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TRANSLATION

PRIMARY PROCESSES OF RADIATION LESION

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Introduction

Ionizing radiations (X- and γ-rays, electrons, neutrons, and heavy nuclear particles) possess high chemical activity; they are capable of breaking any chemical bonds and inducing prolonged reactions. The biological activity of these radiations involves their chemical activity. Initial reactions play a large role in the biological activity of nuclear radiations. A characteristic of the biological activity of ionizing radiations is the high radiosensitivity of the biological objects. Upon the interaction of the radiant-energy quanta with the biosubstrate, active centers of radiochemical processes are born in the process of converting this energy into chemical energy.

As a result of the development of aftereffect reactions, the volume of chemical changes in the organisms after irradiation is much greater than those chemical effects which are observed immediately upon irradiation. Therefore, in the pathogenesis of radiation lesion the determining reactions are the primary ones which develop with high ion yields and which involve in the chemical conversions hundreds and thousands of molecules not immediately affected by the radiation, and by this they significantly increase the total volume of destruction in the cells of the organisms.

The primary radiochemical reactions in the cells, developing relatively slowly, destroy the structural elements of the cells. And when the volume of destruction becomes signigicant, the correlation of the exchange reactions and the dynamic equilibrium is destroyed and, as a consequence, the picture of radiation lesion develops.

The primary radiochemical reactions developing time with self-acceleration have the same significance in radiation pathogenesis

as do the first stages of development of bacteria and viruses infectious diseases during the incubation period.

Interest in the study of the mechanisms of the primary reactions is explained by the fact that, knowing this mechanism, we can block the reactions by various inhibitors and thus localize the radiation effect.

The principal event of the interaction of nuclear radiations with the substrates is ionization, during which the electrons separate from the atoms, ions form, and, in addition, excited atoms develop and radicals appear. These active molecules and fragments of molecules induce various reactions in the biosubstrates of organisms.

The principal difference between ionizing radiations and other active radiations, particularly chemically active ultraviolet radiation, is that the UV quanta, causing only atom excitation, does not cause ionization in the biological substrates. The excited atoms and molecules also possess chemical activity. In most cases both UV and ionizing radiations cause identical chemical changes. However, the energy of the excited atoms is not sufficient, for the development of certain reactions: this explains the qualitative differences in the development of the primary reactions under the effect of UV and ionizing radiations.

In a number of investigations it was established that UV damage to bacterial cells can be completely removed with subsequent irradiation by the visible region of the spectrum. Under the effect of ionizing radiations recovery in the light does not occur.

Radiation injury of the organism is the destruction of the coordination of biochemical processes and the selective injury of certain systems. Here it should be pointed out that in the primary

event the biochemical components of the cells cannot selectively absorb the ionizing radiations since this process depends not on the molecular structure but only on the atomic number of the elements making up the molecules. In biochemical compounds, however, these indicators are very close. Therefore, the sensitivity of the effect depends on the chemical peculiarities of the primary radiochemical reactions occurring in the different substrates of the cells, the rate of the ionic yield, etc. The appearance of new active ions and radicals in the primary event can not only induce new radiochemical reactions unnatural to the organism, but even change the rate of the reactions occurring naturally in the various components of the intermediary metabolism. The change of this rate disrupts the coordination of the reactions, destroys the stationary equilibria which exist in the cells and shifts them to another level, unusual for the organism, and even causes an unsteady development of the metabolic reactions. All this can lead not only to an immediate destruction of the coordination in the intermediary metabolism, but also to an accumulation of toxic products in the organism.

Under the influence of the ionizing radiations in the biological substrates various reactions occur; their significance, for the development of radiation lesion, however, is different. Some of them die out quickly, others are limited only to conversions in one molecule for one ionization event. Only the active centers, producing autocatalytic-type reactions during which thousands of molecules are involved in the process and which by their nature should develop with selfacceleration, have foremost value in raidation lesion. Competition arises between these reactions for the maximum harmful effect. Not one but several such reactions occur in the cells of the organism.

