrosine decomposition equals 2.1 molecules per 100 ev, the yield of 3,4-DOPA is 0.75 molecule per 100 ev. When cysteine hydrochloride is introduced into an irradiated tyrosine solution, a decrease in amino acid decomposition and 3,4-DOPA accumulation is observed. The protective effect was determined at four proportions of molar concentrations of tyrosine and additive: 10:1, 5:1, 1:1 and 1:2; the amino acid concentration in all the experiments conducted was $5 \cdot 10^{-4}$ M. As seen from Fig. 1, cysteine's protective effect increases with an increase in its concentration.

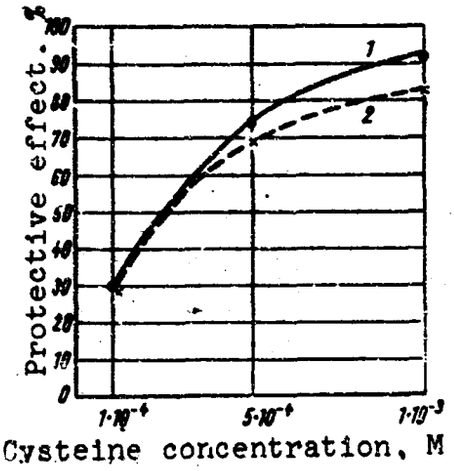

Fig. 2. Dependence of protective effect on concentration of cysteine hydrochloride. 1) Protection of total tyrosine decomposition; 2) protection of 3,4-DOPA accumulation.

The dependence of the protective effect in percentages of the concentration of the additive is presented in Fig. 2. Tyrosine decomposition begins to decrease after the introduction of rather small amounts of cysteine hydrochloride: 29.9% from the addition of $1 \cdot 10^{-4}$ M cysteine [5:1] and 75% at an additive concentration equal to $5 \cdot 10^{-4}$ M [1:1]. From introduction of $1 \cdot 10^{-3}$ M cysteine [1:2] the protective effect equals 93%, that is, practically complete protection of tyrosine is observed. The nature of the effect of the addition of cysteine on the formation of the toxic product, 3,4-DOPA, as seen from Fig. 2, is reminiscent in general outline of its protective effect on tyrosine decomposition. Some difference in the values of the protective effect of cysteine hydrochloride on tyrosine decomposition and 3,4-DOPA accumulation

indicated that although the bulk of the added substance is consumed in a competitive reaction with OH radicals which go into the hydroxylation of tyrosine's phenol ring with the production of 3,4-DOPA, some of it goes into inhibition of other processes of the radiation-chemical conversion of tyrosine, in particular, evidently into the radiation deaminiation processes.

The results obtained showed that substances which are good acceptors of free radicals have a high protective effect and prevent the formation of certain products of amino acid radiolysis with a toxic effect.

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THE ROLE OF LIPID RADIOTOXINS IN THE TOXIC RADIATION EFFECT

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It is well known at the present time that nuclear irradiation is capable of causing the injury and death of any organ, any living cell. Therefore, problems of radiobiology are general biological problems touching upon the interests of biologists with the most diverse specialities.

Over the course of the 70-year history of radiobiology a tremendous amount of material pertaining to the general response reaction of organisms to irradiation has been accumulated. It is known that radiation injury is a set of complex and interconnected changes in the organism. In the final analysis the changes affect the entire organism as a whole and, to one or another degree, all of its organs and tissues. However, in spite of the fact that the general biological changes in an irradiated organism have been well studied, present-day radiobiology has relatively little information on the primary physico-chemical changes and the triggering mechanisms of radiation injury.

For example, there are still no convincing answers to the following questions:

1) Why is the energy of deadly doses of ionizing radiation so small that it is not capable of causing significant changes in molecules at the moment of the cells' irradiation?

2) What are the intensifying mechanisms which bring about the slight primary injuries in the cells and lead sometimes a long time after the irradiation to profound pathological disturbances and to death?

3) What is the mechanism of action of radioprophylactic substances which are capable of protecting organisms from radiation injury only when introduced before irradiation?

The resolution of these questions will make it possible to interpret specific primary reactions of radiation injury and intensifying mechanisms which gradually lead to death of the irradiated organisms. An explanation of the nature of the primary reactions and the intensifying mechanism of radiation injury will create a

basis for effective prophylaxis and treatment of radiation injury.

At the present time there are various hypotheses and theories pertaining to the primary and triggering reactions of radiation injury. Among them the radiotoxin (RT) theory has been put forward many times by various authors. According to this theory, biologically active toxic substances responsible for the development of radiation injury are accumulated in the organs and tissues of the irradiated organism.

For example, as early as 1905 Linzer and Helber [1] found that toxic substances, "leucotoxins," causing destruction of the leucocytes and leading to leucopenia are produced in the blood of irradiated animals. In 1912 Werner [2] expressed the hypothesis that the development of radiation injury is caused by the effect of lecithin radiolysis and the formation here of a toxic product, choline. In the forties Ellinger [3] introduced the "histamine theory of the biological effect of irradiation." Recently, much work has been carried out by Krichevskaya [4] who studied the role of histamine in radiation injury. In the opinion of Klemparskaya [5], Sverdlov [6], Balik and L'vitsyna [7], products of protein decomposition can play a large role in the development of the pathogenesis of radiation injury.

On the basis of numerous experiments on changes in the biological properties of the blood of irradiated animals, Gorizontov [8] presented convincing evidence of the entry of biologically active substances into the blood which promote the development of radiation toxemia. He came to a conclusion concerning the presence in the blood of irradiated animals of various toxic components in different sections of the organism and in different periods of the radiation lesion.

It was shown by the work of A.M. Kuzin (see [9] and this collection, page 4) and his colleagues that *ortho*phenols, which in living tissue easily undergo reversible oxidation forming toxic quinones, increase in the organs and tissues of irradiated plants, as well as in the liver of animals. The data obtained by Kuzin indicate the large role of oxidation reactions in RT production and their indisputable significance in the development of the pathogenesis of radiation injury.

Considering the great importance of oxidation processes in the development of the primary reactions of radiation lesion, Tarusov [10, 11] created the theory of the development of chain oxidation reactions in the biolipids of irradiated cells and tissue. Principal attention in this theory is given to the problem of the formation of the primary toxic substances.

In our work, begun in 1953, toxic lipid substances have chiefly been investigated.

The basic task of the given work was a study of the role of various toxic substances in radiation lesion and establishment of the leading mechanisms in the toxic radiation effect. The work was performed by a group consisting of graduate students, probationers,

port-graduate students and colleagues of the Soil Biology Department of Moscow State University.

The materials and methods of the studies are set forth in other papers from our department which are published in this collection.

Some background indications of the degree of severity of radiation lesion of animals. As is known, the death rate of the affected animals can serve as the most general indication of the injurious effect of high doses of ionizing radiation. Even at the same dose, death of the animals occurs at different times after irradiation, that is, the degree of severity of the injury is different in different animals. Therefore, in selecting animals for an experiment the degree of severity of the developing radiation lesion was controlled and the experiments were conducted against this background. The animals' physiological state, a change in body weight, a change in the amount of the regular blood elements, erythrogram curves and others were recorded as such background indications in our experiments.

Thus, it is seen from Fig. 1 that for each form of radiation lesion of rats there are characteristic changes in the erythrograms and in the same form of radiation lesion, depending on the time passed after irradiation, regular changes occur in the erythrograms. The experiments on rats carried out in the present work confirm data obtained by Terskov and Gitel'zon [12] on rabbits.

The change in the level of various biologically active substances during the development of radiation lesion. Along with the background indications of the degree of severity of radiation lesion, kinetic curves of the change in the level of biologically active substances in various organs and tissues of animals (rabbits, mice and rats) irradiated with different types of ionizing radiation were traced.

As an example we present data from a study of the change in the level of histamine, choline, quinones, protein decomposition products and the activity of lipid RT in the liver of rats irradiated with γ-radiation in a dose of 800 r (acute form of radiation lesion).

It is seen from Fig. 2 that changes in the amount of biologically active substances in the liver of irradiated rats change in a wave-like manner over the course of the whole period of acute radiation lesion. In the first phase of acute radiation lesion (from 5 min to 1-2 days after irradiation) intensified accumulation of histamine, choline and quinones, as well as the inhibition of protein decomposition processes occurs. Then all these changes arrive at the normal level and in the period of manifest clinical changes a decrease in the level of histamine, choline and quinones in the liver is observed. This decrease phase sets in at different times for different toxic substances: earliest for the quinones, then for histamine and in a later period for choline. In the period of manifest clinical changes a progressive increase in protein decomposition products occurs. In distinction to the described phase

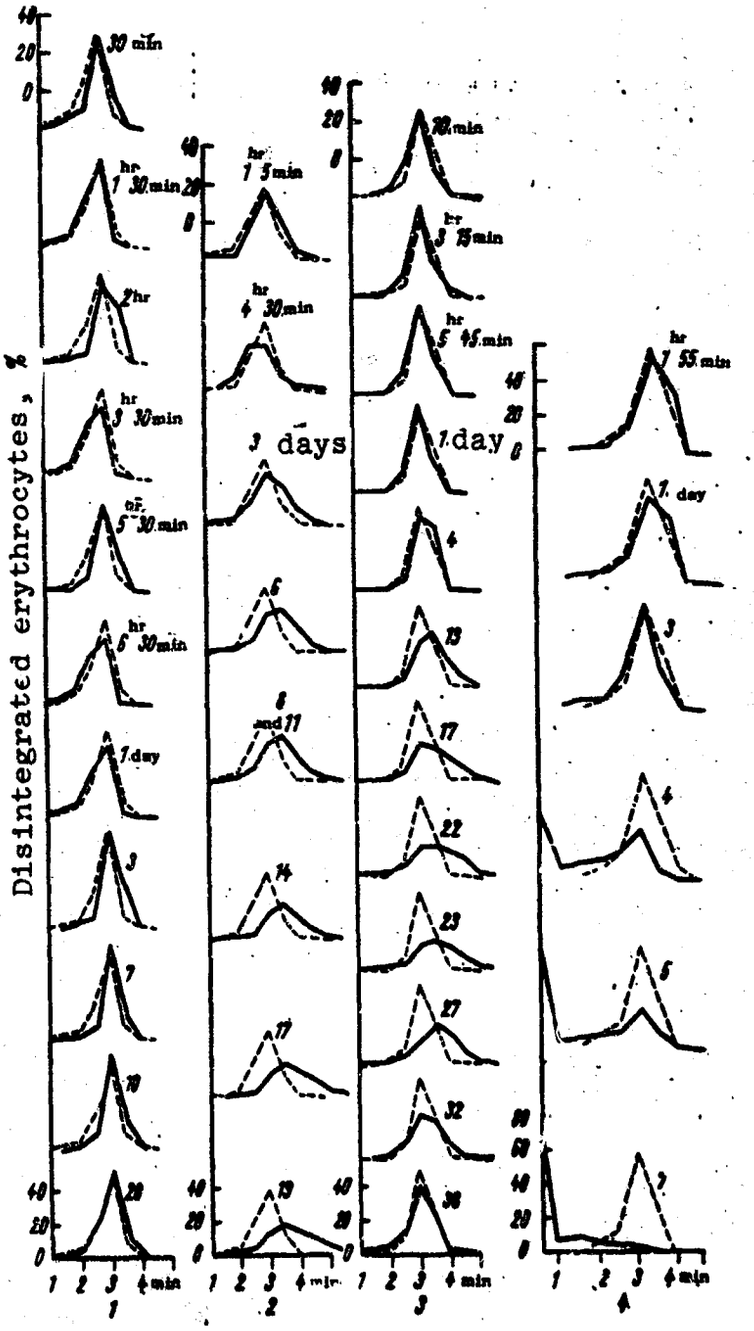


Fig. 1. Change in erythrograms of rats irradiated with different doses of γ-rays. 1) Irradiation with a dose of 200 r; 2) irradiation with a dose of 600 r (animal died on 19th day after irradiation); 3) irradiation with a dose of 600 r; 4) irradiation with a dose of 800 r (animal died on 7th day after irradiation). Dotted line is erythrogram of unirradiated rat; smooth line is erythrogram of same rat after irradiation with γ-rays. The time which passed after irradiation is noted near each erythrogram.

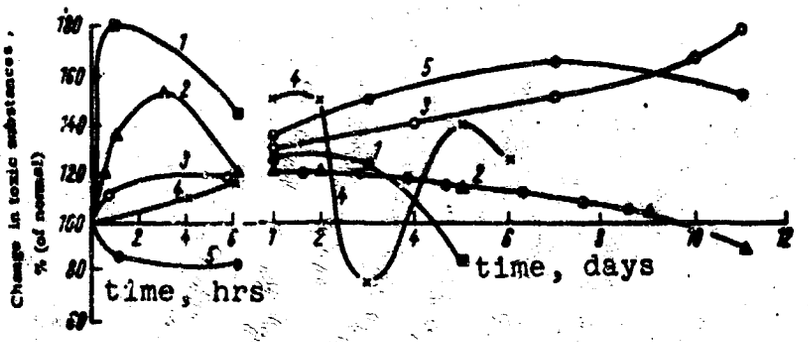


Fig. 2. Change in RT level in rat livers after irradiation in a dose of 800 r at different periods of the radiation lesion (100% – control). 1) Histamine; 2) choline; 3) hemolytic activity; 4) quinones; 5) protein decomposition products.

changes in the level of biologically active substances, lipid RT are found over the entire period of the radiation lesion (see Fig. 2).

The greatest activity of lipid RT is found in the liver of irradiated animals, and it also can be found in other organs and tissues (see this collection, page 126). Data on the detection of lipid RT in the blood are of great interest; this clearly can be used for purposes of radiation diagnostics. Subsequently, it was shown in the Department of Biophysics of Moscow State University that in the intricate complex of the toxic radiation effect lipid RT perform a leading role. In connection with this a study of the chemical nature of lipid RT was carried out, a search for them in various biological specimens was made and the characteristics of the effect of lipid RT on various biological systems were determined.

The chemical nature of lipid RT. Earlier it was found in the department that lipid RT go over completely into the free unsaturated fatty acid fraction (Table 1). Gorkin [13, 14] used this chemical separation method for identifying nonimmune tissue hemolysins which form during the intensification of autolytic reactions. The data which we obtained indicate that lipid RT are very close to the tissue hemolysins studied by Gorkin in chemical properties.

Later, by using the methods of thin-layer chromatography and polarography it was possible to show (see this collection, page 126) that lipid RT are oxidation products of unsaturated fatty acids, and peroxides and epoxides are the principal substances responsible for the toxic effect.

By using oxidized oleic acid [OOA] (OOK) as a model, it was possible to show that OOA is similar in biological effect to the effect of lipid RT.

In our opinion, mainly the unsaturated fatty acids and lecithin which are part of all cells are the principal suppliers of the lipid

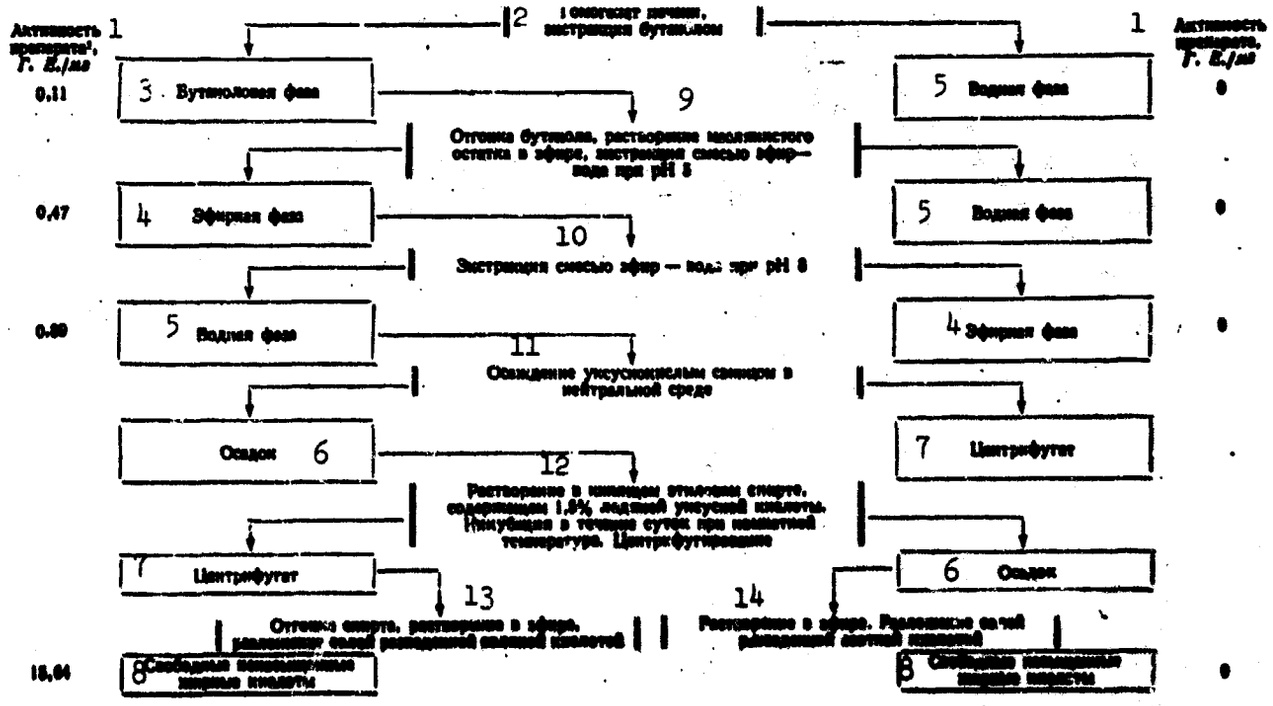
RT.

It is necessary to reexamine Werner's theory [2] about the liberation of choline as a result of lecithin radiolysis and concerning choline's leading role in radiation lesion from the standpoint of the production of the mentioned unsaturated fatty acid oxidation products.

It is assumed in the present work that as a result of the action of radiation on cells intensified decomposition of lecithin occurs and the unsaturated fatty acids which are liberated in this case are easily oxidized. Other unsaturated fatty acids contained in the cells also undergo oxidation. The demarcation of the primary physicochemical radiation effect of lipid RT production and secondary effects which result in a disturbance of normal biochemical reactions is very important in a study of the formation of lipid RT.

TABLE 1

Purification of Hemolytic Factor Produced in the Liver of Rats Irradiated with a Dose of 650 r (Third Day after Irradiation)



*In connection with the fact that at the first stages of the preparation's purification its hemolytic activity reaches 100%, the need arose to express the preparation's activity not in percentages (as was done for water-salt tissue extracts), but in hemolytic units, G.Ye., per mg of wet weight of the original tissue. The hemolytic unit was used in the purification of tissue hemolysins by Gorkin [13, 14] in 1953; it designates the minimal amount of original substance in 1 mg of wet tissue which causes 50% hemolysis.

1) Activity of preparation[1], G.Ye./mg; 2) liver homogenate, extraction with butanol; 3) butanol phase; 4) ether phase; 5) aqueous phase; 6) precipitate; 7) centrifugate; 8) free unsaturated fatty acids; 9) removal of butanol, dissolving of oily residue in ether, extraction of mixture with ether-water at pH 5; 10) extraction of mixture with ether-water at pH 8; 11) precipitation with lead acetate in neutral medium; 12) dissolving in boiling ethyl alcohol containing 1.5% glacial acetic acid. Incubation for 24 hrs at room temperature. Centrifugation; 13) removal of alcohol, dissolving in ether, separation of the salts with dilute hydrochloric acid; 14) dissolving in ether. Separation of salts with dilute nitric acid.

Initially and secondarily forming lipid RT. It was possible to show in experiments on yeast cells (see this collection, page 144) that lipid RT can be found immediately after irradiation. Biochemical processes are strongly inhibited in yeast cells cultured in an aqueous medium. However, in spite of this, prolonged (for 24 hrs and more) maintenance of the cells in water in a nondividing state leads to a decrease in the activity of the lipid RT previously formed in them. Placing the yeast cells in nutrient must lead to intensification of the secondary biochemical reactions and RT activity increases. Lipid RT also was found in organs and tissues of irradiated animals immediately after irradiation. Experiments (see this collection, page 133) showed that in the first hours after irradiation lipid RT are found in a free-radical state: introduced acrylamide is polymerized in the active fraction of oxidized unsaturated fatty acids. In a later period of the development of radiation lesion, in spite of an increase in RT activity, unsaturated fatty acid oxidation products were not found in a radical state.

All these data make it possible to speak about the existence of lipid RT in at least two active forms: primary – free-radical form and secondary – nonradical, stable form.

To explain the question of the formation of primary active products in unsaturated fatty acids, experiments [15] made at the molecular level were carried out on the radiolysis of substances dissolved in oleic acid. β-carotene, which is soluble in oleic acid, whose concentration can be rapidly determined by the photoelectrocolorimetric method, is most convenient to use as such a substance.

The results of the experiments are depicted in Fig .3, from which it is seen that solutions of β-carotene in oleic acid in contrast to other carotene solutions are very radiosensitive. A crystalline β-carotene preparation has high radioresistance. In these experiments [15] it was also possible to obtain for solutions of carotene in oleic acid an effect known from the literature for aqueous solutions under the name of "radiolysis independence of concentration effect" (or, differently, Deyl's effect). In a specific range of carotene concentrations in equal volumes of test samples irradiated with the same dose, the radiolysis of an equal number of carotene molecules occurs (Fig. 4). Preliminary irradiation

of oleic acid and subsequent dissolving of β-carotene in it does not lead to destruction (oxidation) of the dissolved substance.

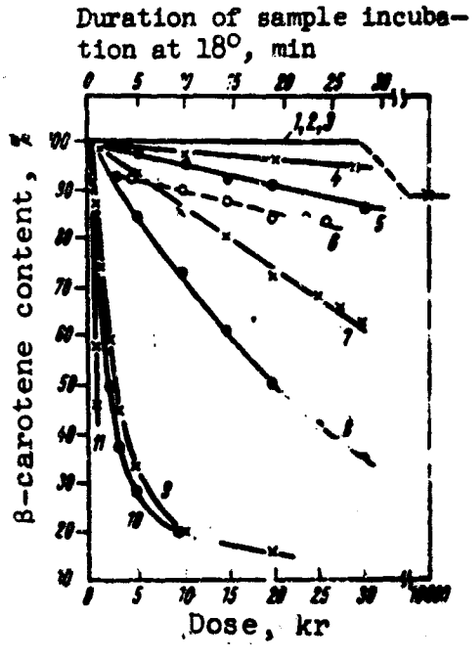


Fig. 3. Radiolysis of carotene in test samples depending on dose of γ-irradiation. 1) Irradiation of crystalline β-carotene at 18° (dose rate 1-5 kr/min); 2, 3 and 4) oxidation of unirradiated β-carotene at 18° in butanol, petroleum ether and oleic acid, respectively; 5 and 6) radiolysis of β-carotene at −196° (dose rate 1 kr/min) in butanol and oleic acid, respectively; 7 and 8) radiolysis of β-carotene at 18° (dose rate from 0.011 to 1 kr/min) in butanol and petroleum ether, respectively; 9, 10 and 11) radiolysis of β-carotene in oleic acid at 18° at dose rates of 1000, 660 and 11 r/min, respectively.

All this can indicate that active short-lived products (radical type) are produced in oleic acid as the solvent. The production of primary lipid RT of radical type evidently occurs as a result of the direct action of radiation on an unsaturated fatty acid.

As subsequent experiments showed (see this collection, page 157) the primary lipid RT which are produced are capable not only of being active with respect to dissolved molecules, but also cause destruction of cells. Evidently, primary lipid RT are capable of causing the production of stable secondary products, previously named "natural radiomimetic."

The effect of lipid RT on various biological specimens and systems. The first work on lipid RT was devoted to a study of their effect on erythrocytes [16]. Extracts and water-salt infusions isolated from tissues of irradiated animals caused hemolysis of erythrocytes which had been added to them. Therefore, the active substances

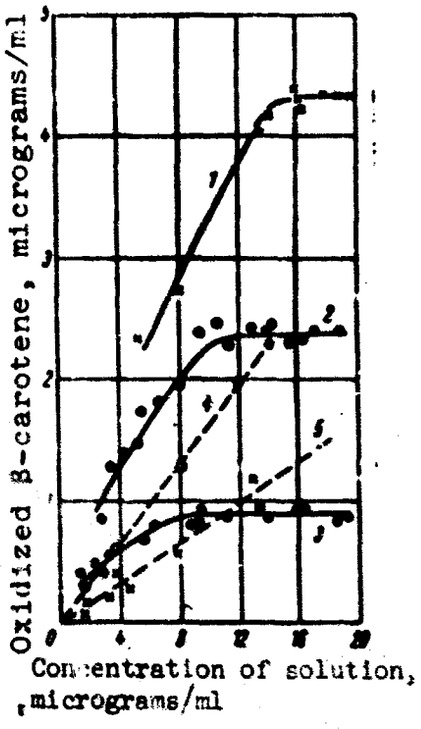


Fig. 4. Radiolysis of β-carotene depending on concentration of solution. 1, 2, 3) Irradiation of β-carotene solution in oleic acid with doses of 10, 5 and 2 kr, respectively; 4 and 5) irradiation of β-carotene solution with a dose of 15 kr in petroleum ether and butanol, respectively. Dose rate of irradiation 1 kr/min, volume of samples, 2 ml.

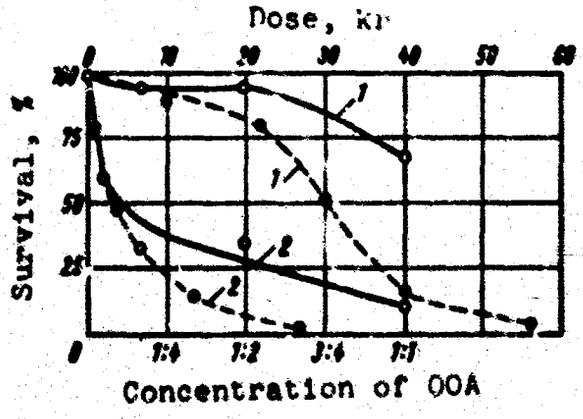


Fig. 5. Survival rate of yeast cells after addition of lipid HT (smooth curves) and γ-irradiation (broken curves). 1) Diploid cells; 2) haploid cells.

found in extracts and infusions of organs of irradiated animals were called the "hemolytic factor."

Later, in experiments [17] carried out on various biological specimens and systems, it was possible to show that lipid RT have the ability to imitate many manifestations of radiation lesion (hence the name "natural radiomimetic").

Thus, it was shown on yeast cells that lipid RT cause injury of yeast cells (Fig. 5), disturbing division processes with the development of the pathological forms and colony sizes so characteristic of radiation injury.

It is very important to note that histamine, choline and some other toxic products were not capable of causing inactivation of yeast colonies, similar to radiation inactivation.

After the introduction of RT into animals, depending on the dose and the method of the preparations' introduction, acute, extremely acute and chronic forms of lesion , similar to radiation lesion, develop [17]. Lipid RT, introduced into animals, cause physiological, morphological and histochemical changes, similar in many details to changes occurring after irradiation of animals with ionizing rays.

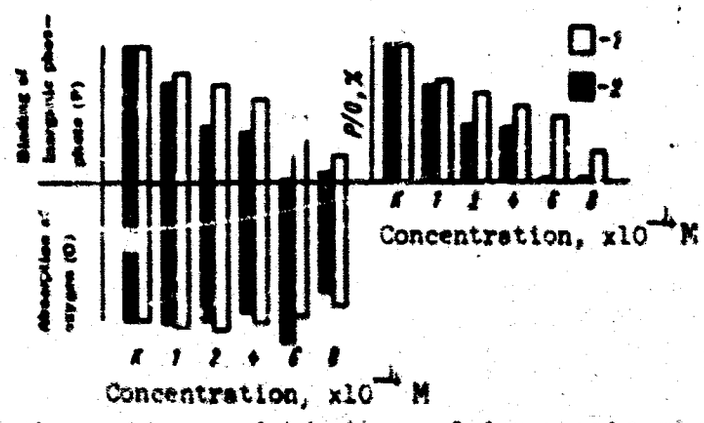


Fig. 6. Oxygen absorption and binding of inorganic phosphate in isolated mitochondria from the effect of lipid RT in different concentrations. 1) Addition of lipids from liver of healthy (unirradiated) rabbits; 2) addition of lipid RT. K) Control (intact mitochondria).

Data obtained from a study of the change in the microstructure of intestinal, liver, kidney and myocardial tissue, as well as from histochemical studies of these organs (ribonucleic acid, neutral fat and lipase content) are presented in this collection (see page 169). On the basis of these data a conclusion is drawn concerning the similarity of morphological and histochemical changes caused by the action of lipid RT and radiation (see this collection, page 169).

A study of hematological changes showed that here a radiometic effect is also observed in the action of lipid RT. Changes are

observed in the physicochemical structure of the erythrocytes and shifts in erythrograms which are usual for radiation lesion occur [17]. In addition to the pathological changes mentioned, lipid RT cause changes in oxidative phosphorylation processes (Fig. 6) in isolated mitochondria and a disturbance in autolytic processes [17] analogous to those due to ionizing radiation.

Data obtained on the ability of lipid RT to cause the production of other biologically active substances which participate in the toxic radiation effect – histamine (see this collection, page 220), choline (see page 214) and quinones (see page 201) are very important. On the other hand, it was not possible to cause the production of lipid toxic substances after the introduction of histamine, choline or quinones even in high, nonphysiological doses in the given experiments.

The data obtained make it possible to speak, first, about the radiomimetic effect of lipid RT and, second, about their leading role in the general radiation effect.

The conclusion concerning the leading role of lipid RT found additional confirmation in a series of papers devoted to a study of the participation of lipid RT in the primary reactions of radiation lesion.

It is well known [9, 13] that a decrease in a cell's oxygen content, as well as the addition of radioprophylactic substances promotes inhibition of the development of the primary reactions. It is shown in the experiments which were conducted that in contrast to a number of other toxic substances, lipid RT are capable of participating in the primary reactions of radiation lesion. Thus, the oxygen radiomimetic effect and the decrease in the activity of lipid RT by radioprophylatic substances were found [17]. The protective effect was thoroughly studied on various biological specimens and systems – in animals, yeast cells and erythrocytes.

The protection of erythrocytes from the radiomimetic effect of a lipid RT model – oxidized oleic acid (OOA) – is illustrated in Table 2. On the basis of these data, as well as previously mentioned data on the free radical state of the primary lipid RT, it can be considered that lipid RT are capable of participating in primary radiation reactions. Radiomimetic models for rapid and preliminary evaluation of radioprotectors were created on the basis of this conclusion [17]. The necessity for such models is urgently dictated by the fact that evaluation of new radioprotectors is associated with large expenditures of time and resources. The radiomimetic models which we have suggested in combination with other methods [17] make it possible to make an orienting evaluation of preparations in a few hours, thereby facilitating preliminary selection of new synthetic radioprotectors.

The following conclusions can be drawn on the basis of what has been stated above:

TABLE 2

Effectiveness of Some Chemical Compounds with Respect to Oleic Acid Hemolysis (from Kudryashov and Kakushkina)

	1 Химическое соединение	2 Концентрация, *M* (или %)	3 Активность ООК $t_{50\%}$[a]	4 Защитный коэффициент $t_{50\%}$ (опыт) / $t_{50\%}$ (контроль)	5 Защита животных (мыши)[b]
6	Цистеамин[c]	$1 \cdot 10^{-2}$	1,5—2,0	4,0—6,0	Высокая 26
7	N-Диэтилмеркаптоэтиламин	$1 \cdot 10^{-2}$	1,0—1,5	4,0—6,0	Средняя 27
8	N-(β-Меркаптоэтилпиридин)	$1 \cdot 10^{-2}$	1,0—1,5	4,0—6,0	Не защищают 28
9	Цистамин[c]	$1 \cdot 10^{-1}$	1,0—1,5	2,5—3,5	Высокая
10	NN-Тетраметилцистамин	$1 \cdot 10^{-1}$	1,0—1,5	1,5—3,0	Средняя
11	*бис*-(*E*-Аминоэтил)-дисульфид	$1 \cdot 10^{-1}$	1,0—1,5	Не защищают	Не защищают
12	S, β-Аминоэтилизотиуроний[d]	$1 \cdot 10^{-2}$	1,0—2,0	2,0—3,0	Высокая
13	N-Метил-β-аминоэтилизотиуроний	$1 \cdot 10^{-2}$	1,0—2,0	2,0—3,0	Средняя
14	N-Диметил-β-аминоэтилизотиуроний	$1 \cdot 10^{-2}$	1,0—2,0	2,0—3,0	Не защищают
15	Тиазолидин	$1 \cdot 10^{-2}$	1,5—2,0	2,0—3,0	Низкая 29
16	β-Аминоэтилтиосерная кислота	$1 \cdot 10^{-2}$	1,5—2,0	1,0—1,5	Высокая
17	Пропилгаллат (пропиловый эфир галловой кислоты)	10^{-6}	1,0—2,0	1,0—1,5	Средняя
18	N-Диэтиламиноэтиловый эфир 3,5-диметилгалловой кислоты	$1 \cdot 10^{-3}$	1,0—2,0	Не защищают	Не защищают
19	N-(3,5-дибутил-4-окси)-бензиламиноэтилизотиуроний	$1 \cdot 10^{-5}$	1,0—2,0	1,5—2,5	Низкая
20	3,5-Дитретбутил-4-окси-α-метилбензиламин	$1 \cdot 10^{-2}$	0,5—1,5	3,0—5,0	Средняя
21	2,4,8-Триметил-3-оксихинолин	$1 \cdot 10^{-2}$	0,5—1,5	Не защищают	Низкая
22	Гиалуроновая кислота	$1 \cdot 10^{-2}$	0,5—1,5	Не защищают	Низкая
23	Аскорбиновая кислота	$1 \cdot 10^{-2}$	4,0	1,4	Не защищают
24	α-Аланин	$1 \cdot 10^{-2}$	1,5—2,0	Не защищают	» »
25	Плазма крови	10%	0,5—1,5	2,0—6,0	» »
30	Липополисахарид из дрожжей	0,1%	0,5—1,5	1,5—2,0	Средняя
31	Липополисахарид из *Esher coli*	0,03%	0,5—1,5	1,3—2,0	»
32	Липополисахарид из *Abortus equi*	0,03%	0,5—1,5	1,5—2,5	»
33	Мукополисахарид (селезенка быка)	0,1%	0,5—1,5	1,5—2,5	»
34	Мукополисахарид (слизистая желудка быка)	0,1%	0,5—1,5	1,3—1,8	»

a $t_{50\%}$ – time of onset of 50% hemolysis.

b Literature data.

c Aminothiols and aminodisulfides are used in the form of hydrochloride salts.

[d] Isothiuronium derivatives are used in the form of hydrobromide salts.

[e] Compounds poorly soluble in water.

1) Chemical compound; 2) concentration, M (or %); 3) OOA activity $t_{50\%}$[a]; 4) protection coefficient $t_{50\%}(exp.)/t_{50\%}(control)$; 5) protection of animals (mice)[b]; 6) cysteamine[c]; 7) N-diethylmercaptoethylamine; 8) N-(β-mercaptoethylpiperidine); 9) cystamine[c]; 10) NN-tetramethylcystamine; 11) bis-(E-aminopentyl)-disulfide; 12) S,β-aminoethylisothiuronium[d]; 13) N-methyl-β-aminoethylisothiuronium; 14) N-dimethyl-β-aminoethylisothiuronium; 15) thiazolidine; 16) β-aminoethylthiosulfuric acid; 17) propylgallate (propyl ester of gallic acid); 18) N-diethylaminoethyl ester of 3,5-dimethylgallic acid; 19) N-(3,5-dibutyl-4-oxy)-benzylaminoethylisothiuronium; 20) 3,5-ditertiary butyl-4-oxy-α-methylbenzylamine; 21) 2,4,8-trimethyl-3-oxypyridine; 22) hyaluronic acid; 23) ascorbic acid; 24) α-alamine; 25) blood plasma; 26) high; 27) average; 28) does not protect; 29) low; 30) yeast lipopolysaccharide; 31) B. coli lipopolysaccharide; 32) Abortus equi lipopolysaccharide; 33) mucopolysaccharide (bovine spleen); 34) mucopolysacchardide (bovine stomach mucosa).

1. Ionizing radiation causes in irradiated organisms the production of a group of toxic substances which brings about intensification of the radiation effect. The following biologically active substances are part of this group: lipid RT, quinones, histamine, choline and protein decomposition products.

2. A phase change is observed in the amount of quinones, choline, histamine and protein decomposition products in animal organs and tissues depending on the time passed after irradiation with ionizing radiation. Lipid RT activity is found over the course of the whole period of acute radiation lesion.

3. Lipid RT are one of the substances which play a leading role in the development of radiation toxemia since they a) are capable of participating in the primary reactions of radiation lesion (primary lipid RT); b) being present immediately after irradiation, are capable of causing the accumulation of other biologically active substances (quinones, choline and histamine) and causing intensified protein decomposition.

4. In chemical nature lipid RT are a group of oxidized products of unsaturated fatty acids – mainly peroxides and epoxides. Primary lipid RT are found in a free radical state, secondary – in a stable, nonradical state.

5. In acting on various biological specimens and systems, lipid RT are capable of imitating radiation lesion in various indices.

6. Lipid RT can be used for a preliminary rapid evaluation of various groups of radioprophylactic substances.

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THE CHEMICAL NATURE OF LIPID RADIOTOXINS AND THEIR DISTRIBUTION IN ORGANS AND TISSUES

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It has been shown by the work of Mochalina [1], Kudryashov [2] and others that after irradiation a "hemolytic factor" which has the capacity to destroy erythrocytes of healthy rats in vitro develops in various animal (mouse and rat) organs and tissues.

The hemolytic activity of the tissue extracts was determined by the visual method. In studying the nature of the hemolytic factor, Kudryashov [3] showed that the hemolysin under investigation is thermostable and dissolves well in diethyl ether and butanol and upon extraction with an ether-water mixture and at pH 8 moves into the aqueous phase. At pH 5 it is found in the ether. This indicates that the active substance is a fatty acid. Upon separation of unsaturated fatty acids from saturated fatty acids, the hemolytic activity moves into the unsaturated fatty acid fraction.

Oxidized oleic acid, which in different biological systems and on various specimens caused changes similar to those arising from the action on these systems and specimens of a hemolytic factor isolated directly from the organism of irradiated animals, was used in subsequent work [4] as a model of the hemolytic factor. Similar data served as further evidence of the similarity of lipid radiotoxins [RT] (PT) and oxidized oleic acid.

Terskov and Gitel'zon's chemical (acid) erythrogram method [6] modified by Yu.B. Kudryashov and M.L. Kakushkina was subsequently used for an objective quantitative study of the hemolytic activity of lipid fractions of the liver of irradiated animals [5]. In connection with this it became necessary to investigate at a new methodological level, which excludes the effect of autolytic and oxidation processes, the distribution of lipid RT in various organs and tissues of irradiated animals. Moreover, with a combination of the erythrogram method and the present-day method of determining the chemical nature of substances – thin-film chromatography – it became possible to continue and to broaden the study of the chemical nature of lipid RT. This paper is devoted to these two questions.

The hemolytic activity of the liver, intestine, stomach, kidneys, testicles, muscles, brain, omentum and blood of irradiated and unirradiated rabbits was studied. The animals were irradiated with a dose of 800 r in GUBE-800 equipment. The animals were decapitated on the third day after irradiation. Removal of the tissues, their homogenization and extraction were carried out in a cold chamber at a temperature of 3-8°. Diethyl ether (8-fold volume) was used as the extractant. The diethyl ether was first freed from peroxides (according to Yur'yev's method). For best extraction the mixture of homogenate and solvent was shaken on a mechanical shaker for 2-3 hrs and then left overnight at a low temperature (3-8°). The extracts obtained were filtered from the homogenate and the solvent removed under vacuum in a film evaporator which eliminates the possibility of intensive oxidation of the samples due to rapid distillation, decreased temperature and decreased pressure. The lipid fractions obtained were freed from phospholipids by precipitating them with a 2.5-fold volume of acetone.

Determination of the samples' hemolytic activity was carried out by a modified [5] acid erythrogram method. The chemical nature of the hemolytically active lipid RT products was determined by the method of thin-layer chromatography in a fixed silicogel-gypsum layer [7]. A hexanediethyl ether-acetic acid system (in a proportion of 85:15:1) was used. A 10% alcoholic solution of phosphomolybdic acid was used as the developer. To test hemolytic activity, the obtained fractions were scraped off together with the adsorbent, dissolved in diethyl ether, the sediment filtered on a glass filter (No. 3) and the solvent removed in a film evaporator. The hemolytic activity in the samples obtained was determined by the erythrogram method.

TABLE 1

Hemolytic Activity of Acetone Fraction of Lipids Isolated from Various Organs and Tissues of Irradiated and Unirradiated Rabbits (dose of irradiation 800 r, third day after irradiation; reliability of experiment 99%)

1 Ткань	2 Концентрация липидов, мг/мл	3 Время 50%-ного гемолиза, мин	
		4 опыт	5 контроль
Печень (6)	0,125	1,680±0,158	2,62±0,29
Кишечник (7)	0,312	2,030±0,065	2,710±0,032
Кровь (8)	0,25	2,070±0,155	2,760±0,216
Семенник (9)	1,0	2,32±0,06	2,86±0,05

1) Tissue; 2) lipid concentration, mg/ml; 3) time of 50% hemolysis, min; 4) experimental; 5) control; 6) liver; 7) intestine; 8) blood; 9) testicles.

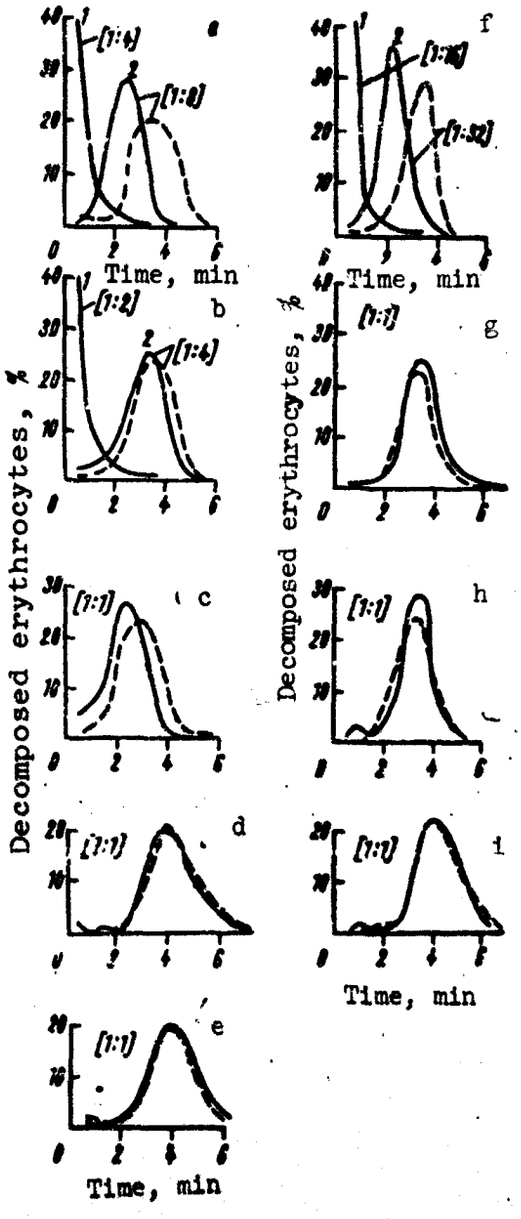

Fig. 1. Hemolytic activity of lipid fractions isolated from various rabbit organs. Dotted line – control; smooth line – experimental. 1) Exp. No. 1; 2) exp. No. 2. a) Liver; b) blood; c) testicles; d) muscle; e) brain; f) intestines; g) kidney; h) omentum; i) stomach.

As already noted, the toxicity of the lipid fractions was studied by determining their hemolytic activity. The results of the experiments are presented in Fig. 1 and Table 1.

Erythrogram curves characterizing the hemolytic activity of various organs are depicted in Fig. 1. As seen from the figure, the time at which a maximum is reached on erythrogram curves pertaining

to various tissues of unirradiated healthy rabbits, as a rule, varies around 3-4 min. In samples isolated from certain tissues of irradiated animals, the time at which a maximum is reached is shifted to the left in comparison with normal. Thus, in liver it is 2.2 min at a dilution of 1:8. In testicle, intestine and blood tissue, similar shifts to the left are found in the erythrograms characterizing an increase in hemolytic activity in irradiated samples in comparison with the control.

Statistically treated data on the time of reaching 50% hemolysis are presented in Table 1. It is seen from the table that for liver, intestine, testicle and blood tissues of irradiated animals ("experimental") the time of 50% hemolysis is decreased, which indicates an increase in the hemolytic activity of various tissues, it can be seen that it will be greatest for liver and smallest for testicles. We did not succeed in finding considerable differences in the hemolytic activity of the kidneys and omentum by the given method. It is also seen from Fig. 1 that the hemolytic activity of muscle, brain, stomach and omentum tissues of irradiated rabbits does not differ from the hemolytic activity of healthy unirradiated rabbit tissues.

The experimental data on the distribution of lipid RT in various organs and tissues generally agree with previously obtained results on the different accumulations of hemolytically active RT in individual organs and tissues.

The absence of a statistically reliable increase in the hemolytic activity in certain tissues in which hemolytic activity was previously found after irradiation (muscle, kidney and brain) may have several explanations.

First, this may be connected with the fact that the new method of obtaining and determining lipid RT reduces secondary factors which affect its activity (autolysis and oxidation) to a minimum. In this case, it must be considered that lipid RT are not found in vivo in muscles, kidneys and brain and their hemolytic activity by the visual method of determination is associated with autolytic and oxidative processes which, however, proceed more intensively in vitro in tissue homogenates of irradiated animals.

Second, this may be connected with the different evaluations of hemolytic activity, which in the visual method is calculated per unit weight of tissue and in the erythrogram method - per unit weight of the lipid fraction. However, it is known that after irradiation the percentage content of the lipid fraction in organs and tissues is increased with respect to the total weight of the organs. In this case the results obtained by the visual and erythrogram methods do not contradict each other.

Further experiments should show which explanation should be considered correct. With regard to such radiosensitive organs as liver, testicles and intestines, an increase in the hemolytic activity of these organs is found both by the visual and by the erythrogram method and evidently can occur not only through an increase in the amount of the hemolytically active fraction, but also

by means of specific chemical changes, since the increase in hemolytic activity occurs per unit weight of the lipid fraction.

The results obtained agree well with the data of work carried out earlier [7] which showed an increase in oxidation products in the lipid fraction (peroxides, epoxides, aldehydes and ketones) of the liver of irradiated animals.

The detection of hemolytic activity in the blood is interesting and new. As is known, many authors using the visual method did not find lipid RT in the blood [8, 9]. Determination of hemolytic activity in the blood by the erythrogram method upon further development can serve as material for diagnosis of radiation lesion (see this collection, page 112).