Although in general all nuclear radiations produce the same ionization effect in the substrates, various types of radiations, have different effects. This is explained by the fact that for different ionization densities the relations between the number of radicals and their local densities are changed somewhat, in connection with which changes occur in the relation of the rates of the primary radiochemical reactions (change of their yields), and as a result of this the value of a certain primary reaction in radiation sickness is increased or decreased and qualitative differences occur.

The active radicals forming during irradiation possess very high chemical activity and they react with any molecules capable of being oxidized or reduced. Their presence, however, still is no proof that they can induce the appearance and development of primary reactions, since along with the chemical reaction there is recombination of the radicals into elementary neutral molecules. If there are no chemically active molecules in the medium or if they are not able to react within a short period of time, chemical changes and formation of reactions will not occur. For instance, pure carbon dioxide does not change under the effect of radiations, since the radicals CO and O which form during irradiation immediately recombine. If to the carbon dioxide we add mercury vapor, for instance, it reacts with the oxygen ions formed during their radiation; as a result, mercuric oxide and an oxide are formed. In the biosubstrates there are also more reactive and less reactive components and, naturally, the primary reactions emerge sooner in the former.

Explanation of the nature and mechanisms of the primary radiochemical reaction in the cells of organisms is not a simple task. The elementary volume of primary radiochemical reactions with ordinary

biologically effective doses is small, and therefore it is very difficult to detect the primary products which have formed, since this is outside the limits of sensitivity of normal chemical analyses. Many researchers have attempted to by-pass this difficulty by studying biochemical changes during irradiation with force doses. During this, however, there is acceleration of the appearance of not only the primary reaction but secondary reactions as well and since the experimental time is reduced it is rather difficult to differentiate them. In an analysis of the mechanisms of the primary reactions the kinetic methods of investigations, and investigations both in vivo and in vitro should be fundamental.

The fundamental criteria for judging the nature of the primary reactions can be:

1) peculiarities of the occurrence of various reactions of the lesion (biological, pathological, biochemical, physicochemical) in time;

2) the possibility of initiating separate reactions of the lesion by products obtained from irradiated organisms or radiochemical reactions;

3) the physicochemical nature of the inhibitors or activators of the radiation lesion and kinetics of their activity;

4) the presence and nature of the active products appearing in the biochemical components of the organisms (radicals, peroxides);

5) the principal possibility for the emergence of reactions with high quantum yields in the given biochemical substrate and its type;

6) the possibility of obtaining a reaction on simple and complicated models applicable to conditions in the cells;

7) the quantitative dependences of the occurrence of the reactions for the lesion on the dose, irradiation regime, and ionization density; and

8) direct information on the occurrence of the given reactions in living cells of the organisms with respect to optical, electric, and other indicators.

The radiosensitivity of the organisms varies greatly. There are organisms which perish from very large doses (10^6r); in the majority of cases, however, these doses are much smaller.

Previously it was considered that the lower organisms are very resistant, since a dose was considered lethal when it caused destruction of the individual immediately under the ray or a very short time after irradiation. When calculating the process, the aftereffects of the dose dropped significantly. The absolute lethal dose for higher animals is 300-900 r, which corresponds to absorbed energy of the order of $2\text{-}5 \cdot 10^4$ erg/g. Such an amount of energy can increase the temperature of the tissue by 0.01° in all.

Such radiation doses cause very insignificant chemical effects if only normal reactions occur, not autocatalytic and chain reactions. It was calculated that under the most optimum conditions (recombination does not occur) in each cell of the organism with a lethal dose not more than 10^{-9}-10^{-10} chemical bonds are broken. When under the effect of radiation reactions with high quantum yields (autocatalytic and chain) do not develop, during such doses it is impossible to detect the products of the radiochemical reactions, since this is outside the limits of the most sensitive analytical methods. Here it must be taken into consideration that by far not all the absorbed energy in the biological substrates is spent on chemical conversions.