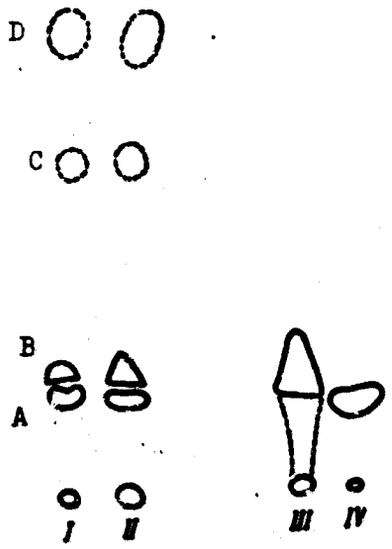

Fig. 2. Diagram of separation of lipid liver fraction by thin-film chromatography. I) Liver lipids of control rabbit; II) liver lipids of experimental rabbit; III) oleic acid; IV) cholesterol. A) Cholesterol fraction; B) free fatty acid fraction; C) triglyceride fraction; D) cholesterol ester fraction.

A study of the chemical nature of lipid RT was carried out on liver tissue which, as was shown, has the greatest hemolytic activity after irradiation. We were able by the method of thin-film chromatography on a fixed silicogel-gypsum layer to separate the samples which were first freed of phospholipids (precipitation with acetone) into four fractions (Fig. 2): cholesterol, unsaturated fatty acids, triglycerides and cholesterol esters. Identification of the fractions obtained was determined both with "markers" - chemically pure compounds (cholesterol, unsaturated fatty acid, oleic acid and triglycerides) and by means of a comparison with the literature data [1]. In a determination of the hemolytic activity of individual fractions it was possible to establish that all the hemolytic activity found in the original sample (before dilution) goes into the free fatty acid fraction (Fig. 3). We did not suc-

ceed in finding statistically reliable differences between the experiment and control in the cholesterol, cholesterol ester and triglyceride fractions. The reliability of the obtained data was determined by the criterion of signs, which makes it possible to judge the reliability from the number of results with the same direction (Table 2). As seen from Table 2, the direction of the changes in the time of 50% hemolysis in the experimental with respect to the time of 50% hemolysis in the control indicates high hemolytic activity of the unsaturated fatty acid fraction in the experimental in each individual case.

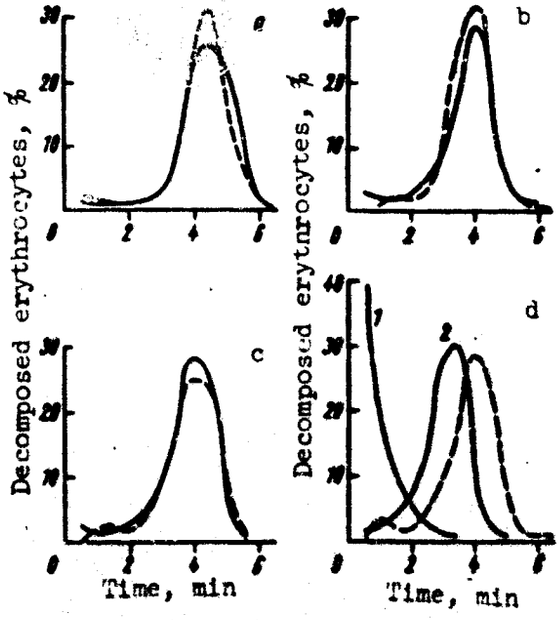

Fig. 3. Hemolytic activity of various lipid fractions of liver. Dotted line – control; smooth line – experimental. 1) Exp. No. 1; 2) exp. No. 2. a) Triglycerides; b) cholesterol; c) cholesterol esters (dilution 1:1); d) unsaturated fatty acids. Dilutions of 1:64 in experiment No. 2 and control, dilution of 1:32 in experiment No. 1.

Thus, data obtained by using new, more perfected methods both in determining the hemolytic activity of the preparations (modified acid erythrogram method) and in the chemical determination of lipid products (method of thin-film chromatography) confirm the previously drawn conclusion that lipid RT belong to the unsaturated fatty acids.

By using the polarographic method, the authors jointly with Z.Ya. Baltbarzdys found that the lipid RT activity belongs chiefly to oxidized products of unsaturated fatty acids (peroxides, epoxides, aldehydes and ketones) and represents a complex mixture of these substances.

TABLE 2

Hemolytic Activity of Unsaturated Fatty Acid Fraction of Liver Lipids of Irradiated and Unirradiated Rabbits
(radiation dose 800 r, third day after irradiation; dilution of 1:32)

1 Время 50%-ного гемолиза, мин		4 Направленность изменений в опыте по отношению к контролю	5 Достоверность опыта, %	1 Время 50%-ного гемолиза, мин		4 Направленность изменений в опыте по отношению к контролю	5 Достоверность опыта, %
2 Контроль	3 Опыт			2 Контроль	3 Опыт		
3,25	2,36	+	>95	2,55	2,0	+	<97
2,9	1,6	+	—	1,25	1,0	+	—
2,8	2,0	+	—	2,2	1,2	+	—

1) Time of 50% hemolysis, min; 2) control; 3) experimental; 4) direction of changes in experimental with respect to control; 5) reliability of experiment, %.

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THE FREE RADICAL STATE OF LIPID RADIOTOXINS

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One of the urgent problems of present-day radiobiology is the question of the role of toxic agents in the development of radiation lesion of organisms of animal and plant origin. At the present time there are several hypotheses concerning the mechanisms of the toxic substances' formation from the action of ionizing radiation on the organism. It has been shown in recent years that unsaturated fatty acids which are produced in the tissues of irradiated animals can have strong toxic and radiomimetic activity [1-3].

By toxic substances are usually meant compounds having high chemical activity with respect to cell components. Therefore it can be assumed that those compounds which are either active intermediates or final products of complex physicochemical and biochemical reactions occurring during irradiation in living organisms will have a toxic function in the primary reactions of cell injury by ionizing radiation.

As is known, free radicals which have a high reactive capacity and essentially change the direction of processes in diverse systems can be the most active intermediate products in various physicochemical reactions.

It seemed of interest to establish whether lipid toxic substances of natural origin which are produced during the irradiation of animals have the properties of free radicals.

White male mice weighing 20 g and chinchilla rabbits weighing 2.5-3.5 kg which were kept under the usual conditions of care and nutrition served as the material for investigation. The animals underwent one general irradiation with rays with a dose of 800 r; the dose rate for rabbits was 50 r/min and for mice was 178 r/min. The irradiation was carried out in a GUBE-800 apparatus. The presence of free radicals in the animal tissues was judged by acrylamide [AA] (AA) polymerization according to References [4-6]. It was established in preliminary experiments that maximum copolymerization of AA with natural tissue components begins after 4 hrs.

Two series of experiments were set up: injection of AA 30 min before irradiation and at different times after irradiation 4 hrs before decapitation of the animals. The acrylamide was injected intraperitoneally in an amount of 20 microcuries per mouse and 150 microcuries per rabbit.

The degree of copolymerization of radioactive carbon labeled AA ($CH_2 = CH - C^{14}ONH_2$) was evaluated by the radiometric method [7].

After decapitation of the animals, the kidneys, liver, spleen and brain were removed. The tissues under investigation were homogenized and an 8-fold volume of ether added to the homogenates. The homogenates with the ether were placed on mechanical shakers and extraction was carried out for 2 hrs with constant mixing of the samples. Then the ether of the "ether fraction" was evaporated and acetone added to the oily residue (in the same amount as the ether). The samples were carefully mixed and left for 2 hrs. The precipitated phosphatide residue was removed (phospholipid fraction) and the supernatant liquid was again evaporated, giving the "acetone fraction" (natural radiomimetic – NR (EP)) [3].

The experiments which were carried out showed that in all irradiated rabbit tissues studied into which AA was injected 30 min before irradiation, no difference from the control was found on the third day in the content of free radical states of NR. Evidently, in this case the activity of the AA injected into the animal was insufficient, and moreover, the greater part of the AA was excreted from the tissue by this time; therefore, further experiments were carried out on mice only.

In experiments on mice a statistically reliable difference in the concentration of the free radical states of NR in irradiated and unirradiated tissues (brain, spleen and kidneys) was not observed. The liver was an exception. As is known, the most active lipid radiotixins were found in the liver (see this collection, page 126), whereas in the brain, kidneys and spleen they either were not found or they were weakly active. Therefore, of the data which we obtained and the results of the work of Astakhov and others (see this collection, page 126), only the liver of irradiated animals was subjected to further study.

Curves of the formation of free radical states of NR in the investigated tissues at different times after irradiation of mice are presented in Fig. 1. As seen from this figure, immediately after irradiation the concentration of free radical states of NR in the liver exceeds the norm by 5-6 times. However, in the first 24 hrs the concentration of free radicals decreases sharply. Later it falls slightly. It can be concluded from the exponential nature of the curve that irradiation causes the production of free radical forms of NR which later disappear from the irradiated animal tissue, evidently being converted into other oxidation products.

The decrease in the concentration of free radical states of NR can be represented in the form of an empirical formula.

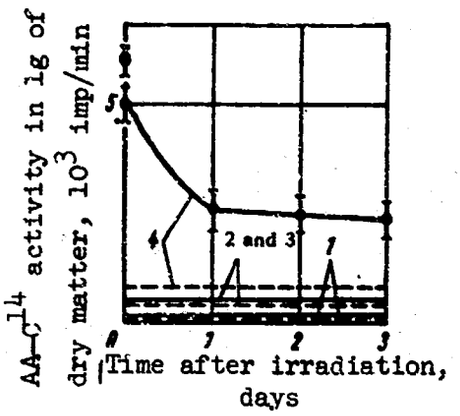

Fig. 1. Production of free radical forms of NR at different times after irradiation of animals. Smooth line – experimental; dotted line – control. 1) Brain; 2) spleen; 3) kidneys; 4) liver.

$$\frac{d\Pi}{dt} = \sqrt{\Pi^2 + w_0} - \Pi,$$

where Π is the intermediate product (free radical state of NR), t is time and w_0 is the rate of chain formation (dimensionless value).

It is well known that peroxides, aldehydes, ketones and epoxides can be intermediate products of the oxidation of unsaturated fatty acids. Similar products were also found in the tissues of irradiated animals [8-10].

Experiments were set up on the demonstration of such products by the method of electrochemiluminescence [ECL](ЭХЛ) of a methanol-sodium citrate system. Recording of the ECL was carried out with an FEU-42 photoelectric multiplier, operating without cooling in a photon counter system and an attached standard chemiluminescence apparatus.

The methanol-sodium citrate system which luminesces during electrolysis produced in the course of 30 and more min a stable luminescence with an intensity of 10,000 ± 50 imp/sec at an electrode voltage of 10 mv and a current strength of 3 ma.

An evaluation of the effect of the test substances on ECL luminescence was made from the value of the inhibitory activity [InA] (ИНА). In this case by the InA value was meant the difference between the background luminescence of the methanol-sodium citrate system and the stationary level at which the luminescence settles 5 min after the introduction of the corresponding substances into the cell.

In the present work the InA of oxidized oleic acid [OOA](ООК) and unoxidized oleic acid [OA](ОК) was studied. It is seen from Fig. 2 that at the same concentrations the InA of OA is lower than that

of OOA. Fig. 3 shows the dependence of the inhibition of the ECL of OOA and OA on their concentrations. The effect observed is probably connected with the presence in OOA of decomposition products which, according to Vasil'yev's data [11], themselves have the effect of exciting luminescence which causes their lower InA.

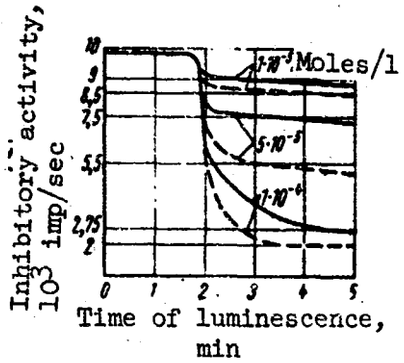

Fig. 2. Kinetics of the inhibition of the electrochemiluminescence of a methanol-sodium citrate system at different concentrations (indicated on the curves) of oxidized (smooth line) and unoxidized (dotted line) oleic acid.

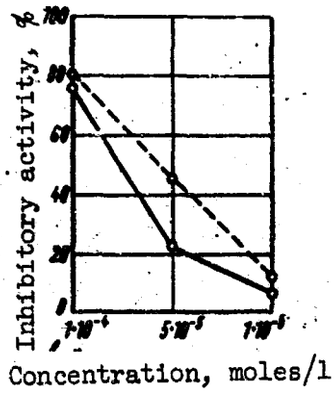

Fig. 3. Dependence of inhibition of electrochemiluminescence of methanol-sodium citrate system on concentration of oxidized (smooth line) and unoxidized (dotted line) oleic acid.

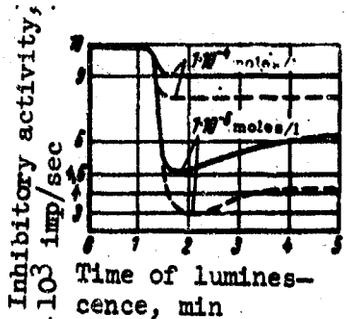

Fig. 4. Kinetics of the inhibition of the electrochemiluminescence of methanol-sodium citrate system by various concentrations of a natural radiomimetic obtained from irradiated (smooth line) and unirradiated (dotted line) rabbits.

It is of interest to compare the InA of OA and NR obtained from rabbit liver on the third day after irradiation of the animal. In calculating the NR concentration, its molecular weight was conditionally equated to the molecular weight of OA. It is seen from Fig. 4 that the InA of NR obtained from an irradiated animal is less than the InA obtained from control animals at the same concentrations. This is in accordance with the concept that [1-3] the NR of irradiated animals may be a mixture of unsaturated fatty acids which contain different oxidation products capable, as in the case of OOA, of exciting luminescence in the methanol-sodium citrate system.

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THE FORMATION OF LIPID RADIOTOXINS IN ANIMALS AFTER IRRADIATION WITH FISSION NEUTRONS, HIGH ENERGY PROTONS AND Co^{60} γ-RAYS

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Papers have appeared in recent years which indicate the important role of toxic products in the pathogenesis of radiation injury of animals [1-3]. However, up until now great difficulties have been encountered in evaluating the significance of various toxic products in the development of radiation injury. This question can be analyzed by comparing the effects of different types of ionizing radiation in equivalent doses. As is known, ionizing emissions which differ in ionization density or, moreover, in linear stopping power [LSP] (ЛПЭ) have both specific characteristics which are chiefly determined by the nature of the primary reaction of the radiation injury (for example, oxygen effect, protective effect, dose rate effect, etc.) and general changes characteristic of acute radiation sickness (for example, death of the cells of radiosensitive organs and changes in biochemical, physiological and other indices, etc.). Therefore, in comparing the toxic effects in the case of the action of ionizing emissions which differ in LSP, there is the possibility of evaluating the significance and characteristics of the formation of one or another toxic agent. In the present work the formation of toxic products of lipid nature was studied, whose important role in the toxemia of radiation lesion is seen from References [1, 4] (see also this collection, page 111).

The experiments were carried out on 46 white male rats weighing 150-180 g and 19 chinchilla rabbits weighing 1.5-2.0 kg. Accumulation of lipid oxidation products in rat livers after irradiation was evaluated by the thiobarbituric acid [TBA](ТБК) method. At different times after irradiation the rat was killed by decapitation, the liver quickly removed and 5 g of liver ground with a homogenizer in 50 ml of a mixture of *n*-butanol and glacial acetic acid (in a proportion of 4:1) for 3 min. Then the sediment was removed by filtration through a No. 4 glass filter and 1 ml of a 1% TBA solution added to 5 ml of the extract. The mixture was placed in a test tube, well shaken and heated in a boiling water bath for 30 min. After the tubes were chilled their contents were analyzed colorimetrically on an FEK-52 apparatus with a No. 5 filter. The value for the "blind" samples which contained *n*-butanol in place of extract was subtracted from the extinction results of the experimental samples.

The weight of the lipids in the extracts was determined by evaporating 10 ml of extract in weighing bottles. The amount of lipid oxidation products was expressed in extinction units, calculated per g of lipids.

Determination of the activity of the natural radiomimetic [NR] (EP) isolated from the liver of rabbits killed on the third day after irradiation was carried out by the method of "loosening" the hemolytic stability of human erythrocytes, which was described earlier [1].

Irradiation of the rats was carried out with protons with an energy of 660 Mev and fission neutrons in equivalent lethal doses of 800 rem. The rabbits were irradiated with protons with an energy of 130 Mev, fission neutrons and Co^{60} γ-rays in equivalent lethal doses of 1000 rem. The synchrotron at the Joint Institute of Nuclear Research [OIYaI, Dubna] (ОИЯИ) was the source of the high energy protons. The dose rate of the protons was 50-300 rad/min. The method of irradiation and the dosimetry has already been described [5, 6]. The IBR (ИБР) reactor (OIYaI) was the source of the fission neutrons. The rabbit and rat cells were placed several meters from the reactor's active zone. The dosimetry was carried out from the induced activity in indium plates, taking a specific absorbed area of a value of $1.2 \cdot 10^{-9}$ rad·cm^2/neutron for the given neutron spectrum [7]. The dose rate of fission neutrons was approximately 400 rad/hr. The GUBE-800 apparatus at the USSR Academy of Sciences Institute of Biophysics was the source of the Co^{60} γ-rays. The dose rate of γ-rays was about 16 rad/min.

The relative biological efficiency [RBE] (ОБЭ) of protons with energies of 130 and 660 Mev and of fission neutrons, taking Co^{60} γ-rays as the standard emission, is about 1.0 and 3.6, respectively [5]. This difference in the RBE of the ionizing emissions is determined by their difference in LSP; high energy protons and Co^{60} γ-rays belong among the rarely ionizing emissions (LSP of the order of 0.3 kev/micron) and fission neutrons to densely ionizing emissions (LSP of the order of 30.0 kev/micron).

Data on the accumulation of lipid oxidation products in the liver of rats irradiated in a dose of 800 rem with protons with an energy of 660 Mev and fission neutrons are presented in Tables 1 and 2, respectively.

As can be seen from these data, the change in the amounts of lipid oxidation products in both cases occurs in approximately the same way. In the first hours after irradiation the amount of these products increases somewhat; however, this difference from the control is not statistically reliable. One day after irradiation the amount of lipid oxidation products decreases and then again begins to increase up until the death of the animals. In the terminal period of the radiation injury in both cases the amount of lipid oxidation products reliably exceeds the control. A similar change in the amount of lipid oxidation products isolated from the liver of irradiated rats was described earlier for the case of γ-rays [8].

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TABLE 1

Accumulation of Lipid Oxidation Products in Liver of Rats Irradiated in a Dose of 800 rem with Fission Neutrons at Different Times After Irradiation

	1 Контроль	2 10 ч	3 1 сутки	4 3 суток	5 суток
Количество крыс . . 5 . . .	8	6	3	4	4
Величина экстинкции, рассчитанная на 1 мг липидов . . 6	16,4±1,3	18,2±1,5	15,9±3,2	20,6±2,1	21,8±2,5

1) Control; 2) 10 hrs; 3) day; 4) days; 5) number of rats; 6) extinction value calculated per mg of lipids.

TABLE 2

Accumulation of Lipid Oxidation Products in Liver of Rats Irradiated in a Dose of 800 rem with Protons with an Energy of 660 Mev at Different times After Irradiation

	Контроль 1	3 ч 2	18 ч	1 сутки 3	3 суток 4	5 суток	7 суток
Количество крыс . 5. . . .	8	3	2	4	4	5	3
Величина экстинкции, рассчитанная на 1 мг липидов . . 6	16,4± ±1,3	18,9± ±2,5	18,1± ±3,0	12,7± ±1,8	19,4± ±1,7	19,3± ±1,0	22,1± ±2,4

1) Control; 2) hrs; 3) day; 4) days; 5) number of rats; 6) extinction value, calculated per mg of lipids.

The results of experiments on NR activity of rabbits irradiated with protons with an energy of 130 Mev and fission neutrons are presented in Fig. 1. As can be seen from this figure, in both cases toxic products of lipid nature capable of decreasing the hemolytic stability of human erythrocytes are accumulated in the rabbit livers. The NR activity in both cases is approximately the same and corresponds to the activity of NR obtained from the liver of rabbits irradiated with Co^{60} γ-rays (Fig. 2).

By comparing the data on the dynamics of the formation of oxidized lipid products and of NR accumulation (see this collection, pages 112 and 161), it can be noted that the activity of stable lipid radiotoxins found during the development of radiation lesion is connected with the production of oxidized products of unsaturated fatty acids. In the first period of radiation lesion considerable accumulation of stable lipid oxidation products is not found by the TBA method, which confirms the data of Kolomiytseva and Kuzin [9].

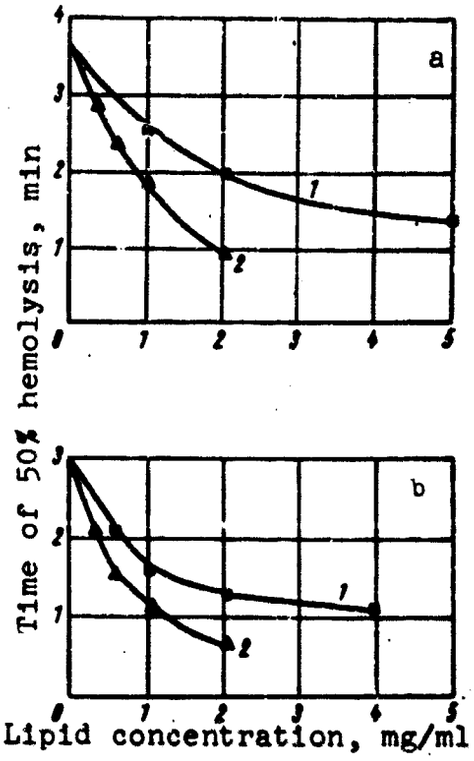

Fig. 1. The effect of lipids isolated from the liver of control rabbits (curve 1) and NR (curve 2) isolated from the liver of rabbits irradiated in a dose of 1000 rem with protons with an energy of 130 Mev (graph a) and fission neutrons (graph b) at the time of 50% erythrocyte hemolysis (by the electrogram method).

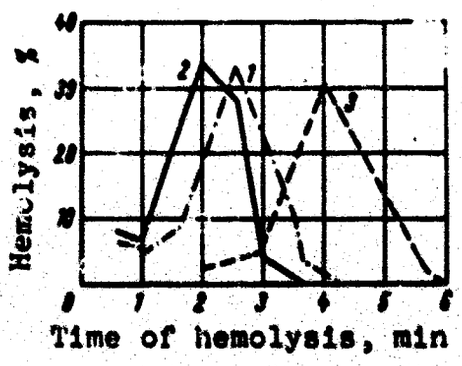

Fig. 2. Erythrograms with lipids of control animals (curve 1) and with NR of rabbits which received doses of 1000 rem of Co^{60} γ-rays (curve 2), as well as without the addition of lipids (curve 3).

As the experiments showed (see this collection, page 112), lipid radiotoxins of the initial stages of radiation lesion are unstable short-lived products of radical type, capable of producing the indirect and remote effects of ionising radiation (see this collection, page 112).

Ionizing radiation with different LSP values when acting in equivalent lethal doses causes generally similar changes in the dynamics of oxidized lipid product formation. The activity of NR isolated from rabbit livers on the third day after superlethal irradiation with ionizing radiation with different LSP is also approximately the same. Thus, certain differences in the primary reactions of radiation lesion for rarely and densely ionizing emissions are not connected with the general activity of lipid radiotoxins, but evidently depend on the local concentration of active molecules and radicals in the tracks of the ionizing particles. Further study of the question of NR distribution in the organs and tissues of animals irradiated with ionizing emissions which differ in LSP will be an interesting task, considering the observed differences in the mortality periods and the susceptibility of the different systems to injury (for example, the hemogenesis system and the intestines) [5].

The following conclusions can be drawn from the results obtained:

1. The accumulation of lipid oxidation products in the liver of rats irradiated in a dose of 800 rem with protons with an energy of 660 Mev, fission neutrons and Co^{60} γ-rays takes place in the same way.

2. The activity of NR isolated from the liver of rabbits irradiated in a dose of 1000 rem with protons with an energy of 130 Mev, fission neutrons and γ-rays is also approximately the same.

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FOOTNOTES

139 [1]The decrease in proton energy from 660 to 130 Mev is caused by the need to dilute the proton beam in order to obtain a sufficiently uniform field of irradiation. 130 and 660 Mev protons are approximately the same in LSP and biological efficiency [5, 6].

THE LIPID RADIOTOXINS OF YEAST CELLS

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It has been shown in previous work (see [1] and this collection, pages 112 and 138) that radiation sickness of animals is accompanied by the accumulation of active toxic products of lipid nature in organs and tissues.

Yu.B. Kudryashov and co-workers in 1962 named the acetone fraction of a lipid infusion of livers of irradiated animals "natural radiomimetic," taking into account that it is capable of causing changes similar to those which ionizing radiation produces [1]. In studying the mechanisms of the appearance and action of lipid radiotoxins [RT](PT) in the animal organism, one of the most unclear questions was that of the origin of the natural radiomimetic. The existence of several methods of its production can be assumed a priori, for example, the decomposition of lipoprotein complexes of injured cells [1], redistribution of the supplies of unsaturated fatty acids in the organism [3], a disturbance in the biosynthesis of proteins and carbohydrates with a switch to fatty acid synthesis [4-6] and others. The complexity of this question is due to insufficient study of the physiological, hormonal and other regulators of lipid metabolism both in the normal and the irradiated animal organism. In connection with this, a search for lipid RT in isolated cells in which there are no such methods of metabolism regulation seemed of interest.

Haploid and diploid yeast cells were used as the subjects in this work. These cells are frequently used as the subject in studying effect of ionizing radiation (see, for example, Reference [7]). Many investigators have observed lipid accumulation in irradiated yeast cells [6, 3, 9]. The nature of the lipophanerosis of irradiated cells still remains unclear [9]. In the opinion of some authors [9, 10], lipids play a protective role in the radiation injury of yeast cells. On the other hand, it has been shown [11, 12] that oxidized oleic acid products when added to yeast cells have a strong toxic effect. Oxidation products of methyl linoleate which gave a characteristic reaction with thiobarbituric acid showed the same effect in an experiment with yeast cells [13]. In the experiments of Kovyazin et al. [14], the addition of weakly oxidized oleic acid to yeast cells sensitized them to the effect of x-ray irradiation.

The principal goal of the present work was an attempt to find toxic lipid products in yeast cells irradiated with different doses (first series of experiments). Since there already is data [9] concerning the change in the lipid metabolism of irradiated yeast cells which have been transferred to nutrient medium, the second series of experiments concerned the activity of lipid RT in yeasts transferred after irradiation into brewer's wort. A third series studied the activity of lipid RT in yeasts kept after irradiation under conditions favoring postradiation recovery [7, 15].

Haploid and diploid yeast cells of the Saccharomyces cerevisae strain were grown in sufficient numbers in a 100-liter fermeter in Rider's nutrient medium for 48 hrs. After this the yeast cells were removed from the medium by separation with subsequent centrifugation. A 10 g sample of the wet residue was irradiated in a GUT-10-400 apparatus at a dose rate of 1 krad/min in doses of 10, 60 and 120 krad. After irradiation the cells either were immediately used for lipid extraction or were placed for 1 and 10 hrs in brewer's wort or for 20 hrs in tap water. Before extraction of the lipids the cells were subjected to "osmotic shock," for which they were transferred to 10 ml of a 10% NaCl solution for 5 min and sedimented by centrifugation, then the supernatant liquid was poured off and 50 ml of distilled water added. After a second sedimentation of the cells by centrifugation and removal of the supernatant liquid a four-fold volume of a chloroform-methanol mixture (in a proportion of 2:1) was added to the cells and mixed on a shaker for 4 hrs. Then the sediment was removed by centrifugation and discarded, and the supernatant liquid was evaporated under vacuum in a film evaporator. The lipids extracted by the chloroform-methanol mixture were poured into 10 ml of acetone and after mixing were left overnight at a temperature of 5°. In the morning the phospholipids which had gone into the sediment were removed by filtration and the filtrate again evaporated under vacuum. The toxicity of the lipid products of the acetone fraction which were isolated was evaluated by the erythrogram method [1].

The survival rate of the yeast cells was determined in all the samples before extraction of the lipids by the microcolony method [16].

Data on the survival of diploid and haploid yeast cells after irradiation and maintenance under different conditions are presented in the table.

Since the yeast cells were irradiated in the form of a very dense suspension, conditions were created for manifestation of the "concentration effect" which, as is known [19], is a particular manifestation of the "oxygen effect." As a result of the anoxia of the cells during irradiation, their survival corresponds to the survival of cells irradiated with sufficient aeration with 2.5-3 times smaller doses.

The survival of diploid yeast cells in our experiments exceeds the survival of haploid yeast cells by several times, which corresponds to the literature data [7] on the radiosensitivity of cells with different numbers of chromosomes. Incubation in all

The Survival Rate of Diploid and Haploid Yeast Cells After Irradiation and Maintenance Under Different Nutrient Conditions (95.0 ± ± 0.5% in the Control)

1	2 Выживаемость при разных дозах облучения, %					
	10 крад 3		60 крад		120 крад	
	4 диплоидные	5 гаплоидные	диплоидные	гаплоидные	диплоидные	гаплоидные
6 После сразу после облучения	91,00±5,55	63,00±5,72	30,00±3,54	23,00±3,60	20,00±1,81	2,00±0,18
7 Инкубация перед посевом:						
8 в сусле 1 ч	93,00±6,80	49,00±4,45	56,00±5,03	14,00±1,27	14,00±1,20	4,00±0,36
9 в сусле 20 ч	85,00±7,71	46,00±4,18	43,00±3,90	16,00±1,45	21,00±1,90	5,00±0,45
10 в воде 20 ч	98,00±7,10	47,00±4,80	72,00±6,70	17,00±2,30	46,00±4,80	4,00±0,50

1) Conditions of maintaining yeast cells after irradiation until inoculation on nutrient agar; 2) survival rate at different doses of irradiation, %; 3) krad; 4) diploid; 5) haploid; 6) inoculation immediately after irradiation; 7) incubation before inoculation; 8) in wort 1 hr; 9) in wort 20 hrs; 10) in water 20 hrs.

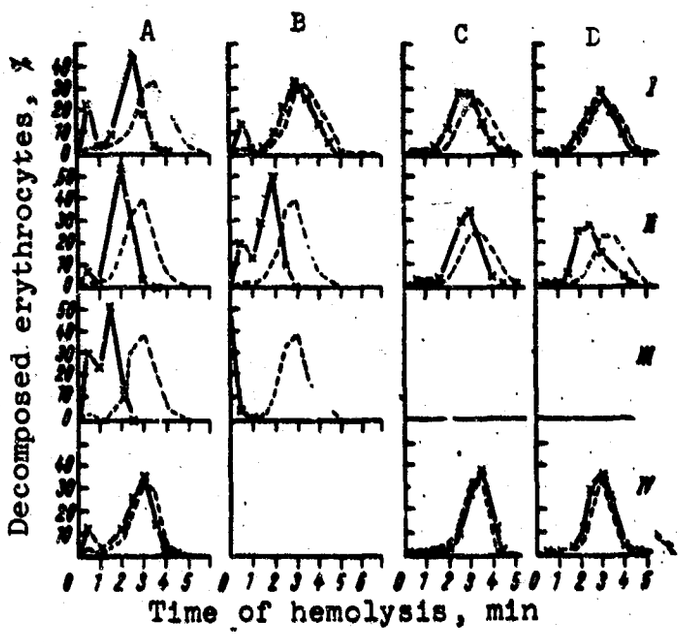

Fig. 1. Activity of lipid RT isolated from irradiated diploid yeast cells. I) Extraction immediately after irradiation; II) extraction after 1 hr incubation in wort; III) after 20 hrs in wort; IV)after 20 hrs in water. A, B, C and D) Doses of 10, 40, 60 and 120 krad, respectively.

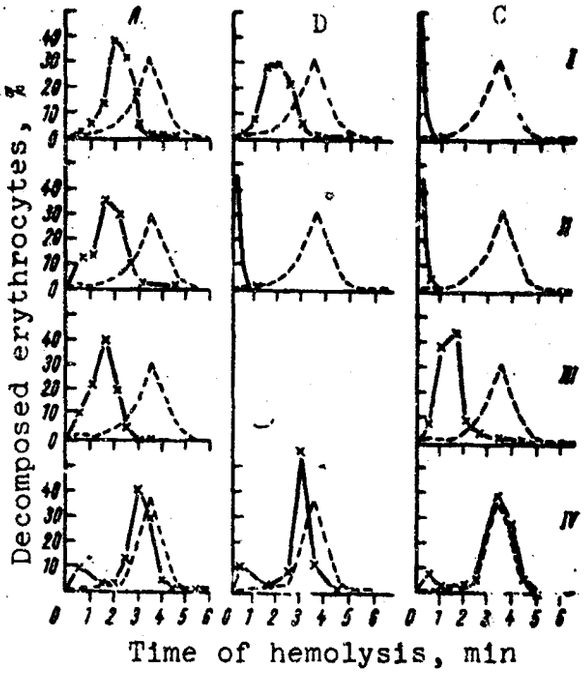

Fig. 2. Activity of lipid RT isolated from irradiated haploid yeast cells. (Notation same as in Fig. 1).

three variants of the experiments before inoculation of the haploid yeast cells had practically no effect on their survival. Incubation of diploid yeast cells in wort for 1 and 20 hrs also produced little difference in survival. When diploid yeast cells were incubated in tap water after irradiation an increase in their survival occurred as a result of manifestation of the phenomenon of "post-radiation recovery."

Data on the hemolytic activity of lipid products isolated from yeast cells are presented in Figs. 1 and 2. Two erythrograms are presented for each dose of irradiation and all incubation conditions of the yeast cells. The erytnrogram for erythrocytes incubated with lipids isolated from yeast cells is shown by the dotted line, and the erythrogram for erythrocytes which were first incubated for 15 min at 37° with lipids isolated from irradiated yeast cells by the unbroken line.

It can be seen from Fig. 1 that irradiation of diploid yeast cells leads to the appearance of active lipid products which sensitize erythrocytes to the hemolytic effect of hydrochloric acid. With an increase in the irradiation dose the activity of the extracted lipids increases. The activity of the extracted lipids increases still more if the cells are incubated in wort for 1 or 20 hrs after irradiation. The maintenance of diploid yeast cells after irradiation in tap water somewhat decreases the hemolytic activity of the lipids extracted from them.

A similar picture is observed in the case of the irradiation of haploid yeast cells (Fig. 2). Some differences in the results presented in Fig. 1 and 2 can only be noted in the effect of the irradiation dose on the activity of extracted lipids: above 40 krad the activity of the extracted lipids does not increase, and even becomes less than from irradiation with a dose of 10 krad. It can be assumed that this is connected with the fact that such irradiation doses are very high for haploid cells and, consequently, only budding cells which are always present in the population, comprising approximately 20-30% of the total number of cells, survive. However, in experiments on haploid yeast cells after irradiation the appearance of active lipid products, whose activity increases during incubation in wort, is observed quite clearly. The activity of the lipid products isolated from haploid cells incubated after irradiation in tap water, changes little in comparison with the activity of lipids isolated from unincubated haploid cells.

Thus, the experiments showed that irradiation of isolated cells leads to the appearance of toxic lipids in them. The activity of the lipids extracted from diploid yeast cells increases with an increase in the irradiation dose. For haploid and diploid strains of cells, placing the cells after irradiation in nutrient medium leads to an increase in the activity of the lipid RT. Placing the diploid and haploid cells in tap water after irradiation does not produce a noticeable change in the activity of the lipid products.

It can be assumed that the production of lipid RT under the conditions of our experiments occurs by two methods. The lipid RT extracted from cells immediately after irradiation appear as a result of physicochemical processes leading to the decomposition of lipoprotein complexes with the liberation of active products of the unsaturated fatty acid type and their oxidation products. When the cells are transferred to a nutrient medium after irradiation active lipid products also appear as a result of a disturbance in lipid metabolism.

Lipid RT are isolated from yeast cells by the same method as NR is obtained from irradiated animal tissue and are similar to them in effect on human erythrocytes. Evidently this is connected with the fact that they both are similar products of lipid oxidation. Characteristic data on the appearance of the thiobarbituric acid reaction which is specific for products of unsaturated fatty acid oxidation with lipid products isolated from yeast cells also confirm this assumption.

The following conclusions can be drawn from this work:

1. After γ-irradiation of diploid and haploid yeast cells, toxic lipid products appear in them.

2. The activity of lipid RT isolated from diploid yeast cells increases with an increase in the irradiation dose.

3. Incubation of irradiated yeast cells in nutrient medium leads to an increase in the activity of the lipid RT extracted from

them.

4. Incubation of irradiated yeast cells in tap water has little effect on the activity of the lipid RT extracted from them.

5. Lipid RT isolated in experiments with yeast cells give a specific reaction to products of unsaturated fatty acid oxidation, which speaks of the similarity of their chemical nature to that of RT isolated from animal tissue.

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THE SPECIFICITY OF THE PRODUCTION OF TOXIC LIPID SUBSTANCES (THE EFFECT OF VIBRATION, ELECTRONARCOSIS AND RADIATION)

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One of the interesting and important questions of radiation biology and medicine is the question of the combined action of factors of nonradiation nature and ionizing radiation on living organisms.

It has been shown in previous work [1, 2] that ionizing radiation causes the production of toxic substances responsible for the development of radiation injury. It has also been established that lipid radiotoxins [RT](PT) capable of causing the formation of other toxic agents play a leading role in general radiation toxemia.

However, the question of the specificity of lipid RT production has still not been resolved. Are other physical factors capable of causing the production of toxic lipid substances? Is lipid RT production a nonspecific reaction of the organism to external influences?

The purpose of the present research was to study the effect of electrical narcosis and vibration on radiation injury and the change in some radiation effects, in particular, the production of toxic lipid substances, from their effect.

The experiments were carried out on white male rats weighing 120-140 g, which were kept under the usual vivarium conditions. Electroimpulse generators of the EI-1 and UEI-1 types were used to create electric narcosis. The electric current was supplied through platinum electrodes with a cross-section of 5 × 5 mm attached to the temporal lobes of the cranium and continually moistened with physiological solution. Electrical narcosis was created in a wide frequency range (from 200 to 2500 cps) at a current strength of 4-20 ma and impulse duration of 0.1-0.5 msec. The method of electronarcosis was developed jointly with K.A. Iordanis. Vibration of the animals was carried out on a vibrastand with a frequency of 70 cps at an acceleration of 10-12 g; the duration of the vibration was 2 hrs.

The oxidation-reduction potential was measured on the shin triceps muscle on a LP-57M tube potentiometer with a polished platinum needle electrode. A saturated calomel half cell served as the comparison electrode. The value of the oxidation-reduction potential, *Eh*, was expressed in the form of ΔEh in millivolts. In studying the "oxygen effect" the oxygen pressure was measured with a pair of the same electrodes on an LP-60 type polarograph with a self-recording unit.

Isolation of lipids from the liver of rats subjected to electronarcosis, vibration and radiation and their extraction was carried out according to the well-known method [3]. The activity of the preparations isolated was determined by the erythrogram method, modified for analysis of tissue lipids in Kudryashov's laboratory [4].

As seen from Table 1, electronarcosis can be produced in rats only under specific experimental conditions. Low current frequencies (25-100 cps) caused instantaneous death of the rats.

The deepest electronarcosis occurs at a frequency of 800 cps, current strength of 10-12 ma, length of created rectangular impulses 0.2 msec and duration of current transmission 10 or more min (see Table 1). At these parameters of electronarcosis the most profound and short-lived decrease in the oxidation-reduction potential occurs in muscle tissue (200 mv), as well as a more prolonged decrease in the oxygen level (40%) (Fig. 1).

TABLE 1

Change in the Oxidation-Reduction Potential From Various Conditions of Electronarcosis (duration of narcosis, 10 min)

1	2	3	4	1	2	3	4
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
200	8	0.2	—40	400	4	0.5	—100
200	5	0.2	—15	800	10—12	0.2	—200
400	6	0.5	—	800	10	0.2	—370

1) Current frequency, cps; 2) current strength, ma; 3) duration of impulses, msec; 4) ΔEh in muscle, mv.

The described electronarcosis causes a short-lived flare in the production of toxic lipid substances (Table 2) which are oxidised products of unsaturated fatty acids in nature - mainly peroxides and epoxides (Fig. 2).

The toxic lipid substances are produced during the first 8-10 min after electronarcosis, and it was not possible to find them later.

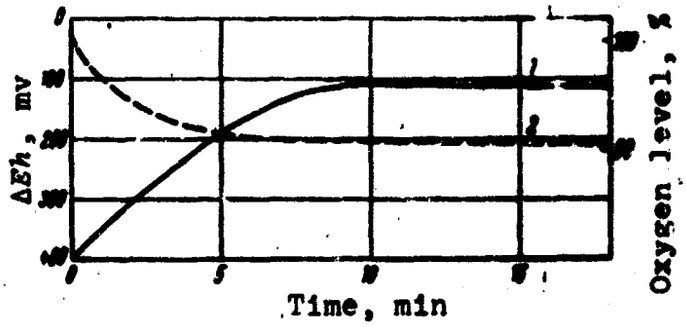

Fig. 1. Change in *Eh* and oxygen level in rat muscle after electronarcosis. 1) Oxygen level; 2) oxidation-reduction potential.

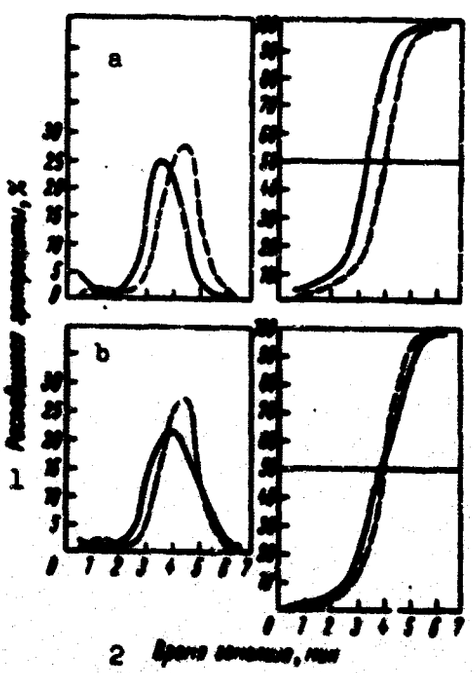

Fig. 2. Hemolytic activity of rat liver lipids after electronarcosis in the first 2 min (a) and after 5 min (b). Unbroken line – experimental; dotted line – control. 1) Decomposed erythrocytes, %; 2) hemolysis time, min.

However, after vibration, short-lived production of toxic lipid substances also occurs in the animals in later periods. After vibration of rats for 2 hrs, just as after electronarcosis, a decrease is found in the oxidation-reduction potential and in the oxygen level of the tissues.

As seen from Fig. 3, the greatest activity of the toxic lipid substances is observed in the first 24 hrs after vibration, and more accurately, in the first 2 hrs. In the next period toxic lipid substances are not found. For comparison of the data obtained the dynamics of the change in the level of toxic lipid substances after

TABLE 2

Activity of Lipid RT Isolated from Rat Livers After Effect of Electronarcosis

1 Растворитель	0–6 мин 2			10–60 мин		
	3 Опыт	4 Контроль	5 Разность	Опыт	Контроль	Разность
6 Смесь хлороформа с метанолом	3.5 3.0 3.4 3.9	3.9 2.9 3.6 4.1	+ — + +			
7 Хлороформ	3.5 1.5 2.5 3.4 3.5 3.8 1.8 4.9	4.1 3.5 4.5 3.9 3.9 3.9 3.9 4.7	+ + + + + + + —	3.7 3.3 4.0 3.8 3.9 3.8 4.5 4.9	3.9 3.9 3.9 3.9 3.9 3.9 4.7 4.8	+ + — + 0 + + —
Хлороформ	4.6 3.8 4.5 3.1 3.1	4.8 4.7 4.8 3.2 3.2	+ + + + +	4.2 4.6 4.8 3.6	4.7 4.7 4.8 3.2	+ + 0 —
8 Петролейный эфир	2.4 3.0 3.1 3.1	3.1 3.1 3.2 3.2	+ + + +	3.2 3.6	3.1 3.2	+ —
9 Достоверность	10 Достоверно с вероятностью от 95 до 97.5%			11 Недостоверно		

1) Solvent; 2) min; 3) experimental; 4) control; 5) difference; 6) mixture of chloroform and methanol; 7) chloroform; 8) petroleum ether; 9) reliability; 10) reliable with probability of from 95 to 97.5%; 11) not reliable.

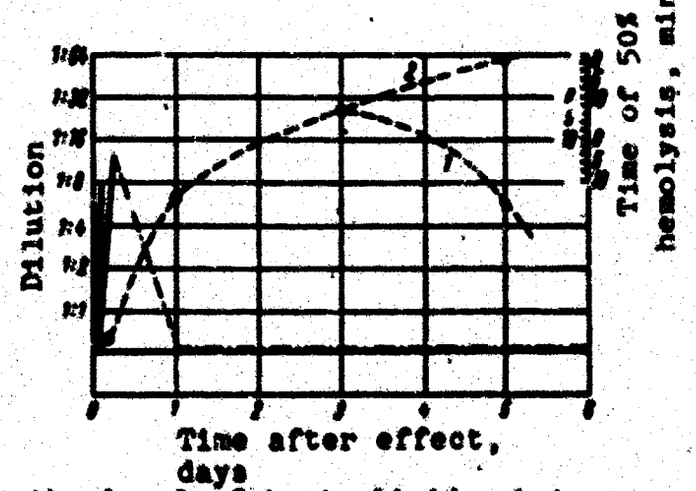

Fig. 3. Change in the level of toxic lipid substances after the effect of radiation, vibration and electronarcosis. 1) Irradiation; 2) control. Dot and dash line – vibration; unbroken line – electronarcosis.

irradiation of the rats with ionizing radiation, the effect of vibration and electronarcosis is presented in Fig. 3. As can be noticed on this figure, lipid RT are found throughout the entire period of radiation injury.

In the surviving animals the lipid RT gradually decrease their activity (curve 1), and in the terminal period the activity which is found increases (curve 2). The results on the change in toxic lipid substances in animals during radiation injury completely confirm data already known from the literature [1, 2].

It follows from the results obtained that various electrical and mechanical factors are capable of causing the production of toxic lipid substances. However, in contrast to ionizing radiation, the production is short-lived and is evidently connected with typical stress changes. The specificity of the production of toxic lipid substances after the action of ionizing radiation lies in the fact that in radiation injury production of lipid RT which increases with time occurs.

It is interesting to note that if the animals are subjected to the effect of electronarcosis or vibration before irradiation with ionizing radiation (or at the moment of irradiation), a slight radioprotective effect is observed. The best conditions for survival of the animals in the case of vibration are: 1 hr before irradiation the dose increase factor [DIF] (ФУД), that is, the ratio of the semilethal doses in the experimental to the control is increased to 1.2, in 3 hrs – to 1.09. From the effect of electronarcosis at the moment of irradiation the DIF corresponds to a value of 1.2. On the basis of the data obtained it is interesting to note that the degree of the decrease in the value of the oxidation-reduction potential from electronarcosis is more than from known chemical radioprotectors [5]. However, this decrease in the potential is short-lived (see Fig. 1). Considering this, as well as noting the stress effect of electronarcosis which is additional to radiation, it is evidently possible to explain the slight prophylactic protective effect of the electrical factor.

In the case in which the stress agents act after radiation, it is possible to observe an intensification of the radiation injury which is manifested in an increase in the number of dead animals.

Evidently, the effect of protection by preliminary electronarcosis or vibration can be explained by the condition of hypoxia which develops in the rat organism and, consequently, by the oxygen effect. The intensification of the radiation injury is evidently connected with an intensification of the primary processes of the radiation injury which are manifested in the lipids.

The following conclusions can be drawn:

1. Electronarcosis and vibration cause a drop in the oxidation-reduction potential and a prolonged decrease in the oxygen level in the tissues.

2. The effect of stress agents causes the production of toxic lipid substances, however, their production is short-lived.

3. The specificity of the production of toxic lipid substances after the effect of ionizing radiation lies in the fact that in radiation injury in contrast to the effect of electronarcosis and vibration, the production of lipid RT increases with time.

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THE JOINT EFFECT OF RADIATION AND AN UNSATURATED FATTY ACID ON ERYTHROCYTES

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Data on the possible participation of lipid radiotoxins [RT] (PT) in the primary reactions of radiation injury have been presented previously [1]. It was shown on a chemical model [2] that solutions of β-carotene in oleic acid have high radiosensitivity in comparison with solutions of the same compound in various other substances. The hypothesis was expressed that an indirect effect of radiation on β-carotene molecules occurs as a result of the production of short-lived primary RT in the solvent – oleic acid. It can be assumed on the basis of these data that primary lipid RT are also capable of causing the injuries of living irradiated cells. In order to verify this hypothesis experiments were set up on a study of the joint effect of radiation and oleic acid on erythrocytes.

Oleic acid, first freed of impurities by vacuum distillation, was used in the experiments. An emulsion of oleic acid in buffer solution, isotontic blood plasma, was prepared according to a previously described method [3]. Human erythrocytes were always washed out of the plasma on the day of the experiment. Irradiation was carried out in doses of 0.5-1.5 and 78 kr with Co^{60} γ-rays in GUT-Co-400 equipment.

The stability of the cells after the applied effects was recorded by the erythrogram method [4]; the reading of the reaction time in all the experiments was carried out from the moment of the addition of oleic acid. In the case in which the erythrocytes were treated with sublytic concentrations of the fatty acid, damage of the erythrocytes was evaluated by their capacity for subsequent hydrochloric acid hemolysis.

In the first series of experiments sublytic concentrations of oleic acid of the order of 10^{-5} M were used. The suspension of erythrocytes in oleic acid was irradiated with a dose of 78 kr and incubated at a temperature of 37°. After different time intervals the change in the stability of the erythrocytes to a standard hemolytic agent – hydrochloric acid – was examined. Erythrocyte suspensions, irradiated without oleic acid, as well as unirradiated cells with oleic acid, served as the control.

TABLE 1

Hydrochloric Acid Hemolysis After the Joint Effect of Radiation and Oleic Acid on Erythrocytes (the time of 50% hemolysis is indicated in half minutes)

1 Номер опыта	2 Время инкубации, мин	3 Воздействие: 4 радиации	5 олеиновой кислоты	6 суммарное	7 «Слепая» проба	8 Дозы, кр
1	15 30 60 120	7,8 6,7 7,4 —	6,6 7,3 7,2 5,4	6,8 4,5 5,5 —	10,5	25
2	15 30	6,3 —	5,1 3,6	4,9 0,0	8,5	65
3	15 30 60	7,2 6,6 —	8,4 8,6 —	7,6 6,2 6,7	9,9	78
4	15 45 120	7,6 6,4 7,5	6,0 5,7 4,9	4,4 4,6 3,3	9,9	78
5	30 180	7,9 7,1	5,2 4,0	5,0 0,0	10,6	78
6	30 240	7,0 7,2	6,7 5,2	6,6 3,3	7,9 7,2	78

1) Exp. No.; 2) incubation time, min; 3) effect; 4) of radiation; 5) of oleic acid; 6) total; 7) "blind" sample; 8) doses, kr.

As seen from Table 1, the resistance to hydrochloric acid of erythrocytes irradiated in the absence of oleic acid at the dose which we selected did not change in the first 3 hrs of the experiment. The resistance of unirradiated erythrocytes after their incubation with oleic acid also did not change significantly during the same time. In contrast to this, in those experiments in which the erythrocytes were irradiated in the presence of oleic acid, upon subsequent incubation for 3 hrs the cells' resistance to hydrochloric acid decreased sharply. In this case it was possible to establish a considerable change in the resistance of erythrocytes to oleic acid after their preliminary irradiation.

Considering the significant intensification of oleic acid hemolysis of irradiated erythrocytes which takes place, in the next series of experiments 0.5-1.5 kr doses of radiation were used, but lytic concentrations of oleic acid ($1 \cdot 10^{-4}$ M) were taken. According to the data presented in Table 2, the effect of radiation on erythrocytes during their irradiation in the presence of oleic acid is stronger than the injury of the cells by radiation and

TABLE 2

Effect of Radiation on Oleic Acid Hemolysis (time of 50% destruction of cells is indicated in half minutes)

1 Номер опыта	2 Действие олеиновой кислоты на эритроциты		Облучение системы эритроциты/ с олеиновой кислотой 5	1 Номер опыта	2 Действие олеиновой кислоты на эритроциты		Облучение системы эритроциты с олеиновой кислотой 5
	необлучен. 3 мин	облучен. 4 мин			необлучен. 3 мин	облучен. 4 мин	
1	15,5	10,8	11,5	4	25,4	18,4	13,7
2	15,5	10,8	8,7	5	24,3	18,0	12,2
3	13,4	11,5	8,0	6	18,0	12,2	10,5

1) Exp. No.; 2) effect of oleic acid on erythrocytes; 3) unirradiated; 4) irradiated; 5) irradiation of erythrocyte-oleic acid system.

oleic acid in the case of their consecutive action. However, this difference is not great and was observed for oleic acid concentrations between $5 \cdot 10^{-5}$ and $5 \cdot 10^{-4}$ M.

It is interesting to note that the energy of activation of the reaction of destruction of erythrocytes irradiated in the presence of oleic acid, in comparison with their destruction from the effect of oleic acid without irradiation of the system is approximately 20% lower, namely, in the first case it has a value of approximately 5 kcal/mole, and in the latter – about 6-7 kcal/mole. The effect of an inhibitor of free radical systems, 3,5-ditertiarybutyl-4-oxy-2-methylbenzylamine[1], on the erythrocyte destruction reaction in the case of the joint action of oleic acid and radiation was investigated. The experiments showed that injury was decreased by the preliminary addition of the above-mentioned compound. As seen from the figure, the decrease in injury is greater in this case than the decrease in injury of the erythrocytes by oleic acid without irradiation of the system.

The data obtained indicate that in the presence of oleic acid an intensification of radiation's effect on erythrocytes occurs. The decrease in the energy of hemolysis activation in an irradiated mixture in comparison with an unirradiated mixture, as well as the greater effectiveness of free radical inhibitors during irradiation may testify in favor of the production of active products in the samples during their irradiation, and in particular, in oleic acid. It is possible that these active products produced in oleic acid are capable of carrying out an indirect mechanism of radiation's effect on the cell, being primary RT.

It should be noted, however, that the use of cell models introduces additional difficulties in evaluating the mechanism of the production and effect of lipid RT in comparison with models at the molecular level. This is connected with the fact that, in addition to RT production in oleic acid, disturbances also occur in the ir-

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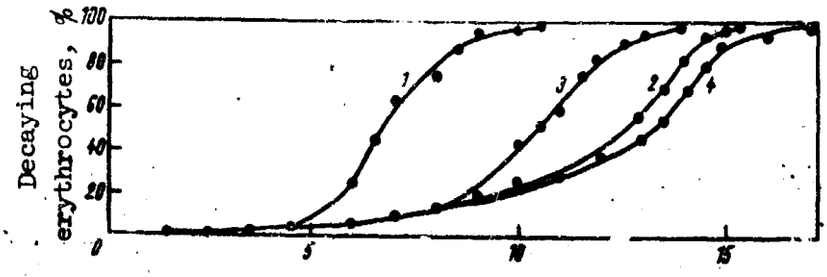

Kinetic curves of hemolysis of irradiated (curves 1 and 2) and unirradiated (curves 3 and 4) erythrocytes from the effect of oleic acid in the presence of 3,5 ditertiarybutyl-4-oxy-2-methylbenzylamine (curves 2 and 4) (dose of 1.5 kr, inhibitor concentration $1 \cdot 10^{-3}$ M, oleic acid concentration $1 \cdot 10^{-4}$ M).

radiated erythrocytes. It is possible that the effect of the active products of irradiated oleic acid is manifested even more strongly after a radiation change in the stroma of the erythrocytes. Further experiments must be devoted to an explanation of this question.

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FOOTNOTES

159 We should like to express our gratitude to Ye.B. Burlakova for the inhibitor preparation.

THE TOXICITY OF LIPIDS IN ANIMAL RADIATION SICKNESS

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According to numerous papers presented in this collection, various toxic compounds affect the development of injuries of living irradiated organisms. The work of Kudryashov et al. [1] in which evidence is presented that radiotoxins [RT](PT) of lipid nature participate in primary processes occurring during the radiation effect is of great interest to this question. Recently, toxic lipid compounds have been found in tissues of animals irradiated with radiation of different ionization densities (see this collection, page 138). Hence it is clear that a thorough study of the question of the role of the toxic factor of lipid nature in the development of animal radiation injury is of special importance.

In broad aspect the question of the role of toxemia in the development of radiation sickness of various living forms is closely connected with the study of the mechanisms through which the physicochemical changes occurring at the molecular level cause disturbances in biological functions. It has been established that in vitro irradiation of lipids in an oxygen atmosphere causes the development of oxidation reactions which depend on the composition of the unsaturated fatty acids [2]. The literature also contains indications that peroxides [3, 4] and free radicals [5] are also produced in lipid fractions of irradiated animal tissues. Moreover, destruction of tissue antioxidants has been found [6, 7].

Considering the physicochemical changes in the lipid components of the tissues of irradiated organisms, the widely known fact that lipids occupy one of the leading places in the structural organization, energetics and functional activity of every living cell should also be noted. Numerous data in the literature indicate the dynamic nature and lability of the tissue lipids, including in stress states caused by diverse factors [8, 9]. Disturbances in lipid metabolism frequently are attributed to early and radiosensitive reactions of living irradiated organisms [10, 11]. Thus, radiation damage of tissue lipids of the animal organism, on the one hand, to a certain degree is determined by the chemical reactivity of these cell components, and on the other hand, it probably should have a specific effect on their function-

al activity. The question of the toxicity of lipids in animal radiation sickness must be approached from these standpoints.

Toxic substances of lipid nature in the tissues of irradiated mice and rats were first detected and studied by a group of B.N. Tarusov's co-workers [12], who used the hemolytic test to determine their activity, while Kudryashov [13] showed that in chemical nature these hemolytically active compounds are unsaturated fatty acids. Unfortunately, as a rule these authors used a method of recording the activity of the tissue hemolysins of irradiated animals which involved prolonged preliminary incubation of the tissue homogenates with erythrocytes. For a long time the very fact of the production of a toxic factor of lipid nature in an irradiated organism caused doubt in the literature. The opinion was expressed that the detection of hemolytically active compounds is only a result of the intensification of irradiated tissue decomposition during its posthumous treatment [14]. To explain the question of the production of a toxic factor in the lipids of irradiated animal organisms, the following goals were established in the present work: 1) to study the hemotoxic properties of an unsaturated fatty acid (oleic) and its oxidation products, and 2) to investigate the conditions of the production of toxic lipids in irradiated animal organisms and attempt to evaluate their role in the development of the sickness.

Lipids from the livers of irradiated and healthy (as a control) animals served as the material for study. Oleic acid, unoxidized and oxidized in an air current at a temperature of 60° was used in model experiments. There was a total of about 100 rabbits in the experiments.

The hemolytic test was selected as the principal biological criterion of toxic activity. The erythrogram method developed by Terskov and Gitel'zon [15] was used. In addition, the effect of oleic acid and tissue lipids on the state of phosphorylation combined with oxidation in mitochondria isolated from the pectoral muscle of pigeons by methods described in Reference [16] was examined. In order to study the mechanisms of toxic lipid production, their hemolytic activity during autolysis, as well as the antihemolytic properties of tissue antioxidants - phospholipids - were determined. Autolytic breakdown of the tissues during incubation (at 37°) was judged from the rate of free fatty acid (by the titration method) and amino acid accumulation [17].

The experiments showed that oleic acid is a strong hemolytic agent - it causes hemolysis at concentrations of the order of $1 \cdot 10^{-4}$ M, that is, in amounts higher by a factor of 10^2 than of the choline and histamine RT known in the literature. At concentrations of $1 \cdot 10^{-6}$ M, this same fatty acid, while not destroying the cells, considerably decreases their resistance to the subsequent action of another hemolytic agent -- hydrochloric acid (at a concentration of $4 \cdot 10^{-3}$ M).

Oxidation of oleic acid increases its hemolytic effect, changing the form of the erythrogram and kinetic hemolysis curves

(Fig. 1). Subthreshold lytic concentrations of oxidized oleic acid and, in addition, of chemically pure peroxides, aldehydes and ketones changed the resistance of erythrocytes treated with them to subsequent hemolysis by hydrochloric acid, in the same way as did the unoxidized acid. The effectiveness of sublytic concentrations of the fatty acid and its oxidation products on the degree of erythrocyte resistance remained constant in the temperature range of 0-37° and did not depend on the volume of liquid in the incubated mixture. These facts made it possible to assume that the hemolytic activity of oleic acid depends on its fixation on cellular structures. This assumption is supported by data from the literature [18, 19] on the irreversibility of fatty acid hemolysis and its dependence on the number of erythrocytes and their structural properties. The phenomenon which was found made it possible to determine objectively the hemolytic activity of compounds in lipid tissue extracts.

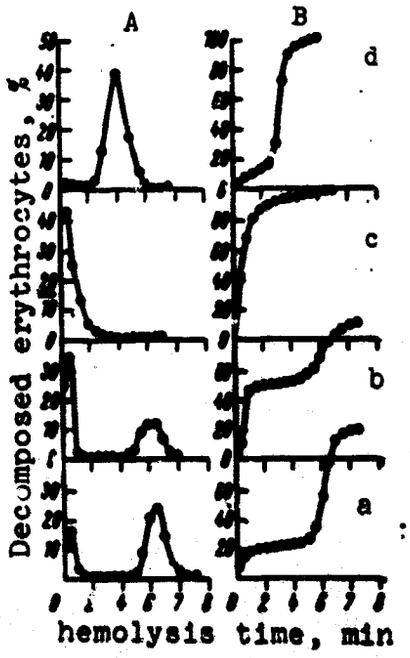

Fig. 1. Hemolytic activity of oleic acid at different oxidation stages. a) 2-4 hr oxidation; b) 24 hrs; c) 3 days; d) 7 days. A) Erythrogram; B) differential curves.

A quantitative determination of the toxicity of different lipid fractions of rabbit livers showed that a a concentration of 0.5-2.0 mg/ml lipids extracted by ether were weakly active, while their hemolytic activity increased considerably after precipitation of the phospholipids. The precipitated phospholipids hardly changed the rate of destruction of intact erythrocytes, whereas they sharply decreased the destruction of cells treated with active lipids.

As seen from the table, the toxicity of lipids obtained after precipitation of the phospholipids from the liver of irradiated rabbits increases in comparison with the control. In this case the

increase in the hemolytic activity of the tissue lipids corresponded to the increase in unsaturated fatty acids, while the decrease in the toxic properties of the lipids from the addition of antioxidants (phospholipids and cysteamine) indicates a certain dependence of these toxic properties on the oxidation of the lipids.

Hemolytic Activity of Rabbit Liver Lipids

Время после облучения 1	Гемолитическая 50%-ная активность, мин 2		Время после облучения 1	Гемолитическая 50%-ная активность, мин 2	
	3 Контроль	4 Опыт		3 Контроль	4 Опыт
1—2 ч 5	3.2±0.2	2.0±0.4	2 сутки 7	2.8±0.4	1.5±0.4
1 сутки 6	2.7±0.2	2.5±0.4	3 »	2.7±0.2	1.6±0.3

1) Time after irradiation; 2) 50% hemolytic activity, min; 3) control; 4) experimental; 5) hrs; 6) day; 7) days.

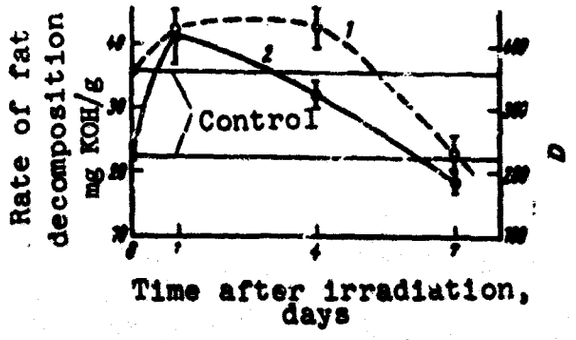

Fig. 2. Change in the rate of proteolysis (curve 1) and lipolysis (curve 2) in the liver in radiation sickness of rabbits (irradiation dose 700 r, rate of processes measured during 2 hrs incubation of the tissue at a temperature of 37°). *D*) Rate of protein decomposition.

At the same time it was shown that protein (proteolysis) and fat (lipolysis) splitting in the livers of irradiated rabbits increases in the first period of the lesion's development, and at the time of the animals' death the rate of these processes is decreased in comparison with normal (Fig. 2).

The results of the experiment clearly make it possible to assume that the increase in enzymatic processes (in particular, lipolysis) in irradiated animal tissue can to a certain degree affect the toxicity of the tissue lipids, at least in the first period of the lesion's development. It is interesting that during tissue autolysis the antihemolytic properties of the phospholipids decrease very rapidly which indicates their great lability. It is seen from Fig. 3 that the antihemolytic properties of the phospholipids decrease sharply in the first hour of the tissue's incubation, whereas an increase in the hemolytic activity in this tissue as well as in fatty acid concentra' 'on is noticeable only after

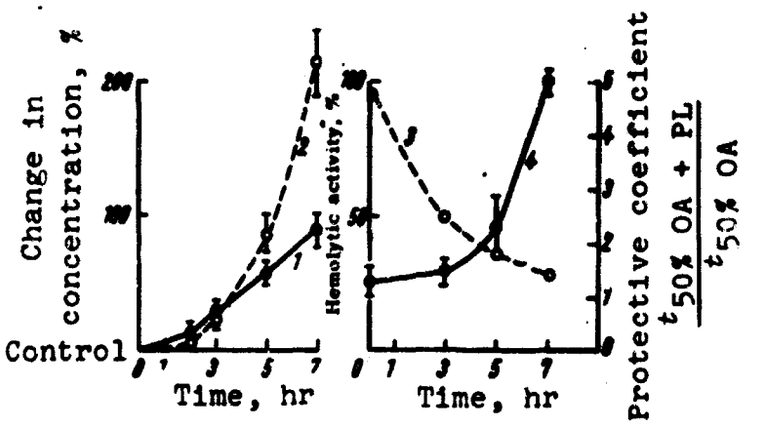

Fig. 3. Change in concentration of amino acids (curve 1), fatty acids (curve 2), antihemolytic activity of phospholipids (curve 3) and hemolytic activity of lipids (curve 4) during incubation of rabbit livers (rate of hemolysis evaluated from time of 50% cell distruction; the effect of the phospholipids judged from the degree of drop in rate of hemolysis).

2-3 hrs of incubation. It is logical to assume that there is a certain connection between the change in the rate of the enzymatic (autolytic) processes in irradiated animal tissues and changes in tissue antioxidants (phospholipids). Hence it is natural that the rate of spontaneous fatty acid oxidation, and consequently, their toxicity, must depend in some way on the rate of the autolytic process. It is known from References [20, 21] that fatty acids and their oxidation products are inhibitors of proteolytic enzymes. It is possible that this latter circumstance by a type of feedback is capable of causing inhibition of autolytic processes in irradiated animal tissues, which in the present work was observed before their death. Thus, in discussing the question of the production of the toxic factor in the tissues of irradiated animals it is clearly necessary to take into account not only the rate of enzymatic processes and the degree of spontaneous lipid oxidation, but also the reciprocal effect of these factors.

The fact that an increase in the toxic properties of the tissue lipids occurs during irradiation of animals is confirmed by testing their effect on isolated mitochondria. Tissue lipids not only decrease the hemolytic resistance of erythrocytes, but also change the functional state of the mitochondria by primarily inhibiting phosphorylation. These data confirm the correctness of our conclusions concerning the intensification of the toxic properties of lipids as a result of irradiating animals, and not only their hemolytic activity. It is interesting to note here that the level of oxidative phosphorylation is also decreased by oxidized acid and chemically pure cumene hydroperoxide, while an antioxidant - cysteamine - decreases the dissociative effect of oleic acid.

On the basis of our own data and material in the literature, it seems of interest to evaluate the role of toxic lipids in animal radiation sickness. There is still not sufficient clarity on the question of the place of toxic lipids in the overall set of pathological symptoms caused by the radiation effect: whether the observed changes are caused by physiological mechanisms or are chiefly due to chemical activity of the radiation, leading, in particular, to the oxidation of unsaturated fatty acids.

Recently, the opinion that the existence of unesterified fatty acids in the organism normally provides for the performance of some physiological functions has become more and more common. There is a hypothesis that fatty acids destroy aging erythrocytes [22] and, in addition, participate in regulating the level of the coupling of oxidation with phosphorylation [23]. The concentration of unesterified fatty acids in the animal organism, according to the data of a number of authors [8, 9], increases during the development of the "stress" adaptation syndrome.

A number of symptoms of radiation sickness, some investigators believe (see, for example, [24]) are a nonspecific reaction of the whole organism to an injurious action. There are indications [24] that protein decomposition increases during the development of the adaptation syndrome. It can be expected that the intensification of autolytic processes and the simultaneous increase in hemolytically active fatty acids in irradiated animal tissues noted in our experiments, as well as in the experiments of other authors [25], will turn out to be connected in some way with the adaptation syndrome.

According to data in the literature [18, 19, 25], under physiological conditions fatty acids have an effect on properties of the lipoprotein membranes of cells and tissues (for example, of mitochondria and erythrocytes). In connection with this it is interesting to note that in our joint experiments with Yu.B. Kudryashov and E.E. Slava the preliminary irradiation of erythrocytes even with such a small dose as 1 kr noticeably weakened their resistance to subsequent oleic acid hemolysis. The effect of oleic acid on erythrocytes, as the experiments showed, depends to a certain degree on its fixation on cellular structures and on oxidation. Evidently, for purposes of further study of the role of the toxic factor of lipid nature in the development of radiation sickness of living organisms a thorough investigation of the radiation damage of lipoprotein membranes of living cells, the interaction of fatty acids with them and the effect of these processes on the properties and biological functions of irradiated organisms is necessary.

In conclusion the following conclusions can be drawn:

1. The effect of oleic acid on erythrocytes depends on its oxidation, as well as on its fixation on cellular structures.

2. After irradiation of rabbits with a dose of 1 kr an increase occurs in the toxic properties of the liver lipids in act-

ing on erythrocytes as well as on isolated mitochondria.

3. An increase in the toxicity of the lipids occurs both in the first 2-4 hrs after the effect and immediately before the death of the animals. The rate of enzymatic autolytic processes increases in the first period after the effect and is lower than normal before the animals' death.

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MORPHOLOGICAL AND HISTOCHEMICAL CHANGES IN THE ORGANS OF ANIMALS SUBJECTED TO THE ACTION OF LIPID RADIOTOXINS

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Clarification of the question of what fine morphological and cytochemical changes occur in animal organs from the effect of radiation and lipid RT (PT), so-called natural radiomimetics, is of great theoretical and practical importance [1]. The question of similarities and differences in their effect on structural cell components and their cytochemical characteristics remains open.

In the present work a number of rat organs were examined after irradiation with γ-rays in a dose of 800 r, action by fast neutrons in a dose of 600 rad at an energy of 1 Mev and the injection of lipid RT obtained from the livers of rabbits irradiated with γ-rays and neutrons. Highly radiosensitive organs – intestines, liver and kidney and a less radiosensitive organ –the myocardium were investigated.

The effect of the indicated factors on cell structure and certain aspects of ribonucleic acid and lipid metabolism, as well as on the morphology of mitochondria, with which, as is known, oxidation, oxidative phosphorylation and ATP (АТФ) production are associated, was studied by cytological and histochemical methods.

There are numerous data in the literature on histological changes in tissues of irradiated animals [2-4]. Hemorrhages in the liver, cellular tissue, heart, lungs and intestines are observed in acute radiation sickness. Protein and fat decomposition occur in the liver and kidneys, radiation nephritis may develop and nephron filtration and resorption mechanisms are disturbed. Focal necroses develop here.

The severe effect of ionizing radiation shows up in the digestive tract [2, 3, 5-7] and the outcome of the illness depends much on the degrees of its damage. If, for example, the intestines are shielded, the survival of animals after irradiation increases. Macroscopic changes in the intestines are manifested in edema of the

intestinal tissues, hemorrhages and necrosis of sections of the intestinal and stomach walls. The mucous membrane of gastrointestinal tract is subject to very severe changes. The earliest and most significant changes occur in the crypts where mitotic activity of the cells is very high [8-11]. In this case the rhythm of cell division is disturbed, mitotic activity decreases considerably and some of the cells die. At large doses of irradiation (up to 10 kr) destruction and death of a large number of epithelial cells of the small intestine occurs, new cell production ceases, the physiological process of cell replacement is disturbed and the submucous membrane becomes exposed. This in turn disturbs the intestinal barrier, as a result of which conditions are created for the development of infectious processes [2]. In connection with the increase in the penetrability of the intestinal wall, severe dehydration of the organism occurs [12]. Radiation acts in an inhibitory way on the immunobiological properties of the organism [13], suppressing antibody production and phagocytosis.

Many authors have considered the cardiac muscle to be radioresistant, however, it has been shown recently [14, 15] that severe changes also occur in it. Swelling, hypertrophy or atrophy of the muscle fibers, flabbiness of the cardiac muscle and partial disappearance of transverse striation are described in these papers. Sometimes the cardiac muscle fibers undergo granular decomposition and vacuolization.

Work in which the effect of toxic substances formed in irradiated organisms has been studied is of great interest. Substances causing the destruction of intact erythrocytes which were added to them were found in liver homogenates of irradiated mice; these products are called the "hemolytic factor" [16-26]. It has been demonstrated that the hemolytic factor also has a toxic effect on other cells [25, 26]. In connection with this it was given the more general name of "cytotoxic factor" [20], and then – "natural radiomimetic" [1]. It has been established by biochemical isolation of the cytotoxic factor that it contains a large quantity of unsaturated fatty acid oxidation products of the oleic type [1, 16, 27, 28]. After introduction of oxidized oleic acid into an organism, changes similar to changes during radiation sickness occurred [27-30].

A preparation of the cytotoxic factor (natural radiomimetic) isolated from irradiated rabbit livers has a toxic effect on various biological systems, similar to the effect of radiation [1, 25, 26, 31), and, depending on the dose injected, most acute, acute and chronic forms of the lesion can develop.

Cytological and histochemical changes in organs from the effect of radiation have still been insufficiently studied and research on the effect of lipid RT on cells and tissues is actually just beginning.

In the present work adult white male rats were taken for investigation. A total of 150 animals was used in the experiment. Twelve hrs before fixation of the material the rats were deprived of food and given water. Eighty two rabbits were used for isolation

of the unsaturated fatty acids (lipid radiotoxic substances) which served as the radiomimetic.

For histochemical purposes pieces of liver, kidney, small intestine and heart were fixed in Becker's (calcium-formol) and Carnoy's fluids and in 10% formalin. Material fixed by the Carnoy method was embedded in paraffin and sections 5 μ thick were made. A reaction for ribonucleic acid [RNA](РНК) was carried out on such sections by Brashe's method. Control sections were treated with ribonuclease. Pieces of organs fixed by Becker's method also were embedded in paraffin. The thin sections were stained with Heidenhain's hematoxylin stain for mitochondria.

Tissues fixed with formalin were stored in the cold in 7% formalin, then sectioned on a freezing microtome and stained with sudan III for neutral fat. The lipase reaction was carried out on part of the frozen sections by the "twin-80" method suggested by Gomori. The effect on the sections of Lugol's solution or a phenol solution which suppress lipolytic activity in the sections was used as a control.

Along with the histochemical demonstration of lipase, its activity was studied biochemically. Demonstration of lipase activity in the livers of control and experimental animals was carried out by Zhdanov and Ivanova's method [32].

Several samples were taken for investigation 30 min and 1, 2, 3, 4, 5 and 6 hrs after the mixing began.

The procedure of examining the samples was the following. To 40 ml of the mixture was added 20 ml of distilled ether (freed of peroxides and water by Yur'yev's method), the mixture was extracted and the insoluble fat removed by filtration. The filtrate was divided into four equal parts: two were titrated with an 0.02 M KOH solution into alcohol with phenolphthalein, two were left in open weighing bottles until the ether had completely evaporated and the weight of the fat was determined in these two on an analytical balance with accuracy to the fourth place.

Calculations were carried out according to the formula

$$X = \frac{a-b}{m} T,$$

where X is the acid number (mg KOH/g); A is the amount of KOH solution which went into titrating the sample containing fat (ml), b is the amount of KOH solution which went into titrating the control sample (without fat) – the "blind" sample; m is the weight of the fat, T is the titer of the KOH solution (mg KOH/ml) (KOH was titrated with an 0.1 M HCl solution).

The acid number shows the number of milligrams of base necessary to neutralize the free fatty acids in a 1 g sample of the test substance.

In the course of our work, irradiation with γ-rays and fast neutrons, as well as lipid RT, were used.

Irradiation with γ-rays. Rats were subjected to one total irradiation in a GUBE-800 unit in a dose of 800 r at a dose rate of 55 r/min. The irradiation was carried out with four Co^{60} preparations uniformly located around the animal. The rats were killed on the 7th-8th day after irradiation – at the climax of the manifest clinical changes.

Irradiation with fast neutrons. Irradiation with fast neutrons with an energy of more than 0.1 Mev was carried out totally in an IBR-2000 fast impulse reactor. The rats were irradiated with neutrons with a dose of 600 rad at an energy of 1 Mev. The animals were also killed on the 7th-8th day after irradiation.

The extraction of lipid RT [1] and their injection into rats. The lipid RT (natural radiomimetic) preparation was isolated from the liver of rabbits irradiated with γ-rays and neutrons under the same conditions as the rats. Two to three days after irradiation, the rabbits were killed by decapitation, the liver removed and pulverized in a homogenizer for 3-5 min at 1000 rpm. All the operations were carried out in the cold room (at a temperature of 2-4°). A four-fold volume of diethyl or petroleum ether (the ether was first distilled in the presence of Fe^{2+} to remove such impurities as peroxides) was poured over the homogenate obtained. The homogenate with the ether was placed on a mechanical shaker. The extraction was carried out with constant mixing of the samples for from 2 to 24 hrs. Two hrs after the beginning of the mixing the four-fold volume of ether was poured off, and a two-fold volume poured on. The mixture was filtered after 24 hrs and the ether fraction obtained was evaporated in a film evaporator in a vacuum. Acetone (in an amount of 1 ml per weighed portion of the tissue taken) was added to the oily residue in order to free it from phospholipids which are insoluble in acetone. The samples were mixed carefully and left overnight in the cold room. The phospholipid precipitate was removed by filtration and the supernatant liquid again subjected to rapid evaporation. The oily preparation obtained ("acetone fraction") was weighed and used in experiments as the lipid radiomimetic.

The toxicity of the preparation was studied preliminarily by the erythrogram method [33]. The work was carried out in an FEK-M or FEK-52 apparatus. Curves (erythrograms) were constructed on the basis of the data obtained which reflect changes in the stability time of erythrocytes taken from animals. Changes in the erythrograms give an idea of the toxicity of the radiomimetic used. Erythrograms obtained after the action of an 0.004 *n* hydrochloric acid solution on erythrocytes served as the control.

The activity of the NR (EP) preparation was determined from the erythrograms obtained [1]. The same degree of hemolysis occurs from the effect of 0.004 *n* hydrochloric acid in physiological solution (in a dilution of 1:40), a radiomimetic obtained from animals subjected to neutron irradiation (1:12,800) and a radiomimetic ob-

tained from animals subjected to γ-ray irradiation (1:16,000). Preparations of the indicated activity were used for injection into animals.

There was one intraperitoneal injection in a dose of 0.6-0.8 ml, which corresponds to γ-ray irradiation with a dose of 700-800 r [1].

In the experiments rats were irradiated with γ-rays (in a dose of 800 r) or subjected to the effect of a flux of neutrons with an energy of 1 Mev (in a dose of 600 rad). Animals were also injected with 0.7-0.8 ml of radiomimetic obtained from animals after γ-ray or neutron irradiation. The animals were dissected on the 7th-8th day.

The observations described below were made during the experiment.

Macroscopic changes caused by the effect of lipid RT and irradiation with γ-rays and neutrons. After the injection of lipid RT and irradiation of the rats symptoms of radiation sickness appeared: appetite decreased, disorder of the gastrointestinal tract and anemia of the mucosa developed. The animals began to react sluggishly to stimuli and noticeably lost weight. The following changes were noted upon autopsy in the animals affected by irradiation: foci of hemorrhages appeared in the lungs and sometimes local necrotic foci were encountered. Anemia of the cardiac tissue, intestines, kidneys and other organs developed. Necrotic foci appeared in the intestines. Focal hemorrhages were seen in a number of organs.

After the injection of lipid RT considerable changes were observed in the internal organs, particularly the liver. It was enlarged and became anemic, its lobes coalesced and commissura of the liver with a number of adjacent organs were observed. Adiposis and sometimes punctate hemorrhages developed in it.

The stomach changed in the cardiac section of the cicatrix; the intestines, particularly the small intestine, had necrotic foci. The transitional section of the small intestine was severely distended into the large intestine. The spleen decreased in size, became firm to the touch and was covered on top with a fatty deposit. The kidneys were full of punctate hemorrhages. The right kidney was often adherent to the liver. The mesentery was flaccid, the testicles were porous and fat was deposited on them. Sometimes the site of the injection was seen since a necrotic patch developed around it.

Cytological and histochemical study of the liver of control rats and animals subjected to irradiation and the effect of lipid RT. The parenchymatous cells of normal rats have a polygonal shape; the cell borders and the nuclei which contain 2-3 nucleoli are clearly visible. The venous capillaries are lined with comparatively small compressed Kupffer cells. Plasmatic cells, as a rule, are not encountered. RNA is found in the cytoplasm of the parenchymatous cells in the form of clumps; the intensity of the reaction is average. The nucleoli are rich in RNA. The Kupffer cells have a low basophilia in

comparison with the parenchymatous cells (due to the presence of RNA) and dense oval nuclei.

The hepatic cells of animals irradiated with γ-rays (dose of 800 r) undergo atrophic, dystrophic and necrotic changes and the capillaries are dilated, the parenchymatous cells contract. Many Kupffer cells are swollen, disintegrated and fall into the lumen of the capillaries. The RNA content of the hepatic cells increases; the basophilic masses become coarser. In many cells disappearance of the nucleoli and karyolysis is observed. Plasmoblasts and plasmocytes appear in the liver.

From the effect of radiomimetic obtained from animals subjected to irradiation with γ-rays (we shall call it γ-radiomimetic for short), some cells, just as from irradiation, undergo atrophic and necrotic changes. In some cases the basophilic masses in the cytoplasm become more pronounced, in other cells fatty inclusions are accumulated, and fragments of cytoplasm rich in RNA are distributed around the periphery of the cells and in the perinuclear zone. The nuclei of the parenchymatous cells are shriveled and condensed. Many plasmatic cells which strike the eye with their clear basophilia appear in the liver; neutrophils appear in the vessels and stroma of the liver. Mitoses, rare in a normal liver, are encountered in the parenchymatous cells.

From the effect of fast neutrons (dose of 600 rad, energy of 1 Mev) focal necroses are found in the liver. The cytoplasm of a majority of the cells is lumpy and basophilic, especially around the periphery of the cell. The Kupffer cells are severely swollen and some of them fall into the lumen of the capillaries.

Radiomimetic obtained from animals subjected to irradiation with neutrons ("neutron radiomimetic") also leads to the development of dystrophic and necrotic changes in the liver. The cytoplasm of the parenchymatous cells has considerable lumpiness. The RNA content of the lumps is high. Some of the cells are vacuolized, the boundaries between the cells are obliterated and they are decomposed. In some cells the nuclei are hypertrophied; the nucleoli are also enlarged. Sometimes the nucleoli emerge from the nuclei. The capillaries are distended; numerous neutrophils are seen in them. The number of plasmatic cells with clearly basophilic cytoplasm also increases.

A study of lipase activity by the "twin-80" method showed that lipase is absent from the cytoplasm of the parenchymatous cells of the control animals. There is a weakly positive reaction in a few nuclei in which small dark granules of PbS are distributed along the nuclear membrane. The lipase reaction is positive in the nuclei and cytoplasm of the Kupffer cells, as well as in the peripheral sections of the erythrocytes (Fig. 1).

After irradiation with γ-rays the lipase activity of the hepatic tissue increases considerably. Granules of PbS are found in the cytoplasm and especially in the nuclei of parenchymatous cells. Enzymatic activity is also intensified in the endothelium of the liver capil-

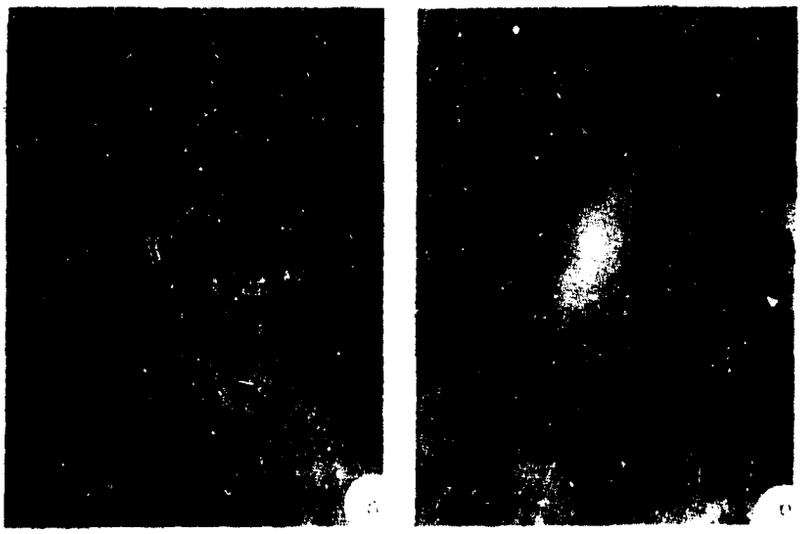

Fig. 1. Liver cells of normal rats and rats irradiated with γ-rays. (The "twin-80" method for lipase.) a) Hepatic cells of normal animal (lipase does not appear in the parenchymatous cells; a positive reaction is found in the endothelium and erythrocytes); b) parenchymatous cells of liver after irradiation with γ-rays in a dose of 800 r (granules of PbS are seen in the nuclei of the hepatic parenchyma). Magnification 7 × 90.

1	2		5	
	3	4	6	7
0,5	0,0721	0,0707	2,18	1,41
1	0,2872	0,1244	2,27	1,72
2	0,2231	0,2857	2,32	1,91
3	0,2216	0,2502	2,45	1,98
4	0,2502	0,1871	2,41	2,05
5	0,2739	0,2425	2,46	2,02
6	0,2227	0,2549	2,62	1,95

1) Incubation time, hrs; 2) weight of fat isolated from the animal livers, mgs; 3) injected; 4) intact; 5) acid number of sample; 6) experimental; 7) control.

laries and erythrocytes (Fig. 1).

Similar changes occur in the lipolytic activity of the hepatic tissue after neutron irradiation and the injection of γ- and neutron radiomimetics. Lipase activity increases very strongly in the nuclei of the parenchymatous cells (PbS granules clog the nuclei); PbS granules are also encountered in the cytoplasm. Lipolytic activity is also intensified in the Kupffer and plasmatic cells and in the erythrocytes.

The following results were obtained from a biochemical deter-

mination of the lipolytic activity of the liver tissue of control rats and animals after the injection of γ-radiomimetic (see table).

As can be seen from these data, in the experimental animals an increase in lipase activity occurs in the first hours of incubation, then there is a slight lag and again a jump after 5 hrs of incubation. In the controls the activity increases in the first 2 hrs, and then during the next hours remains relatively constant. It is important that at the beginning of the experiment the lipase activity of the control animals was considerably lower than that of the experimental animals. (Thirty rats were used in the experiment.)

A study of the mitochondria in the liver of normal rats showed that they have a granular shape, sometimes are slightly elongated. Large numbers of them fill the cytoplasm of the parenchymatous cells (Fig. 2).

After γ-ray irradiation the morphology of the mitochondrial apparatus changes considerably. In some cells the mitochondria generally disappear, in others they become few in number, but large, rounded, oval or rod-shaped. Finally, cells are encountered which almost without exception are clogged with large clumps which evidently are conglomerate swollen mitochondria and condensed particles of cytoplasm which have undergone coagulation. Some cells retain the mitochondria unchanged.

From the injection of radiomimetics obtained after irradiation of the animals with neutrons and γ-rays, the mitochondria in the parenchymatous cells of the liver for the most part are fused into large irregular masses, frequently located around the nuclei. In some cells the mitochondria are swollen, spherical or considerably elongated. In the latter case the ends of the mitochondria probably stick together to form a conglomerate (see Fig. 2).

Neutral fat is contained in the liver of normal rats which were starved for 24 hrs before they were killed, in the form of rather small droplets scattered throughout the cytoplasm of the parenchymatous cells. After irradiation with γ-rays and fast neutrons the number and size of the fatty droplets in the cells increase. This phenomenon was also observed after injection of the animals with radiomimetics.

Cytological and histochemical study of the small intestine of control rats and animals subjected to irradiation and the action of lipid RT. In the intestines of normal rats the epithelium lining the villi and crypts is a layer of cylindrical cells. There are numerous mitoses in the crypts. Basophilia of the cytoplasm of the epithelial cells due to the presence of RNA is comparatively low. It is lower in the epithelium of the villi and somewhat higher in the crypt cells. The nucleoli are basophilic, especially in the nuclei of the crypt cells.

Considerable changes occur in the rat intestines on the 8th day after γ-ray irradiation: destruction of the epithelial cells of the apices of the villi which are desquamated into the lumen of

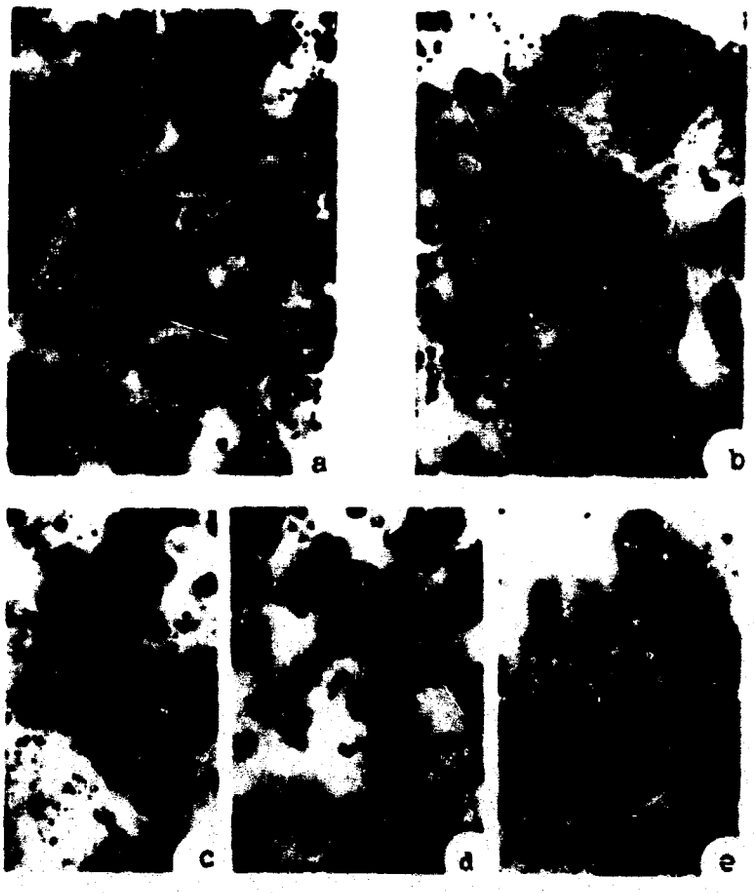

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Fig. 2. Mitochondria in liver cells of normal and irradiated rats. (Heidenhain's iron hematoxylin stain). a) Mitochondria in hepatic cells of normal animals; b) mitochondria in liver after injection of neutron radiomimetic; c) mitochondria in liver after irradiation with γ-rays; d and e) mitochondria in liver cells after injection of γ-radiomimetic. Magnification 7 × 90.

the intestine. Sometimes whole epithelial layers fall off, and in some cases destruction and decomposition of even whole villi occurs. The stroma of the retained villi is swollen and becomes edematous. The number of plasmatic cells increases in it.[1] The cavities of the crypts are considerably enlarged, the crypt cells are condensed, mitoses are rarely encountered. The RNA content of the epithelium of the villi and crypts increases. The nuclei and nucleoli are frequently hypertrophied, especially in the crypts. Sometimes the nucleoli emerge from the nuclei.

After the injection of γ-radiomimetic a very considerable growth of the connective tissue in the villi occurs in the intestines. Here the number of plasmatic cells and lymphocytes increases. Mitoses are encountered in the crypts - more frequently than after γ-ray irradiation, but considerably more infrequently than normally.

Some of the epithelial cells of the villi, and sometimes whole epithelial layers are desquamated into the lumen of theintestines. The RNA content of the epithelial cells of the villi and crypts increases considerably. The necrotizing cells gradually lose their basophilia, the nuclei decompose and the cells pass into the detritus.

Irradiation with fast neutrons causes severe destruction of the intestines. Frequently the epithelial layers of the mucosa and even whole villi are torn away into the lumen of the intestines and the submucous membrane becomes exposed. In the epithelium of the villi, if it is retained, the borders between the cells are obliterated and the cytoplasm is vacuolized. The beaker-shaped mucous glands swell, the cells get too full of secretion and disintegrate. The intensity of the basophilia of the cytoplasm of the intestinal epithelial cells increases. The number of plasmatic cells rich in RNA increases.

After the injection of neutron radiomimetic, destruction of some of the epithelial cells of the villi also occurs. The borders between the cells are obliterated. In the crypts the changes are comparatively small: mitoses are retained and the number of dead cells is small. Beaker-shaped mucous cells secrete intensely and partially disintegrate. The number of plasmatic cells in the connective tissues of the villi is increased. The RNA content in the epithelium of the villi and crypts is increased in comparison with normal.