Part of the energy is dispersed and each ionization event by far does not result in decay of the molecules. Therefore, in most cases the number of chemical products should be negligibly slight. At the same time, doses tens and hundreds of times weaker have a noticeable biological effect (genetic, stimulative, etc.).

Such a high radiosensitivity long ago gave grounds for assuming that during the primary interactions of the ionizing radiations with the biological substrates, amplifying mechanisms should exist.

TARGET THEORY

The target theory is the first theory to attempt to explain the disproportion between the amount of energy absorbed by an organism and the biological effect. It is based on the quantitative principles detected during the effect of different doses of ionizing radiation on the population of organisms, causing their destruction and genetic mutation phenomena [1-3].

Numerous investigations conducted on the simplest organisms showed that the functional dependence between the amount of absorbed energy of different ionizing radiations (dose) and the lethal result is expressed by the exponential curve shown in Fig. 1 (Curve 1).

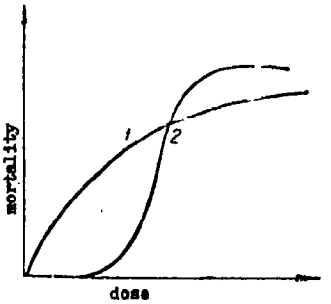

Fig. 1. Mortality of the organisms vs. the irradiation dose (types of destruction curves): 1) one-hit cell; 2) multihit cell.

Data are often cited which characterize the reverse dependence, i.e., the dependence between the amount of surviving organisms and the dose (these data have been during the irradiation, with different doses, of colonies of bacteria, viruses, and mold). Data are also cited which characterize the dependence between the irradiation dose and the amount of genetic mutations appearing. From these data it follows that as the irradiation dose decreases the number injured organisms is reduced. With the smallest doses, however, when several ionization events react on the cell, individual cells are damaged.

Calculations results have shown that certain cells can die as a result of absorbing a negligibly slight amount of radiation energy. In some investigations it was stressed that one ionization event is sufficient to kill a bacterial cell. In a number of cases the curves have another form – sigmoid (see Curve 2 in Fig. 1) and the data obtained for some of the simplest organisms are characteristic of higher organisms. To destroy a cell it is necessary to accumulate a certain amount of energy, and until the energy is accumulated up to a certain limit, the biological effect is not manifested. In this case a threshold is observed on the curves, the curves do not come from the coordinates origin, and several ionization events are needed to destroy the cell. Formal calculations have shown, however, that the number of ionization events occurring in the cells is more than the minimum amount which can cause destruction. Upon irradiation of a great number of the simplest organisms or cells of a higher organism, the radiation quanta evenly intersect the irradiation field and there is the probability that they will get into the cells; this probability increases with the dose. The cell, however, does not always undergo lesion during ionization events. For instance, with 5000-r

irradiation of yeast no less than 1000 ionization events occur in each cell, while only 10% of the number of irradiated cells is destroyed. At the same time certain cells are destroyed as a result of 4-6 ionization events.

To explain the quantitative regularities occurring under the influence of ionizing radiations, the founders of the target theory have made the following assumption. Since the amount of energy of the ionizing radiations producing a biological effect is very small and destruction of the cells in the populations is subordinated to statistical regularities, a large part of the energy of the ionization events is lost in vain and only those few quanta which hit some target have a biological effect. This means that in each cell there is a sensitive volume (target), usually many times less than the volume of the cell; the entire remaining part of it is practically insensitive to radiation. Only those ionizing particles which fall into this sensitive volume and produce its ionization have a harmful effect. Entry into the target is subject to the laws of probability. According to this theory, the sensitive volumes are very diverse in size and shape. This determines the radiosensitivity of the cells. Since it was impossible to explain the different character of the destruction curves by one assumption alone, a second assumption was made, to the effect that these volumes have a different sensitivity to radiation. In certain cases cell damage occurred during one ionization event in the sensitive volume (exponential damage curves); in other cases it occurred during several events. Using this mathematical device (the probability theory) the destruction curves are calculated. Of basic significance here is the Poisson distribution to which the principles of strike (lesion) distribution are subject:

$$y/y_0 = 1 - e^{-\alpha D}\left(1 + \alpha D + \frac{(\alpha D)^2}{2} + \ldots + \frac{(\alpha D)^{n-1}}{n-1}\right),$$

where y/y_0 is the ratio of the number of destroyed cells to the total number of irradiated cells; α is the probability that the given ionization event will produce a lesion; D is the irradiation dose.

The probability of the destruction of organisms and individual cells can be calculated from this formula when the survival curve has an exponential or sigmoid character. In the first case the probability of destruction is expressed by the first term of this formula. The second term of the series expresses the probability of destruction in those cases when two ionization events are required to produce the effect.

The survival and destruction curves can be calculated quite well by an equation of this type. The Poisson equation is the main basis of the target theory. Many researchers have modified this formula by introducing various constants into it and thereby attained better coincidence between experimental and test data.

Better coincidence of theoretical and experimental data is observed when the survival curves start from the origin. This corresponds to those cases when the lesion is caused most likely by one ionization event, although the cell is permeated by many electrons which cause thousands of ionization events in their paths.

As we know, Poisson distribution is asymmetrical, but in many cases it is rather difficult to distinguish it from normal Gauss distribution [4, 5].

In certain papers, however, it has been shown with reasonable accuracy that the destruction distribution is characterized not by the Poisson equation but by the regular symmetric curve of Gauss

distribution. For instance, this has been shown very clearly by G. V. Sumarukov [7] who studied the statistical distribution of the destruction of granary weevils under the influence of various doses of γ-radiation. Crowther [6], who correctly applied the Poisson equation to calculations of lesion curves, also detected no deviations from Gaussian distribution and proposed the use of Poisson distribution, since with a change in the coefficient we can pass from an exponential to a sigmoid curve.

The destruction and survival principles for organisms show that the statistical processes which, in accordance with the target theory, depend only on the entry of quanta into the target, are of important significance. It should be pointed out, however, that this statistical character is not a specific peculiarity of the action of the ionizing radiation. Such curves, however, are obtained under the action on the cell of such factors as, for instance, the temperatures of the various toxic substances (mercuric chloride, phenols); they are the result of general biological variability of the cells in relation to different external effects. Here it was noted that the dependence between the mortality and the concentration of the substance (by the dose) is characterized by an exponential curve under the effect of poisons not possessing a cumulative effect, and by a sigmoid curve when the poison possesses a cumulative effect.

The sole basis for the target theory is the statistical principles for distributing the destruction and other manifestations of the biological effect of radiations. To interpret this effect a formal model for the probability of hitting the target is proposed. Actually, the statistical principles of destruction and the manifestations of mutations indicate only that the determining event on which

the biological effect depends is caused by the statistical principles.

A study of the biological reactions of radiation as a function of time, temperature, and oxygen pressure shows that the lesion processes have kinetic principles of chemical reactions.

In investigations devoted to the applicability of the target theory, the development of time processes and their dependence on external conditions is ignored and the dependence of the effect on the dose is assumed as the basis.

It is well known, however, that the dose is not the absolute magnitude which determines the biological effect. The dose is a time function and its magnitude depends on the period of time after irradiation when the destruction in connection with the presence of the reaction of the aftereffect is calculated.

The determining event occurs in the process of the radiochemical reactions developing with time.