A study of the lipase activity in normal intestinal tissues showed that the enzyme is found mainly around the periphery of the cell nuclei of all layers of the intestinal wall. PbS granules are also deposited in small amounts in the cytoplasm of the villi epithelium. The lipase activity is lower in the epithelial cells of the crypts. There is very high lipase activity in the plasmatic cells, and PbS granules are found in the nuclei and in very large amounts in the cytoplasm.

After γ-ray irradiation, the lipolytic activity of all layers of the intestines increases. The nuclei become clogged with granules. Many granules appear in the cytoplasm of the epithelial, muscle and other cells. Lipase activity is retained at a very high level in the plasmatic cells.

The injection of γ-radiomimetic leads to increased lipase activity of intestinal tissues. The content of PbS granules in the nuclei of epithelial and muscle cells increases particularly heavily, the activity in the cytoplasm also increases. Lipase activity is increased in the endothelium of the capillaries and in the erythrocytes which almost without exception become black with the PbS granules which clog them. The lipase content of the plasmatic cells is high. Irradiation with neutrons, as well as the injection of radiomimetic obtained after neutron irradiation causes similar changes in the intestinal tissues of the injected rats. In the epithelium of the villi the PbS granules fill the nuclei and are found in the cytoplasm in a greater amount than normally. There is very high enzyme

activity in the plasmatic cells, endothelium and erythrocytes.

The mitochondria in the epithelial cells of the intestines of normal rats are small threads or granules. There are especially many of them in the apical parts of the cells.

After γ-ray irradiation the mitochondria in the apical sections of the cells are considerably swollen and fuse into beaded formations or irregular conglomerates. These conglomerates are sometimes encountered in the basal sections of the cells. It is quite difficult to distinguish individual mitochondria in such conglomerates.

The injection of γ- and neutron radiomimetics leads to similar results: the mitochondria in the epithelium of the intestines are swollen and fuse into supernuclear conglomerates. Conglomerate mitochondria are sometimes encountered under the nucleus. In dead cells mitochondria frequently are not seen at all.

Neutral fat in the epithelium of normal rat intestines is found in a few cells in the form of fine droplets (the rats, as was indicated, were starved for 24 hrs). After irradiation with γ-rays and neutrons and the injection of both types of radiomimetic droplets of neutral fat appear in the epithelium in large amount, and some cells are diffusely stained with sudan III.

Cytological and histochemical study of the kidneys of control rats and animals subjected to irradiation and the action of lipid radiotoxins. Uninjured convoluted and straight tubules and renal bodies in which the area between the outer and inner leaflets of Shumlyanskiy's capsule is small and slit-like are clearly seen in the kidneys of the control rats. RNA is found in the cytoplasm of the tubule cells and Malpighian bodies in a comparatively low concentration.

From the effect of γ-rays and neutrons changes which primarily affect the convoluted tubules occur: granular dystrophy which changes to necrosis arises. In such cells the nuclei often disappear, the cytoplasm becomes lumpy, the apical membrane of the cell disintegrates and pieces of cytoplasm with mitochondria and pycnotizing and lysing nuclei fall into the lumen of the tubules. The kidney glomeruli often are shriveled and the space between the outer and inner leaflets of the capsule is increased. Basophilia of the cytoplasm of the tubule and glomeruli cells is somewhat increased.

The injection of γ- and neutron radiomimetics causes changes primarily affecting the convoluted tubules of the kidney, similar to radiation.

Lipase activity in the glomeruli cells of normal rat kidneys is low. In the tubules lipase is found in the nuclei and numerous PbS granules are also found in the cytoplasm, chiefly located in the vicinity of the walls.

Lipase activity intensifies in the glomeruli and especially in the convoluted tubules from the effect of γ-rays and neutron radio-

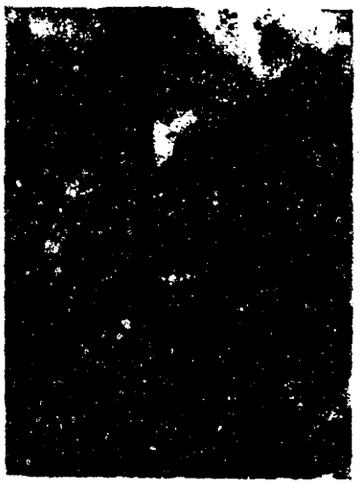

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Fig. 3. Cells of renal tubules after injection of γ-radiomimetic. Lipase reaction. PbS granules are seen in the cytoplasm and nuclei of the parenchymatous cells. Magnification 7 × 90.

mimetic. In the latter the PbS granules clog the nuclei and are scattered in large numbers in the cytoplasm (Fig. 3). In lysing nuclei the lipolytic activity disappears, and small uniformly distributed PbS granules appear in the cytoplasm of such necrotizing cells.

Mitochondria in the renal tubule cells of normal rats have different shapes in different parts of the nephron. In the convoluted tubules they are primarily elongated and located chiefly in the basal parts of the cells, whereas in the insertion and connective sections they are mainly granular. In Henle's loops the mitochondria are small granules or are in the form of rods.

After irradiation with γ-rays, in cells which have been most strongly subjected to the action of radiation, the mitochondria are swollen, are fused into strands or, on the contrary, are very fine. In disintegrating cells the mitochondria can be seen in the bits of cytoplasm which fall into the lumen of the tubule. In other sections of the nephron the changes in the mitochondria are less considerable.

The injection of γ- and neutron radiomimetics also leads to fusing and swelling of the mitochondria, chiefly in the convoluted tubules of the kidney (Fig. 4).

Neutral fat which is found in the form of fine droplets in rare cells of the nephron, after the action of radiation and radiomimetics appears in the form of larger droplets and in a large number of cells.

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Fig. 4. Cells of disintegrating renal tubules after injection of neutron radiomimetic. Iron hematoxylin stain. Swollen mitochondria and detachment of pieces of cytoplasm with nuclei in the lumen of the tubule are seen. Magnification 7 × 90.

Cytological and histochemical study of the myocardium in control rats and animals subjected to radiation and the action of lipid RT. In the myocardium of normal rats the transverse striation of the myofibrillae is clearly seen; the centrally located nuclei have 2-3 small nucleoli; the connective tissue layers between the muscle trabeculae are thin. RNA is found in the muscle cells in a low concentration; the nucleoli also contain RNA.

After the effect of γ-rays and neutrons separation of the muscle fibers occurs, in many places (especially from the effect of γ-rays) the transverse striation disappears and vitreous segments devoid of transverse striation are formed. In some places granular decomposition of the muscle fibers is seen. The nuclei are partially lysed, and some are shriveled and become turbid. Basophilia of the cytoplasm of the muscle cells in a majority of cases increases; only in fibers which have undergone considerable necrotic changes can a decrease in the RNA content be observed. In some sections of the myocardium hyperemia and focal hemorrhages are seen.

The injection of γ- and neutron radiomimetics causes changes which are very reminiscent of the effect of radiation. Separation of the muscle fibers also occurs, sections of coagulated necrosis, foci of polyemia and hemorrhages appear. The transverse striation, especially after the effect of γ-radiomimetic, becomes poorly visible in places. Neutron radiomimetic often causes coarsening of the myofibrillae and a kind of "coagulation" of the A-disks which are converted into dense granules. Some nuclei are deformed and disappear. The RNA content of the muscle cells mainly increases, the nucleoli become more basophilic and sometimes emerge from the nucleus.

Lipase activity in the cardiomuscular tissue of intact animals is low. Fine PbS granules mainly appear around the periphery of the nuclei. In the cytoplasm of the muscle fibers single grains are en-

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Fig. 5. Cardiomuscular fibers of a rat after injection of γ-radiomimetic. "Twin" reaction for lipase. Intensely positive reaction is seen in the capillaries. Magnification 7 × 90.

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Fig. 6. Lipase reaction in erythrocytes of myocardial capillaries (marked with arrow) after injection of γ-radiomimetic. Magnification 7 × 90.

countered, and in the endothelium of the capillaries enzyme activity is somewhat higher. Lipase is also encountered around the periphery of the erythrocytes, whereas in the deep sections of the erythrocytes enzyme activity is not found.

After irradiation with γ-rays lipase activity increases in the muscle nuclei and in the cytoplasm. The content of granules in the endothelium of the vessels also increases considerably. The erythrocytes become clogged with granules. Lipase activity also increases

after neutron irradiation.

The injection of γ- and neutron radiomimetics leads to intensification of lipase activity, especially in the nuclei of the myocardium, capillary endothelium and erythrocytes (Figs. 5 and 6).

The mitochondria in the cardiac muscle of normal rats are arranged in regular chains along the myofibrillae and have an oblong oval shape.

After γ-ray irradiation many mitochondria begin to swell and fuse with each other, forming long strands, frequently not separated into individual mitochondria or with a barely noticeable separation.

After injection of γ- and neutron radiomimetics the mitochondria also frequently swell and fuse into long strands. In some muscle fibers the mitochondria are not found. Sometimes they are difficult to distinguish from rows of "coagulating" A-disks of the myofibrillae which, in deforming, take the shape of densely stained granules.

Neutral fat is not encountered in normal cardiomuscular tissue (droplets of it can be seen only in the fat cells of the connective tissue layers). After the action of radiation and radiomimetics fine fat droplets located chiefly in the zone around the nucleus sometimes appear in the muscle fibers.

Discussion of results. The data obtained indicate that from the effect of radiation and lipid RT very similar morphological and histochemical changes occur in the organs studied.

In liver, intestinal, kidney and myocardial tissues dystrophic and necrotic changes, focal hemorrhages, polyemia in some sections and anemia in others are observed.

It was possible to show by histochemical methods that in all organs dystrophic changes are accompanied by an increase in the basophilia of the cytoplasm, and in some cases of the nucleoli of various cells.

These data, however, do not agree with the results of the investigations of some authors [34] who noted a decrease in the RNA content of tissues after irradiation with γ-rays. Evidently, during the development of radiation sickness the nucleic acid content of the tissues changes, and following a certain increase characteristic of a state of paranecrosis, a decrease in the RNA content of the cell occurs. In segments with necrosis less basophilia of the cytoplasm can actually be seen. However, it has not been excluded that the increase in the basophilia of the cytoplasm in this work from irradiation and the effect of lipid RT reflects not so much an increase in RNA content as an increase in the number of phosphate groups released during disintegration of RNA, – proteides. The work of Tsanev [35] who studied phosphate group and pentose content by biochemical and cytochemical methods in the connective tissue after injury speaks of such a possibility. Tsanev showed that intensified

production of phosphate groups causing an increased basophilia of the cytoplasm occurs after injury of the connective tissue by strong pressure.

Both from irradiation and from the effect of RT an increase in lipase activity was observed. This was shown by both cytochemical and biochemical methods. The only method of demonstrating lipase ("twin") used in cytochemistry perhaps does not reflect very accurately the intravital location of the enzyme. Many histochemical reactions for enzymes suffer from this shortcoming, in which the intermediate or final reaction product can diffuse into the cell, being absorbed secondarily on a number of its structures.

It has been determined by biochemical methods that lipase in cells of the pancreas is localized in the secretory granules, and in the liver in the mitochondria [36]. It is still not clear whether lipases are located in the nuclei.

Although the incubation period (4 hrs) was shortened in the present work and a rapidly penetrating substrate was used, and insoluble calcium soaps were also obtained as a result of the reaction, that is, conditions for retaining the intravital location of the enzyme were observed, the authors are not completely satisfied that lipase is actually located in the nuclei. If this were confirmed, it would be one more demonstration of the severe vulnerability of the nuclei and of a change in their metabolism from radiation and the injection of RT. Further development of the method and a study of lipase in various cell fractions are necessary.

However, in order to demonstrate that diffusion of the final product is not great, the fact that in endothelial cells and plasmatic cells lipase is always found in the cytoplasm of intact and experimental animals, while in the epithelium of the intestines and kidneys the enzyme appears primarily in the nuclei can be cited. It is doubtful that the conditions for diffusion here are so different that such a difference in their location is always obtained.

While one can argue about the site of the enzyme's location, the fact of intensification of lipase activity seems undoubted and indicates considerable changes in lipid metabolism both due to irradiation and to the injection of lipid RT. It is interesting to note that lipase activity increases considerably in the endothelium of the capillaries and the erythrocytes which perhaps is one of the reasons for a change in their penetrability and increased vulnerability.

The accumulation of neutral fat in the cells was also one of the manifestations of disturbances in lipid metabolism in the preparations in the given experiments.

The importance of the lipids in the formation of submicroscopic membrane structures of the cell is well known. Under the influence of radiation, protein-lipid compounds of the membrane can disintegrate [37]. A disturbance in lipid metabolism can thus cause considerable changes in cell structure. The considerable changes in

the structure of the nuclei, cytoplasm, mitochondria and fibrillar formations which were noted in the present work can serve as indirect proof of disturbances in lipid metabolism. The mitochondria which draw fatty acid oxidation products into the Krebs cycle are connected to a certain degree with lipid metabolism.

The possibility of the participation of the mitochondrial lipids in the mechanism which provides for linking of electron transfer in the respiratory chain with ATP synthesis, has also not been excluded. As is known, it is precisely the structure of the mitochondria with distribution of certain enzyme complexes on the outer and inner membranes which provides for the fulfillment of the principal function of the mitochondria - ATP production. Damage to the structure of the mitochondria must invariably cause a disturbance in their functions.

In all the tissues studied both from radiation and from the injection of RT, structural changes in the mitochondria and their conglomeration, swelling or even disappearance in the injured cells were observed. It is evident that these changes disturb the functions of the mitochondria.

Thus, there is a very great similarity in the cytological and cytochemical changes in cells from the effect of γ-ray and neutron irradiation and the injection of lipid RT.

Some differences in macroscopic changes after the injection of lipid RT are observed in comparison with the effect of radiation. The formation of commissures between organs occurs; a fatty deposit on the surface of organs is observed. Such a reaction is characteristic of intraperitoneal injections of other fatty products.

Microscopic differences between these two effects are manifested in some revival of the connective tissue and especially in an increase in the number of plasmatic cells in response to the injection of lipid RT. After the effect of radiation the answering inflammatory changes in the tissues are weakly expressed. The number of plasmatic cells, with whose activity antibody production is connected, can increase in the early periods after irradiation [30] but later decreases.

The traumatizing effect of lipid RT on mitosis is less sharply expressed.

It should be noted that in investigations on the effect of oxidized oleic acid (artificial radiomimetic) on the cytology and histochemistry of a number of rat organs which were carried out earlier [30], very similar results were obtained from the effect of this substance and natural lipid RT.

So, in nature of action on cells lipid RT have a very great similarity with γ-ray and neutron irradiation.

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FOOTNOTES

177 In previously conducted investigations we established that in later periods after irradiation (on the 12th-17th day after γ-ray irradiation) the number of plasmatic cells decreases.

THE CYTOGENETIC EFFECT OF LIPID RADIOTOXINS

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The development of structural changes in the chromosomes is one of the specific cell reactions to irradiation [1]. It is clear from the literature on radiation genetics that the observed chromosomal aberrations are not the direct result of irradiation. Reversible potential defects whose fate is affected by various factors develop first [2, 3]. A number of substances, used not only before irradiation, but also after it, is capable of modifying the effect of irradiation. Such substances as adenosine triphosphate, alanine, cysteine and others, used after irradiation, decrease the percentage of affected cells [3-6]. Ethylenediamine tetraacetate, on the other hand, intensifies the lesion [7]. It has been established that these substances affect potential defects, promoting the recovery of normal functions or turning hidden defects into visible ones [6].

It has been shown in the work of a number of authors [8, 9] that toxic lipids called natural radiomimetic [NR](EP) because of their capacity when injected into healthy unirradiated animals of causing a picture similar to that of radiation sickness (death of the animals, change in protein autolysis, hemolysis of intact erythrocytes, inhibition of mitosis, etc.) develop in the organism of irradiated animals. NR and oxidized oleic acid [OOA](OOK) which simulates the effect of NR, caused chromosomal aberrations in Ehrlich's ascites carcinoma cells [9]. This fact deserves special examination since NR is a substance which forms in an irradiated organism to a greater degree than in an unirradiated organism. The amount after irradiation increases with time [10]. It has remained unclear whether NR acts directly on the cell's genetic material, causing aberrations, or promotes manifestation of the already present hidden defects. (There is always a high percentage of spontaneous aberrations in Ehrlich's ascites carcinoma cells.) The purpose of the present work was to answer this question.

OOA, prepared in the form of an emulsion (0.5 ml of OOA in 100 ml of distilled water), was used as the NR model.

The experiments were performed on Ehrlich's ascites carcinoma

cells and on loach (Misgurnus fossilis) ovum cells.

The ascites cancer cells were subjected to the action of OOA in different concentrations for 30 min with constant mixing. The mixture was injected intraperitoneally into unbred white mice in an amount of 0.5 mg per animal. The irradiation was carried out in GUT-Co-400 equipment in doses of from 50 to 1000 r at a dose rate of 100 r/min. The preparations were stained with methylene blue. Chromosomal aberrations were counted in the first fission after injection of the ascites, 21-22 hrs after injection of the cells.

Experiments with normal cells were performed on artificially fertilized loach ova, developing at a temperature of 12.5°. The developmental stage was determined according to a system worked out by Neyfakh [11]. The conditions of treating the ova were the same as in the experiments with Ehrlich's ascites carcinoma cells. The material was stained with acetocarmine; the chromosomal abnormalities were counted in the anaphases and telophases in the first fission after the treatment. The total percentage of cells with chromosomal aberrations, the number of cells with fragments and the total number of fragments were taken into account in analyzing the defects. The average number of fragments per cell with fragments was computed on the basis of these data, which served as a quantitative evaluation of the number of initially affected cells according to a method suggested by one of the authors [12].

The effect of irradiation and OOA on Ehrlich's ascites carcinoma cells. As seen from the figure (see graphs a and b), OOA, like γ-rays, considerably increases the percentage of chromosomal aberrations. But there is a definite difference between the effects of both factors. OOA increases chromosomal aberrations approximately to 40%, and at all concentrations except 1:16, the number of injured cells remains at the same level. From the effect of γ-rays, chromosomal aberrations continually increase with the dose, reaching 80% at 1000 r. The pattern obtained from the effect of OOA might be explained either by poor penetrability of this substance for the cell or by the fact that OOA does not act directly on the chromosomes, but brings out already present hidden defects in the chromosomes of the cancer cells.

A further analysis of the defects showed that the number of fragments per cell with fragments from the effect of OOA not only does not increase with an increase in the latter's concentration, but remains at the same level as in the control (Table 1).

These results speak in favor of the fact that OOA acts only as a developer of hidden defects, since in the opposite case the increase in the total defect rate should have been accompanied by an increase in the degree of defectiveness of individual cells [1, 2] which occurs in the case of irradiation.

The results of calculating the percentage of initially injured cells are also depicted in the figure (see graph b) and confirm the conclusion which was drawn.

TABLE 1

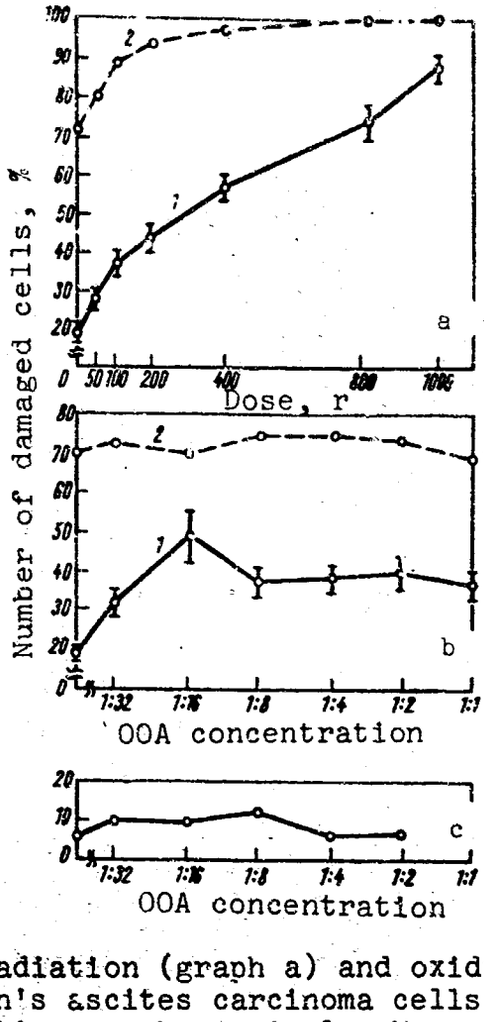
Number of Fragments per Cell with Fragments in Experiments with Ehrlich's Ascites Carcinoma

Вариант опыта 1	Концентрация ООК и доза облучения 2	Число фрагментов 3	Вариант опыта 1	Концентрация ООК и доза облучения 2	Число фрагментов 3
Контроль 4	—	1,79			
ООК 5	1:32	1,69	Облучение + + ООК 7	9 400 р + 1:32	3,00
	1:16	1,77		1:16	4,45
	1:8	1,73		1:8	5,38
	1:4	1,86		1:4	4,10
	1:2	1,85		1:2	6,10
	1:1	1,83		1:1	4,00
Облучение 6	50 р	2,03	ООК + + облучение 8	1:32	3,42
	100 р	2,35		1:16	4,00
	200 р	2,89		1:8	3,91
	400 р	3,49		1:4 + 400 р	6,35
	800 р	6,19		1:2 9	8,0
	1000 р	6,37		1:1	9,8

1) Type of exp.; 2) OOA concentration and irradiation dose; 3) number of fragments; 4) control; 5) OOA; 6) irradiation; 7) irradiation + OOA; 8) OOA + irradiation; 9) r.

The effect of OOA on normal loach ova cells. It is seen from preliminary data obtained in experiments with loach ova (see graph c) that with an increase in OOA concentration the effect, if it is increased in comparison with the control, is only very slight and remains at the same level at all concentrations. The 1:1 concentration which proved toxic for the ova is an exception. At this concentration the number of dividing cells decreases sharply, the chromosomes lose their usual appearance and fuse with each other, forming a shapeless mass. The bridges stick together, forming thick strands between two groups of chromosomes. Chromosomes which have been left behind oriented in the direction of the fission spindle, are often observed. Similar phenomena (multiple stuck together bridges and residual chromosomes) are described in Pankova's work [13] on loach ova at a dose of 1000 r which causes the death of 90% of the embryos and deformities in the rest. The absence of an increase in the cytogenetic effect on loach ova with an increase in OOA concentration up to the toxic concentration testifies in favor of the fact that OOA evidently does not act on normal chromosomes and the effect obtained on cancer cells is connected with the development of hidden defects which existed in the cells before the action of OOA.

Joint effect of OOA and γ-ray irradiation. For a further analy-



Effect of γ-ray irradiation (graph a) and oxidized oleic acid (graph b) on Ehrlich's ascites carcinoma cells and the change in the number of defective anaphases in loach ova from the effect of oxidized oleic acid (graph c). 1) Defective anaphases; 2) initially damaged cells.

sis of the cytogenetic effect of OOA experiments were conducted on the joint effect of this substance and γ-rays. A constant irradiation dose of 400 r which causes defects in approximately half of the cells and varying OOA concentrations were used in these experiments. The oxidized oleic acid was used either immediately before irradiation or immediately after. The results of the experiments are presented in Table 2.

As seen from this table, from the action of OOA against a background of irradiation, the effect increases at first, but, beginning with a concentration of 1:16, remains at approximately the same level. The picture is similar to that which is observed from the effect of OOA alone, with the sole difference that from the joint effect of OOA and γ-rays the percentage of defective anaphases is correspondingly higher. With the reverse order of the interaction (OOA + irradiation) the effect gradually increases, reaching almost 100% at the highest concentration (1:1).

To analyze the data obtained, we calculated what effect should be expected if irradiation and OOA act independently and if a simple addition of their effects is observed. These expected values are also presented in Table 2, from which it is seen that the use of OOA after irradiation gave results which correspond to the expected, whereas its use before irradiation caused (at high concentrations) a considerably greater effect. The results of a statistical treatment are presented in the same table and confirm this conclusion.

TABLE 2

Percentage of Abnormal Anaphases in Ehrlich's Ascites Carcinoma Cells from the Joint Action of OOA and γ-irradiation

Концентрация ООК 1	Варианты опыта 2		Ожидаемые величины для аддитивного действия обоих факторов 5	Сравнение отдельных экспериментальных точек с теоретически ожидаемыми 6			
	400 р + ООК 3	ООК + 400 р 4		400 р + ООК 3		ООК + 400 р 4	
				x^2	p	x^2	p
1:32	54,00±4,74	67,00±2,67	38	0,602	$4,6 \cdot 10^{-1}$	17,39	$1 \cdot 10^{-4}$
1:16	66,00±3,33	59,50±3,48	73	10,56	$1,0 \cdot 10^{-3}$	19,49	$1 \cdot 10^{-4}$
1:8	70,00±3,75	66,50±2,46	61	6,68	$1,8 \cdot 10^{-2}$	7,56	$5 \cdot 10^{-3}$
1:4	75,00±3,48	77,00±2,97	82	10,..	$1,5 \cdot 10^{-3}$	16,84	$1 \cdot 10^{-4}$
1:2	74,00±3,10	78,50±2,46	69	2,35	$1,3 \cdot 10^{-1}$	11,37	$9 \cdot 10^{-4}$
1:1	68,50±3,28	99,50±0,35	61	4,3	$3,0 \cdot 10^{-2}$	380	$1 \cdot 10^{-7}$
Сравнение экспериментальных кривых с теоретически рассчитанной кривой . . 7				6,28	0,4	42,18	$1 \cdot 10^{-7}$

1) OOA concentration; 2) type of exp.; 3) 400 r + OOA; 4) OOA + 400 r; 5) expected values for additive effect of both factors; 6) comparison of individual experimental points with theoretically expected points; 7) comparison of experimental curves with theoretically calculated curve.

Thus, OOA when used after irradiation does not interact with the effect of radiation and when used before irradiation causes a considerable intensification of the effect.

The data obtained make it possible to consider that OOA present in the cell at the moment of irradiation takes some part in the initial reactions caused by irradiation. Based on the data obtained above relative to OOA's properties as a developer of potential defects, it can be assumed that OOA affects only part of the initial injuries caused by irradiation or affects only the early links of the chain of radiobiological reactions.

Conclusions. It can be stated on the basis of the experiments which have been conducted that OOA, in causing chromosomal aberrations in cancer cells, is a developer of hidden spontaneous defects existing in the chromosomes of these cells.

OCA does not have an effect on uninjured chromosomes of normal cells. If it is in the cell at the time of irradiation, OOA takes part in the initial reactions of radiation injury.

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THE TOXIC EFFECT OF WATER-SOLUBLE OXIDATION PRODUCTS OF IRRADIATED LINOLENIC ACID

A.S. Mochalina

Active intermediate radiation-chemical oxidation products (peroxides, organic peroxides, *o*-quinones, oxidation products of unsaturated fatty acids, aldehydes, etc.) can arise in a cell from the effect of irradiation. The high biological effectiveness of these compounds is known from their toxic, mutagenic and antimitotic activity [1-15].

Among the work which has been conducted in this direction, individual studies of the biological effect of aqueous extracts of oxidized lipids are of considerable interest.

It has been shown that water-soluble oxidation products of lecithin and methyl linolenate inhibit Rous sarcoma viruses [9], inhibit respiration and glycolytic activity of Ehrlich's ascites carcinoma cells [10], cause abnormal development of fertilized sea urchin and annelid eggs [11] and inhibit the division of yeast cells [14].

The intraperitoneal injection of mice with aqueous extracts of oxidized linolenic acid leads to the death of occasional individuals in the first 4 days. In the animals which survive falling out of the hair close to the injection site and destructive changes in the liver are observed [15].

In the given studies the aqueous extracts were obtained from lipids oxidized by ultraviolet rays. In this case it was established that the water-soluble oxidation products interact with thiobarbituric acid [TBA](TBK) with the production of a red pigment. The nature of the substances responsible for the above-indicated biological effects still remains unexplained. There are only assumptions about the presence of unsaturated carbonyl compounds and peroxides in the aqueous extracts [14-16].

At the present time there is no information in the literature concerning investigations directed toward a study of water-soluble TBA-positive lipid oxidation products from the effect of ionizing radiation. At the same time, they may have an essential role in the radiobiological effect.

In connection with this, the purpose of the present investigation was to demonstrate with the help of TBA the production of water-

soluble oxidation products after irradiation of unsaturated fatty acids [UFA](НЖК) and to study their biological effect.

Linolenic acid [LA](ЛК),[1] which has three double bonds in its molecule, was selected for these experiments.

An 0.5% alcoholic solution of LA was prepared from 96% ethyl alcohol for the experiments.

An original method of oxidizing LA, absorbed on a large surface, was used in this work. (We had already used this method in previous work on a study of the kinetics of the production and accumulation of water-soluble products of LA autooxidation in the presence of oxygen from the air.)

For this purpose 0.6 ml of an 0.5% alcoholic LA solution was deposited on large-pored filter paper (diameter 9 cm) and after evaporation of the alcohol (in 5 min) the filter paper with the adsorbed LA was subjected to irradiation with Co^{60} γ-rays in the presence of atmospheric oxygen. Two series of investigations were carried out: in the first series LA was irradiated in doses of from 1 to 5 kr at a dose rate of 190 r/min (EGO-2 equipment); in the second – in doses of from 10 to 100 kr at a dose rate of 10 kr/min (EGO-4 equipment). The filter papers with the applied LA were irradiated in Petri dishes. The experiments were carried out in the cold to inhibit autooxidation. Filter papers with LA, kept under the same conditions, but without irradiation served as the control.

Immediately after irradiation the oxidized LA products which formed were washed off with physiological solution (0.15 M, pH 6.3). For this purpose the filter paper was submerged in 5 ml of physiological solution and eluted for 5 min. This led to almost complete washing off of the oxidized LA products. The eluates obtained were completely transparent solutions. TBA was used to demonstrate oxidation products in them.

It is known from the literature that the reaction of TBA with oxidation products of polyunsaturated fatty acids is extremely sensitive. For example, 0.2 microgram of oxidation products in 1 ml of LA can be determined colorimetrically [the intensity of the color D_{maks}(532 mμ) = 0.1] [17, 18].

After pouring together 2 ml of eluate and different volumes of an 0.6% aqueous TBA solution and subsequent heating in a boiling water bath for 15 min, colored solutions were obtained. The optical density of these solutions was measured on an FEKN-57 apparatus using two light filters: green (532 mμ) and blue (455 mμ). The absorption spectra in the visible region were recorded on a recording SF-2M spectrophotometer.

The colored solution possessed a characteristic, well reproducible absorption spectrum in the regions of 532 and 455 mμ. The band with λ = 532 mμ is the most intensive, whereas λ = 455 mμ is less intense and more diffuse. The intensity of the solution's rose color is directly related to the total dose of γ-irradiation. The

[1]/200

yield of LA oxidation products responsible for the bands both at 532 mμ and at 455 mμ increases with an increase in the dose. At the same time the dose rate of the irradiation plays an important role in the production of TBA-active oxidation products. For example, in experiments with a dose rate of 10 kr/min, the appearance of these products in LA irradiated with 10 kr was not found. Irradiation of the filter paper containing LA in a dose of 20 kr led to the formation of TBA-positive oxidation products, close in optical density value to a dose of 5 kr at a dose rate of 190 r/min. These experiments show that the time factor is an important condition for the production and accumulation of LA oxidation products.

It should be noted that a two-fold increase or decrease in the LA concentration leads accordingly to greater or lesser production of TBA-positive oxidation products as a result of the direct action of ionizing radiation on LA molecules.

The measurement of peroxide numbers by iodometric titration was carried out at the same time in the eluates obtained. An increase in peroxide numbers in accordance with an increase in the irradiation dose was noted.

In order to characterize the oxidation products formed, experiments on their resistance to various temperature conditions were carried out. The following experiments were set up for this purpose: eluates in sealed ampules were heated for 60 min in a boiling water bath and then the TBA reaction was carried out. The results obtained showed that such treatment leads to a 5-fold decrease in the substances responsible for the 532 mμ band and to a two-fold decrease in substances responsible for the 455 mμ band in comparison with freshly prepared eluates.

In this case the peroxide numbers decrease significantly.

A slower destruction of these compounds in the solution is observed when the eluates are stored in the refrigerator at a temperature of 2°. After 24 hrs, the intensity of the color D_{maks} (532 mμ) decreases 2-fold and remains for a long time at this level. The decrease in the D_{maks} (455 mμ) under these conditions is slight. The results obtained may indicate that the 532 and 455 mμ bands are responsible for the production of various LA oxidation products after irradiation in the presence of atmospheric oxygen.

The importance of products of the radiation-chemical oxidation of UFA in the development of radiation injury made it necessary to explain the biological effect of the above-described water-soluble TBA-positive LA oxidation products. For this purpose the effect of the eluates on erythrocytes and the infusoria Paramecium caudatum was investigated.

Mouse erythrocytes in a volume of 0.005 ml were added to 1 ml of eluate and incubated for 30 min at a temperature of 38°. After centrifugation the degree of hemolysis was determined from the yield of hemoglobin on an FEKN-57 apparatus. A sample containing erythrocytes in physiological solution served as the control.

The results obtained showed that water soluble LA oxidation products have a hemolytic effect. Complete hemolysis occurs from the effect of eluates obtained by irradiating the LA in doses of 5 kr and above [D_{maks} (532 mμ) > 0.1].

A study of the survival and rate of division of the infusoria was carried out by a method described in a number of papers [19-22]. For these experiments elution of the oxidation products was carried out with 1/120 M phosphate buffer with a pH of 7.2. The eluates were poured into holes and 16 infusoria were transferred into them for 60 min. Paramecia kept under the same conditions simply in buffer served as the control. After 60 min each paramecium was transplanted into a hole with fresh nutrient medium. The survival and rate of division were recorded after 24 hrs.

It was established that the presence in the medium of TBA-positive LA oxidation products irradiated in doses of 2-3 kr causes inhibition of paramecium division. With an increase in the LA irradiation dose the toxic effect of these products increases, manifested in large-scale death of the infusoria.

Thus, the production of water-soluble LA oxidation products and their high biological activity gives a basis for studying and evaluating the role of these products in the development of radiation sickness.

The determination of the chemical nature of the UFA oxidation products and the conditions of their production is an essential link in these studies.

At the present time individual papers on this subject are being published. Among the most important is the work of Frankel et al. [23, 24], in which the authors, by using various modern fractionation methods, isolated from autooxidized LA (at a temperature of 37° in an oxygen environment) four isomeric hydroperoxides at carbon positions 9, 12, 13 and 16. These hydroperoxides differed from each other in physical and chemical properties. The hydroperoxides in positions C_9 and C_{13} were most resistant to high temperature and further oxidation. The authors [23, 24] also showed that splitting of the primary hydroperoxides leads to the production of monoaldehydes, dialdehydes and hydroperoxide aldehydes.

Some authors point to the great similarity of the reactions during UFA autooxidation and oxidation under the influence of ionizing radiation in the presence of atmospheric oxygen [25-27].

The question of which of the above-indicated LA oxidation products are responsible for the development of the color in the reaction with TBA remains definitely unexplained. The notion exists that the principal TBA-reactant is a water soluble tricarbon fragment, identified as a malondialdehyde. The absorption spectrum of a colored solution of malondialdehyde with TBA is characterized by one band in the region of 532 mμ [28-30]. However, recent investigations using thin-film and gas chromatography showed the presence of various TBA-active substances in water extracts of LA

oxidized by ultraviolet rays which possess high biological activity. It has been suggested that these are unsaturated carbonyl compounds [14-16].

In analyzing the literature data and comparing them with the results of our own investigations, it can be assumed that the water-soluble TBA-positive substances obtained from the radiation-chemical oxidation of LA are close to the conjectural compounds of carbonyl nature.

In future investigations great attention must be paid to a detailed study of the chemical nature of identified UFA oxidation products and their toxic effect.

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Footnotes

196 The linolenic acid was obtained in a sealed ampule from the "Chemical Reagent" plant in L'vov.

Transliterated Symbols

196 макс = maks = maksimal'nyy = maximum

RATE OF ACCUMULATION AND INTERRELATION OF LIPID RADIOTOXINS AND QUINONES

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The toxic radiation effect is a complex process in which a group of radiotoxins [RT](PT) which are formed participate. As has been established, among the various RT lipid RT and quinones play a leading role (see this collection, pages 4 and 112).

It has been shown that lipid RT and quinones develop after irradiation in various organs and tissues of plant and animal organisms, as well as in single cells (see this collection, page 4).[1]

In connection with the fact that lipid RT and quinones have much similarity in acting on various biological specimens and systems (see this collection, pages 4 and 112) and they are all extracted by lipid solvents (in the first stage of treatment), the question arose of whether the effect of one of the RT referred to is due to the presence of the other as an impurity in the fraction under investigation.

In order to answer this question, both a parallel investigation of the rate of production of one or another RT and a careful study of the fractions isolated containing one RT in the presence of another were undertaken on the same animals in the present work.

The work was carried out on more than 70 rabbits weighing 2-3 kg and 400 white male rats weighing 150-170 g. The animals were irradiated in GUEE-800 equipment in a dose of 800 rad at a dose rate of 50 rad/min. There was one total irradiation.

The quinone preparations were obtained from the livers of irradiated rabbits (see this collection, page 37), as well as by incubation of equal amounts of tyrosine and tyrosinase solutions at a temperature of 30° for 30 min according to a previously developed method (see page 53). The lipid RT preparations and their activity were obtained and determined by methods developed by Yu. B. Kudryashov and co-workers.[2]

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[2]/206

Oxidized oleic acid served as a model of lipid RT. Oxidation was carried out for 3 days at a temperature of 60° in a current of air. The oxidized oleic acid was injected intraperitoneally once in an amount of 0.2 ml of the preparation per rat.

The experimental animals were killed by decapitation at different times after the effect (irradiation or injection of various preparations). The liver was removed from the animals and the quinone and lipid RT content determined. Intact animals, kept under conditions similar to those of the experimental animals, were always used as the control.

Extraction of the quinones from the tissue homogenate was carried out with a five-fold volume of alcohol (ethanol) acidified to pH 3.3 for 2 hrs at room temperature. Then the extract was filtered and a quantitative determination of the quinones was carried out in the obtained solution by two methods: polarography and spectrophotometry. The polarographic determination of the quinones was performed in the alcohol solution (filtrate) at $E^{\frac{1}{2}} = -0.35$ v (background – phosphate buffer, 0.15 M, pH 6.8) on LR-60 and PA-2 polarographs according to a method developed earlier. For the spectrophotometric determination the quinones from 15 ml of the alcoholic extract were adsorbed on aluminum hydroxide at pH 8.2-8.4. The aluminum hydroxide was removed by centrifugation and washed twice with alkalized water. The quinones were eluted with 5 ml of 0.04% hydrochloric acid for 1-2 min, then centrifuged and the quinones in the solution determined at pH 7.4 on an SF-4 spectrophotometer at a wavelength of 255 mμ.

The rate of quinone and lipid RT accumulation was of primary interest. It has been shown in the work of Plyshevskaya et al. (see this collection, page 37) that a rapid increase in the quinones occurs in rat liver in the first 4-8 hrs after irradiation and then there is a further rise on the 2nd day. The rate of quinone accumulation was not investigated further. In the present work the rate of quinone and lipid RT accumulation was studied in the livers of rabbits and rats irradiated in a dose of 800 rad for 6 days. The results obtained are presented in the table and in Fig. 1.

As seen from the data presented, in experiments on rats, following the initial, previously indicated (see this collection, page 37) rise in quinone content, on the 3rd-4th day the content drops sharply with a subsequent rise on the 5th day. The nature of the curve for rabbits is the same in principle with a certain shift in the fluctuation phases.

The curve of lipid RT accumulation, whose amount in rabbit livers always remained above the normal level, is of a different nature. The different courses of the curves make the assumption that the activity of a fraction containing one of the toxins is caused by contamination by another unlikely. In fact, by using spectrophotometric and polarographic methods, it was possible to show in the present work that the lipid RT preparations studied do not contain quinones. As seen from Fig. 2, the absorption maximum characteristic of quinones is not found in the range of the

Quinone Content (in % of control) in Rat and Rabbit Livers after Irradiation and After Injection of Lipid RT (at different times after the effect – irradiation or injection of lipid RT; control 100%)

1 Объект исследования	2 Метод исследования[1]	3 4 ч	4 1 сутки	5 2 сутки	5 3 сутки	5 4 сутки	5 5 сутки	5 6 сутки
6 Крысы (облучение в дозе 800 *рад*)	I	109	149	150	75	97	141	124
	II	114	124	163	96	83	—	106
7 Крысы (введение липидных РТ)	I	124	178	124	93	110	124	129
	II	199	158	—	84	80	92	105
8 Кролики (облучение в дозе 800 *рад*)	I	133	71	93	128	—	—	—
	II	129	71	92	128	—	—	—

[1] I – polarographic; II – spectrophotometric.

1) Subject of investigation; 2) method of investigation[1]; 3) hrs; 4) day; 5) days; 6) rats (irradiated in a dose of 800 rad); 7) rats (injection of lipid RT); 8) rabbits (irradiated in a dose of 800 rad).

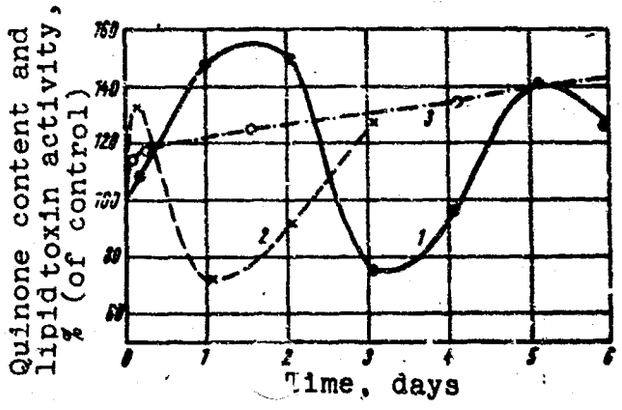

Fig. 1. Dynamics of change in quinone content and lipid RT activity after γ-ray irradiation in a dose of 800 rad. 1) Quinone content in rat livers; 2) quinone content in rabbit livers; 3) lipid RT activity in rabbit livers; control – 100%.

absorption spectra of lipid RT. The absorption maximum, found in lipid RT preparations in the region of 310 mμ, corresponds to the absorption of toxically inactive impurities present in the preparations. Thus, the quinones and lipid RT studied are individuals and do not contain toxically active impurities. This conclusion was made after a study of all the preparations isolated from livers of rats and rabbits in different stages of radiation sickness

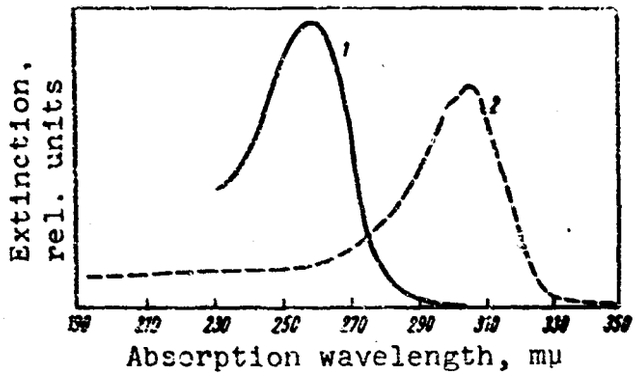

Fig. 2. Absorption spectra. 1) Quinones; 2) lipid RT preparations.

caused by different doses of ionizing radiation.

The data obtained indicate that in spite of the similarity (in many features) in the biological effect of quinones and lipid toxic substances (see this collection, pages 4 and 112), these RT are capable of participating independently in the development of the toxic radiation effect: the quinones – in the initial period and in the period of expressed clinical changes, and the lipid RT – throughout the entire radiation sickness.

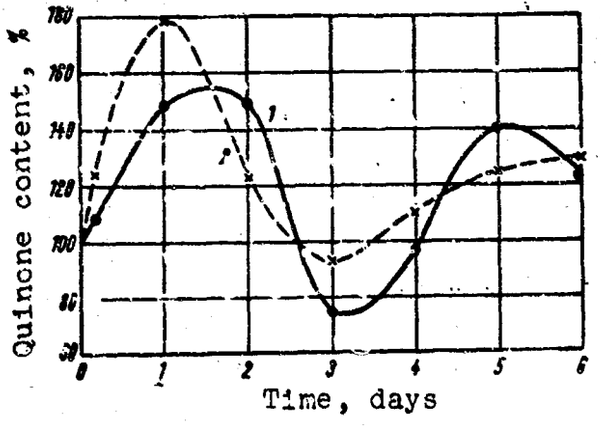

Fig. 3. Change in quinone content in rat livers. 1) After γ-ray irradiation in a dose of 800 rad; 2) after injection of lipid RT; control – 100%.

From a study of the characteristics of the biological effect of both RT, the question of the interrelation of their production in an irradiated organism arises. In order to determine this, the possibilities of the appearance of toxic quinones after the injection of lipid RT (and their model – oxidized oleic acid) in an amount of 0.2 ml per rat, as well as the production of toxic lipid substances after injection of quinones isolated from the livers of

irradiated animals (and their model – tyrosine treated with tyrosinase) were studied.

The experiments which were conducted indicate that lipid RT injected in a sufficient amount into rats cause a phase change in the quinone level analogous to that which occurs after the action of ionizing radiation (Fig. 3).

Thus, a radiomimetic effect of lipid RT is found from the change in the amount of quinones in animal livers.

The injection into rats of quinones isolated from liver or formed during the oxidation of tyrosine treated with tyrosinase (even in amounts causing death) did not cause the production of toxic lipid substances.

These data do not contradict the conclusion concerning the independent initial production of the toxic substances under consideration. It is doubtful whether it is possible to explain the initial rapid increase in the quinones by lipid RT alone, whose concentration on the first day is still insignificant. Perhaps, the secondary rise in their amount is largely connected with the effect of lipid RT. Evidently there is no basis for assuming that lipid RT are formed under the influence of the quinones.

The investigation which has been carried out, it seems to the authors, confirms the hypothesis concerning the production in an irradiated organism of several toxic substances, and the different rates of their accumulation cause the complexity and many-faceted development of the symptoms of radiation sickness.

The following conclusions can be drawn from the results obtained:

1. Quinone and lipid RT preparations isolated from the tissues of irradiated animals do not contain toxically active impurities.

2. Phase variations in the quinone level in rat and rabbit livers in different periods of acute radiation sickness and a gradually increasing lipid RT level are found.

3. The injection of lipid RT into animals causes a change in the quinone level in the liver analogous to that which occurs from the effect of ionizing radiation.

4. The injection of extracts of quinones in test doses into animals did not cause changes in the level of the toxic lipid substances.

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Footnotes

201 [1]See also Kurdyashov, Yu.B. et al., Zh. obshchey biologii

[J. of Gen. Biology], 25, 3 (1964).

201 [2]See footnote [1] above.

THE MECHANISM OF DAMAGE TO THE SUPERFICIAL ERYTHROCYTE LAYER BY UNIRRADIATED AND IRRADIATED UNSATURATED FATTY ACIDS

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The theory of the toxic effect of penetrating radiation on an organism, as is known, is open to quantitative substantiation. Bacj and Alexander [1] present the following calculation: if a radiotoxin [RT](PT) with a molecular weight of 1000 is formed with an ion yield equal to one, then after total irradiation in a dose of 500 r, 50 mg of RT will be formed in the human organism. This amount, with uniform distribution of the RT in the irradiated organism, corresponds to a toxin concentration of the order of $1 \cdot 10^{-6}$ g/ml.

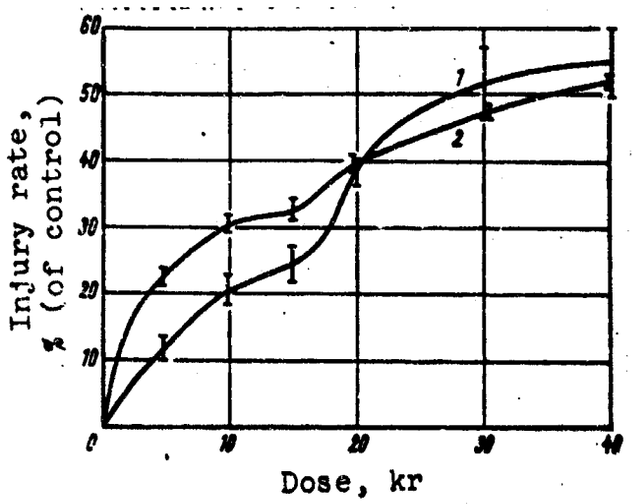

Fig. 1. Dependence of damage to erythrocytes from the blood of newborn (curve 1) and adult (curve 2) rats on the dose of γ-irradiation.

In the present work for the purpose of comparing the injurious effect of radiation and radiomimetics, the kinetics of the damage to erythrocytes suspended in physiological solution was investigated after the effect of radiation and unirradiated and irradiated unsaturated fatty acids which, as is assumed, have radiomimetic pro-

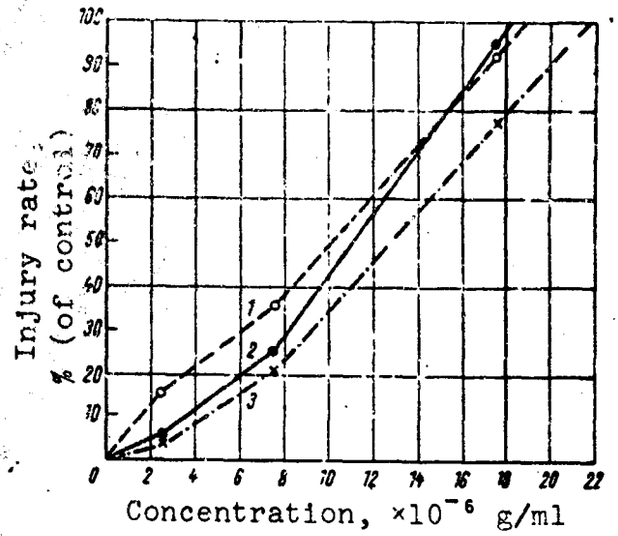

Fig. 2. Dependence of injury of rat erythrocytes on concentration of unsaturated fatty acids. 1) Linoleic acid; 2) linolenic acid; 3) oleic acid.

perties [2]. The injurious effect of unsaturated fatty acids on erythrocytes was investigated at those concentrations in which RT can be produced in an irradiated organism.

Heparin-treated fresh rat blood was diluted with physiological solution in a proportion of 1:100. The erythrocyte suspension thus obtained was subjected to γ-irradiation in doses of 10, 20, 30 and 40 kr. In another series of experiments erythrocyte suspensions were incubated for 1 hr at a temperature of 32.0 ± 0.2° with unsaturated fatty acids in concentrations from $1 \cdot 10^{-6}$ to $18 \cdot 10^{-6}$ g/ml. Since unsaturated fatty acids under oxidation conditions have radiomimetic properties [2], it seemed of interest to compare the dose dependence of erythrocyte injury by radiation and by unsaturated fatty acids. The effect of cell injury was investigated by a photoelectrocolorimetric method which we developed: the change in optical density of the erythrocyte suspension with time from the effect of isotonic alkaline buffer was determined. The shift in the kinetic curves of the experimental samples in percentages relative to the position of the control curve served as the measure of erythrocyte damage [3, 4].

Two dose curves of the radiation injury of erythrocytes from the blood of newborn [1] and adult [2] rats are shown in Fig. 1. The obtained dependence of the injurious effect in percent of the control on the irradiation dose can be interpreted on the basis of classical target theory [5]. As seen from the figure, the curves start out in the form of an exponential function, change to an S-shaped segment (which is more expressed for erythrocyte suspensions from the blood of newborn rats) and end up asymptotically parallel to the abscissa. An analysis of these curves given in another paper [6] made it possible to establish three different components of rad-

iation injury in the erythrocyte membrane. These components which we called α-, β- and *K*-components form populations of targets in the erythrocyte membrane which differ sharply from each other in degree of radiosensitivity: the α-components are single-hit targets, while the β- and *K*-components are multiple-hit targets.

We shall now examine the dose curves, obtained by the same method of isotonic alkaline hemolysis, after the action of various unsaturated fatty acids on erythrocyte suspensions.

Samples of oleic and linolenic acid, produced domestically, and of linoleic acid produced by the British Miller firm were used for measuring hemolytic activity. After opening the samples were stored under nitrogen at a temperature of 0°. The dependence of the degree of erythrocyte damage on the concentration of unsaturated fatty acids is shown in Fig. 2.

As seen from the course of the dose curves, the degree of erythrocyte damage has a linear dependence on the concentration of the unsaturated fatty acids in the range from $1 \cdot 10^{-6}$ to $2 \cdot 10^{-5}$ g/ml. By extrapolating the curves in the direction of a decrease in injurious effect, the absence of a "threshold" (inactive) dose is found: all the curves begin with the zero value and reach approximately the same upper limit, corresponding to 100% damage of the erythrocytes. The linear dependence of the effect of cell damage on the concentration in the absence of a "threshold" concentration of the injurious agent is proof of the fact that the interaction between molecules of the injurious agent (unsaturated fatty acids) and the substrate (superficial layer of the erythrocyte) is a zero order reaction: it is as if the unsaturated fatty acid dissolves in the injury substrate.

The data concerning the absence of a "threshold concentration" of the hemolytic agents – unsaturated fatty acids – indicate that an erythrocyte is injured from a single action of the injurious agent, that is, from the action of one molecule. One molecule of an unsaturated fatty acid, coming into contact with the cell surface at any place, causes injury of the erythrocyte. This mechanism, as is seen, differs radically from the mechanism of "target" injury by penetrating radiation.

The question arises of what determines the limiting concentration of unsaturated fatty acids which causes 100% erythrocyte injury. Since one molecule of unsaturated fatty acid injures an erythrocyte at any contact site, it can be assumed that the limiting concentration of the injurious agent corresponds to that number of molecules at which the whole surface of the erythrocyte is covered with molecules of unsaturated fatty acids. At this limiting concentration the injury of the erythrocyte will be maximal which is manifested in instantaneous hemolysis, whereas at lower concentrations of the unsaturated fatty acids the time of the onset of hemolysis will be greater the smaller the concentration of the injurious agent.

There are data in the literature that powerfully acting hemoly-

tic agents which cause "instantaneous" hemolysis act at a concentration at which the surface of the erythrocyte is completely covered with molecules of the hemolytic agent [7, 8]. Maximal injury of erythrocytes was found, for example, from the action of linolenic acid in a concentration of $18 \cdot 10^{-6}$ g/ml. It can be assumed that this concentration of the injurious agent corresponds to the number of molecules at which the surface of the erythrocyte is completely covered with linolenic acid molecules. To verify the correctness of this assumption we shall make the following calculation.

The concentration of erythrocytes in rat blood is approximately $6 \cdot 10^9$ cells/ml [9]. The radius of the rat erythrocyte $r_{er} = 3.1$ μ [10]; hence the surface of the erythrocyte P_{er}, if it is considered to be equal to the surface of a sphere with approximately the same radius, equals

$$\Pi_{sp} = 4\pi r^2_{sp} \approx 120 \cdot 10^8 \text{ Å}^2. \quad (1)$$

There was a one hundredfold dilution of the rat blood in the experiments, that is, the erythrocyte concentration was

$$\sim 6 \cdot 10^7 \text{ cells/ml}. \quad (2)$$

The surface of all the erythrocytes P_{er} in 1 ml of blood at this concentration equals on the basis of (2) and (1)

$$\Pi_{sp} \approx 120 \cdot 10^8 \times 6 \cdot 10^7 = 7{,}2 \cdot 10^{17} \text{ Å}^2. \quad (3)$$

We shall now calculate the surface which all the molecules of linolenic acid occupy at the limiting concentration causing 100% injury of all the erythrocytes available in the given subject. The number of molecules of linolenic acid at the limiting concentration equals

$$18 \cdot 10^{-6} \text{ (g/ml)} \approx \frac{18 \cdot 10^{-6}}{280} M/\text{ml} \approx \frac{18 \cdot 10^{-6}}{280} \cdot N \text{ molecules/ml} \approx$$
$$\approx 3{,}6 \cdot 10^{16} \text{ molecules/ml}. \quad (4)$$

where 280 is the molecular weight of linolenic acid and N is Avogadro's number ($N = 6 \cdot 10^{23}$).

One molecule of fatty acid, independently of the length of its carbon chain, takes up on the lipid layer with its lipophilic end an area of approximately 22 Å^2 [11]. Hence, on the basis of (4) we obtain the area P_l which all the molecules of linolenic acid in 1 ml occupy:

$$\Pi_l = 3{,}6 \cdot 10^{16} \cdot 22 \text{ Å}^2 = 7{,}9 \cdot 10^{17} \text{ Å}^2. \quad (5)$$

By comparing Eqs. (3) and (5) it can be seen that the numerical values of the total surface of all the erythrocytes P_{er} in 1 ml and the total surface of the lipophilic segments of all the linolenic acid molecules P_l in 1 ml differ from each other by no more than 8-9%, that is,

$$\Pi_{ep} \approx \Pi_{e}. \qquad (6)$$

It follows from Eq. (6) that the limiting concentration of linolenic acid which causes maximal injury of the erythrocytes is the concentration at which the surface of the erythrocytes is completely covered with molecules of the injurious substance. At this limiting concentration the number of linolenic acid molecules which completely cover one erythrocyte equals

$$\frac{\Pi_{ep}}{22\ \text{Å}^2} \approx \frac{120 \cdot 10^8}{22} \approx 5 \cdot 10^8 \text{ molecules.}$$

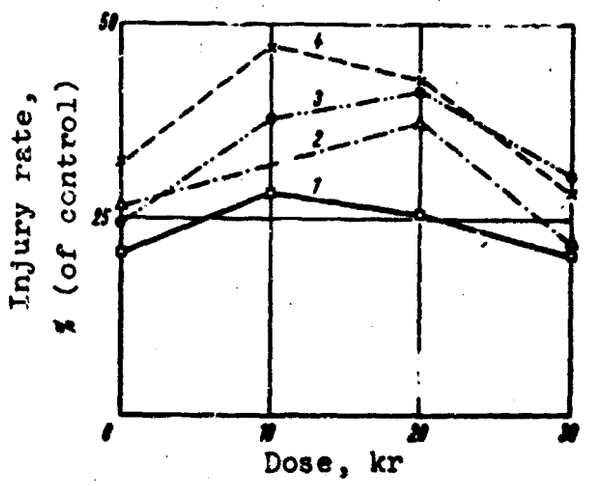

Fig. 3. Dependence of erythrocyte injury on effect of irradiated linolenic acid (1-4 – experiment number; acid concentration $7.5 \cdot 10^{-6}$ g/ml).

It seemed of interest to examine the injurious effect of linolenic acid subjected to γ-irradiation in doses of from 10 to 30 kr. An unsaturated fatty acid under oxidation conditions contains, as is known, radiomimetically acting peroxides, whose concentration can change from irradiation.

In Fig. 3 are shown the results of four experiments obtained from the action of irradiated linolenic acid in a concentration of $7.5 \cdot 10^{-6}$ g/ml on erythrocytes. As seen from these data, the injurious effect of the unsaturated fatty acid increases as a result of irradiation, reaches a maximum in the region of doses of 10-20 kr and decreases to the initial value at 30 kr.

The question arises of whether the intensification of the injurious effect of irradiated linolenic acid is connected with an increase in the concentration of peroxides in the irradiated samples. To answer this question, the peroxide content in unirradiated and

Content of Peroxides in Linolenic Acid

Доза, кр 1	2 Концентрация перекисей, $\times 10^{-6}$ моль/мл			
	3 Опыт 1	3 Опыт 2	3 Опыт 3	3 Опыт 4
4 Контроль	10,5	9,6	9,9	19,2
10	9,6	9,3	8,4	18,0
20	9,6	9,3	9,0	20,2
30	9,3	9,6	10,5	21,0

1) Dose, kr; 2) peroxide concentration, $\times 10^{-6}$ mole/ml; 3) experiment; 4) control.

irradiated (with different doses) linolenic acid was investigated. Peroxide concentration was determined by Hartmann and Clavind's method [12]. The calibration curve was obtained with succinic acid peroxide synthesized according to Guben [13]. The accuracy of the measurements in micromoles of peroxide per ml was $\pm$10%.

Data on the peroxide content in linolenic acid after irradiation in doses of 10, 20 and 30 kr are presented in the table.

As seen from the data presented, the peroxide content does not increase from the effect of γ-irradiation in doses of 10, 20 and 30 kr and, consequently, there is no correlation between the detected effect of an increase in the hemolytic activity of irradiated linolenic acid and the peroxide content.

Thus, our investigations showed that the injurious mechanisms of penetrating radiation and hemolytic agents are subject to different principles. Whereas the injury of a biological specimen by penetrating radiation occurs according to Poisson statistics, that is, the statistics of random and extremely rare events, the kinetics of the injury of a biological specimen by hemolytic agents corresponds to a zero order chemical reaction: the hemolytic agent acting in an extremely small amount, but in the form of a stable substance, as it dissolves in the injury substrate, whose mass is practically infinitely large in comparison with the mass of the toxin. Unsaturated fatty acids are not, therefore, radiomimetics, since the kinetics of their injurious effect on erythrocytes is subject to a different principle than the kinetics of erythrocyte injury by penetrating radiation.

The following conclusions can be drawn:

1. The kinetics of the injury of erythrocytes, suspended in physiological solution, by penetrating radiation can be interpreted on the basis of target theory, whereas the injury of erythrocytes by unsaturated fatty acids is controlled by the law of the kinetics of a zero order chemical reaction.

2. The maximal concentration of oleic, linolenic and linoleic acids which causes 100% injury of erythrocytes lies in the range of $(18-22)\cdot10^{-6}$ g/ml; at this concentration the surface of the erythrocyte is completely covered with unsaturated fatty acid molecules, which corresponds to $5\cdot10^{8}$ molecules on one cell.

3. From γ-irradiation of linolenic acid in doses of 10-20 kr its hemolytic activity increases. This increase, however, is not connected with the peroxide content, whose concentration does not increase from the effect of irradiation in doses of 10, 20 and 30 kr.

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210 эр = er = eritrotsit = erythrocyte
210 п = p = poverkhnost' = surface
210 л = l = linolenovyy = linolenic

CHOLINE PRODUCTION IN ANIMAL RADIATION SICKNESS

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Much attention has been paid to the radiation toxic effect in radiobiological literature. It is expressed by the effect of a group of biologically active substances whose production in animal organs and tissues is closely interrelated. The question of choline's role in radiation sickness, which arose in the initial period of radiobiology's development [1], still continues to attract attention [2-5].

Choline's physiological and biochemical significance has been well studied [6], therefore an explanation of the change in the level of the choline content in tissues and organs of irradiated animals is of great interest in solving the problem of radiation toxemia.

However, the dynamics of the change in the choline level (particularly in the initial period) during radiation sickness has still not been completely studied. Moreover, it is still not clear at the present time how choline production is connected with other biologically active substances. In the present work the dynamics of the changes in the level of free and total choline, as well as the connection of its production with the appearance of other lipid radiotoxins [RT](PT) – the so-called natural radiomimetics – was studied.

White male rats weighing 130-150 g were used in the work. Irradiation was carried out in an RUM-3 unit in a dose of 800 r (filters: Al – 1.0 mm and Cu – 0.5 mm). There was one total irradiation.

Preparations of oxidized oleic acid which served as a model of lipid radiotoxins (RT) were obtained by a previously described method [7]. The OOA (OOK) preparations were injected once intraperitoneally into the animals in a dose of 0.25 mg per rat. Intact animals, kept under the same conditions as the experimental ones, served as the control. At different times after injection of the preparation or irradiation, the rats were killed by decapitation, the test organs were extracted and the amount of total and free choline in them was studied.

For determination of total choline the test organs were dried in an incubator at a temperature of 100-105°, then they were ground and placed in a Soxhlet apparatus. Extraction was carried out with absolute methanol for 8-24 hrs. The methanol was removed by evaporating the extract almost to dryness with subsequent hydrolysis of the residue with a boiling aqueous $Ba(OH)_2$ solution. The hydrolysate was neutralized with acetic acid and after filtration through an asbestos filter, a solution of Reinecke salt in methanol was added to the filtrate. Then after precipitation of the choline, the solution was filtered through a No. 4 glass filter and the residue obtained dissolved in acetone. The amount of choline reineckate was determined electrophotocolorimetrically (with a No. 2 green filter [8]).

For determination of free choline the tissue being analyzed was pulverized in the cold and an equal volume of a 10% trichloracetic acid solution added to it. The solution obtained was filtered and the lipids extracted three times with diethyl ether. The ether residue was evaporated in a water bath. The choline was precipitated with a 2% solution of Reinecke salt in methanol and the precipitate of choline reineckate was dissolved in acetone. The concentration of the choline reineckate was determined electrophotocolorimetrically (with a No. 2 green filter).

Changes in the choline level of various rat organs occur at the very beginning of radiation sickness (Fig. 1).

Thus, in the first period after one total irradiation in a dose of 800 r the amount of total choline in the liver, spleen, kidneys, heart, muscles and testicles of the rats changes noticeably in comparison with normal. These changes are not the same for different tissues. In the spleen, testicles and kidneys an increase in choline is observed, while in the skeletal muscles and myocardium there is a decrease.

There are data in the literature pertaining to changes in the choline level of various organs of irradiated animals. However, the change in total choline content in the initial periods of radiation sickness was not studied by the authors of these papers [2-5], but only the dynamics of the change in choline 24-38 hrs after the action of radiation was studied. In the opinion of some authors [3], an increase occurs in the first 24 hrs after irradiation with sublethal and lethal doses; in the opinion of others [4, 5], there is a decrease in the total choline in various organs. Thus, the data available in the literature are contradictory. This can evidently be explained both by the difference in the methods used by different authors and by dissimilar evaluation of the degree of severity of the radiation sickness (dependence of the changes on time after irradiation). And in fact, it is seen from our data (see Fig. 1) that, depending on the time after irradiation, both an increase and a decrease in the amount of choline can be observed in comparison with normal.

As seen from Fig. 2, the amount of choline in the first period of radiation sickness (first hours) increases, while in later periods (on the fifth day) a drop in the choline level below normal can be observed in the rat livers. There is no data in the

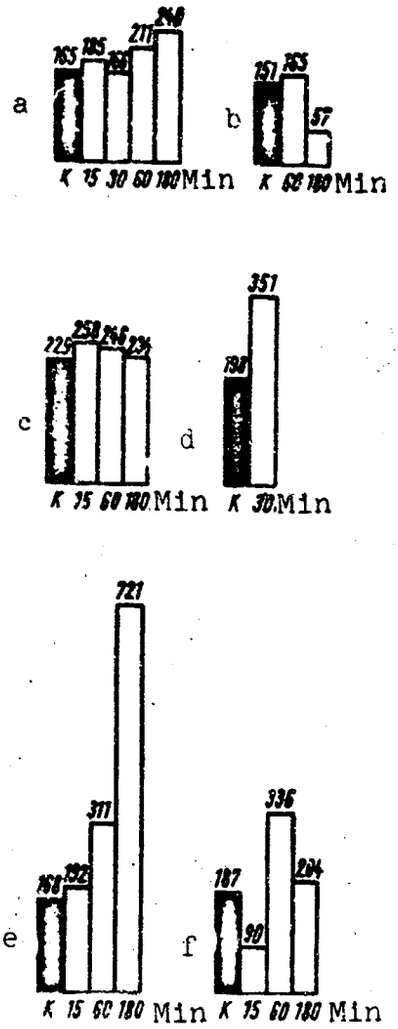

Fig. 1. Average content of total choline in organs and tissues of rats irradiated in a dose of 800 r. a) Liver; b) muscles; c) kidneys; d) testicles; e) spleen; f) heart; *K*) control. The time after irradiation is given along the abscissa.

literature about a change in free, biologically active choline during radiation sickness. In the experiments conducted, in contrast to the change in total choline content, a sharp increase in free choline occurs both in the first period (1 hr after irradiation) and on the third day.

It can be assumed that the change in the free choline content is clearly connected with intensification of autolytic reactions during radiation sickness. Indirect data in the literature indicate this. For example, it has been shown that processes of tissue autolysis can increase sharply in certain periods of radiation sickness [9]. On the other hand, it is believed that free choline can be produced in the tissues as a result of intensification of autolytic processes [10].

It was shown in the experiments conducted that during

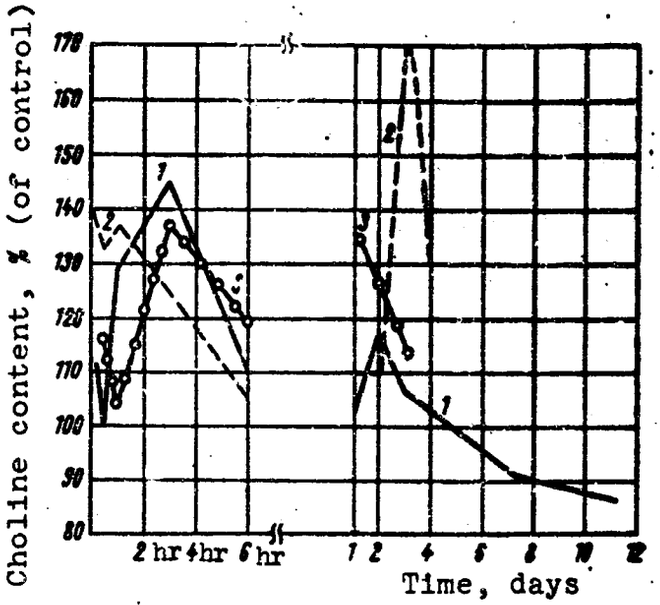

Fig. 2. Content of free and total choline in rat livers after irradiation and OOA injection. 1) Amount of total choline after irradiation; 2) amount of free choline after irradiation; 3) amount of total choline after OOA injection.

autolysis the amount of free choline in irradiated tissues increases rapidly (Fig. 3).

The increase in free choline and the gradual decrease in total choline on the third day after irradiation evidently are related to the intensification of autolytic processes in the liver of irradiated animals at this time. In the first period of radiation sickness the increase in free and total choline evidently occurs through its regeneration.

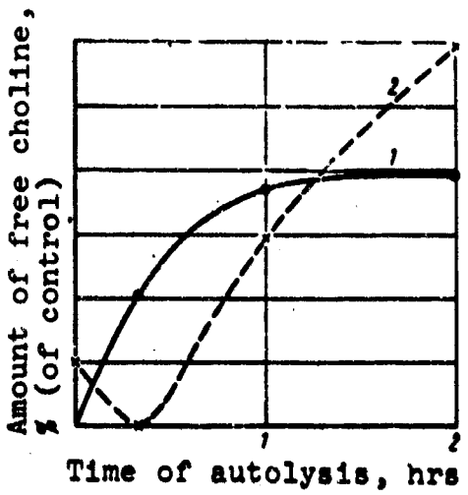

Fig. 3. Change in amount of free choline in the livers of rats irradiated in a dose of 800 r during autolysis. 1) Control (normal; 2) irradiated rats.

As is known (see this collection, page 161) the production of other toxic substances – lipid RT – is also connected with an intensification of autolytic reactions. On the basis of these data the question arises: what is the connection between choline and lipid RT?

To answer this question, the effect of lipid radiotoxic substances on the change in the total choline level was studied.

It is seen from Fig. 2 that lipid RT cause a change in the choline level: an increase in the first period after injection and then some decrease in the choline level; however, the amount of total choline remains considerably above normal for 3 days. The obtained phase changes in the level of total and free choline are similar to what is observed during radiation sickness. However, they do not always coincide in time after the effect.

Thus, lipid RT are capable of causing the production of another biologically active substance – choline, which, in the opinion of a number of authors [1, 4], participates in some manifestations of radiation sickness.

The following conclusions can be drawn from the investigations which have been presented:

1. Phase changes in total and free choline level in various organs and tissues of rats irradiated in a dose of 800 r are observed.

2. An increase in total choline in the spleen, liver and kidneys occurs in the first period of radiation sickness; an early decrease can be found in the skeletal and cardiac muscles.

3. An increase both in free and in total choline occurs in the liver of irradiated rats in the first period of radiation sickness, evidently through its regeneration.

4. In the period of the further development of radiation sickness an increase is observed in the free choline level and a decrease in the total choline level. This is evidently connected with intensification of autolytic processes in the tissues.

5. The injection of lipid RT leads, like radiation, to phase changes in the amount of choline in the liver.

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THE CHANGE IN THE FREE HISTAMINE LEVEL IN RAT TISSUES FROM THE EFFECT OF PHYSICAL AND CHEMICAL FACTORS

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The prevention and treatment of radiation sickness is the fundamental problem of contemporary radiobiology. Therefore, the study of the toxic substances formed in the organs and tissues of biological specimens irradiated with ionizing radiation is of exceptionally great importance at the present time.

This problem has attracted many investigators: Tarusov [1], Kuzin [2, 3], Gorizontov [4], Ellinger [5], Krichevskaya [6], Sverdlov [7], Kudryashov [8] and others. Various toxic substances differing in chemical nature have been found by the investigators: histamine, choline, oxidized products of unsaturated fatty acids, organic peroxides, quinones and others. All these substances are capable of causing individual symptoms characteristic of radiation sickness. However, there are no data on the connection and sequence of production of these substances during radiation sickness in the literature.

Kudryashov et al. [9] found in the tissues and organs of irradiated animals products of unsaturated fatty acid oxidation which are accumulated with time and depend on the size of the irradiation dose. The authors called these products "natural radiomimetics" [NR] (EP), since they are formed in the living irradiated organism and are capable of imitating radiation sickness in various biological specimens and systems. It has been shown that NR is capable of participating in the primary reactions of radiation sickness; other toxic substances – histamine and quinones – as is known from the literature [1, 2], do not participate in the primary reactions.

The question naturally arises of whether NR when injected into the animal organism is capable of causing the production of secondary toxic substances. In the present work the widely known toxic substance – histamine – was selected for this investigation.

All the work performed over the course of two decades by various investigators did not divert attention from the old question raised by Ellinger of the significance of histamine in radiation sickness. And when in recent years the possibility of protection against radiation sickness by histamine was established, this evoked new studies of histamine's dual role. According to the literature data, the free histamine level can increase under the

influence of various factors. Sel'ye [10] attempted to explain the complex changes occurring in radiation sickness by a "stressor effect on the organism."

In connection with this, we studied the dynamics of free, biologically active histamine accumulation during radiation sickness and after NR injection to determine the interrelation of NR and histamine production. In addition, we attempted to establish whether the change in the free histamine level is specific for radiation sickness. Vibration was selected as a nonspecific factor differing from ionizing radiation. Thus, in our work the question of the production of one of the radiotixic substances, namely histamine, under the influence of NR, radiation and vibration was investigated.

Products of oleic acid oxidation [OOA] (OOK) were used as the NR model; the oxidation of oleic acid was carried out for 48 hrs at a temperature of 60° by an air current in the dark. White male rats weighing 150-160 g were the subject of investigation. The OOA was injected once into the animals intraperitoneally in an amount of 0.2 ml per rat. The irradiation (one total) of the animals was carried out in a GUBE-800 unit in a dose of 800 r. The rats were killed by decapitation at different times after the OOA injection or irradiation. The organs and tissues were removed from the animals and their content of free biologically active histamine, as well as the histaminopexic capacity determined by chromatography [5]. Each figure presented in the tables was obtained by statistical treatment of the data from 10-20 rats, and the free histamine level in the tissues was determined in 4-6 parallel samples. The free histamine level and the histaminopexic capacity of the tissues of healthy, intact rats served as the control.

The NR was obtained from rabbit livers (third day after irradiation in a dose of 1000 rad) by extraction of the lipids with ether and subsequent removal of the phospholipids with acetone [9]. The NR was injected intraperitoneally once into rats in an amount of 0.7-1.0 g per rat. Vibration of the rats was carried out in VUS-70/200 as well as VU-15 vibrounits with the following parameters: frequency 70 cps, amplitude 0.5-0.6 mm, vibro-overload 11-12 g, duration of vibration 1 hr.

Krichevskaya's experiments [6] are confirmed by data obtained from the study of the free histamine level in irradiated rat tissues and organs: the histamine level increases in the initial period and decreases in the ensuing period.

As seen from Table 1, 5 min after irradiation a considerable increase occurs in the histamine content of the skin and kidneys; the increase is less noticeable in the stomach, while in the liver the histamine level remains unchanged in this period. On the third day after irradiation, the amount of histamine in the skin, kidneys and stomach drops, while the free histamine content of the liver is increased 1.5 times.

After the injection of OOA into rats, a picture of the free histamine content is observed which is very similar to that of

radiation sickness: the same phase change in the skin, kidneys, stomach and liver. This indicates a similarity in the action of NR and ionizing radiation.

NR preparations when injected into rats cause statistically reliable changes in the free histamine content of the tissues studied: skin, kidneys and liver. In the initial period of the illness after NR injection, a sharp increase in histamine occurs; in the terminal period the histamine is decreased in comparison with normal. Each tissue has its own specific changes. Thus, in

TABLE 1

Effect of Irradiation, OOA and NR on Free Histamine Content of Tissues

1 Вариант опыта	Число опытов 2	3 Содержание свободного гистамина на 1 г ткани, мкг			
		4 Кожа	5 Печень	6 Почки	7 Желудок
8 Контроль (норма)	30	12,7±0,6	6,3±0,5	4,4±0,2	12,5±0,3
9 5 мин после облучения	12	24,0±1,7	6,3±0,9	7,5±0,4	13,8±0,7
10 3 суток после облучения	10	9,6±1,4	10,0±1,1	6,2±0,3	6,5±0,2
11 1 ч после введения ООК	14	17,7±0,9	8,8±0,2	8,9±0,5	17,7±0,7
12 3 суток после введения ООК	17	9,0±0,2	13,0±0,5	4,9±0,4	10,4±0,4
13 Начальный период поражения (1 ч после инъекции ЕР)	14	26,0±1,6	9,0±1,2	7,5±0,3	—
14 Терминальный период (3 суток после инъекции ЕР)	16	8,0±0,9	5,0±1,4	3,5±0,2	—

1) Variant of experiment
2) Number of experiments
3) Free histamine content per g of tissue, μg
4) Skin
5) Liver
6) Kidneys
7) Stomach
8) Control (normal)
9) 5 min after irradiation
10) 3 days after irradiation
11) 1 hr after OOA injection
12) 3 days after OOA injection
13) initial period of illness (1 hr after NR injection)
14) terminal period (3 days after NR injection)

the skin which normally has a greater amount of free histamine (12.7 ± 0.6 μg per g of tissue) than other tissues, the sharpest variation in its level occurs: in the initial period of the illness the free histamine level increases more than two-fold, while in the terminal period it decreases more than three-fold (in comparison with the initial period). Less sharp variations are observed in the liver of affected rats. All these changes are similar to those which occur during acute radiation sickness. The great similarity in the dynamics of the range in the free histamine level in the rat tissues studied after the effect of NR, OOA and ionising radiation indicates a similarity in the action of the chemical substances and radiation, that is, radiomimetic activity of products of unsaturated fatty acid oxidation.

The radiomimetic effect of a change in the free histamine level in organs and tissues is accompanied by a change in the histaminopexic capacity of the tissues. Krichevskaya [6] believes

that the histaminopexic capacity of the tissues is one of the defense mechanisms which regulate the level of free histamine in the organism and is suppressed in animals after irradiation. These conclusions were also confirmed by the experiment (Table 2).

TABLE 2

Effect of Irradiation and OOA on Histaminopexic Capacity of Tissues (in per cent of histamine binding)

1 Вариант опыта	2 Число опытов	3 Гистаминопексическая способность: 4 Почки	5 Кожа
6 Контроль (норма)	22	89.0±1.5	79.0±1.2
7 1—2 ч после облучения	15	48.0±3.2	57.0±2.8
8 3 суток после облучения	11	—	42.0±4.1
9 1—2 ч после введения ООК	33	74.0±1.9	69.0±2.3
10 3 суток после введения ООК	11	39.0±3.5	39.0±4.1

1) Variant of experiment
2) Number of experiments
3) Histaminopexic capacity
4) Kidneys
5) Skin
6) Control (normal)
7) 1-2 hrs after irradiation
8) 3 days after irradiation
9) 1-2 hrs after OOA injection
10) 3 days after OOA injection

Inhibition of the tissues' capacity to fix histamine occurs in the kidneys and skin 1-2 hrs after irradiation. OOA affects the histaminopexic capacity of the skin and kidneys in the same way as radiation sickness. The kidneys and skin - the tissues in which an increase in the histamine level from radiation and radiomimetic effects (see Table 2) occurs most rapidly and clearly - were selected for a study of the change in histaminopexia. A gradual decrease in the capacity to bind histamine occurs in the tissues over the course of 3 days after the injection of OOA. In 3 days the histaminopexic capacity of the tissues decreases two-fold. It can be concluded that OOA causes the change in the free histamine level of the tissues and in histaminopexic capacity in the same way as occurs in radiation sickness.

TABLE 3

Effect of Vibration on Free Histamine Content of Tissues

1 Вариант опыта	2 Число опытов	3 Содержание свободного гистамина в тканях, мкг: 4 Кожа	5 Почки	6 Печень	7 Желудок
8 Контроль (норма)	43	12.7±0.5	4.4±0.2	6.3±0.5	11.5±0.5
9 5 мин после вибрации	21	24.5±0.9	8.8±0.2	7.7±0.3	16.0±0.7
10 1 ч после вибрации	11	12.0±0.8	4.8±0.3	6.7±0.4	11.9±0.5
11 3 суток после вибрации	10	12.8±1.0	3.8±0.3	5.8±0.4	10.8±1.5

1) variant of experiment; 2) number of experiments; 3) free his-

tamine content per g of tissue, μg; 4) skin; 5) kidneys; 6) liver; 7) stomach; 8) control (normal); 9) 5 min after vibration; 10) 1 hr after vibration; 11) 3 days after vibration.

Changes were also found in the level of free histamine in the rat tissues in a study of the effect of vibration on the free histamine level of rat tissues (Table 3).

Vibration changes the free histamine level. However, these changes differ from the effects of radiation and radiomimetics. After the effect of vibration a short-lived increase in the histamine level occurs in the tissues studied; this increase was the same as that from the effect of radiomimetics and radiation. A histamine decrease phase was not observed: 1 hr after vibration the amount of histamine in all the tissues studied reaches normal and does not change in the future. Thus, the dynamics of the change in the free histamine level after the effect of vibration differs from the effect observed from radiomimetics or radiation.

The following conclusions can be drawn:

1. The injection of rats with OOA (a model of toxic substances developing during radiation sickness) causes phase changes in the free histamine content of organs and tissues. Sharp inhibition of the histaminopexic capacity of the organs and tissues also occurs.

2. After the injection of rats with lipids isolated from irradiated rabbit livers (NR), a change is observed in the free histamine level bearing a phase character.

3. The changes observed are similar to those of radiation disturbances in the free, biologically active histamine content and the histaminopexic capacity of organs and tissues.

4. The radiomimetic effect of a change in the free histamine level of rat tissues makes it possible to draw the conclusion that NR causes the production of a secondary toxic substance, histamine, during radiation sickness.

5. The dynamics of the change in the free histamine level of the tissues from another effect - vibration - differs from the dynamics observed after the action of radiation or radiomimetics.

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THE ROLE OF INCREASED SENSITIVITY TO A NUMBER OF BIOGENIC FACTORS IN THE DEVELOPMENT OF RADIATION TOXEMIA

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The profound discoordination of various metabolic processes caused by ionizing radiation inevitably leads both to the development of specific radiotoxins [RT] (PT) and to a disturbance in the level of biologically active substances inherent to the organism, which is relatively constant under physiological conditions. In the case of the accumulation of the latter in an irradiated organism in abnormally high concentrations they also can become the cause of certain radiation reactions.

Moreover, in studying the development of radiation toxemia it is necessary to take into account not only the level of these normal or abnormal metabolites, but also the possibility of a different, more severe pathological reaction of the irradiated organism.

There are rather numerous indications in the literature at the present time concerning such increased sensitivity of irradiated animals, their individual organs or systems to a whole series of humoral factors. However, the intimate mechanisms lying at the basis of this interesting phenomenon are still far from clear. Korchemkin and Rayeva [1] observed on an electrocardiogram an intensification of adrenalin's toxic effect on irradiated dogs. They recorded block, extrasystole and arhythmia. They also noted in irradiated animals excitation of the sympathetic nervous system.

Hyperactivity of the myocardium to the injection of adrenalin was found in irradiated dogs by Danysh [2]. According to his data, the pressor reaction to adrenalin also intensifies as the radiation sickness develops. However, Danysh did not note higher adrenalin toxicity in mice irradiated with a sublethal dose.

The literature data concerning a change in the reactivity of irradiated animals with respect to acetylcholine are somewhat contradictory. According to Danysh, the sensitivity to acetylcholine of the cholinergic receptors of the small intestine and uterus of guinea pigs irradiated with a sublethal dose considerably decreases. On the other hand, according to Smirnov and Shikhodyrov's data [3], the sensitivity to acetylcholine both of the small and of the large intestine of rats after irradiation in a dose of 1000 r is considerably increased. It is possible that

these differences can be explained both by species specificity and by the dose of irradiation used. In older studies [4, 5] a considerable increase was noted in the sensitivity of irradiated rabbits and dogs to adenosine, trypsin and histamine. Sensitivity to the latter becomes greatest 2-2.5 hrs after irradiation [4, 5]. When the skin histamine test was used, a sharp increase was also noted [6] in the sensitivity of irradiated guinea pigs to histamine.

It is possible that the high death rate of rats from the joint action of irradiation and small doses of oxidized oleic acid in Goncharenko and Kudryashov's experiments [7] should be considered not only as an example of the synergic effect of ionizing radiation and natural radiomimetic, but also as a manifestation of the increased sensitivity which is evidently characteristic of irradiated animals.

With this convincing experience of previous investigators in mind, the authors attempted to obtain their own data on this subject. The investigations were carried out on white unpedigreed mice and rats which were subjected to x-ray irradiation in a dose of 800 r at a dose rate of 50 r/min with 0.5 Cu + 0.75 Al filters.

In determining the changes in the sensitivity of the cholinergic receptors from the effect of ionizing radiation, instead of the very unstable acetylcholine, its analogue, carbocholine, which is not subject to the action of cholinesterase in the organism, was used in the experiments.

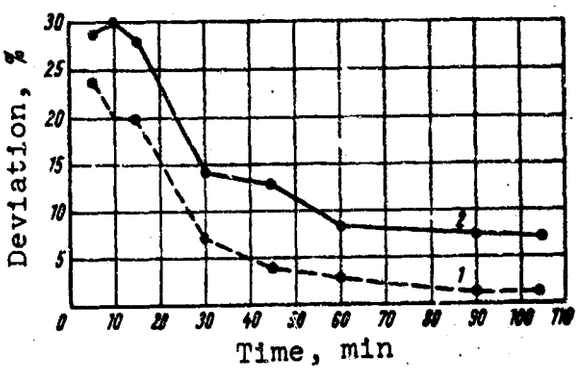

Fig. 1. Effect of ionizing radiation on the sensitivity of rats to carbocholine. 1) Control (normal); 2) after irradiation.

The electrocardiographic studies (Fig. 1) which were carried out showed that the slowing down of the rhythm of the heart contractions after the intraperitoneal injection of rats with 25 µg of carbocholine is considerably more strongly expressed in irradiated animals than in normal animals. The difference in the effect's duration is especially sharp. In the control rats 45 min after the effect, the frequency of the heart contractions becomes practically normal, while in the irradiated rats after 105 min it

still remains statistically reliably slowed down.

The sensitivity of irradiated rats to histamine increases to an even greater degree. In fact, normal rats withstood the intraperitoneal injection of 25 mg of histamine well. In the case of preliminary irradiation in a dose of 800 r the injection of the same amount of histamine immediately afterward caused a severe state of shock in a majority of the animals. Prostration, an expressed pilomotor reaction and cyanosis of the extremities was observed. In the first hours after the effect 65% of the irradiated animals died.

In view of the considerable accumulation of histamine in the irradiated organism [8], it was assumed that the increase in sensitivity to this highly active biogenic factor can be the cause of a whole series of further radiation reactions. In view of this, it seemed necessary to investigate in detail the cause and mechanism of the given phenomenon.

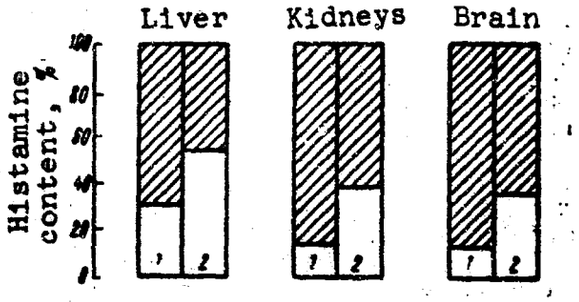

Fig. 2. Effect of ionizing radiation on the distribution in organs of histamine from the general circulation between its free and bound fractions. 1) Control (normal); 2) 45 min after irradiation. Light column) free histamine; cross-hatched column) bound histamine.

First of all a comparative study of the distribution of histamine injected into the blood in the various organs of normal and irradiated animals was required. However, a determination of only its total content in the tissues was insufficient for the purposes of this experiment. Since the cause of histamine's high toxicity for irradiated animals was especially interesting, it was necessary to investigate the level of its free biologically active, that is, highly toxic, fraction.

It was established from an analysis of the data obtained that in normal animals the greater part of the histamine injected into the general circulation is very quickly (in 5 min) absorbed by the tissues of various organs in complete correspondence with the data of Halpern et al. [9], who injected very small doses of labeled histamine and observed its extremely uneven distribution in the tissues of individual organs.

It is clear that in accordance with the different penetrabilities of the histohematic barriers [HHB] (ГГБ), the order of

the organs investigated should be the following: kidneys, liver and skin. Histamine injected into the blood does not penetrate at all into the brain of normal rats. Here, as seen from Fig. 2, the greater part of the histamine which penetrated into the organs from the general circulation in normal rats appears to be in a bound, that is, biologically inert, state. It changes very sharply from the effect of ionizing radiation. Evidently, as a result of the considerable suppression of the histaminopexic capacity of the tissues [10] the histamine which penetrated into the organs of irradiated animals is found primarily in a free state. This one circumstance – the sharp increase in the free histamine level in the tissues due to their low histaminopexic capacity after irradiation – should, it would seem, explain histamine's high toxicity for irradiated animals. However, the investigations which were conducted disclose still another extremely important fact – a disturbance in the hemato-encephalic barrier [HEB] (ГЭБ) for histamine, which shows up clearly in the rats 45 min after irradiation.

With the extremely high sensitivity of the nerve centers, such a significant increase (approximately two-fold) in the normal level of free histamine in the central nervous system [CNS] (ЦНС) cannot but have serious consequences.

Thus, ionizing radiation evidently disturbs the whole defense system which the organism has available during the detoxification of histamine. If it is assumed that the HHB and especially the HEB are the first defense mechanism regulating the penetration of histamine from the blood into the tissue which is normally completely impenetrable to histamine in rats, the second defense mechanism is the histaminopexic capacity of the tissues. It is likely that both these mechanisms are equally disturbed during irradiation. Which of them is the principal mechanism which determines the development of the given radiation reaction?

The existence of a clear species specificity in this respect in mice and rats unexpectedly helped in solving this problem. Mice are even more resistant to histamine. Normally, they withstand well the injection of even such high doses as 500-1000 mg per kg, that is, 10-20 mg of histamine per mouse and, which is very important, their attitude toward histamine is completely unchanged after irradiation. The given radiation reaction, thus, is completely absent in them.

To determine the cause of this species difference, the effect of ionizing radiation on the same mechanism was investigated in mice. Therefore the investigations carried out on rats were repeated under exactly identical conditions on these mice.

In this case it was found that the histamine injected into the general circulation is distributed in the peripheral organs of normal animals just as in rats. The histaminopexic capacity of the tissues is similarly suppressed by ionizing radiation, as a result of which histamine injected into the blood which had penetrated into the organs is found in irradiated mice, as in rats, primarily in free biologically active form. However, in mice the HEB remains impenetrable to histamine after irradiation.

Is this difference actually decisive, that is, can the increased sensitivity to histamine of irradiated animals be considered only as a central reaction caused by histamine excitation of the corresponding centers?

The complete absence of any toxic reaction on the part of normal rats to the suboccipital injection, that is, bypassing the HEB, of even 50 µg of histamine is the answer to this question. This dose exceeds by approximately four times the amount of histamine which penetrates into the CNS when injected into the general circulation through the HEB disturbed by irradiation.

Thus, although the injection of histamine directly into the cerebrospinal fluid undoubtedly disturbs the relative constancy of the immediate internal environment of the CNS, the conclusion suggests itself that by itself in the obligatory absence during irradiation of a high level of free histamine in the peripheral organs, it still does not create conditions incompatible with life. Hence it follows that both components of the given radiation reaction are equally necessary for its development, and in the absence of one of them, as occurs in mice, increased sensitivity to histamine does not develop.

The last series of experiments in which it was possible to incite the given radiation reaction in mice by simultaneously injecting 10-20 mg of histamine intraperitoneally and 10-20 µg suboccipitally, bypassing the HEB, into irradiated animals proves this hypothesis. Under these experimental conditions, as in rats, death of 64% of the irradiated animals was observed. In normal rats with high histaminopexic capacity of the tissues, even such a combined histamine injection does not cause death.

The following conclusions can be drawn:

1. Ionizing radiation causes an increase in the sensitivity of rats to carbocholine and histamine.

2. The development of increased sensitivity of irradiated rats to histamine is due to suppression of the histaminopexic capacity of the tissues and to a disturbance in the HEB for histamine.

3. The absence of the given radiation reaction in mice which is an interesting example of species specificity is a result of the disconnection of these two mechanisms. In the case of their artificial combination, it is possible to induce this phenomenon in mice.

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THE PARTICIPATION OF CERTAIN BIOLOGICALLY ACTIVE SUBSTANCES IN RADIATION DISTURBANCES OF THE PENETRABILITY OF THE HISTO-HEMATIC BARRIERS

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Many years of investigations by L.S. Shtern and her colleagues have shown the importance of a relative constancy of the immediate internal environment of the organs and tissues for a normal course of physiological processes. One of the principal mechanisms which determines and maintains constancy of the environment is the functioning of the histo-hematic barriers [HHB] (ГГБ). In addition to preserving the constancy of a given organ's internal environment, the HHB play an important role in providing a humoral link between various organs. Therefore, in a discussion of the role of radiotoxins [RT] (РТ) in the development of radiation sickness in regard to their remote effect it is impossible not to touch upon the question of the HHB.