The statistical principles are easily explained by the probability processes during the chemical reactions. Probability processes play a large role in the formation and development of complex reactions with high quantum yields or chain-type reactions. Not every ionization event initiates a reaction, since the ions and radicals which have formed can be destroyed as a result of secondary recombination. Therefore, the occurrence of ionization events does not initiate a reaction in each cell. The possibility of development of a reaction is also a probability process, since the reaction can be stopped and will not go to the end. The probability of the formation and development of reactions can also be described by Poisson distribution.

The geometric shape of the target and its size are hypothetical.

The curves of destruction vs. dose are the basic element for this calculation. In a number of cases we achieved good formal coincidence of the results. However, attempts to associate the quantitative theory with the structural elements actually existing in the cells did not yield positive results [8].

At first, the entire nucleus was considered to be the sensitive element in the cell. But its geometric dimensions proved to be too large, and therefore the nucleoli were taken as the sensitive elements of the cells. After the emergence of the chromosome theory of heredity, the point of view that the chromosomes and the genes included in them are the sensitive centers gained widespread popularity. On the basis of the target theory we attempted to solve the opposite problem, i.e., to calculate the dimensions of the genes as a target from the amount of mutations caused by the ionizing radiations [9]. These calculations, even from the point of view of the chromosome theory of heredity, ceased to be satisfactory since the amount of genes increased as they were investigated and therefore much less space was assigned to them in the chromosomes than that which was determined using the Poisson formula from the radiomutagenic effect. To explain these inconsistencies we attempted to use the mechanism of energy mutation, detected in the crystal system. To harm a gene, a secondary electron need not hit the sensitive volume. Those electrons which strike the zone around the sensitive volume of the gene have a harmful effect; from this zone they can migrate to the gene. From this there emerged a variation of the target theory called the hit theory. This assumption, which emerged in order to reconcile the contradictions of the target theory, is of a purely declarative nature, since it allows a complete arbitrariness

in evaluating the hit field. Here it is necessary to take into account that there are as yet no specific data on the possibility of migration of the energy of ionizing radiations, i.e., on the migration of the ionization event without losses.

Therefore the entire target theory, resting as it were on a quantitative basis when we are dealing with formal statistical regularities, becomes particularly qualitative when the question is raised about the specific cell organoids which are the targets. Taking these limitations into account, the proponents of the target theory [?] concluded that use of the target theory to explain the mechanism of the biological effect should be limited only to viruses, bacteria, and the mutagenic process for which the dependence of destruction on dose is expressed by an exponential curve. During this the large molecules in the chromosomes and the viruses as a whole fulfill the role of the targets.

The chromosome apparatus and the nucleic acids of which they are constructed naturally play an important role in the cells. The assumption, however, that this apparatus should be injured immediately in the initial event is highly doubtful and questionable. It naturally is injured, but the mechanisms of this lesion can be direct. In recent years much material has been gathered indicating that the important systems are damaged indirectly. Chemical protection, the oxygen effect, and influence of temperature and amount of water on the lethal and genetic effects testify to this. Facts testifying to the fact that the important vital structures are damaged by secondary reactions in no way discredit the importance of these systems. For instance, on irradiation of higher organisms, the hemogenic system, not the nervous system, is damaged first, but this

diminishes in no way the role of the nervous system. Nevertheless, many investigators are striving to prove that the elements of the nucleus are more sensitive than other parts of the cells, and they see in this confirmation that the hypothetical target is located there. There are quite a few works in which it is proved that the nucleus is more sensitive than protoplasm to radiation. This conclusion is drawn on the basis of experiments with large cells in which the section of the cell with the nucleus and the sections of unnucleated protoplasm were irradiated separately. These experiments, however, do not reflect the actual relations existing in the cells during general irradiation. If we irradiate the heart of an animal with a stream exceeding the lethal dose and which is absolutely lethal for cells, the organism will die. If we irradiate the extremity of the animal with the same stream or with one many times more powerful, the animal will survive. It dose not follow from this that the cells in the tissue of the extremity are more resistant than the heart cells.