It has been established by numerous investigations [1-5] that ionizing radiation causes a change in the penetrability of the HHB. However, the mechanisms of these disturbances at the present time have still not been finally disclosed. It has been shown in a whole series of papers that some chemical substances are capable of changing the state of the HHB and of affecting the development of pathological processes within them.

However, it is known that toxic substances are formed in the organism of irradiated animals: choline [6], histamine [7, 8], quinones [9], lipid RT [10, 11] and others which clearly take a significant part in the development of certain secondary changes observed during radiation sickness. Leaving aside the question of the possible effect of a whole series of substances united under the general name of "radiotoxins" on the penetrability of the HHB, the effect of lipid RT on the penetrability of the HHB and their connection with free, biologically active histamine was investigated in the present work.

The penetrability of the HHB of the liver, muscles and the brain in different forms of radiation sickness (doses of 0.6, 5 and 10 kr) from the effect of lipid RT and their model, oxidised oleic acid [OOA] (ООК), was investigated. The lipid fraction from the liver of unirradiated, healthy rabbits served as the control. The lipid RT were obtained by a method described in the literature [12].

Penetrability was determined by the isotope method. A radioactive phosphorus isotope was used as an indicator of penetrability. The ratio of the tissue radioactivity to the radioactivity of blood removed at the same time, expressed in per cent, served as the index of HHB penetrability. A chromatographic method, modified by Krichevskaya [13, 14], was used to determine free histamine.

Effect of Irradiation, OOA and Lipid RT on HHB Penetrability

Вариант опыта 1	Доза 2	Время после воздействия, сутки 3	Отношение радиоактивности тканей к радиоактивности одновременно взятой крови, % 4 — Мозг 5	Мышца 6	Печень 7
8 Облучение	13 0,6 кр 0,6 » 5 » 5 »	1 3 1 3	28,0±2,2 11,0±1,3 35,0±3,1 5,0±0,9	112±9 110±7 178±12 13,1±1,1	2340±204 1048±113 1788±182 175±43
9 ООК	14 0,06 мл 0,06 » 0,2 » 0,2 »	1 3 1 3	14,0±1,2 6,0±0,8 16,0±1,5 3,0±0,4	212±21 82±6 89±8 11±1	1091±125 728±97 826±95 344±49
10 Липидная РТ	14 1 мл 1 »	1 3	16±1 5,0±0,7	111±14 44±8,5	1001±112 667±89
11 Контроль	14 1 мл	1 и 3	6,0±0,8	69±10	622±46
12 Норма	—	—	6,0±0,5	74±9	630±82

1) Variant of experiment
2) Dose
3) Time after effect, day
4) Ratio of tissue radioactivity to radioactivity of simultaneously removed blood, %
5) Brain
6) Muscle
7) Liver
8) Irradiation
9) OOA
10) Lipid RT
11) Control
12) Normal
13) kr
14) ml

The results of the study of HHB penetrability under different influences are presented in the table.

After one total γ-irradiation, a definite pattern is observed in the change in the HHB penetrability for all forms of radiation sickness investigated. This pattern, as seen from the table, consists in the fact that in the first period of radiation sickness an increase is observed in the HHB penetrability in the tissues examined. In the ensuing period of radiation sickness a noticeable decrease in penetrability occurs. The degree of the change in the HHB penetrability depends on the size of the irradiation dose. From irradiation with a dose of 0.6 kr, the increase in the penetrability is greater and the decrease is less than after irradiation with a dose of 5 kr.

Injection of the animals with lipid RT, as was shown earlier [12], leads to different severities of the illness depending on the amount of the substance injected, in the same way as different doses of γ-radiation cause different forms of radiation sickness. The change in the HHB penetrability is presented in the table. In the initial stage of the illness an increase is observed in HHB penetrability. As the illness develops HHB penetrability decreases and in the most acute form of the illness (in the terminal period), it falls sharply below normal, that is, a pattern characteristic of radiation sickness is observed. The injection of rats with lipids isolated from unirradiated, healthy rabbit livers, as seen from the table ("control"), does not lead to noticeable changes in HHB penetrability.

Taking into consideration the fact that the biological activity of lipid RT is connected [15] with the oxidation products of unsaturated fatty acids, in the next series of experiments the HHB penetrability of the tissues of rats which were first injected with OOA was studied. Different doses caused different degrees of sickness of the animals. As seen from the table, the injection of OOA also leads to various disturbances in penetrability, depending on the doze and time passed after injection of the preparation. In the initial period of the illness the HHB penetrability of the tissues is increased, in the next period it decreases and in the terminal period (in the most acute form of the illness) it falls sharply below normal.

Thus, the disturbance in the HHB penetrability of the tissues from the injection of rats with lipid RT evidently depends on their content of oxidized products of unsaturated fatty acids.

It is possible to conclude on the basis of the data presented that lipids isolated from tissues of irradiated animals have a definite radiomimetic effect on HHB penetrability and reproduce well both stages of the change in penetrability observed during radiation sickness. It can be assumed on the basis of these facts that substances of a lipid nature which appear during radiation sickness participate in the disturbance of HHB penetrability.

However, it is known that the level of another biologically active substance, histamine, which also, as is known [8], affects HHB penetrability, changes during radiation sickness.

The question arises of whether these toxic substances act individually on HHB penetrability or whether their production and effect are interconnected. Work carried out by the author jointly with Bilushi and Kudryashov on the mechanism of histamine production during radiation sickness (see this collection, page 220), in which it was shown that lipid RT and its model (OOA) when injected into rats in minimum lethal doses change the level of free, biologically active histamine, it seems, brings some clarity to the solution of this problem. The dynamics of these changes, just as from irradiation, has a phase character. In the initial period of the illness the level of free histamine increases in all the organs and tissues studied, in the next period the level decreases. The rate of decrease in histamine level, just as after irradiation, is different in different organs. As is known [8],

one of the defense mechaniams which regulate the free histamine level is the histaminopexic capacity of the tissues which is suppressed after irradiation. We succeeded in showing that the histaminopexic capacity of the tissues of animals injected with lipid RT also decreases continually during the entire period of the illness. The data obtained make it possible to speak of a definite connection between the two biologically active substances – lipid RT and free histamine – during the development of radiation sickness and of their role in the radiation changes in HHB penetrability. A number of investigators suggest [16-18] that lipid RT are one of those substances which are capable of participating in the primary processes of radiation sickness. It is also known that histamine does not participate in the primary processes of radiation sickness. Consequently, it is possible to assume that the lipid RT which have developed in the organism of irradiated animals are capable of changing the level of free, biologically active histamine, that is, the lipid RT promote the appearance in the irradiated organism of secondary toxic substances which in turn can aggravate the course of the radiation sickness, including pathological changes in HHB penetrability.

Thus, one possible mechanism of action of ionizing radiation on HHB penetrability may be radiation's indirect effect thanks to the production of primary toxic substances – lipid RT and secondary substances – histamine.

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SOME DATA ON THE NATURE OF TOXIC FACTORS IN RADIATION SICKNESS

P.D. Gorizontov, G.M. L'vitsyna and Yu.D. Balika

The effect of ionizing radiation on the organism is very complex. In addition to an immediate, direct effect on cells and tissues, an indirect effect on the organism takes place through the nervous and primarily the humoral system.

The blood is of fundamental importance in the transmission of the radiation effect. It is well known [1-6] that the blood in an irradiated organism acquires new biological properties. This condition of the blood has been called toxemia.

Toxicity of the blood appears in the early periods after irradiation (1 day) [4]. It has also been shown [2] that the blood flowing out of different organs has different biological properties, that is, it is assumed that it contains various radiotoxins [RT] (PT) [2]. The fact that in *in vivo* as well as *in vitro* experiments the blood from an irradiated animal caused changes in a number of systems and organs of a healthy (unirradiated) organism was promising for a study of toxemia in radiation sickness. It has been shown [2, 4] that "irradiated" blood has an inhibitory effect on the hemogenesis system. In this case the changes in hemopoiesis were analogous to changes occurring in the organism after irradiation.

Radiotoxins which develop from the effect of ionizing radiation on the organism not only affect the hemogenesis system but also cause by humoral means complex correlative changes in a whole series of organs and systems. Experiments on rat-parabionts established [7] changes in the biocurrents of the brain of the unirradiated partner. The investigations of M. Fedotova showed the effect of the toxemic factor on the intestinal and liver tissue of the unirradiated parabiont [8].

Thus, in the first stage of the work it was possible to obtain convincing evidence of the role of the humoral component as one of the pathogenetic mechanisms of the development of radiation sickness. However, the question of the nature and mechanisms of the development of toxemia in radiation sickness has remained practically unstudied up to the present time.

In the present work the role of the protein component in the onset and development of "radiation" toxemia was investigated. Among the few investigations in this direction, there are indirect data indicating a protein nature of the humoral factors which cause the development of toxemia. Linzer and Hellber, Kershman and Haupp inactivated the serum of irradiated animals, depriving

it of the most toxic properties, by heating it to a temperature of 60° for 0.5 hr.

Kuznetsova [9] in a study of the humoral factors of the irradiated organism also found that the substance investigated is thermolabile and is contained in the albumin and globulin fraction. It turned out that these factors do not go into the filtrate during ultrafiltration and into the dialysate during dialysis of the serum.

The facts which have been presented are only approximate since the investigators did not conduct a thorough study of the protein components under investigation, but only limited themselves to a determination of the proteins' resistance to heating and the capacity for filtration of the biologically active solutions through semipermeable membranes.

There is no information at all in the literature on the question of what types of proteins are responsible for the toxemic effect of the blood of irradiated animals. There are only Pett and Erass' indications of a dependence of the toxemic effect on polypeptides and peptones [cited by Ludwig].

Individual immunological studies speak in favor of the protein nature of the toxemic factor. Sverdlov (see this collection, page 250), by perfusing an irradiated rabbit ear, found a change in the antigenic qualities of the perfusate by the Zil'ber method of anaphylaxis with desensitization.

The data of Klemparskaya et al. [10], who established in experiments, by screening the extremities during total irradiation of animals, that fewer cytolysins are produced in the blood of protected animals than in unprotected, deserve special attention. We are inclined to consider this increase in cytolysins which Klemparskaya and colleagues pointed out as one possible cause of the toxemia. Moreover, the biological properties of the cytolysins are already known. The increase in the cytolysin content of irradiated tissues is connected with the appearance of certain protein agents.

The assumption concerning the specific importance of the cytolysins – antibodies – in toxemia is also in accord with the fact of the appearance of antileucocytic antibodies in the organism in chronic radiation sickness [11].

In recognizing the protein nature of the toxemic agents, it is impossible to forget about immune mechanisms as consequences of the appearance of the indicated agents. An explanation of this possible means of realizing the "toxemic" effect is very important since the data of immunohematology concerning the detection of autoantibodies in various diseases of the blood system are fully applicable to an explanation of the disturbances in the hemopoiesis of healthy recipient dogs observed in a study of toxemia.

These facts stimulated the authors to study the biological activity of the perfusate, to determine the mechanisms of its

toxic effect and to investigate the proteins which were washed out of the tissues of irradiated animals. Principal attention was given to the globulin fraction of the perfusate: fundamental importance is attached to globulin in immunology since the globulin fraction contains the bulk of the antibodies.

A method used for studying the mechanism of toxemia – the tissue perfusion method – was used in the experiments. We carried out perfusion of the extremities of irradiated and unirradiated dogs. The dogs received one total irradiation in a three-tube x-ray apparatus in a dose of 800 r. The dose rate was 17 r/min at a voltage of 180 kv and a current strength of 14 ma; the filters consisted of an 0.5 mm copper and 1 mm aluminum layer. The perfusion fluid was collected and the amount of cellular elements was determined in individual portions of the perfusate.

There are statements in the literature [1-3] that humoral factors which cause one of the manifestations of toxemia during radiation sickness – changes in the blood, are produced in irradiated myeloid tissue. Moreover, the idea of the possible role of the cellular elements of the blood and bone marrow appeared on the basis of data concerning a decrease in the cell content of irradiated bone marrow as a result of perfusion. It was assumed that very intensive washing out of elements of the peripheral blood from the irradiated tissues occurs and that at the same time bone marrow cells appear in the perfusate.

In order to confirm the assumptions which were made, the morphological composition of the perfusate was studied qualitatively and quantitatively during the operation which was conducted.

Observation of the cellular composition of the perfusate did not establish any changes in the leucocytic formula due to perfusion or the appearance of bone marrow cells in the perfusate (Fig. 1).

In comparing various data, it can be assumed that the toxic factor does not have to be whole bone marrow cells, but products of their massive decomposition, as well as cells changed in an antigenic respect.

On the basis of data on the possibility of demonstrating disturbances in hemopoiesis by means of immune reactions, the authors assumed that these humoral factors may be autoantigens which develop in myeloid tissues after irradiation. It is already known [12, 13] that autoantigens are produced in certain tissues of an irradiated organism. However, similar indications with respect to the myeloid tissues have still not been encountered.

The antigenic properties of the myeloid tissue were demonstrated by the Zil'ber method of active anaphylaxis with desensitization. Bone marrow of dogs removed before irradiation and 3 days after irradiation was used as the antigen. The results of the experiments showed that after irradiation the bone marrow of the dogs acquires new antigenic properties.

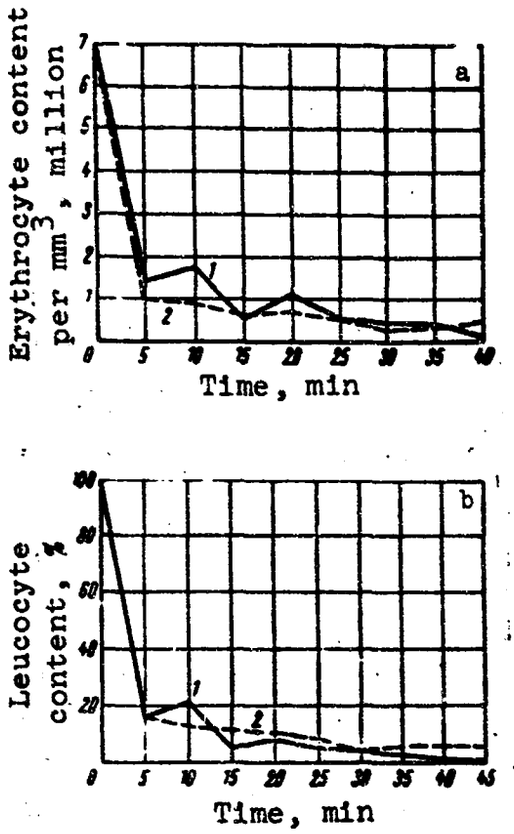

Fig. 1. Content of erythrocytes (graph *a*) and leucocytes (graph *b*) in perfusate. 1) Irradiated dogs; 2) unirradiated dogs.

It is known that the presence of cytolysins in the organism can have an injurious effect on the tissues. Based on the data of Klemparskaya et al. [8-10] on the appearance of cytolysins in irradiated animals, an attempt was made to determine the cytological activity of the perfusate. Klemparskaya's method, in which cytolytic activity was demonstrated by the perfusate's effect on the leucocytes of the donor dog itself, was used (Fig. 2). A significant increase in cytological activity of the perfusate was found during the 35 min of washing which is clearly connected with the washing out of cytolysins from the deep regions of the tissues.

The available indications concerning the protein nature of the toxic factors and our own observations on an increase in the cytolytic activity of the perfusate stimulated the authors to investigate the proteins washed out of the tissues and contained in the perfusate.

The globulin fraction was obtained by the method of salting out with ammonium sulfate at 50% saturation of the solution.

It has been established by the investigations of a number of authors [12-14] that there is a change in the antigenic properties of certain tissues of irradiated dogs.

In connection with this it was of interest to study the antigenic properties of the γ-globulin isolated. The antigenic properties of the protein were studied by three methods: in the model of Zil'ber general anaphylaxis with desensitization in guinea pigs, by reproducing local anaphylaxis in rabbits (the Artyus-Sakharov phenomenon) and by determining antibody production in a complement fixation reaction.

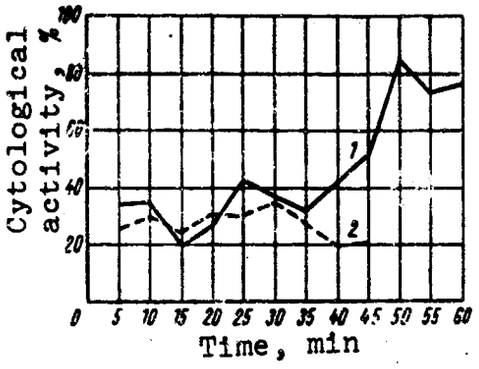

Fig. 2. The cytological activity of the perfusate with respect to leucocytes. 1) Irradiated dogs; 2) unirradiated dogs.

In the experiments on anaphylaxis with desensitization a comparison was made of the antigenic properties of globulin isolated from the same dog before and after irradiation.

We succeeded in establishing by experiments on reproduction of local anaphylaxis as well as in experiments with general anaphylaxis a decrease in the antigenic properties of the γ-globulin fraction isolated from a perfusate of irradiated dog tissues. This was expressed in the fact that in guinea pigs sensitized with the globulin from a perfusate of irradiated dogs [GPID] (ГПОС), complete desensitization could be achieved by the injection of the globulin from a perfusate or unirradiated dogs [GPUD] (ГПНС) and on the other hand, in the impossibility of obtaining desensitization in pigs sensitized with GPUD when injected with GPID. After desensitization the guinea pigs sensitized with GPID did not react to injection of the antigen. At the same time, a positive reaction was always noted in guinea pigs sensitized with GPUD in response to a resolving injection of GPUD.

A decrease in the content of the antigens in the globulin fraction of the perfusate of irradiated dogs is also shown in experiments on the reproduction of the Artyus-Sakharov phenomenon. Rabbits were subcutaneously injected at 4-day intervals with GPUD or GPID. In the experiments 1 and 5 mg samples were used which were dissolved *ex tempore* in 1 ml of physiological solution. The time of the appearance of positive reactions depended on the antigenic properties and the amount of globulin injected. In the case in which the rabbits were injected with 5 mg of GPUD, there was a positive reaction to its fourth injection, whereas GPID did not produce a reaction even on the ninth injection. One mg samples of GPUD caused a positive reaction after the seventh injection, whereas GPID did not produce a reaction after the tenth injection.

It was possible to compare the observed changes in antigenicity with the organism's capacity to produce antibodies against the homologous globulin which was injected. Six dogs from one litter were immunized with globulin in an amount of 16 mg per kg of weight. Three dogs were immunized with GPUD and the other

three with GPID (the GPID and GPUD, as indicated above, were isolated from the same donor dog). The antibodies were determined in a complement fixation reaction. The same globulin which was used for immunization (dissolved *ex tempore* in physiological solution) was used as the antigen in the reaction. The working dose of globulin was 0.004 mg/ml.

The results of the experiments are presented in the table.

Complement Fixation Reaction [CFR] (PCK) in Dogs After Injection of Globulin Fraction*

1 Время постановки РСК после введения глобулина, недели	2 РСК после введения глобулина		1 Время постановки РСК после введения глобулина, недели	2 РСК после введения глобулина	
	3 «необлученного»	4 «облученного»		3 «необлученного»	4 «облученного»
2nd	0/3	1/3 (1 : 320)	5th	0/3	3/3 (1 : 160)
3rd	1/3 (1 : 20)	2/3 (1 : 320)	6th	0/3	2/3 (1 : 80)
4th	0/3	3/3 (1 : 640)	7th	0/3	2/3 (1 : 10)

*Numerator – number of positive samples; denominator – number of samples set up.

1) Time of CFR after globulin injection, weeks
2) CFR after globulin injection
3) "Unirradiated"
4) "Irradiated"

It is seen from the table that antibodies were not produced to the injection of GPUD. After the injection of GPID antibodies were found between the 2nd and the 6th weeks after immunization.

In addition to a determination of the antigenic characteristics of the γ-globulin isolated from a tissue perfusate of irradiated dogs, the study of the biological effect of the globulin isolated on healthy dogs was of special interest. A determination of the biological properties of "irradiated" globulin in comparison with the effect which the injection of a globulin fraction from unirradiated dogs causes could produce fundamental information concerning the significance that this protein has in the development of toxemic manifestations during radiation sickness.

The present investigation had as its goal the determination of the biological effect of the globulin fraction with respect to hemopoiesis and the immunobiological reactivity of the organism. Samples of γ-globulin in an amount of 16 mg per kg, dissolved *ex tempore* in 40 ml of physiological solution, were injected intravenously into healthy dogs. Six pairs of dogs were used in the experiment: three pairs were from the same litter, the other three pairs were selected according to weight, sex and peripheral blood indices. Six series of globulin preparations were tested (the γ-globulin was isolated from a tissue perfusate of the same dog before irradiation and 3 days after irradiation).

The state of hemopoiesis and the immunobiological reactivity of the organism was studied in detail in the dogs before and

after the globulin injection.

Soon after the γ-globulin injection a definite reaction was noted in 11 of the 12 dogs. It consisted in the mucosa becoming pale, sluggishness, slight dyspnea and sometimes vomiting. Only one dog did not show any reaction to the injection of GPUD. The reaction described was brief and on the following day, as a rule, the dogs' general condition was good. In a comparison of the general state of the animals which received GPUD and GPID, there were certain differences. They consisted in the fact that the dogs which received GPID withstood the procedure which was carried out less well. In these dogs, in addition to the symptoms indicated, the body temperature rose to 40° on the 1st, 2nd, 6th and 7th days, diarrhea appeared and conjunctivitis developed.

The blood picture changed after the globulin injection. In the first 3 hrs after the injection of each of the globulins, a decrease was noted in the amount of leucocytes in the peripheral blood. After 6 hrs the number of leucocytes equaled the initial number, and after 24 hrs it increased to 210% of the initial number (Fig. 3). In this case, the leucocytosis in response to the injection of GPID was much less (170%) after 24 hrs. On the 3rd day the number of leucocytes returned to the initial level and did not change considerably in the future.

In a calculation of the leucocytic formula, an increase in the absolute number of eosinophils was noted in five of the six animals which received GPID. The eosinophil content was increased 2-3 times from the 5th day of the experiments in comparison with their number before the beginning of the experiment. (The initial state was judged from 4-5 analyses.) After the injection of GPUD only one dog out of the six showed an increase in the number of eosinophils. In the rest of the dogs a decrease was observed in comparison with the initial data. As an illustration, the eosinophil content of one pair of dogs in which one dog was injected with GPUD and the other with GPID is presented in Fig. 4.

The injection of γ-globulin caused both in the experimental and control groups of dogs a decrease in the number of thrombocytes 24 hrs after the beginning of the experiment. The number of thrombocytes changed in four out of the six dogs in each group. In the rest of the animals the index remained at the original level or, on the other hand, exceeded it. Later, the number of blood platelets was gradually reduced. In this case the recovery of the dogs which received GPID proceeded slowly. Average data on the thrombocyte content in the two groups of dogs is presented in Fig. 5.

Specific disturbances were found in computing the myelograms of the bone marrow punctures of these dogs. The bone marrow of three control and three experimental animals was examined. The nature of the changes was the same in all three dogs in each group. The state of erythropoiesis is shown in Fig. 6. In animals which received GPID, the number of cells of early generations, young forms and the total number of erythroblasts decreases. Regeneration processes (regeneration index) were temporarily retarded although the transformation of the cells (maturation index)

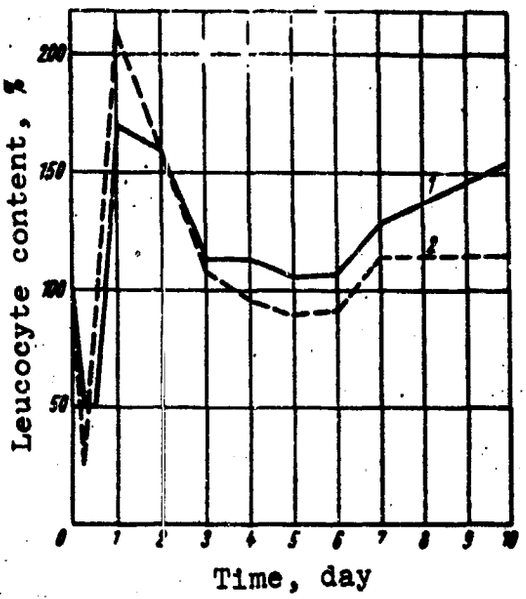

Fig. 3. Leucocyte content. 1) After GPID injection; 2) after GPUD injection.

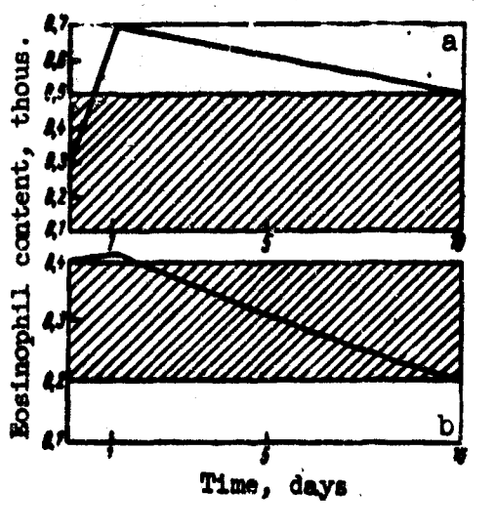

Fig. 4. Eosinophil content. a) After GPID injection; b) after GPUD injection. Cross-hatched area – control.

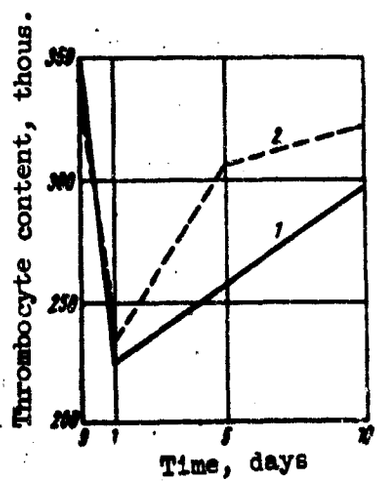

Fig. 5. Thrombocyte content. 1) After GPID injection; 2) after GPUD injection.

did not change. Changes in the number of reticulocytes in the periphery corresponded to the disturbances in the bone marrow. In one case, on the 5th day of the experiment reticulocytes were not found in the smear. There was no change in the number of erythrocytes. The amount of other cellular elements changed slightly and irregularly.

The organism's immunobiological reactivity is considerably inhibited during irradiation: the skin's bactericidal capacity is

decreased [15], the phagocytic activity of the blood neutrophils is suppressed [16] and lysozyme production in the saliva decreases [15].

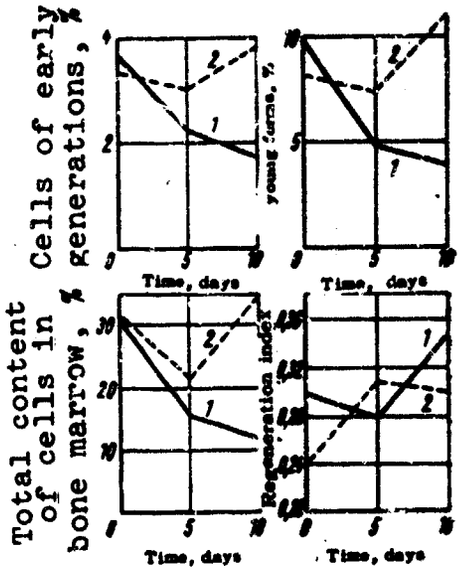

Fig. 6. State of erythropoiesis. 1) After GPID injection; 2) after GPUD injection.

In connection with this, it seemed of definite interest to study the changes in the immunological reactivity of healthy dogs after the injection of the globulin of a perfusate of irradiated animals. The state of immunobiological reactivity was judged from the phagocytic activity of the blood neutrophils and the bactericidal properties of the skin. Determination of the phagocytic activity of the blood neutrophils with respect to a living culture of *Staphylococcus albus* "Lepin" was carried out by the standard method as modified by Alekseyeva [17]. The percentage of phagocytosing neutrophils per hundred counted was calculated, as well as the phagocytic index – the number of cocci phagocytized by one neutrophil. The skin's bactericidal properties were studied by Klemparskaya's method [17]. Impressions were taken from the lateral surfaces of the dog's trunk.

In the study of these indices both the general and the different features of the change in the organism's immunobiological reactivity depending on the qualitative characteristics of the globulin injected were demonstrated.

The phagocytic activity was determined on the day of the experiment 1, 3 and 6 hrs after the globulin injection, then for 10 days at intervals of 2 days and after this, once a week. Observations were carried out for 4-5 weeks after the globulin injection.

The general nature of the change in phagocytic activity was observed in the period of 1-3 days. Immediately after the injec-

tion of GPID and GPUD activation of phagocytosis was noted. It was greater after the injection of GPUD. After 3 days the phagocytic activity returned to the initial level in dogs which had been injected with GPUD and was lower than the original background in dogs after the injection of GPID. The activation which was noted was short-lived. From the 3rd day clear differences in this index were demonstrated: in dogs which were injected with GPUD, the phagocytic activity decreased to normal and later varied within the range of the original values, whereas in dogs after GPID injection, a significant suppression of phagocytosis was noted which was retained up to the 10th day of observation. On subsequent days the phagocytic activity of the blood neutrophils returned to the original level.

Data on the changes in the percentages of actively phagocytizing neutrophils in dogs after the injection of GPUD and GPID are presented in Fig. 7. The phagocytic indices are not presented here since they always corresponded to the changes in the percentage of actively phagocytizing neutrophils. Statistical treatment confirmed the reliability of the results obtained. A comparison of the values of the phagocytic activity of the two groups of dogs showed that 5 and 10 days after the injection of globulin $t > 3$ and $p > 95\%$.

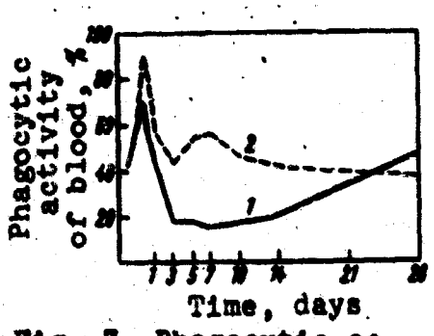

Fig. 7. Phagocytic activity of blood in dogs. 1) After GPID injection; 2) after GPUD injection.

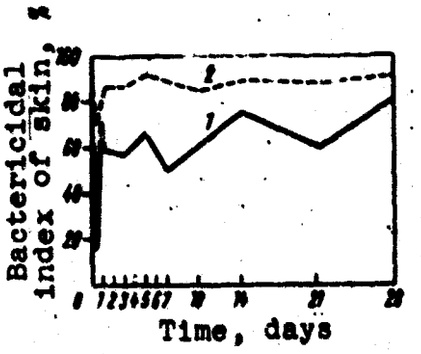

Fig. 8. Bactericidal index of skin. 1) After GPID injection; 2) after GPUD injection.

The bactericidal properties of the dogs' skin were determined in the same periods. Data concerning changes in the bactericidal index (percentage of dead colonies 5 min after application of *B. coli* to the skin) in the two groups of dogs are presented in Fig. 8. Several general patterns were also demonstrated from a study of the bactericidal index of the skin of dogs after their injection with GPUD or GPID. During the 1st day, especially 1 hr after injection of the globulins, a decrease was observed in the skin's bactericidal properties in all the animals: slight in those animals which were injected with GPUD and sharp in dogs after the injection of GPID. In these dogs the bactericidal index of the skin 1 hr after globulin injection decreased 80% in comparison with the original values, whereas in dogs after the injection of GPUD, the decrease was no more than 20%. Later the bactericidal properties of the skin became normal. In dogs which were injected

with GPUD, it did not change significantly over the course of the entire experiment.

Other changes were observed in the animals after the injection of GPID. The bactericidal properties of the skin which had increased somewhat after 1 day of the experiment (however, they nevertheless remained below the original data) again decreased on the 5th-10th day and then on the 21st day after injection of the globulin. Thus, the injection of GPID significantly changed the immunobiological reactivity of healthy dogs.

The investigations which were conducted first of all showed a change in the antigenic properties of the myeloid tissue after irradiation. This, in our opinion, can be one of the causes of the development of toxemia during radiation sickness. It can be assumed that the disturbances in hemopoiesis developing after irradiation are caused not only by the direct effect of radiation but also by immune processes through the production of antibodies against bone marrow cells which are changed in an antigenic respect.

We did not succeed in establishing a washing out of the morphologically recorded cells of the donor's bone marrow in a study of the perfusate. It is most likely that the toxemic factors are cell decomposition products, namely, their protein components. On the one hand, the increase in the leucolytic activity of the perfusate itself, and, on the other, the biological effect which was observed in *in vivo* experiments after GPID injection indicate this.

The increase in the cytolytic activity of a perfusate of irradiated tissues clearly can be considered as the washing out of antibodies (cytolysins) against cells which are antigenically changed from the donor's bone marrow.

After the injection of GPID a change in hemopoiesis (erythroblastic part) was noted in healthy dogs, which coincided with those disturbances which were observed earlier in the recipient dogs after their perfusion with blood from irradiated donor tissues. The injection of GPID into healthy animals also caused a decrease in the immunobiological reactivity of their organisms which was expressed in a decrease in the phagocytic activity of the blood neutrophils and the skin's bactericidal properties. All these changes are similar to the change in the organism's reactivity during radiation sickness.

Thus, the experiments which were conducted compel one to think that the toxemic factor in radiation sickness can be the globulin fraction of the irradiated organism's blood.

It was shown in experiments on anaphylaxia with desensitization that GPID contains fewer antigenic complexes than GPUD. Experiments on the reproduction of local anaphylaxis (Artyus-Sakharov experiments) confirm these data. Our ideas in this respect agree with the investigations of Chudnovskikh and Rodionov [18], who by a detailed analysis of the protein structure of irradiated and unirradiated dogs showed the absence of three peptides from

the serum globulin of irradiated dogs. The decrease in antigenicity observed has a sufficient effect on the isoantigenicity of the GPID since, on being injected into the same species of healthy animals, it causes the production of antibodies against itself.

As has been shown, an increase in the absolute number of eosinophils occurred in response to the injection of GPID into the blood of healthy dogs. Considering the literature data, it can be thought that this to some degree indicates allergization of the organism.

Thus, the data obtained indicate the real possibility of the development of autoimmune reactions through the protein part of the perfusate. The data obtained also reinforce our opinion that these autoimmune reactions are part of the general mechanism of toxemia in radiation sickness. However, it is still impossible to say at the present time whether the leading role in the development of toxemia belongs to the changed globulin fraction, since the biological significance of other protein components of the blood and its lipid fraction in the manifestation of toxemia is still unknown.

It is quite likely that the substances causing the toxemic effect in radiation sickness are heterogeneous in nature. Ludwig's data [19] from a study of the remote effect during local tissue irradiation speak for this. According to his information, such effects as tumor regression, retardation of mitosis and inhibition of thymonucleic acid synthesis can be caused by different factors.

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THE PARTICIPATION OF HUMORAL TOXIC AGENTS IN THE DEVELOPMENT OF SOME SYNDROMES OF RADIATION SICKNESS

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Thanks to recent studies [1-7] in radiobiology, concepts of the pathogenetic significance of toxic agents produced during irradiation have been confirmed. It has become clear that radiotoxins [RT] (PT), whatever their nature may be, are included as one link in the chain of pathological changes leading to radiation sickness. Judging by the literature data, the toxins perform a dual role: on the one hand, they can participate in the development of irradiated cell injury, acting as one of the primary mechanisms of radiation damage [8] and on the other, they can serve as an important mechanism of the indirect (remote) effects of radiation [1-3, 6].

However, the study of the radiation toxic effect on mammals has centered mainly on changes in the morphological composition of the blood and organs of hemogenesis [5, 9-11], whereas the participation of RT in other syndromes of radiation sickness has been little studied.

However, there are data pointing to the pathogenetic role of the toxic factors in many other important manifestations of radiation sickness.

In the experiments which are being described intact rabbits were injected intravenously with perfusates of an irradiated rabbit femur which was isolated in a vascular respect from the organism. Ringer-Locke solution was used for the perfusion. Irradiation was carried out in an RUM-3 apparatus with a dose of 2000 r. (The dose rate was 53 r/min, the hardness of the rays was 180 kv with 0.5 mm copper and 1.0 mm aluminum filters.) The perfusate was collected during the irradiation and for 20 min afterward and injected in an amount of 40 ml per kg of weight. In addition to the usual blood examination, the number of thrombocytes and the coagulability according to Burker's method were determined in the recipients.

In control experiments the recipients were injected with perfusates of unirradiated femur. The experiments were carried out on 45 animals (15 of them were controls). The results are presented in the table and in Fig. 1.

It is seen from the table that in the experimental animals from the 3rd day of the observations a gradual lengthening of the blood coagulation time begins. At the end of the second week this index is increased almost 75% with respect to the original value.

Effect of Perfusate from Irradiated and Unirradiated Femur on Blood Coagulability and Thrombocyte Content

1 Время после введения, сутки	2 Изменение времени свертывания, сек			3 Изменение количества тромбоцитов, тыс.		
	4 Опыт	5 Контроль	6 p	4 Опыт	5 Контроль	6 p
1	1,3±0,5	0,5±0,2	0,6	5,0±1,6	−1±1	<0,01
2	4,9±1,8	1,8±0,9	0,05	27±9	0,5±0,9	<0,01
3	8,4±2,0	1,6±1,0	<0,01	49±14	2,0±0,7	<0,01
5	17,6±3,1	4,1±1,7	<0,01	79±20	3,0±1,2	<0,01
7	21,0±3,3	4,3±1,8	<0,01	97±18	3,7±1,4	<0,01
9	22,6±3,0	3,9±1,6	<0,01	96±19	−1,0±0,8	<0,01
11	25,1±4,2	4,7±1,8	<0,01	91±21	−2±1	<0,01
13	26,8±4,4	4,5±2,0	<0,01	88±16	0±1,1	<0,01

1) Time after injection, days
2) Change in coagulation time, sec
3) Change in number of thrombocytes, thousandths
4) Experimental
5) Control
6) r

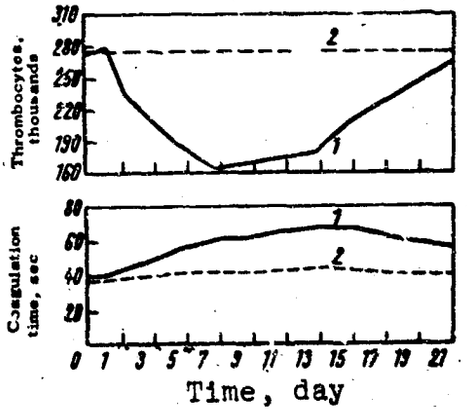

Fig. 1. Change in number of thrombocytes and blood coagulation time from the effect of toxic agents. 1) Experimental; 2) control.

As shown in Fig. 1, a decrease in coagulability is noted for a long time. During the third week the coagulation time begins to shorten, but this process occurs slowly and at the end of the third week it is still far from concluded.

Simultaneously with the decrease in blood coagulability, a decrease is recorded in the number of thrombocytes, reaching its

greatest degree at the end of the first and beginning of the second week. From the middle of the second week of the experiment the number of thrombocytes increases and it reaches the original values at the end of the third week.

Thus, from the effect of RT from irradiated tissues such characteristic components of the hemorrhagic syndrome as a decrease in blood coagulating properties and a decrease in the thrombocyte content appear. It is necessary to add to this that the decrease in coagulability does not develop in parallel with the thrombopenia, and is retained somewhat longer than the latter. This evidently indicates that the toxic factor affects some links of the blood coagulation system besides the blood platelets and is clearly capable by various means of participating in the development of hemophilia under the influence of irradiation.

One of these means may be the effect of the toxic agents on the tonus of the blood vessels. As the observations showed, the toxic agents from irradiated tissues, beginning with the moment of irradiation and for 3-4 days, have a vasoconstrictive effect. Vasodilative substances appear on the 5th-6th day. By altering the lumen of the vessels and thus participating in the redistribution of the blood (the efflux into the pulmonary system and into the vessels of the abdominal cavity organs), the toxic agents can be an additional factor favoring the development of hemorrhagia.

Clearly, the toxic agents bear a definite share of the responsibility for inhibition of certain natural immunity factors and, consequently, can participate in the development of the infectious-toxic syndrome of radiation sickness.

Studies of the bactericidal properties of the skin and the phagocytic activity of the leucocytes were conducted on 16 rabbits which were injected with a perfusate from an irradiated femur. Twelve animals injected with a perfusate from an unirradiated femur served as the control.

For a determination of the skin's bactericidal properties by Klemparskaya's method [12], a *B. coli* culture in a dilution of 1:6000 was used. The number of bacterial colonies in each experiment was expressed in percentages of the number of colonies in the first impression from the skin of an intact animal.

A *Staph. albus* culture was used for the studies of phagocytosis.

The results of experiments on the determination of the skin's bactericidal properties at different times after RT injection are represented graphically in Fig. 2. As seen from this graph, the bactericidal properties of the recipients' skin are decreased on the 3rd day. This decrease reaches a maximum on the 5th-7th day and by the 10th day it begins to end. In the control experiments the changes in the skin's bactericidal properties are not statistically significant.

Simultaneously with the decrease in the skin's bactericidal properties in the experimental animals, inhibition of phagocytosis

is noted which is manifested in a 20-25% decrease in phagocytic activity (Fig. 3) and a 20-30% decrease in the phagocytic index. Recovery of these indices occurs in the third week of the experiment. Summing up these data, it can be concluded that RT acts in an inhibitory way on important mechanisms of the organism's antibacterial defenses and, consequently, can participate in the development of infectious complications from irradiation.

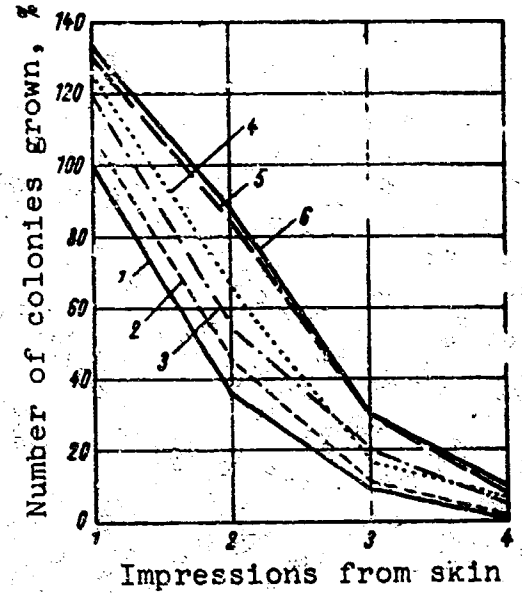

Fig. 2. Effect of toxic agents on skin's bactericidal properties. 1) Intact rabbits (100% on the first impression); 2) control; 3) in 3 days; 4) in 10 days; 5) in 5 days; 6) in 7 days.

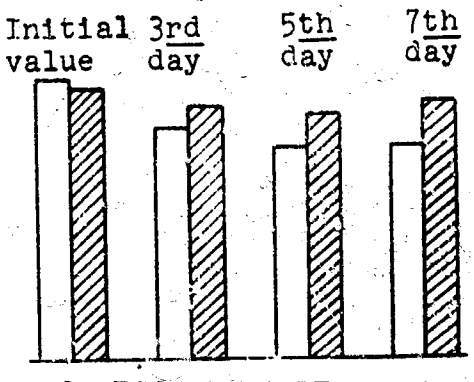

Fig. 3. Effect of RT on phagocytic activity of the leucocytes (relative units). Cross-hatched columns – control.

In evaluating the role of RT in the development of the principal syndromes of radiation sickness, it is important to determine quantitatively the significance of the toxic effects in the whole set of changes caused by radiation injury of the organism. With respect to changes in the bone marrow, opinions on this question differ.

According to Gruzdev's data [9], the role of the remote effects is small, whereas Rozin [11] attributes a share of up to 50% of the damage to them.

We compared blood changes after total irradiation of rabbits and hematological changes caused by the injection of RT by the method described above. In this case it turned out that the significance of the toxic factors for the development of the various manifestations of radiation sickness is dissimilar. If judged by changes in the total number of leucocytes or lymphocytes, the effect of RT is comparable to the effect of total irradiation in a dose of 200-250 r (20-25% of the lethal dose). However, with respect to the number of thrombocytes, the result of the toxic effect is comparable to the result of total irradiation in a dose of 400-500 r, while with respect to blood coagulation time, it is close to the results of irradiation in a still larger dose (800-1000 r). Thus, in our experiments a parallelism in the sensitivity of individual indices to the effect of radiation and of toxic agents does not appear. At the same time, the quantitative significance of the toxic effects with respect to the blood morphology is clearly established.

While the role of the toxic effects in comparison with the direct effect of radiation is sufficiently clear with respect to changes in morphological composition and blood coagulability, it is even more significant with respect to the skin's bactericidal properties and the leucocyte's phagocytic capacity. In periods of development and degree of expression the inhibition of these indices is close to changes occurring from total irradiation of rabbits in a dose of 600-800 r.

By correlating the data presented, it is possible to see that the toxic effects are diverse and reproduce a number of the manifestations not only of the hemopoietic but also of the hemorrhagic syndromes and they also promote a decrease in natural immunity. To this must be added the mutagenic properties of the toxic agents [6, 13, 14].

Considering the early period and duration of toxin production, as well as the quantitative characteristics of the toxic effects, a conclusion concerning their importance in the development of the principal syndromes of radiation sickness seems well founded.

It is likely that the mechanisms of the toxic effects are numerous and varied, as are the toxins themselves. It can be assumed that in addition to a direct effect on cells and tissues, the toxins participate in autosensitization of the organism as well as in disturbances in the neuroendocrine regulation. A more detailed analysis of these mechanisms remains a most important problem. At the same time, the development of measures for detoxification of an irradiated organism as a valuable component of complex therapy of radiation sickness is an important task.

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THE ROLE OF RADIOTOXINS IN ANIMAL RADIATION SICKNESS

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At the present time it can be considered as proved that radiation sickness develops both as a result of the direct interaction of radiation energy with cell structures and indirectly, remotely. The relative role of the remote effect and the mechanisms of its accomplishment remain, however, the least studied. According to the data of Gorizontov [1], Betts [2] and others, disturbances in neuroendocrine and humoral regulation in the broad sense of this word acquire tremendous importance in the accomplishment of the remote effect. In numerous investigations of Kuzin [3], Tarusov [4] and their colleagues the radiotoxic nature of the remote effect is illustrated. The detection of the remote effect in plants made it possible for the authors to explain it unequivocally by the development of abnormal metabolites from the effect of irradiation [3].

As follows from the literature, as well as from the data of the present conference, quinones, unsaturated fatty acids, organic and inorganic peroxides, products of protein decomposition, choline, histamine, protein antigens, various metabolites and cytotoxic agents of undetermined nature can act as radi toxins [RT] (PT).

In order to examine this complex problem, it is necessary to bring clarity to the original concepts. First of all it is necessary to separate the infinite variety of reactions developing in the course of radiation sickness from the primary processes of the radiobiological effect. Such disarticulation of the specific triggering link, no matter how artificial it may seem, is necessary if only for the reason that the injury of a majority of the myeloid or intestinal cells which determines, as is known, the outcome of acute radiation sickness in animals, occurs soon after irradiation. An elementary morphological analysis makes it possible to demonstrate the massive death of a majority of the cells in the first hours after irradiation. On the other hand, the transplantation of a small number of bone marrow cells in the first 24 hrs after the effect makes it possible to prevent a lethal outcome, in spite of the continuing development of the radiation syndrome and consequently, of the toxemia.

It is quite clear from what has been stated that the toxins alone which are produced in the cell itself immediately after the effect being induced during the exchange of energy, can play a

definite role in the acute effect of irradiation in the full sense of the word. The results of experiments which showed the bone marrow's close radiovulnerability *in vivo* and *in vitro* indicate this [5, 6]. Some considerations concerning RT's possible role in the direct and remote effects of irradiation are presented below.

Radiomimetic intoxication as a model of "local cell radiotoxicosis." In a comparative study of the effect of ionizing radiation and radiomimetic agents of the alkylating type which we made jointly with R.G. Kostyanovskiy in the fifties, the hypothesis was expressed that the radiobiological effect may be caused not only by short-lived radicals, but also by more stable intermediate products of their further conversions, whose effect is imitated by the radiomimetics [7, 8]. If this is true, specific chemical reactions whose rate must have a temperature dependence lie at the basis of the radiation effect, just as it does in the radiomimetic effect. At the same time retardation of the course of intoxication caused by nitrogen mustard gas [methyl-bis-(β-chlorethyl)-amine; NH2] was shown in frogs with a decrease in body temperature, just as this occurs after irradiation under similar temperature conditions (Fig. 1). The data obtained make it possible in both cases to interpret this phenomenon unambiguously if it is recognized that in this case a decrease occurs in the reactivity of the hypothetical intermediate RT or HN2.

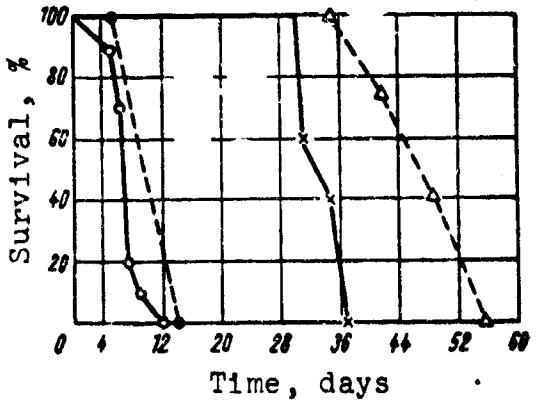

Fig. 1. Temperature dependence of frogs' death from irradiation in a dose of 900 r [9] and from the effect of HN2 (40 mg/kg). O) 0-1°; ●) 18-20°; ×) 5°; Δ) 23°; solid line) NH2; dashed line) irradiation.

A subsequent analysis of the effect of radiation and HN2 made it possible to establish the striking similarity of these agents in a wide range of doses. In Fig. 2 we show the results of one of the experiments [8]; the dependence of the mean duration of the life of mice on the HN2 dose is presented in juxtaposition with certain data of Rayevskiy. Both curves join at a point corresponding to the minimal absolute lethal doses of x-rays and HN2 (750 r and 4 mg/kg, respectively).

A cursory comparison of both curves does not leave any doubt as to the closeness of the phenomena which they represent. A re-

gion of an absence of dependence of the duration of life on the dose (up to 15 mg/kg) – plateau *A* on curve 2, an interval of progressive shortening of life (15-100 mg/kg; segment *B*) and, finally, the region of doses (100-2000 mg/kg, segment *C*) at which death occurs almost instantaneously is also characteristic of the effect of HN2. Here, in each of the indicated intervals the clinical manifestations of the intoxication are similar to the corresponding disorders characteristic of radiation sickness caused by different doses of irradiation. In the region of the doses lying on the plateau, the symptomatology develops only on the 2nd to 3rd day and is characterized by damage to the blood circulation and intestines. In interval *B* death occurs with manifestations of severe disorders of the nervous system (inhibition, which is replaced by convulsions). In interval *C* the illness is extremely acute and is manifested in severe convulsions which occur immediately after the effect and lead to death in 10-30 min analogously to death from radiation. The shift of the entire HN2 curve to the left along the dose axis which is evidently the result of an accelerated course of the intoxication in comparison with radiation sickness, as well as the briefer initial rise caused by HN2's primary damage to the intestines, in connection with which the animals do not live up to the time of bone marrow death, deserves attention.

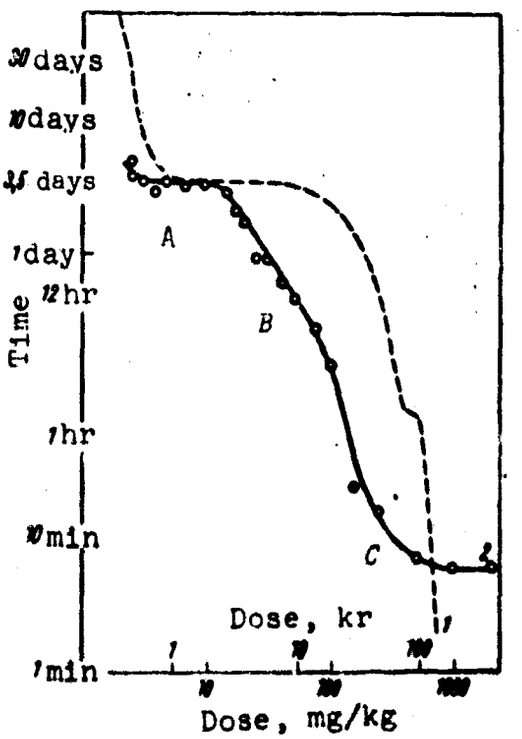

Fig. 2. Dependence of mean duration of life of mice on dose. 1) Rayevskiy radiation method; 2) HN2.

Thus, in spite of the different nature of radiomimetics and ionizing radiation, it is impossible to ignore the basic similarity of the biological effects which they cause which go beyond the bounds of a simple analogy. The equal effectiveness of thiol protectors during both effects also indicates this (table).

Comparative Data on Protection from Irradiation and the Effect of Radiomimetic Agents in Minimal Absolute Lethal Doses by Thiol Protectors*

1 Протектор	2 Вид воздействия	3 Мыши			4 Крысы		
		5 Число	6 Выжило к 30-му дню	7 Выживание, %	5 Число	6 Выжило к 30-му дню	7 Выживание, %
8 Цистеамин	Облучение 12	30	21	70	25	18	72
	HN2	30	20	67	20	18	90
	HN3	—	—	—	25	20	80
9 Цистамин	Облучение 12	30	18	60	20	14	70
	HN2	20	10	50	20	9	45
10 АЭТ	Облучение 12	35	32	91	20	10	50
	HN2	30	24	80	—	—	—
11 Контроль	Облучение 12	30	2	7	36	2	6
	HN2	30	1	3	20	1	5
	HN3	—	—	—	25	—	0

*HN3 — chlorhydrate-β-chlorpropyl-bis-(β-chlorethyl)-amine; protectors in doses of 150 and 100 mg/kg, respectively for mice and rats injected intraperitoneally 15 min before the effect.

1) Protector
2) Type of effect
3) Mice
4) Rats
5) Number
6) Survived to the 30th day
7) Survival rate, %
8) Cysteamine
9) Cystamine
10) AET
11) Control
12) Irradiation

An analogy of this type allows one to admit the possibility of the existence, and perhaps, the leading role of intracellular RT in specific types of cell death. In particular, it is tempting to assume that the massive death of the cells of radiosensitive organs mentioned above which is observed in the first hours after irradiation, that is, long before their division, leading to destruction of the bone marrow, is precisely of such a nature [10, 11]. An explanation of this question is of fundamental importance since now there can be no doubt that injury of cells in the interphase, but not localized mutations and not structural damage of the chromosomes, recorded in the form of aberrations [12], determine the outcome of acute radiation sickness in animals. The latter, while clearly related to the dose, affect, however, a relatively smaller percentage of the cells which die primarily in the first mitosis in proliferating (radiosensitive) tissues and hardly affect the functions of the rest of the somatic cells of the animals because of the latter's heterogenicity [13]. The role of localized mutations in the cells of higher animals with a diploid set of chromosomes is insignificant because of their recessiveness and limited sphere of influence. There is still no satisfac-

tory explanation of the mechanism of interphase death. The attempt to explain it unambiguously by the variety of the dominant lethals [14] cannot be recognized as satisfactory, based on the randomness of the mutation process which determines the low probability of the rigidly controlled events causing the observed massive uniform destructive reaction. This, however, is how matters stand from the standpoint of classical concepts which ascribe the main role in the primary mechanisms of the radiobiological effect to discrete elementary ionization acts [15]. The position is made considerably easier by inclusion of the production of intracellular toxins in the system under discussion. The latter as a result of sufficient concentration can block the synthesis of nucleic acids or enzymes by directed genome damage and also cause concentrated injuries of cytoplasmic structures, also leading to death of the cell. Considering, however, the numerous data concerning the incomparably great radiosensitivity of the nucleus in comparison with the cytoplasm, a genome type of damage seems more likely. Micronecrotic foci, identified by luminescent microscopy of suspensions stained with fluorochromes which bind with the nucleic structures, which appear in the bone marrow in the first hours after irradiation and the effect of radiomimetic substances point to the connection of the observed cell destruction with DNA (ДНК) and RNA (РНК) synthesis [16, 17].

Whatever it is, it is quite clear that interphase death differs fundamentally in nature from structural injuries of chromosomes. It is directly connected with the cell's functional state, determining in the final analysis the tissue's radiosensitivity. For example, we did not succeed in finding indications of destruction in a cytological analysis of a regenerating liver after preliminary irradiation of animals in doses of 150-300 rad, although the level of cells with chromosomal rearrangements hardly differed from their number in the bone marrow after irradiation in corresponding doses [18, 19]. According to other data [10, 20], interphase death was absent from the liver after irradiation in larger doses. However, it is typical of cells of such radiosensitive organs as the bone marrow, spleen, thymus and testicles. Moreover, a morphological demonstration of cell destruction is possible only under conditions of the functioning of the irradiated tissue in the organism. Destruction of bone marrow cells is not found after irradiation *in vivo*, although when such irradiated cells are transplanted, they completely lose the ability to regenerate [5]. After irradiation *in vivo*, the manifestation of destruction of bone marrow cells [21] or spleen cells [5] is delayed until the ligature is applied. Finally, in a fibroblast culture morphological changes were also not observed in cells in the interkinetic phase [22].

All this indicates that the described radiation reaction arises only in cells in a special functional state, not connected with the process of division [10], and its appearance is possible only in an intact organism. Such a phenomenon conforms poorly with the concept of cell death as a result of individual instantaneous events and can be better understood from the standpoint of RT which block the metabolic link responsible for these special functions.

Returning to the effect of alkylating compounds, let us note that in the case of mustard gas intoxication massive death of the hemogenic cells occurs in the first 6-24 hrs [8], whereas losses connected with chromosomal aberrations in mitosis comprise a small share of the total damage.

Thus, the concept of the radiotoxic nature of interphase death in the form of "microtoxicosis" of a specific type of cells deserves attention and verification of its correctness in special investigations. Finally, it can be assumed that a specific role in the development of structural chromosome damage belongs to local RT. In any case, it is impossible to explain from the classical point of view [15] data being accumulated on the possibilities of changes in the radiobiological effect with the help of various modifying factors and even more concerning the reparation of chromosomal damage with a decrease in the dose rate [18, 23, 24].

Radiotoxins and radiation's remote effects. The existence of a remote effect of ionizing radiation in animals is most clearly represented in experiments on the irradiation of limited areas of the body or on the total effect of weakly penetrating emissions. The disturbance which is observed in these cases in the blood system cannot be explained by direct injury of the hemogenic organs and therefore requires the service of indirect mechanisms. Similar conditions are laid down in examining the causes of the leucopenia developing in man during radiation therapy of neoplasms in which restricted areas of the body are subjected to multiple local irradiation, whereas the bulk of the hemogenic tissue remains outside the direct effect of the radiation.

The question arises: what is the nature of the remote effects observed in these cases? Is it also possible here to assume the effect of RT conveyed from the irradiated areas as the source, as is clearly shown in A.M. Kuzin's work on plants? An unequivocal answer to this question does not seem possible at this time. At the present time we, jointly with A.L. Vygodskaya and L.Kh. Eydus, are conducting appropriate investigations in this direction, the first results of which are presented in this collection (see page 270). Here we shall only point out that humoral factors which determine the development of the remote effect can have quite a different origin than the "local radiotoxins." Their development in early stages of the illness evidently is due primarily to a disorder of the neuroendocrine regulation, and later to a disturbance in metabolism.

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THE MUTAGENIC EFFECT OF EXTRACTS OF VARIOUS ORGANS OF IRRADIATED MICE

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The development of chromosomal rearrangements in epithelial cells of the corneas of rat recipients of extracts of carcasses of rats irradiated with a dose of 1500 r has been demonstrated earlier [1]. It has also been found that when the head is shielded and the bodies of the mice are irradiated with the same dose, the frequency of the development of chromosomal rearrangements in the corneal epithelium increases approximately 10-fold in comparison with intact animals. The assumption has been made that substances of the "radiotoxin" type, capable of causing cytogenetic effects in unirradiated cells, develop in the irradiated animal organism.

It was the purpose of the present work to demonstrate in which organs of irradiated animals the production or accumulation of the active substances occurs.

In the experiments 367 mice of the CC-57 line (brown) were used; they were two-month old males. Fifty of them served as donors. Irradiation was carried out in an RUT-200 apparatus in a dose of 1500 r at a dose rate of 30 r/min with 0.5 mm copper and 1.0 mm aluminum filters; the focal distance was 40 cm. Eighteen mice were irradiated simultaneously. Dose measurement was carried out with a DIM-60 precision dosimeter.

The mice were killed 1, 2 and 3 days after irradiation. For preparation of the extracts, the small intestines, testicles, liver, spleen, brain and fatty tissue were removed; heparinized blood was also used. The organs were washed with Ringer's solution after which 4 g weighed samples were taken and were carefully pulverized in 8 ml of Ringer's solution in a glass homogenizer. After 2 hrs of extraction in the cold the homogenate was centrifuged for 10 min at 5000 rpm, filtered and 0.3 ml of the filtrate injected intraperitoneally into the recipient mice. The control mice were injected with extracts of organs of intact animals prepared in the same way. The recipient mice were killed 24 hrs after the injection of the extracts, the eyeballs were fixed for 3-4 hrs, after which they were stored in 70% alcohol. Staining of the prepared corneas was carried out with Böhmer's hematoxylin. The chromosomal rearrangements were counted in both corneas of each animal.

TABLE 1

Cytogenetic Effect of Radiotoxins on Mouse Corneal Epithelial Cells in an *in situ* Experiment

1 Вид воздействия	2 Вариант опыта	3 1-е сутки: 6 Число животных	7 Число нор. митозов	8 Число перестроек	9 Перестройки, %	4 2-е сутки: 6 Число животных	7 Число нор. митозов	8 Число перестроек	9 Перестройки, %	5 3-и сутки: 6 Число животных	7 Число нор. митозов	8 Число перестроек	9 Перестройки, %
10 Интактные животные	—	5	1213	0	0	—	—	—	—	—	—	—	—
11 Экстракт из жировой ткани	Контроль 18	5	1663	0	0	—	—	—	—	—	—	—	—
	1500 *p* 19	5	1131	2	0,13±±0,01	10	3748	5	0,18±+0,06	10	3414	2	0,060±±0,003
12 Экстракт из головного мозга	Контроль 18	10	2825	0	0	—	—	—	—	—	—	—	—
	1500 *p* 19	20	5013	11	0,19±±0,01	20	4862	13	0,29±±0,02	17	5658	13	0,26±±0,01
13 Экстракт из тонкого кишечника	Контроль 18	5	1200	0	0	—	—	—	—	—	—	—	—
	1500 *p* 19	5	945	1	0,18±±0,43	5	1255	0	0	5	1700	1	0,08±±0,02
14 Экстракт из печени	Контроль 18	5	1020	0	0	—	—	—	—	—	—	—	—
	1500 *p* 19	5	1555	0	0	5	887	0	0	—	—	—	—
15 Экстракт из селезенки	Контроль 18	5	1613	0	0	—	—	—	—	—	—	—	—
	1500 *p* 19	5	1069	0	0	—	—	—	—	—	—	—	—
16 Экстракт из семенников	Контроль 18	5	1058	0	0	—	—	—	—	—	—	—	—
	1500 *p* 19	5	1331	0	0	—	—	—	—	—	—	—	—
17 Кровь	Контроль 18	5	1266	0	0	5	1727	0	0	5	1382	0	0
	1500 *p* 19	5	1445	0	0								

1) Type of effect; 2) variant of experiments; 3) 1st day; 4) 2nd day; 5) 3rd day; 6) number of animals; 7) number of normal mitoses; 8) number of rearrangements; 9) rearrangements, %; 10) intact animals; 11) fatty tissue extract; 12) brain extract; 13) small intestine extract; 14) liver extract; 15) spleen extract; 16) testicle extract; 17) blood; 18) control; 19) 5.

TABLE 2

Cytogenetic Effect of Radiotoxins on Mouse Corneal Epithelium in an *in vitro* Experiment

1	2	3 1-е сутки				4 2-е сутки				5 3-и сутки			
Вид воздействия	Вариант опыта	6 Число животных	7 Число нор. митозов	8 Число перестроек	9 Перестройки, %	6 Число животных	7 Число нор. митозов	8 Число перестроек	9 Перестройки, %	6 Число животных	7 Число нор. митозов	8 Число перестроек	9 Перестройки, %
10 Интактные животные	—	5	1213	0	0	—	—	—	—	—	—	—	—
11 Экстракт из головного мозга	Контроль 13	7	1903	0	0				—				
	1500 р 14	10	3436	1	0,03± ±0,02	9	2624	0	0	10	2144	0	0
12 Экстракт из жировой ткани	Контроль 13	5	1200	1	0,13±	—	—	—	—	—	—	—	—
	1500 р 14	10	1643	0	±0,10 0	9	2267	3	0,09± ±0,02	10	2161	3	0,12± ±0,02

1) Type of effect; 2) variant of experiment; 3) 1st day; 4) 2nd day; 5) 3rd day; 6) number of animals; 7) number of normal mitoses; 9) number of rearrangements; 9) rearrangements, %; 10) intact animals; 11) brain extract; 12) fatty tissue extract; 13) control; 14) r.

As seen from Table 1, extracts of organs of intact animals did not have a cytogenetic effect. When fatty tissue extracts were injected, the percentage of chromosomal rearrangements on the 1st day after irradiation was 0.13 ± 0.01%, on the 2nd day 0.18 ± 0.06% and on the 3rd day 0.060 ± 0.003% with complete absence of rearrangements in the control material. However, in all these cases the difference from the control is not statistically reliable.

When brain tissue extracts were injected, the percentage of chromosomal rearrangements in the recipients changed more significantly: 0.19 ± 0.01% on the 1st day after irradiation, 0.29 ± ± 0.02% on the 2nd day and 0.26 ± 0.01% on the 3rd day with complete absence of rearrangements in the control. The difference from the control material is reliable in all variants of this experiments. The extracts prepared from other organs did not have any effect.

In analyzing the results from individual animals, considerable individual variability was observed in the organism's reaction to the effect of the extracts, which also occurred in previous experiments [1].

The next series of experiments was carried out for purposes of demonstrating the significance of the complete organism in the production of radiotoxins. Mice of the same line, sex and age were used in the experiment. The brain (whole) and fatty tissue from the abdominal cavity were removed from the killed animals. The tissues were washed with Ringer's solution, placed in the solution in Petri dishes and irradiated in an RUM-5 apparatus in a dose of 1500 r at a dose rate of 32 r/min. Filters consisting of 0.5 mm of copper and 1.0 mm of aluminum were used during the irradiation; the focal distance was 40 cm.

Immediately after irradiation the organs were placed in fresh Ringer's solution. All the operations were carried out in the cold. The organs were stored for 3 days at 4° in vessels with ground-glass stoppers. After 1, 2 and 3 days extracts were prepared from the irradiated organs, 0.3 ml of which was injected into the recipient mice.

Intact organs stored under the same conditions served as the control. The mice were killed 24 hrs after the injection of the extracts. The eyeballs were removed and corneal preparations were made.

It is seen from Table 2 that the injection of the extracts did not cause the appearance of chromosomal rearrangements, and all the observed deviations from the control are not statistically reliable.

Data of experiments on irradiation of various tissues *in situ* in a dose of 1500 r showed that a completely reliable cytogenetic effect appeared only after the injection of an extract prepared from brain tissue. At the same time, the same extract, but prepared from brain tissue irradiated outside of the living organism, did not have a cytogenetic effect. This fact indicates the

important role which various organismic factors play in the production of radiotoxins. Evidently, besides primary reactions of the radiation oxidation of brain tissue biolipids [2-4], it is as if the radiolytic products which developed in other organs of the irradiated organism can be adsorbed in this organ (the increase in the effectiveness of an extract prepared on the 2nd and 3rd day after irradiation indicates this last possibility). Being involved in the general metabolism, these products can be converted into a number of toxic substances of the type of unsaturated fatty acids, epoxides and products of their further decomposition [5]. Irradiated fatty tissue may be one of the active sources of these harmful metabolites [6].

The following conclusions can be drawn from the results of the work which was carried out:

1. A reliable cytogenetic effect of a water-salt extract of the brain of irradiated mice when injected into intact recipients has been demonstrated.

2. A similar, but statistically unreliable effect appeared after the injection of a fatty tissue extract. Extracts of intestine, liver, testicles and spleen, as well as heparinized blood, did not produce an effect.

3. The extracts proved to be effective only when the organs were irradiated *in situ*. When the same organs were irradiated with the same dose *in vitro*, it was not possible to find an effect.

4. It is possible that the observed remote mutagenic effect of radiation is caused by products of biolipid radiolysis which develop in the irradiated organism. The inclusion of these products in the chain of metabolic processes probably leads to the accumulation of mutagenic substances in the organism. It will be the task of future investigations to determine the relative significance of such chemical mutagenesis after irradiation with different doses of ionizing radiation.

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THE REMOTE EFFECT OF RADIATION ON THE HEMOPOIETIC ORGANS

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It has been shown at the present time that, in addition to the direct injury of organ tissues by ionizing radiation, remote effects play a specific role in the development of radiation sickness. It has been established that toxic products are formed in irradiated tissues, and work on their identification is being carried out in a number of laboratories. There are also a number of indications concerning the important role of pathological changes caused by disturbances in neurohormonal regulation and immunological reactions as well as by metabolic changes.

A study of remote effects in the hemopoietic organs which are highly radiosensitive and are responsible for the damage at average lethal doses is of the most theoretical and practical interest.

The first results of investigations which are being carried out for the purpose of determining the relative role of the remote effect of ionizing emissions in general radiation injury, of a study of the possible mechanisms of the remote effect and of a determination of the theoretical possibility of reducing it are presented below.

In recent years a number of investigators [1-3] have found considerable damage of the bone marrow in a screened extremity after total irradiation of animals in lethal doses. A considerable difference in the number of nucleate bone marrow cells of the irradiated extremity 24 hrs after its irradiation individually or together with other parts of the body was also observed [3, 4].

It should, however, be pointed out that the use of the criterion which was applied in these studies - a decrease in the number of karyocytes - requires great care since the number of nucleate bone marrow cells depends on the animals' age and the conditions of their maintenance and can change from the effect of chronic infections, stress factors and other causes [5].

In connection with this, special attention must be paid to correct determination of the number of nucleate cells in the control animals under the given experimental conditions. For strict equalization of the "background" it is necessary, according to

the available data [5], to use animals of the same line and age, sterile conditions of their maintenance, automatic delivery of water and food, artificial climate, etc. However, it is rather complicated to fulfill all these conditions.

In the experiments which were conducted 306 unbred pubescent male rats of different weights kept under the usual conditions on the usual diet were used.

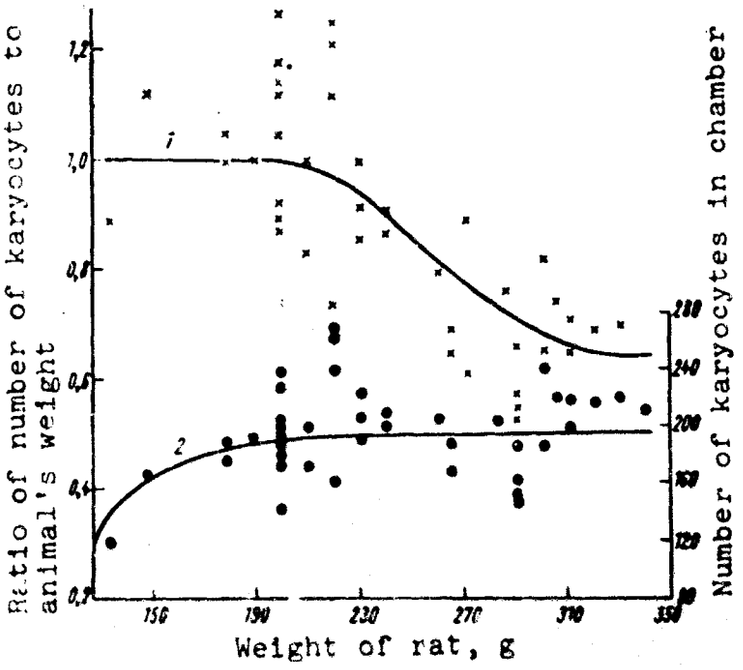

Fig. 1. Dependence of number of karyocytes of the bone marrow on animal's weight.

The change in the number of karyocytes in the bone marrow of the tibiae of irradiated and screened rat extremities after x-ray irradiation of the animals in a dose of 1000 r (RUM-11 apparatus, voltage 210 kv, current strength 20 ma, 0.5 mm copper and 1.0 mm aluminum filters, distance from anticathode 27 cm, dose rate 100 r/min) was studied. The rats were irradiated in pairs in organic glass chambers. One of the rear extremities which extended outside of the chamber was fastened with a soft ligature and covered with a 6 mm thick lead shield. Under these conditions the total dose measured under the shield did not exceed 6 r. The animals were killed 1 hr and 20 min, 3, 6, 24 and 48 hrs after irradiation by decapitation. A quantitative analysis of the bone marrow was carried out in the tibia which was completely shielded with lead. This made for confidence in the fact that the decrease in the number of nucleated cells in the shielded extremity is not the result of the direct effect of scattered radiation which might have been observed in an analysis of the bone marrow of the femoral bone. The whole bone was ground in 15 ml of 3% acetic acid, after which the number of karyocytes contained in the bone marrow of the tibia was determined in a Burker chamber. The num-

ber of karyocytes in each sample was counted twice (in the upper and lower chambers) and the results averaged. The number of nucleate elements in both extremities of control unirradiated animals was determined by the same method. The difference in the number of cells in the right and left extremities of the same animal did not exceed 20% in a majority of cases.

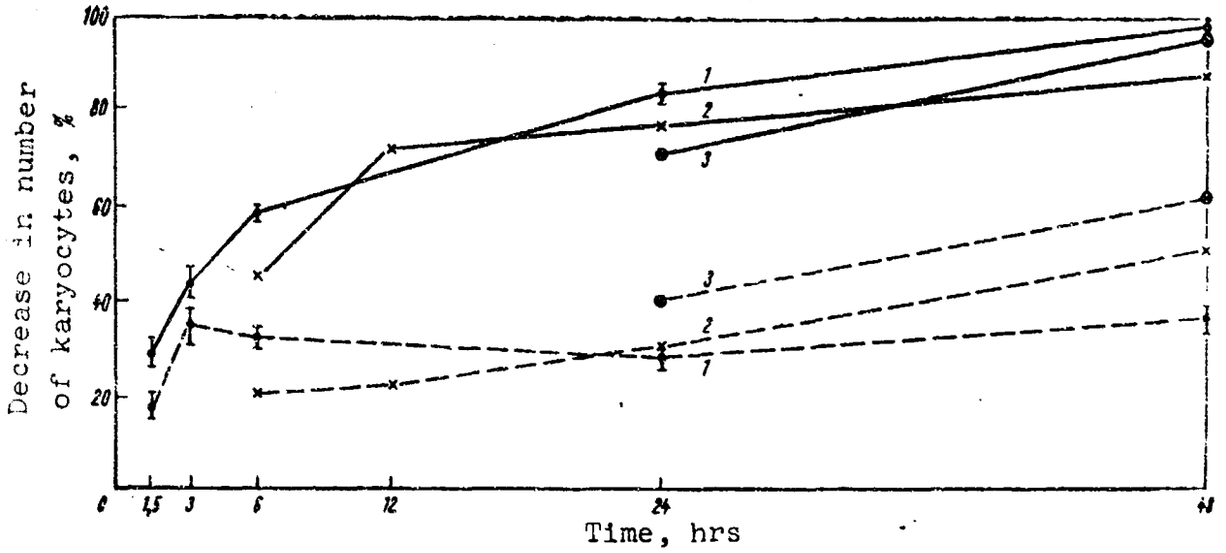

Fig. 2. Change in number of karyocytes in irradiated and shielded extremities. Solid lines) irradiated extremities; dashed lines) shielded extremities; 1) rats, 1000 r (authors' data); 2) rats, 716 r [1]; 3) mice, 800 r [3].

First of all, the dependence of the number of karyocytes of the bone marrow on the animals' weight was established in the 43 control animals. The ratio of the number of nulcear cells of the bone marrow, averaged from the results of counting in both extremities, to the animal's weight is presented in Fig. 1 (curve 1). As seen from the graph, the absolute number of cells increases with the animal's growth up to a certain limit, after which it remains at a constant level (curve 2). In evaluating the decrease in the number of karyocytes in experimental animals of different weights, the values corresponding to curve 1 were taken for the control indices in all the experiments.

In the first series of experiments (161 animals) the change in the karyocyte content of the bone marrow was studied in irradiated and shielded extremities after total irradiation of the rats. The dynamics of the development of the lesion, expressed in a decrease in the number of cells, is depicted in Fig. 2. The sharp decrease in the number of karyocytes in the shielded extremity draws attention. A similar result was observed by other investigators whose data are presented on the same graph. The considerable decrease in the number of cells 1.5-3 hrs after irradiation which was detected is especially interesting.

It is possible, however, that it is connected with the

stress caused by the irradiation conditions which were unusual for the animals: the rats were kept for 12-15 min in a chamber in a fixed position with an extremity extended. To separate the direct effect of irradiation on the shielded extremity from the nonspecific effects, a group of animals was subjected to simulated irradiation, with performance of all procedures connected with this. As seen from the table in which the results of the experiment are presented, 3 hrs after the effect a considerable part of the effect described above is brought about not as a result of irradiation, but is caused by stress factors connected with the irradiation procedure itself.

It is unclear, however, how the effect of simulated irradiation develops with time and what contribution it makes to the total effect in the later periods of observation. Investigators [5] who studied the effect of the procedural factor established that the number of nuclear bone marrow cells 24 hrs after the effect was 16% lower than normal.

Effect of Simulated Irradiation on Number of Bone Marrow Karyocytes

3 ч после облучения (1)			3 ч после мнимого облучения (2)	
Количество животных (3)	Уменьшение количества кариоцитов, % (от контроля) (4)		Количество животных (3)	Уменьшение количества кариоцитов, % (от контроля) (4)
	в экранированной конечности (5)	в облученной конечности (6)		
13	34±3,9	43±3,4	13	21,5±2,8

1) 3 hrs after irradiation
2) 3 hrs after simulated irradiation
3) Number of animals
4) Decrease in number of karyocytes, % (of control)
5) In shielded extremity
6) In irradiated extremity

In the second series of experiments (102 animals) an attempt was made to reduce the injury of the bone marrow in the shielded extremity. Sodium aminoethylthiophosphate (cystaphos) was used as the protective substance. The protector was injected subcutaneously 30 min before irradiation in an amount of 350 mg per kg of weight.

In all periods both in the irradiated and in the shielded extremity a slight protective effect was noted (Fig. 3). If, however, the possible contribution of the procedural factor in these periods after the effect is taken into consideration, the degree of protection increases. At the same time, it is still impossible to exclude the possibility that the protective effect is chiefly due to a decrease by the protector of the effect of procedural

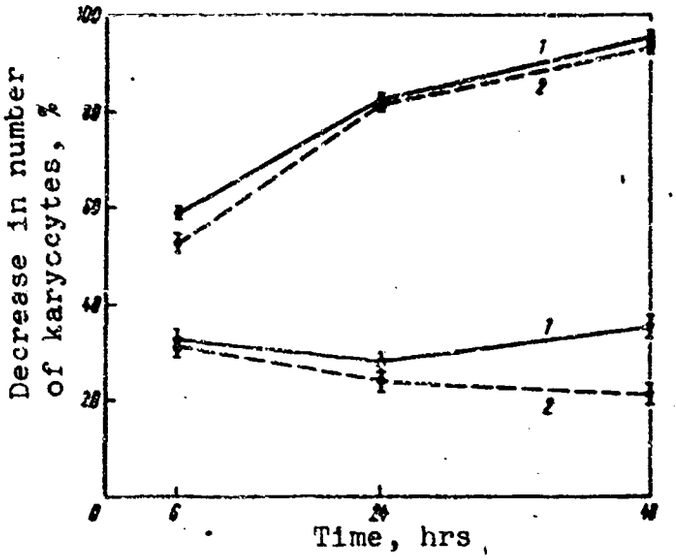

Fig. 3. Effect of protector on change in the number of karyocytes in irradiated (upper curves) and shielded (lower curves) extremities. 1) 1000 r; 2) cystaphos + 1000 r.

stress and not to a decrease in the remote radiation reaction.

Thus, at the present time it can be considered as established without any doubt that under ordinary experimental conditions the number of bone marrow karyocytes is sharply decreased in the shielded extremity.

A prophylactic injection of protector somewhat decreases this injury. A careful analysis of the contribution made by nonspecific reactions is necessary to obtain information about the influence of the true remote effect.

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THE ROLE OF THE METABOLIC PROPERTIES OF REGENERATING TISSUES IN RESISTANCE TO IONIZING RADIATION

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The radiotoxin [RT] (PT) theory which has received broad development in contemporary radiobiology considers radiosensitivity in direct connection with metabolic properties of the object being irradiated. The production in irradiated tissues of RT which cause not only severe functional, but also cytogenetic injuries is directly dependent on primary radiation-chemical processes [1-5]. It has been shown that by increasing in the tissues the amount of substances which decrease the primary radiobiological effect, it is possible to decrease RT production and, consequently, radiation injury of the tissue.

From the viewpoint of the RT theory it is interesting to examine the increase in the radioresistance of tissues during their development of a plastic regenerative condition. It is well known that the metabolism of tissues which are regenerating following injuries differs considerably from the metabolism of tissues in a state of normal functional activity. Proteolysis, lipolysis and other catabolic processes are intensified in regenerating tissues. In addition to this, intensive compensatory reactions develop, the metabolism of proteins, nucleic acids, lipids and other vitally important compounds is accelerated by several times, tissue proliferation begins, leading to filling in of the defect. The capacity of the tissue after various injuries to proceed into this special plastic state, described by Studitskiy [6], represents one of the most important adaptive defense reactions of the organism.

We have shown in a number of investigations that the tissue of an organism brought into a plastic state by an injury is distinguished by considerable radioresistance from normal tissue. For example, the skeletal muscle of mammals irradiated in a dose of 2000 r almost completely loses the capacity for regeneration after mechanical injury. If muscle which is already regenerating after an injury is subjected to irradiation, the regenerative capacity is slightly disturbed and a dose of irradiation of more than 6000 r is necessary to suppress it [7]. A considerable increase in the radioresistance of muscle which is regenerating after denervation has also been shown [8]. There are data in the literature concerning an increase in the radioresistance of regenerating skin epithelia [9, 10], bone tissue [11], thyroid

gland [12] and bone marrow [13-16]. An interesting investigation has been carried out on planarians [17]: a planarian regenerating after being cut into parts is considerably more resistant to irradiation than a normal planarian.

Up to the present time the process of regeneration was considered by a majority of radiobiologists [18, 19] only as a means of accelerating elimination of cells injured by irradiation and recovery of the tissue at the expense of cells which remained uninjured and capable of multiplication. However, it is seen from our own experimental material that the regeneration process not only promotes elimination of the dead cells of the irradiated tissue, but also provides for increased radioresistance of this tissue. The correctness of this position is confirmed especially clearly by data [20] obtained in an investigation of the effect of irradiation on normal and regenerating corneal epithelia.

The magnitude of the radiation injury of the epithelium was judged from objective numerical data characterizing the mitotic index and the percentage of chromosomal aberrations of the epithelial cells. All the observations were carried out on one generation of cells entering into the first mitosis after irradiation. By this the assumption that the detected decrease in radiation damage of regenerating epithelium can be explained by acceleration of the replacement of injured cells by multiplying uninjured cells was removed. It was shown that in an irradiated normal cornea the number of dividing cells with chromosomal aberrations in 5 days of observation is about 85%. At the same time, in a regenerating irradiated cornea after 24 hrs the chromosomal aberrations comprise only 30% and in 3 days – a total of 4.3%. The considerably smaller number of dividing cells with aberrations in the regenerating cornea speaks of the fact that it received less damage in comparison with a normal cornea from irradiation with the same dose. A careful morphological analysis of the material obtained indicates that the cause of the increased radioresistance is the metabolic properties of the regenerating tissue.

Studies of certain metabolic properties of regenerating tissues were carried out in connection with the dynamics of their radioresistance. It is known that in the primary radiobiological effect a specific role belongs to catalase which not only splits hydrogen peroxide but also inactivates active OH and HO_2 radicals produced during irradiation [21]. From a study of the change in catalase activity in muscle tissue regenerating following mechanical trauma or after denervation it turned out that the maximum increase in catalase activity (five-fold) coincides with the period of the greatest radioresistance of these tissues.

Considerable attention is also being paid in contemporary radiobiology to the radioprotective role of sulfhydryl compounds. It has been shown [22] that the mechanism of action of many radioprotectors is connected with the liberation of free sulfhydryl groups. It is interesting that in regenerating tissues the content of reduced sulfhydryl compounds is increased [23, 24]. We have shown that regenerating muscle which has increased radioresistance contains 68% more free SH-groups than normal muscle. Thus, this metabolic characteristic of regenerating tissues is

also in accord with their increased radioresistance.

Moreover, various antioxidants which are produced during metabolism play the known role of radioprotectors in regenerating tissues. The data from investigations [25] in which the low value of the oxidation-reduction potential of regenerating tissues was shown indicates their increased content.

It is likely that the enumerated data do not exhaust all the metabolic characteristics of regenerating tissue connected with its increased radioresistance. However, the available experimental material clearly shows how closely radioresistance is connected with the functional state of the tissue, with its metabolism. As a result of the characteristics of the metabolism in regenerating tissues, conditions are created which decrease RT production, and consequently, the injurious effect of radiation. The concept of radioresistance includes not only vulnerability to ionizing radiation, but also the capacity for regenerative processes after its action. It is entirely possible that during regeneration conditions are created in the tissues which promote recovery processes both at the tissue and at the cellular level.

A more detailed study of radiation-chemical processes in regenerating tissues and the principles of the production and effect of RT will make possible an even deeper understanding of their role in the radioresistance of various organisms and their tissues.

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A STUDY OF THE ROLE OF TOXINS IN RADIATION DAMAGE OF COTTON PLANTS BY MEANS OF GRAFTS

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In recent years more and more significance in the radiation injury of living organisms is being ascribed to a disturbance in metabolic processes in the cell and as a consequence of the accumulation in it of abnormal metabolites (see [1-6] and others).

A convenient method of determining the role of metabolic changes in the irradiated organism and of the toxic metabolic products formed as a result in the biological effect of radiation is grafting of an irradiated plant or its individual organ to an unirradiated plant. In experiments with transplantation of unirradiated wheat germs into irradiated endosperms [7] and in investigations with the grafting of unirradiated potato parenchymatous cones to an irradiated tuber of adult plants [6, 8], a typical picture of radiation injury was observed with the sole difference that the effect was quantitatively less than after direct irradiation of the plants. The given question is also interesting with respect to cotton plants.

For this purpose cotton plants of the 108-Ф variety (*Gossypium hirsutum L.*) grown from ordinary seeds were grafted onto plants grown from irradiated seeds of the same variety and vice versa. Grafts of plants: unirradiated to unirradiated and irradiated to irradiated served as the control (an outline of the experiments is presented in Table 1).

The experiments were carried out in 1964 near Tashkent. Seeds which had been moistened for 24 hrs in water were irradiated with Co^{60} γ-rays in equipment of the UzSSR Academy of Sciences Institute of Nuclear Physics at a dose rate of 24 r/sec. The total dose was 10 kr. Sowing of the irradiated and unirradiated seeds was carried out on the day of irradiation in Wagner vessels with a capacity of 11 kg of soil. Before impaction 3 g of P_2O_5 and 2.5 g of K_2O was added to each vessel and the plants received 2.5 g of nitrogen in the form of supplementary feedings in various growth phases.

The plants were grown under natural conditions on a platform with a humidity of 65% of the complete water capacity. The experiments were repeated five times; two plants were grown in each vessel.

TABLE 1

Outline of the Experiment

Exp. No.	Rootstock	Scion
1	Unirradiated	× unirradiated
2	Unirradiated	× irradiated
3	Irradiated	× unirradiated
4	Irradiated	× irradiated

Grafting of the plants was carried out in the morning (from 6 to 8 AM) and evening (from 8 to 9:30 PM) hours in the 4-5 true leaf phase by the "ligule" method with the lower part of the scion's stem dipped in a test tube containing water. In this case, in order to avoid rapid drying of the tissue surface the region of contact of the rootstock and scion was wrapped with polyethylene tape which was removed as the grafted components grew together. Water was added to the test tubes every day. After complete growing together of the components (7-10 days after grafting) the tapes and the test tubes containing water were removed; 99% of the roots took. Then in order to strengthen the interference of the components, the "mentor" method was used for some of the grafted plants: the generative organs were removed from the rootstock and all the leaves were removed from the scion with the exception of the very top 1-2 rudimentary leaflets.

In all cases, independently of the combination, the following served as criteria of the effectiveness of the toxins produced from the effect of radiation: growth, development, size, morphological change in the bolls and yield of raw cotton in the scion.

TABLE 2

Effect of Irradiated and Unirradiated Rootstock on Scion's Growth and Development

Номер варианта 1	Вариант 2		Высота главного стебля прививки на 10/IX, см 3	Период от посадки до бутонизации, сутки 4
1	Необлученные	× необлученные 5	12.50±1.39	50.00±0.49
2	Необлученные	× облученные 6	15.0±0.7	49.00±0.51
3	Облученные	× необлученные 7	8.33±1.07	55.0±1.3
4	Облученные	× облученные 8	8.50±1.84	54.00±1.91

1) Number of variant; 2) variant; 3) height of main stem of scion on 10 September, cm; 4) period from seedlings to budding, days; 5) unirradiated × unirradiated; 6) unirradiated × irradiated; 7) irradiated × unirradiated; 8) irradiated × irradiated.

TABLE 3

Effect of Irradiated and Unirradiated Rootstock on Size of Bolls and Yield of Raw Cotton in Scion (60 plants)

Номер варианта 1	Вариант 2	Число собранных коробочек 3	Общий вес сырца с одного растения, г 4	Вес одной коробочки, г 5
1	Необлученные × необлученные 6	248	21,1	5,1
2	Необлученные × облученные 7	243	18,1	4,6
3	Облученные × необлученные 8	237	18,9	4,8
4	Облученные × облученные 9	245	16,2	4,4

1) Number of variant
2) Variant
3) Number of bolls collected
4) Total weight of raw material from one plant, g
5) Weight of one boll, g
6) Unirradiated × unirradiated
7) Unirradiated × irradiated
8) Irradiated × unirradiated
9) Irradiated × irradiated

The observations showed (Tables 2 and 3) that irradiation of moistened cotton seeds with γ-rays in a dose of 10 kr considerably suppresses growth, retards development and while not essentially affecting the productivity of the plants, decreases the size of the bolls which decreases the yield of raw cotton (variant 4). When an irradiated plant is grafted on an unirradiated plant, the effect of the radiation is noticeably removed in the scion (variant 2): growth and development occur normally, the weight of the bolls and the yield of raw cotton are decreased to a lesser degree than in the irradiated control (variant 4). Consequently, under the influence of the unirradiated rootstock toxic substances are rendered harmless and normalization of disturbed metabolic processes occurs in the irradiated scion which was reflected in the growth, development and economically valuable properties of the latter. With the opposite combination, on the other hand, under the influence of the irradiated rootstock a noticeable suppression of growth and development, a decrease in boll size and yield of raw cotton occurs in the unirradiated scion.

An interesting, in our opinion, phenomenon is found from a study of the remote effect of radiotoxins on the shape of the bolls. From self-grafting of an irradiated plant on an irradiated (control) plant, deformed bolls are produced both in the scion and in the rootstock (see Figs. 1 and 2). When an irradiated plant is grafted to an unirradiated plant and after artificial removal of all the leaves from the former and the fruiting organs from the latter ("mentor" method), the grafted plant, as a rule, produces morphologically completely normal bolls (Fig. 3). However, if the irradiated scion has but 1-2 of its own leaflets, this is not observed – bolls characteristic of irradiation are

produced on it (Fig. 4). When an unirradiated plant is grafted to an irradiated plant and the indicated "mentor" method is used, externally unchanged bolls are produced on the unirradiated scion (Fig. 5). But again the effect of the rootstock on the morphology of the bolls is not found in the scion if 1-2 of its own upper leaves are left on the latter.

Fig. 1. Boll of unirradiated cotton plant of the 108-Φ variety (control).

Fig. 2. Part of the stem with deformed boll of irradiated plant grafted to an irradiated plant (control).

In connection with what has been stated the question naturally arises of whether the change in the bolls which develops in an unirradiated scion under the influence of the irradiated rootstock is hereditary or is only somatic. In order to answer this question, the seed generation obtained from the divergent bolls of the scion are being studied at the present time.

Thus, the appearance of deformed bolls on an unirradiated scion under the influence of the irradiated rootstock or, vice versa, the production of morphologically normal fruit in an irradiated plant grafted to an unirradiated depends on the presence of the scion's own leaves. Morphological deviations in the bolls or the recovery of their shape occurs only in the absence of the scion's own leaves. This leads to the thought that an unirradiated scion in the presence of its own normally photosynthesizing leaves renders harmless the toxic substances which have entered it from the irradiated rootstock. As a result, the developing fruit receive the usual assimilation products, while in the absence of leaves the scion is deprived of this possibility and abnormal metabolites enter the fruit developing on them from the irradiated rootstock and cause changes in their shape. On the other hand, in the absence of its own leaves from an irradiated scion - the usual compounds enter it from the unirradiated rootstock

and it produces morphologically normal fruit. In an irradiated scion in the presence of its own leaves, the disturbance in the metabolic processes and the production of toxic substances is so strong that the compounds which have entered from the unirradiated rootstock cannot completely normalize the metabolism and block abnormal products in the scion and the latter, entering the developing fruit, cause their deformation.