This experiment in no way testifies to greater radiosensitivity of heart cells, but it confirms the importance of the heart as a communication organ in the physiology of the organism. Theoretically an improper experimental set-up leads to the incorrect assumption that protoplasm is completely insensitive to radiation and its damage has no effect on cell necrosis or on other postirradiation sequelae [8,9]. For instance, on the basis of experiments with separate irradiation of spermatozoa and ova and an analysis of the transmission silkworm characteristics, the conclusion was made that the nucleus is 1000 times more radiosensitive than protoplasm [10]. With similar experiments on another silkworm species it was

established that if we irradiate the ovum cytoplasm before fertilization and fertilize it with unirradiated spermatozoa, those genetic features carried by the spermatozoa are disturbed in the progeny, i.e., the implicit effect of irradiated protoplasm is observed [11].

More valid are tests in which in the evaluation of the radiosensitivity, we at first irradiate the nucleus extracted from the protoplasm, then the cytoplasm, and then the nucleus and protoplasm are brought into contact [12]. Upon irradiation, for instance, of isolated nuclei of frog and salamander ova, morphological changes characteristic for damage are demonstrated at doses of 10-15 c. Similar changes in the nucleus on irradiation of cells as a whole are observed at significantly lower doses [13]. When an unirradiated nucleus is introduced into irradiated protoplasm, similar changes occur in the nucleus as when irradiated.

When the nucleus and protoplasm are irradiated separately and the irradiated nucleus is introduced into unirradiated protoplasm, it was established, on the other hand, that doses leading to damage of the cytoplasm and nucleus are of the same order. Inhibition of cell division was observed when only the protoplasm was irradiated with comparatively small doses, and at large doses division stopped completely.

The adherents to the target theory reached a satisfactory agreement of the formally calculated data of the vulnerability for β- and γ-radiations, however, greater difficulties arise when calculating the destruction curves for α-particles even from a formal point of view. The specific ionization generated by α-particles is appreciably higher than its electrons. Hence, the probability of an α-particle hitting the target under an equal ionization dose is much

less than that of electrons induced by γ-radiation and creating essentially the same ionization as α-particles. Therefore, we could expect from the target theory that α-radiation will be less effective than γ- and β-radiation, particularly when death is caused by one ionization event. Actually, we should note the greater efficiency of α-radiation. To understand this from the target-theory point of view, we need assume that the targets for α-, β-, and γ-radiations are different in size.

Attempts were made to strengthen the target theory by certain chemical and physical concepts and to connect the target size with the size of the high-molecular-weight molecules in the cells. And on the basis of these concepts, the method of determining molecular dimensions is proposed. This, apparently, is possible to do, but only when one molecule is inactivated after one ionization event. When after one ionization event several breaks occur in several molecules, these concepts are inapplicable. For instance, as a result of investigating molecular inactivation in protein and enzyme solutions, it was established [14] that the magnitude of the effect depends on the temperature, and the temperature coefficient here is equal to 2-3. The temperature coefficient mainly indicates the course of chemical reaction, and can indicate other mechanisms only when it is proved that a chemical process is not developing which is very unlikely. The authors of these investigations concluded, however, that with increasing temperature the volume of the molecule increases and hence, the probability of a hit increases. This is difficult to understand from the physicochemical molecular point of view.

One of the consequences arising from the target theory, was the assertion that if the target, i.e., the elements of the chromosome

apparatus, is damaged the cell can live or die only when division ensues, since the radiation-induced disturbances in the chromosome apparatus will lead to abnormal division during which the cell will be destroyed [15].

It has been established that cells can die regularly after several divisions. For instance, when Infusoria is X-irradiated with certain doses they die after 3-4 division [16], while the first divisions occurred completely normally. Necrosis of yeast cells after irradiation occurs during the seventy-eighth division [17]. Of course there are doses at which the cell dies during the first division.