Fig. 3. Irradiated plant grafted to unirradiated. The leaves have been removed from the scion and the fruiting organs from the rootstock; normal bolls were produced on the scion.

Fig. 4. Part of the stems of unirradiated rootstock and irradiated scion with part of the leaves left on the latter. Boll of the scion is deformed.

GRAPHIC NOT REPRODUCIBLE

Fig. 5. Unirradiated plant grafted to irradiated. The leaves have been removed from the scion and the fruiting organs from the rootstock. One of the two developing bolls on the scion is deformed.

According to the data of some authors (for example, [1, 8]), under the influence of radiation, intermediate oxidation products of semiphenol and semiquinone nature are formed in the organism, while according to the results of other investigations (for example, [2, 4, 5]), after irradiation the accumulation of organic peroxides of lipid nature occurs. In our opinion, in addition to these classes of substances any substance is responsible for the radiobiological effect if its amount in the cell inordinately exceeds the norm, since under the influence of radiation the organization and coordination of the activity of enzyme systems is primarily disturbed: the activity of some enzymes is increased, the activity of others is suppressed (see [1, 3, 5] and others). As a result the synthesis or the breakdown of some compounds is intensified, and of others, it is decreased, which creates a disproportion in the amount of various substances in the cells.

An increase in the concentration of substances usually harmless to the cell, even those such as water-soluble carbohydrates and amino acids, beyond certain limits is ballast for the organism and leads to various physiological changes, in particular, it suppresses plant growth [9-13]. We are now conducting appropriate investigations in this direction.

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THE PROPERTIES OF A RADIOTOXIC SUBSTANCE SUFFICIENT TO CAUSE DEATH OF MAMMALS 30 DAYS AFTER IRRADIATION

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Hypotheses that in irradiated organisms toxic substances are produced which are capable by themselves of causing many effects which arise as the result of the action of ionizing radiation are considered as proved at the present time (see [1] and this collection, pages 4 and 90). At the same time, the question of the relative significance of radiotoxic substances in general and those known at the present time, in particular, in the death of mammals in the first 30 days after irradiation has still not been answered.

We believe, and this is our initial assumption, that a radiotoxic substance is of decisive importance in determining the probability of the lethal effect of radiation in mammals in the first 30 days after irradiation. This conviction is based on the fact that is is possible with the help of the radiotoxic substance hypothesis (by ascribing to it four simple natural properties; see below) to explain from a single standpoint, on the one hand, certain still unexplained fundamental results of fractionated irradiation of animals with sublethal doses of radiation, and on the other, some also unexplained quantitative relations between single local and total irradiations with sublethal doses of radiation.

To determine the possible properties of the radiotoxic substance which, according to the initial assumption, plays the decisive role in the development of processes leading to the death of irradiated mammals in the first 30 days after irradiation, we used results of radiation's injurious effect after fractionated irradiation with sublethal doses. We shall present these results briefly.

Under conditions of two irradiations, when only the first dose is sublethal, the probability of death decreases with an increase in the time interval between the two fractions. It is taken for granted that reparation of the injury caused by the first irradiation occurs in the time between the two irradiations, so that "the second dose does not add to the initial dose, but to its decreased value which remains at the moment of application of the second radiation effect." This decreased dose is called the "residual dose."

It turned out that the change in the residual dose with time in many cases is exponential [2-14]. The exponential nature of the reparation of the residual dose at the level of a multicellular

organism, in our opinion, must play the same major role in radiobiology as occurred with respect to the exponential nature of the accumulation of radiation's inactivating effect at the molecular and cellular levels.

To explain the fact of the exponential nature of the residual dose's reparation in time at the level of the multicellular organism, we have suggested and examined a simple phenomenological model of radiation's effect on a multicellular organism [15].

Within the framework of this model, in order that it determine the death of mammals in the first 30 days after irradiation, it is sufficient to ascribe to the radiotoxic substance (in reference [15] it bears the name "essential reparable link") the following four natural properties:

1. The amount of toxic substance formed is directly proportional to the irradiation dose in the range from zero to several thousand roentgens. Saturation sets in at higher irradiation doses so that the amount of toxic substance cannot increase infinitely (see this collection, pages 28 and 37).

It is natural to assume in this case that the amount of toxic substance formed per unit dose and per unit weight of the irradiated tissue area will be different in different irradiated sections of the animal's body. This assumption can be written in symbolic form as follows:

$$\Pi = \sum_i \Pi_i = \sum_i \eta_i m_i D_i \quad \text{at} \quad 0 \leqslant D_i \leqslant D_{i\,\text{крит}}. \qquad (1)$$

where m_i is the mass of the tissue segment being irradiated in a dose of D_i; η_i = const – "the specific amount of toxic substance formed," that is, averaged over the whole mass m_i irradiated in dose D_i in 1 unit mass of the body segment m_i and per unit of the radiation dose; $D_{i\ \text{krit}}$ is the threshold irradiation dose (at radiation doses higher than $D_{i\ \text{krit}}$, saturation sets in the irradiated segment m_i, that is, deviation of the relation between Π_i and D_i from direct proportionality).

2. The time in which the amount of toxic substance reaches its maximum value does not depend on the irradiation dose and the site of its production.

3. After reaching its maximum value, the amount of the toxic substance decreases with time at a rate proportional to its concentration:

$$-d\Pi = k \cdot \Pi \cdot dt, \qquad (2)$$

where $k = k(D) > 0$. The rate k depends on the irradiation dose but the time during which the amount of the toxic substance decreases by half is never less than several days.

4. The organism dies when and only when the total amount of toxic substance in the entire irradiated organism reaches some threshold, or critical, value.[1] (This should occur within the time from zero to 3 days after conclusion of the next irradiation, if the irradiation is fractionated.) This threshold is different for different animals in virtue of biological variability (in order that 50% of the animals die, it is necessary that a threshold be reached greater than that, for example, for 20%):

$$\Pi = \sum_i \Pi_i = \Pi_{крит}. \qquad (3)$$

In other words, when this threshold is reached, some (still unidentified) vitally important system in the organism is "poisoned" and the animal dies independently of the rate at which the toxic substance is removed from the organism.

It is sufficient for the toxic substance to have these four properties for it to be the material substrate which, in undergoing the effect of reparation processes detected by the method of two-fold irradiation, can provide the exponential character of the decrease of the "residual dose" in time – the injury caused by a sublethal dose of radiation.

Some arguments in favor of the correctness of each of the four properties were presented earlier [15], therefore a different method of proving the correctness of the proposed model is given in this article, since this method was presented very briefly in [15]. We are talking about the experimental confirmation of a very unexpected result of two of the indicated properties. The lack of triviality and the unexpectedness of this result are underlined both by the fact that it concerns only one irradiation with lethal doses, on the one hand, and, on the other hand, by the fact that it establishes a completely determined quantitative connection between equivalent local and total irradiations.

Only properties 1, 2 and 4 are needed to derive this result, since property 3 has significance only for fractionated irradiation. Using Relations (1) and (3) we shall attempt to predict, let us say, the $LD_{50/30}$ (ЛД) after a total irradiation, based on the results of local and "supplementary local" (tnat is, irradiation of only that part of the body which was screened during the local irradiation) irradiations.

For local irradiation the $LD_{50/30}$, for convenience denoted in the future by D_1, must be connected with the weight of the irradiated section in the following way:[2]

$$\eta_1 m_1 D_1 = \Pi^{(50)}_{крит}. \qquad (4)$$

Here η_1 is the specific amount of the toxic substance formed after local irradiation, m_1 is the weight of the irradiated part of the animal's body and $\Pi^{(50)}_{krit}$ is the threshold value for the toxic sub-

[1]/293
[2]/293

stance at which 50% of the irradiated animals die.

For the supplementary local irradiation we have analogously

$$\eta_2 m_2 D_2 = \Pi^{(50)}_{\text{крит}} \tag{5}$$

and, finally, for total irradiation

$$\eta_1 m_1 D_T + \eta_2 m_2 D_T = \Pi^{(50)}_{\text{крит}}. \tag{6}$$

We shall carry out successively in Relations (4)-(6) the following operations:

$$\frac{\eta_1 m_1}{\Pi^{(50)}_{\text{крит}}} = \frac{1}{D_1}; \quad \frac{\eta_2 m_2}{\Pi^{(50)}_{\text{крит}}} = \frac{1}{D_2}; \quad \frac{\eta_1 m_1}{\Pi^{(50)}_{\text{крит}}} + \frac{\eta_2 m_2}{\Pi^{(50)}_{\text{крит}}} = \frac{1}{D_T}.$$

We obtain from the thus altered relations (4)-(6) the following evident relation between D_1, D_d and D_t:

$$\frac{1}{D_T} = \frac{1}{D_1} + \frac{1}{D_2}; \tag{7}$$

Using Relations (4) and (5) it is also possible to predict some results of local and nonuniform total irradiations when segments m_1 and m_d are irradiated not with the same dose D_t (as occurs during uniform total irradiation), but with different doses: $D_1'(0<D_1'<D_1)$ and $D_2'(0<D_2'<D_2)$, respectively (of course, here the nonuniform total irradiation must be of equal effect with the local irradiations, that is, lead to the death, for example, of the same 50% of the animals). Under conditions of such nonuniform total irradiation, as one can easily be persuaded, between the values D_d' and D_d (at known D_1') there must be the following quantitative relation:

$$D_2' = D_2\left(1 - \frac{D_1'}{D_1}\right). \tag{8}$$

Thus, Relation (8) reflects the interchangeability of two different local irradiations, namely: if doses D_1 and D_d each lead separately to the death, let us say, of 50% of the animals, the simultaneous irradiation of two parts of the animal with a dose $D_1' = \frac{1}{2} D_1$ (on segment m_1) and a dose $D_2' = \frac{1}{2} D_2$ (on segment m_d) or nonuniform irradiation in any other combinations ($D_1' = \frac{1}{n} D_1$ and $D_2' = \frac{n-1}{n} D_2$ at $n > 1$) also will lead to the death of the same 50% of the animals. Relation (8) thus emphasizes most graphically the correctness of our interpretation of the factor which possesses the four properties indicated above as a radiotoxic substance.[3]

Thus, Relations (7) and (8) represent the desired relations between the $LD_{50/30}$ after total and local irradiations. This re-

lation, as one can easily be convinced, must be observed not only for doses causing the death of 50% of the animals, but also for any other equally effective doses of irradiation.

Connection Between the $LD_{50/30}$ in Rats After Total and Local Irradiations

Область облучения 1	Вес облученного участка, мг 2	$LD_{50/30}$ 3		Литература 6
		в эксперименте 4	рассчитанная по формуле (7) 5	
7 Тотальное облучение	250	650	668	[16]
8 Надчревье	50	2200	2088	
9 Все тело, кроме надчревья	200	950	923	
7 Тотальное облучение	—	650 (700)	614 (709)	[16, 18]
10 Живот	—	980 (1100)	1088 (1077)	
11 Все тело, кроме живота	—	1700 (2000)	2013 (2444)	[17, 18]
12 Цистеамин перед тотальным облучением	—	900	906	[17]
13 Цистеамин перед облучением живота	—	1480	1284	
14 Цистеамин перед облучением всего тела, кроме живота	—	3010	2880	

1) Area of irradiation
2) Weight of irradiated segment, mg
3) $LD_{50/30}$
4) Experimental
5) Calculated from Formula (7)
6) Reference
7) Total irradiation
8) Epigastrium
9) Entire body except epigastrium
10) Abdomen
11) Entire body except abdomen
12) Cysteamine before total irradiation
13) Cysteamine before irradiation of abdomen
14) Cysteamine before irradiation of entire body except abdomen

Some, unfortunately, scanty experimental facts in favor of the correctness of the result which we derived (7) are presented in the table (there are no experimental data in the literature with respect to (8)].

Although the agreement of the theoretical and experimental results is satisfactory in the cases presented, we should like to obtain additional and more convincing data, from which, in particular, it would be seen that this agreement is not a chance, but completely reproducible phenomenon. It is also desirable to verify Formula (7) at sublethal and alethal doses [(8) at all dose levels] as well as in small segments of local irradiation [in order to determine the limits of applicability of Relation

(1)]. If Formulas (7) and (8) are confirmed under these conditions it will be possible to use the concepts presented above with great confidence both in the attempt to identify the factor which, evidently being a radiotoxic substance, gives rise to processes leading to radiation sickness after local irradiations, as well as to a lethal effect after one and fractionated irradiations, and in seeking new methods of treating radiation sickness.

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Footnotes

289 [1]It is precisely this property which is evidently the only character in which the toxic factors generally differ from any others which also lead to a fatal outcome [either without any production of toxic substances at all or through the production (by acting on various parts of the body) of different, that is, not interchangeable in final effect [see Formulas (7) and (8)] toxic substances].

289 [2]With the assumption that m_1 is sufficiently large so that the inequality $D_1 \leq D_{1,\,krit}$ is observed (see property 1). A similar assumption is made in writing Relation (5).

290 [3]Relation (8) can also be used for identifying this radiotoxic substance among known (from the "Survival Rate" test or from other tests) radiotoxic substances. In this case D_1' and D_1 are the respective doses of the test substance injected into the animal and D_d' and D_d are the doses of ionizing radiation.

291 [4]With a decrease in the area of local irradiation the saturation effect can begin to show up (see property 1). In this case we will obtain decreased values for dose D_1 from Relation (7). To obtain more correct values it is necessary to replace Relation (7) with a more general relation derived by taking into account the possible effect of saturation. The derivation of a more general relation taking into account not only the saturation effect but also the possible existence of several radiotoxic substances, several reparation mechanisms of the "residual dose," etc. is beyond the scope of this article.

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Transliterated Symbols

288 крит = krit = kriticheskiy = critical

289 л = l = lokal'nyy = local

290 д = d = dopolnitel'nyy = supplementary, additional

290 т = t = total'nyy = total, whole-body

THE PARTICIPATION OF RADIOTOXINS IN RADIATION'S REMOTE EFFECT

L.M. Kryukova and L.M. Shmakova

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It was shown earlier [1] that after irradiation of one leaf of a whole plant inhibition of mitoses can be observed in shielded growth points of the plant – the stem and roots. Removal of the irradiated leaf immediately after irradiation eliminated the inhibition of mitoses which made it possible to assume the production of radiotoxins [RT] (PT) in the irradiated leaf with their subsequent migration throughout the entire plant [2].

Fig. 1. General appearance of sunflower plants. Left) Experimental plant; middle) upper bud removed without radiation; extreme right) control.

Subsequently, significant morphological changes were observed [3] in the secondarily developing axillary buds after local irradiation of the scar of the removed upper bud.

In the present investigation we repeated these experiments and attempted to detect the appearance of RT in the shielded leaves after local irradiation of the scar of the removed upper bud. The experiments were carried out on sunflowers of the "Pere-

dovik" variety of the 1963 Elite harvest. On the 40th day after the seeds were sown the upper bud was removed from the plants and the scar from this bud was irradiated with x-rays in an RUP-1b/f apparatus with a dose of 25 kr at a tube voltage of 210 v and current strength of 15 ma. All the rest of the plant was shielded with lead. Five plants were used in each variant of the experiment. The typical result of one series of experiments is presented in Fig. 1. It is seen from the figure that in plants in which the upper bud was removed (plant in the middle of the picture) the lower buds developed normally. At the same time, in experimental plants in which the upper bud was removed and the scar from this bud irradiated, deformed flowers developed from the lower buds (plant at the extreme left). The change in the flowers and leaves which occurred from the effect of RT is clearly seen in Fig. 2.

Fig. 2. Change in leaves and flowers from the effect of radiotoxins overflowing from the irradiated area (local irradiation of scar from removed upper bud).

To demonstrate the appearance of RT in these changed leaves from the effect of local irradiation, an extract was prepared from the leaves of experimental and control plants according to a previously described method [2]. The biological activity of the extracts was determined from the vitality of Ehrlich's asciten cancer cells according to a method described by Ayzenman et al. [4].

The data from these investigations are presented in the table.

Effect of Extracts of Control and Experimental Sunflower Plants on the Vitality of Ehrlich's Ascites Cancer Cells (Death of Cells from the Effect of Extracts of the Experimental Plants is Taken as 100%)

Повторность 1	2 Гибель клеток, %	
	контроль (физиологический раствор) 3	под влиянием экстрактов из контрольных растений 4
1	17,6	45,2
2	17,3	41,3
3	26,8	41,0
4	22,6	42,1
5	24,2	44,0
6	29,1	50,0
5 Среднее . .	22,9±2,3	43,9±1,4

1) Repetition
2) Death of cells, %
3) Control (physiological solution)
4) From the effect of extracts of control plants
5) Average

As seen from these experiments, biologically active substances – radiotoxins which kill ascites cancer cells, are actually present in the shielded sunflower leaves which underwent deformation from the effect of irradiation of a distantly located part of the plant.

The investigations which were carried out confirm our hypothesis that the remote effect, manifested in the form of morphological deviations, is caused by RT flowing into the unirradiated parts of the plant from locally irradiated sites. It is likely that in radiation's direct effect no small role in the observed disturbance in morphogenesis belongs to the RT produced in the irradiated tissues.

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DISCUSSION

TARUSOV, B.N.

Very interesting controversies have developed here today on the question of the role of toxic agents in the primary mechanisms of radiation lesion.

A great deal of material in this connection has been presented by A.M. Kuzin's laboratory, which demonstrates on the basis of experiments chiefly on plants that the principal toxic products of endogenic origin in radiation lesion are semiquinones.

Yu.B. Kudryashov and his colleagues have presented a vast amount of material on animals in which they show that the principal toxic substances in radiation sickness are products of biolipid oxidation – fatty acids and their peroxides.

It seems to me that there are no great fundamental contradictions in these two directions.

Ionizing radiation is a very strong physical agent, however, it does not always lead to the development of reactions of the autocatalytic type with high quantum yields which can only cause the observed phenomena of the lesion. The possibility of the development of reactions of this type is determined by the reactivity of the substrate. We have long paid attention to the fact that reactions induced by ionizing emissions in lipid fractions of cell cultures satisfy this condition. The fact that chain oxidation of lipids, from which the principal cellular structures with enzyme systems located on them are formed, occurs in the initial act evidently does not now evoke doubts. Destruction of the lipid base by oxidation disorganizes their coordination. Bacj and Alexander, for example, are now examining the system of the primary lesion in precisely this aspect.

In speaking of this general system, we must take into account that this oxidation reaction takes place in a complex environment of various lipid and liposoluble substances containing oleates, lipovitamins, flavins, etc. Therefore, the reaction is very complicated in nature. Its autoaccelerating nature indicates that it must take place in stages with the production of active intermediate products. These active intermediate products arise and multiply in each chemical act and, acting on intact molecules of the biosubstrate, caus in them (initiate) chemical conversions of the same nature. These active intermediate radicals and peroxides are the primary toxic products. Being accumulated in the initial act of the effect of radiant energy, they can provide for the persistent development of the process.

The data presented by Yu.B. Kudryashov showed quite convincingly in kinetic experiments that the lipid oxidation products which he obtained are such intermediate agents of the chain oxidation of lipids, capable of inducing reactions of this type in the structural biolipids of cells. Along with this, the work of A.M. Kuzin's school, who in operating with other characteristics found toxic substances of the semiquinone type, which in their experiments were capable of initiating oxidation reactions of this type in biolipids, as their joint experiments with Kudryashov showed, appears convincing.

It is impossible to see a contradiction in this. If the same reaction produces two products, then they both participate in one process. It is necessary only to find their place and sequence. Moreover, one must take into account that the concept "semiquinone" has now become very broad and those substances about which A.M. Kuzin is talking are very close in their chemical properties to fatty acids and their peroxide radicals. In any case, it can now be said that in the complex primary reaction which develops from the effect of radiant energy and strikes at the weakest reaction link – the lipid complex, a study of the toxic substances (active intermediate compounds) is very urgent. There are many of these products and we still know very little about them, and after all these are the principal activators of the principal radiochemical reactions in the biosubstrates.

I cannot help but mention one further promising point. This is the demonstration of the mechanism of DNA (ДНК) injury by radiant energy. From the viewpoint of the direct effect of radiation, the damage to this system is inexplicable in connection with the fact that, as a study of radiation reaction activity shows, the DNA system is many times more resistant than lipids. The question arises of whether the active intermediate products which developed during the reaction – toxic substances – will have sufficient energy to initiate oxidative conversions in the DNA molecule. Investigations, which have been reported on here, concerning the fact that the toxic substances produced in the lipids during irradiation can have a genetic effect, are of great interest.

SHTUKKENBERG, Yu.M.

It is extremely tempting to raise the question of the existence and nature of the primary toxins since in this case the entire problem of radiation injury and protection is extremely simplified. However, it is clear that whatever important role individual primary toxins play in radiation lesion, they alone cannot wholly explain the entire complex set of disturbances caused by the action of radiation, since in an unirradiated organism primary toxins begin to act against a background of a normally functioning organism and in an irradiated organism – against a background in which metabolic processes and the functions of all the organism's systems are disturbed to one or another degree. Therefore, the effect of toxins alone in an unirradiated organism cannot cause biological effects which are strictly identical both in a qualitative and a quantitative respect.

It was quite convincingly shown in the group of reports by

Yu.B. Kudryashov and colleagues and A.M. Kuzin and coworkers that at least two classes of compounds exist: lipid toxins and quinones, which are radiomimetics and whose effect on an unirradiated organism causes a whole series of disturbances which are observed during radiation sickness. Therefore, the very raising of the question of primary toxins is quite well-founded and the study of this question is promising both in theoretical and practical respects.

It would be extremely important for further study of this problem to do the following:

1. Compare the concentrations injected into an unirradiated organism with the concentrations of the same toxins produced in the organism after irradiation at which the same or similar biological effects are obtained, taking into consideration the laws of the elimination, distribution and conversion of individual toxins. (It would also be interesting to compare the toxin concentrations when injected into animals irradiated with different doses.) Of course, the concentrations in the first case must be large, but the closeness of these concentrations would be one important and direct proof of the determining role of the primary toxins in radiation sickness.

2. Study the dynamics of the accumulation and conversion of individual primary toxins formed in an irradiated organism, since if the hypothesis of the primacy of the toxins is correct, the time relations of the concentration of such toxins should be similar in irradiated and unirradiated organisms with the same distribution of the toxins in the organism. In connection with this, it is also of interest to study the time-concentration relations of the primary toxins in individual organs and tissues after local irradiations and to connect them with the radiosensitivity of the organism's organs, tissues and systems. It follows from what has been stated that a study of the quantitative kinetic principles, the conversions of the primary toxins, their location after general and total irradiation, as well as a comparative study of the kinetics of the behavior of the primary toxins in irradiated and unirradiated organisms is an important task for future research.

As we have shown with the use of a tritium marker and later with heavy oxygen, periodic metabolic processes occur in the organism which are unequivocally connected with the periodicity of the onset of exacerbations during radiation lesions and the periodicity of the death of irradiated animals; a hypothesis has been introduced that these effects are caused by a periodic change in the concentration of radiotoxins in the irradiated organism. I have given the kinetic principles which explain the periodicity in metabolic processes and the periodicity in the change in the concentration of compounds formed as a result of consecutive metabolic reactions. If radiation lesions and the death of animals are caused chiefly by the effect of lipid toxins and quinones, there must be a correlation between the phase changes in the concentrations of the indicated toxins in the organism and the phase nature of the exacerbation of radiation lesions and the "peaks in the mortality" of irradiated animals.

In the report by Kuzin, Kudryashov, Lebedeva, Baltbardzys and Bilushi some curves of the change in toxin concentration with time having a phase nature were demonstrated. Therefore, a comparison of the kinetic principles of the change in primary toxin concentration in an irradiated organism with the phase nature of the metabolic processes and the phase nature of the course of radiation lesions and mortality is extremely important; the existence of a clear correlation between the indicated phenomena would be one further proof of the important role of the primary toxins in the development and course of radiation lesions and would help to disclose the inner mechanisms lying at the basis of the phase nature of radiation sickness.

On the whole, this conference has shown that further study of the chemical nature and mechanisms of action of the primary toxins and the kinetics of their formation and conversions is an urgent scientific problem of great practical importance, since the solution of these problems will make it possible to give a quantitative evaluation of the role of individual primary toxins in radiation lesions, to intervene actively in processes of the onset and development of radiation lesions and to develop methods of preventing radiation lesions.

SHAL'NOV, M.I.

In the concept of primary radiotoxins great importance is attached to chemically active products which are formed as a result of multiple defects. Therefore, the question naturally arises of the role of these defects together with primary injuries of unique cell structures in the triggering mechanisms of the radiobiological effect.

The radiation-chemical yields from the dissociation of biologically important substances of different natures which have been measured at the present time do not allow one to speak of the primary radiosensitivity of any of them. The ion yields rarely exceed 1 molecule and this means that 1 out of 1000 or 1 out of 10,000 molecules is damaged. Therefore, it is more difficult to connect the high biological effectiveness of radiation with the injury of multiple structures than with the injury of unique structures. This can be explained by a naive, but convincing calculation.

Let us imagine a DNA (ДНК) molecule under whose genetic control a large amount of different protein enzymes is synthesized. The synthesis of each enzyme is controlled by its own gene. Several genes operate simultaneously in the cell – the synthesis of the appropriate number of enzymes takes place. At an ion yield equal to one it is possible to evaluate which is more important – the injury of one molecule of each of the enzymes or of one base on one gene segment of DNA which controls the output of one enzyme.

It is quite clear that primary injuries of multiple structures at the exact information center are reversible and can remain unnoticed. The destroyed molecules will be replaced by newly synthesized molecules. On the other hand, in the case of local

DNA injury (the reaction unit is 1 muton) the synthesis of a normal enzyme can cease and the synthesis of an abnormal one can begin. In other words, local injury of DNA can be intensified during metabolism (biosynthesis) up to a general biological occurrence. This can also be of significance for radiotoxin production. After all, besides toxins which are formed directly in the process of radiolysis and as the result of enzyme activation (liberation from complexes where they are inhibited), radiotoxins can also be produced as a result of a "crisis of overproduction" of enzymes from injury of the cell's gene control. In particular, the overproduction of phenyloxidase can entail an increase above normal in the concentration of substances of quinoid nature, as was noted in a number of papers.

Chain reactions in a defective biosubstrate or multistage consecutive reactions which are transferred from substrate to substrate can be an independent means of increasing radiotoxin concentration. It is difficult to determine at present the initially injured substrate since none of the radiotoxins has been identified. Free amino acids and proteins can be the source of substances of quinoid nature; lipids and carbohydrates can be the source of peroxides, aldehydes and ketones. Altered precursors of the nucleic acids may also make an appreciable contribution as possible radiotoxins.

The unique structures, in particular, DNA, may be the most likely object of attack. It is impossible to believe that a protein casing always insures DNA from attack by radicals. In the post-telophase of the cell and during DNA synthesis, part of the DNA is evidently liberated from all proteins up to histone and becomes available for reactions of any type. The maximum radiosensitivity of DNA biosynthesis falls in just these periods. DNA synthesis (through negative feedback) is inhibited by the abnormal precursors which intrude into the cell in this period. The cytogenetic effect is also associated with the development of latent damage sustained by DNA in these periods.

Radiotoxins can promote the development of latent injuries. The cytogenetic effect of oxidation products of oleic acid which was spoken about in the report by Labzina, Kudryashov and Luchnik seems to be connected with their effect on injured DNA in the G_1- and S-periods. It should be noted that the depolymerization of DNA which evidently lies at the basis of the structural chromosome rearrangements is characterized by post-radiation development. Labile phosphate esters are produced in a DNA chain, irradiated in oxygen or without it, which are decomposed only in the presence of oxygen or oxidizers. Consequently, any oxidizable radiotoxin can act as a developer of potential injuries in DNA which are recorded at the chromosome level in the period of mitosis.

KUZIN, A.M.

M.I. Shal'nov is quite correct when he speaks about the great biological effectiveness of the injury of a unique gene structure (muton) which gives information for the synthesis of one or another enzyme. It is precisely for this reason that we attach such significance to work which showed ease of joining

o-quinones to DNP (ДНП), DNA and histones. However, M.I. Shal'nov makes a mistake, which has been repeated many times before him, in speaking of the ineffectiveness of the damage of multiple structures. The mistake lies in the fact that it is assumed without foundation that the injury of a multiple structure leads to its disappearance. But since these structures are multiple, the conclusion is drawn that the ones that disappeared will be replaced by those which remain or by newly synthesized structures and, thus, the cell will not undergo damage. Actually, the injury, for example, of such a multiple structure as the mitochondrum does not lead to its disappearance, but to a disturbance in its functions, to a change in the coordination of its oxidative processes, as a result of which other substances (abnormal metabolites, radiotoxins) begin to appear in the cell. Whether or not there are other uninjured mitochondria in the cell has no significance for the radiotoxin production by the injured structure. And as a result of the high rate of enzymatic processes the injury of even one mitochondria can lead to the production of huge numbers of radiotoxin molecules in the cell in a short time after irradiation. Its effect on unique nuclear structures leads to further deepening and development of the radiation lesion of the cell.

BRESLAVETS, L.P.

The considerable, almost vast literature on the effect of ionizing emissions on organisms both with the purpose of obtaining mutations and with the purpose of changing the course of growth and development shows the importance which is attached to this factor in the national economy.

However, in spite of the attempts of many authors to give a correct explanation of the mechanism of its action, they have not led to the desired goal or, more correctly, they have had definite significance only in individual cases and for individual objects. A.M. Kuzin's hypothesis concerning the production of special substances in irradiated plant cells, which has been confirmed by experiments, is a step forward. These substances, isolated from irradiated plant tissues, inhibit the division of the cell nucleus, as a result of which retardation or even death of germinants and adult plants is observed, cause anomalies in their morphology or change the course of metabolism. In a word, all the vital processes in the plants are affected. These substances received the name of radiotoxins in the first stages of the investigation.

It seems to us that the term "radiotoxin" is not very apt. Substances which not only injure cell structures but also stimulate vital processes in the cell appear during irradiation. It has already been shown that the so-called radiotoxins in small amounts at first cause stimulation of development, and with an increase in their concentration lead to inhibition of development and death of the plants. All those who attach great importance to the use of ionising emission for increasing productivity are interested in determining the chemical nature of these substances. The name "radiotoxins" narrows this question. Perhaps the name "anomalous metabolites" is also not very apt, but it transmits the very essence of the matter better.

KONOPLYANNIKOV, A.G.

I have several remarks on V.I. Suslikov's report.

The formula

$$\frac{1}{D_T} = \frac{1}{D_L} + \frac{1}{D_A} \qquad (1)$$

can be obtained without resorting to the concept of radiotoxins if it is assumed that the semilethal dose during local irradiation is inversely proportional to the body mass, that is

$$\left.\begin{aligned} D_L &= \frac{D_T}{m_L}, \\ D_A &= \frac{D_T}{m_A}, \\ m_L + m_A &= 1. \end{aligned}\right\} \qquad (2)$$

It is possible to obtain Formula (1) by simple transformations of Expressions (2). This relation will undoubtedly correspond well to the experimental data only in the case in which the irradiated sections of the body contain in approximately equivalent amounts radiosensitive organs responsible for the survival of the animals (primarily the hemopoietic organs and intestines), as occurred in all three cases used for the calculation. The constants η_l and η_d for these cases will differ little from 1.0. In those cases in which areas of the body which differ sharply in their significance for the animals' survival undergo irradiation or when these constants must differ from 1.0, Formula (1) will describe very approximately the relation of doses of general, local and supplementary irradiations. Such situations are possible in local irradiation of sections of the brain, esophagus, intestines, tubular bones and others, when entirely different formulas of radiation injury will be observed. In analyzing the original premises of V.I. Suslikov's calculations, it is also necessary to point out that it is not generally obligatory that the value Π mean the toxin level, but it will be more well-founded to invest in it the concept of the magnitude of the damage to organs which determine the animal's survival. The functional relation of Π and the irradiation dose evidently is also very approximately described by a linear function. As already indicated above, the hypothesis that the value Π_{krit} is a constant during the irradiation of different sections of the body is fundamentally wrong.

The author's hypotheses concerning the nature of the exponential character of the regeneration of sublethal radiation injuries are also very doubtful. In this case such a relation is connected not with the dynamics of the change in the level of the hypothetical toxins, but with the character of the repopulations of damaged radiosensitive organs, primarily of the bone marrow. Such explanations are confirmed by one curious phenomenon which is observed after sublethal irradiation of animals - the phenomenon of "increased radioresistance" which appears approximately

10-15 days after the first nonlethal irradiation. In studying the dynamics of bone marrow regeneration in mice it was shown that in this time it is as if "hyperfunction of the bone marrow" is observed which is expressed in the fact that the number of cells in the bone marrow of the animals which is sharply decreased after irradiation again reaches the values of the control and then reliably exceeds them. A description of this phenomenon in terms of the toxin hypothesis is impossible.

The creation of a mathematical model for describing the results of fractionated irradiation of animals in sublethal doses is possible only after a complete reexamination of the author's original premises – in switching from hypothetical toxins to actually existing cells.

SHTUKKENBERG, Yu.M.

It should be noted that the relation between the irradiation doses of two sections of the body which are complementary in mass, D_1 and D_2, and of the whole body D which cause the same effect of acute injury, obtained by V.I. Suslikov from too formal and simplified considerations, is not justified in a number of cases. In order to obtain the relation $1/D = 1/D_1 + 1/D_2$ it is sufficient to assume that the biological effects (amount of toxins formed in the organism) are proportional to the mass of the irradiated tissue. In this case the radiosensitivity of individual organs and tissues and their role in the functions of the complete organism is not taken into consideration. The indicated equality is approximately achieved when relatively large supplementary sections of the tissue are irradiated. In the opposite case this equality is wrong. When almost all of the organism is irradiated and a very small section of the tissue containing bone marrow (for example, a small section of the skin) is protected, a 50% lethal dose works well, while the dose D_2 which a small section of the tissue can obtain can be very large and not cause the same effect as irradiation of the whole organism. In this case, according to the data in V.I. Suslikov's report, the relation is obtained that $1/D = 1/2D$ (the member $1/D_2$ is relatively small), that is, the equality is not observed.

SUSLIKOV, V.I.

If by factor n, as A.G. Konoplyannikov suggest, the number of injured cells of the bone marrow or intestine is meant, great difficulties arise in explaining the exponential character of damage reparation in terms of the "residual dose." We have previously examined these difficulties in more detail [15].

The increased radioresistance which sets in after the first irradiation and is demonstrable by the method of repeated irradiations which is connected, according to the generally accepted opinion, with the excessive repopulation of cells of the hemopoietic system, can therefore be considered within the framework of the concepts which we are developing as due to the presence of reparation processes which differ in nature from those which cause the exponential character of the change in the "residual dose" with time.

The postulates suggested by A.G. Konoplyannikov, from which Relation (7) can actually be derived, are a particular case of Relations (4)-(6) in the case of the same intensity of production of the toxic substance in different sections of the irradiated animal. If the intensity of radiotoxic substance production is different in different parts of the irradiated animal, the postulates suggested by A.G. Konoplyannikov will be internally contradictory since the values of one and the same quantity, for example, D_1 obtained from Relation (7) from known experimental values for the quantities D_d and D_t (see "epigastrium" in table) and obtained from his second postulate from the quantities D_t and m_1 will be different. Actually, from Relation (7) the quantity D_1 must equal 2058 r, and from the postulate $D_1 = D_t/m_1$, this quantity must be different, namely 3250 r. The experimental value 2200 r is much closer to 2058 r than to 3250 r and this is an argument in favor of our assumption of different intensities of radiotoxin production in different parts of an irradiated animal.

With regard to Yu.M. Shtukkenberg's last remark, I should like to draw attention to the fact that in considering the "saturation" effect (see note' on page 293), the difference between the experimental value of D_1 and that predicted from Relation (7) will decrease and, perhaps, will become completely insignificant.

KUDRYASHOV, Yu.B.

It has been shown in a series of papers by our group that the radiation toxic effect is the result of the multiple effect of various radiotoxins. The dynamics of the production of each of the components of this group is unique and depends on many things: the irradiation conditions, the characteristics of the biological specimen, the specifics of the mechanism of production of the toxic substance and others.

Deciphering of the toxic radiation effect as a whole and its elements individually is of great interest from the viewpoint of the diagnosis and treatment of radiation sickness. Besides the undoubted importance of the study of one or another radiotoxin, the necessity of determining the primary, leading link in the development of the toxic radiation effect has come to a head. Our work has basically been devoted to this problem. Evidently, we have succeeded in separating out this link, which is the primary production of oxidized products of unsaturated fatty acids which have a wide range of action on various biological specimens and systems and cause the production of other radiotoxins. Our work has experimentally confirmed B.N. Tarusov's hypothesis concerning the participating of unsaturated fatty acids in the primary reactions of radiation sickness.

The reports presented by the group from A.N. Kusin's laboratory are very interesting. A central place in this work is given to quinone production. The principal point of application of the quinones is their effect on unique structures of the cell nucleus, on DNA synthesis and on cell division.

The quinones, as the work of A.M. Kuzin and his colleagues showed, are formed as a result of the intensification of oxidative reactions in the irradiated organism.

Thus, the primary mechanism of radiation sickness and consequently, the leading link in the toxic radiation effect is the intensification of oxidation reactions of substrates sensitive to them, unsaturated fatty acids which produce "lipid radiotoxins" as well as phenols which are oxidized to toxically active quinones. The great interest manifested in radiobiological literature in the primary reactions of radiation sickness is closely connected with the development of radioprophylactic agents.

B.N. Tarusov's hypothesis concerning the role of chain oxidation reactions and A.M. Kuzin's detailed outline which shows the role and site of the numerous changes in the biochemical processes and their connection with structural disturbances in the cells during the development of the radiation syndrome have great importance for an understanding of the primary and intensifying mechanisms of radiation sickness.

The work of P.D. Gorizontov and his colleagues should also be mentioned. The important role of toxic substances in radiation sickness was first demonstrated most convincingly in this work and the many-sidedness of their action was shown.

At the present time the question of the toxic radiation effect has ceased to be controversial and has been transformed into a problem which occupies the central place in modern radiobiology.

Manuscript Page No.	Transliterated Symbols
303	т = t = total'nyy = total, whole-body
303	л = l = lokal'nyy = local
303	д = d = dopolnitel'nyy = supplementary, additional
303	крит = krit = kriticheskiy = critical

CONCLUDING REMARKS

The large amount of new factual material presented at this conference convincingly demonstrates the production in an irradiated organism of biologically highly active substances capable of causing many radiation effects. Historically, the name "radiotoxin" has been attached to these substances, but one must agree with L.P. Breslavets who indicated the narrowness of this term which does not reflect, in particular, the fact that in small amounts these substances can stimulate a number of vital processes.

It also follows from the material which has been presented that a whole series of these active substances arises in an irradiated organism at different stages of the development of radiation sickness, which does not allow one to speak of one specific "radiotoxin," and leads to the concept of a change in the concentration in the irradiated organism of a number of biologically active substances which form a supposed class of "radiotoxins." Substances which are quite different in chemical nature belong to this class. Here we are talking about such *o*-quinones as oxidized chlorogenic acid, oxidized tyrosine and other not more closely identified low-molecular quinones. Products of unsaturated fatty acid oxidation of peroxide, epoxide or carbonyl nature possess the properties of radiotoxins. There is no doubt that choline and histamine, appearing under the influence of irradiation in anomalous amounts, also have their effect on the development of radiation sickness.

Finally, it has been convincingly shown that at later stages of the development of radiation sickness the phenomenon of toxemia is closely associated with protein decomposition products or with products of abnormal protein synthesis which is probably connected with a disturbance in the transmission of metabolic information from the cytogenetic structures of the cells.

However, this set of "radiotoxins" can easily be classified into at least three groups.

Such substances as choline and histamine might belong to toxins of low specificity for radiation. They have a very small radiomimetic spectrum, explaining by their appearance only a few characteristics of the course of radiation sickness.

Radiotoxins of protein nature should be placed in the second group. They appear at the climax of radiation sickness as a result of profound primary changes in nucleoprotein metabolism. An exceptionally important role in the outcome of radiation sickness clearly belongs to them. Knowledge of their properties, the causes of their production and the dynamics of their accumulation are

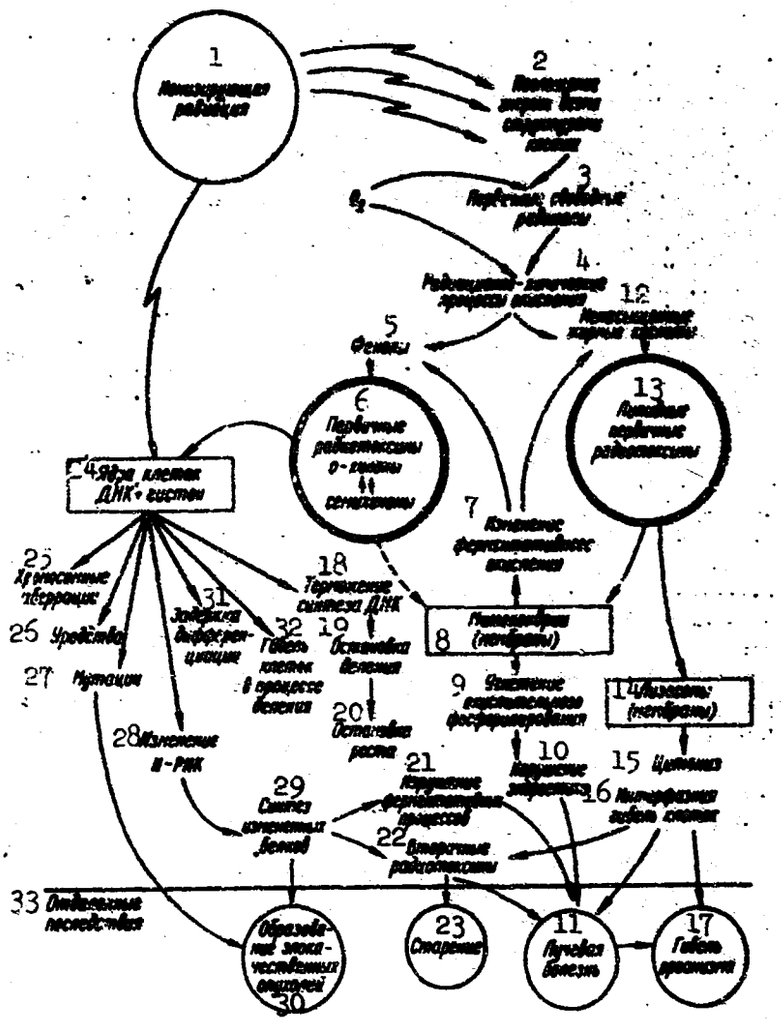
extremely important for the treatment of radiation sickness. However, their appearance undoubtedly is of secondary origin. They are not found immediately after irradiation and it is doubtful whether they can provide an understanding of the primary mechanisms of radiation lesion. The primary processes themselves which lead to their appearance require understanding. It seems to us that the name "secondary radiotoxins" should be given them for greater clarity of the processes occurring in the irradiated organism.

Finally, the biologically active substances about which the most information was given at our conference, belonging to the classes of the *o*-quinones and products of unsaturated fatty acid oxidation must be placed in the third group of radiotoxins. Their production immediately after irradiation, a regular increase with time and, what is especially important, a broad spectrum of radiomimetic properties are characteristics of precisely these substances. These substances should be called "primary radiotoxins" since their production is due to purely radiochemical oxidation processes directly at the moment of irradiation. A special feature of these substances is the post-radiation increase in their concentration through further chain and enzymatic oxidation reactions of the precursors which are always present in the cells.

Evidently, only the "primary radiotoxins" can be considered as substances which cause further development of events in an irradiated cell. It is interesting to note that both products of phenol oxidation (*o*-quinones and free radical semiquinones) and products of unsaturated fatty acid oxidation (peroxides and epoxides) have groups of atoms close in chemical nature and are strong oxidizers.

This explains a number of similar properties of the radiotoxins, for example, the ability to inhibit cell division, to cause cell death, to imitate radiation lesion in animals (drop in weight and number of lymphocytes) and others.

A particularly important property of the *o*-quinones is their indicated capacity to form nucleophilic compounds with nucleoproteins of the cell nucleus. It is precisely thanks to this property that considerable damage to unique cytogenetic cell structures occurs, through which the properties of these substances as typical radiomimetics are manifested. It is well known that the DNA of the cell can be damaged by a direct hit of an ionizing particle on it. However, this is a rate phenomenon and at doses causing the death of mammals it occurs in single cells and it is doubtful whether it can explain, for example, the cessation of division or the mass death of the cells of a number of radiosensitive tissues. At the same time, the *o*-quinones, thanks to the rapid increase in their amount in the first hours after irradiation, reach the concentration at which they react in the same way with the set of cell nuclei, causing inhibition of DNA synthesis, the formation of chromosomal aberrations, the death of the cells after a few divisions and the production of anomalous proteins through a change in the informational properties of DNA.



1) Ionizing radiation; 2) absorption of light energy by cell structures; 3) primary free radicals; 4) radiochemical oxidation processes; 5) phenols; 6) primary radiotoxins *o*-quinones ⇄ semiquinones; 7) change in enzymatic oxidation; 8) mitochondria (membranes); 9) inhibition of oxidative phosphorylation; 10) disturbance in energetics; 11) radiation sickness; 12) unsaturated fatty acids; 13) lipid primary radiotoxins; 14) lysosomes (membranes); 15) cytolysis; 16) interphase cell death; 17) death of organism; 18) inhibition of DNA synthesis; 19) cessation of division; 20) cessation of growth; 21) disturbance in enzymatic processes; 22) secondary radiotoxins; 23) aging; 24) cell nucleus DNA + histone; 25) chromosomal aberrations; 26) deformities; 27) mutations; 28) change in I-RNA (И-РНК); 29) synthesis of changed proteins; 30) formation of malignant tumors; 31) inhibition of differentiation; 32) death of cells during division; 33) remote consequences.

If a leading role in the cytogenetic effects of irradiation can be ascribed to the *o*-quinones, perhaps first place in the cytolytic manifestations of the effect of ionizing radiation belongs to the lipid radiotoxins. Their active influence on cell

membranes can lead to mass interphase cell death soon after irradiation and to a change in the functions of the mitochondria (to the suppression of oxidative phosphorylation) which is very closely connected with the death of organisms from a sufficient dose of irradiation.

The role of the radiotoxins in the development of radiation sickness can be represented in the form of the diagram which is presented here.

The principal importance of the quinones in the injury of cytogenetic cell structures and the role of lipid radiotoxins in disturbing the functions of the mitochondria and lysosomes in cytolytic processes are reflected in the diagram.

We attach special importance to the biochemical intensification of processes of radiotoxin production through their primary effect on the membranes of the mitochondria and distortion of the course of enzymatic oxidation processes in these structures. Perhaps the explanation of the experimentally demonstrated appearance of the quinones from the effect of the injection of lipid toxins into the organism lies here. The primary physicochemical processes arising in the cell biostructures after the absorption of energy are not considered in the suggested diagram since they are beyond the scope of the interests of this collection and were already discussed in our earlier publications.

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