Thus, the cause of cell death is not the one-time disruption of the chromosome apparatus of the target and the event of cell division but the occurrence in the cell substrates of primary radiochemical reactions which develop in time after irradiation, and this leads to numerous chemical conversions during which cell division and gemmation is disrupted.

The main disadvantage of the target theory is that it completely ignores the time factor and is satisfied only by the dependence of the biologic effect on the dose. Therefore, the various primary chemical reactions, for which the time factor plays a basic role and allows us to characterize their nature on the basis of kinetic peculiarities, are not taken into account at all.

Much material has been gathered, showing that cell damage is connected with the development of aftereffect processes and that the biologic manifestation of the effect of ionizing radiations and even radiomutation depend on environmental conditions (temperature, water content, metabolism). These factors act both during and after

irradiation. Hence, the development of damages in time is connected with the development of long-lasting reactions with high quantum yields.

The target theory came under attack with the appearance of the theory of indirect action, which also has substantial shortcoming but is widely recognized. Attempts were made to reconcile the target theory with the theory of indirect action, which reduces all radiochemical processes in the cells to the formation of water and hydrogen peroxide radicals. It was hypothesized that water radicals in the target zone diffuse from their formation site hit the sensitive volume on the basis of the law of probability, and cause reactions there similar to those which the ionization events cause. Using the concepts of the target theory the authors of this hypothesis selected formulas determining the probability of knocking radicals from the strike zone into the sensitive volume, starting with the quantity, diffusion velocity, and their life time [15, 18].

Such a construction is very artificial, since, in addition to the arbitrary assumption of the size and shape of the target and zone near it, the values for the diffusion path of the radicals are very arbitrary and not experimentally substantiated. Their life time is short, therefore, we must assume hypothetical intermediate compounds of the radicals which have a longer lifetime. Such a theory has the same deficiencies as the target theory, since the physicochemical reaction is regarded as a separate preliminary state and essentially ignores the physicochemical mechanisms of the primary reactions determining the biological effect of radiation. This is purely a formal construction.

It should be noted that there are errors in the investigations devoted to calculation and analysis of the experimental data. Typical sigmoid and exponential curves are characteristic only for a limited dose range. For a wider range of doses we obtain complicated curves which, from the target theory point of view, are more complicated to interpret. For instance, when studying the effect of x-rays in a very wide dose range on survival it was established that an exponential shape of the curve corresponding to the one-hit type of death is observed only in the zone of action for high doses, whereas in the zone of action for low doses this curve becomes sigmoid which is again incompatible with the target theory [19]. The shape of this curve, it turns out, depends on the time for calculating death. Previously death was taken into account under the beam and the cells which died were counted, using as the criterion their capacity to divide immediately after irradiation. To obtain the exponent, high doses were usually used. If we take into account remote cell death after several divisions, we usually obtain sigmoid curves (e.g., for yeast after six-seven gemmations) [17]. Data are presently available indicating that the type of survival curve depends on the composition of the culture medium. In contrast to the observations which established that the survival curves for haploid races of yeast are always exponential and curves for diploid races are always sigmoid, it was shown that the differences in the curve types are due to different radiosensitivities. If for haploid it is higher, then with average lethal doses the probability of death is higher. When the external conditions are changed, similar exponential curves can be obtained for diploid strains. The action of radiometric substances, which cause the same genetic changes as ionizing radiations [20-22]

poorly agree with the target theory. At the present time the target theory has lost its value, since it cannot explain the basic manifestations in the development of radiation lesion and, especially, cannot interpret the primary radiation effects. From the point of view of this theory we cannot explain the chemical protection of both somatic and genetic lesions. This theory gives no prognoses with respect to the selection of chemical compounds for prevention and cure. It is not able to explain the causes and character for the course of aftereffect reaction which play a most important role in radiation lesion. This theory adds nothing to an understanding of the reduction reaction which is characteristic both for somatic and genetic lesions.

Thus, the lesion, its magnitude (radiosensitivity), are connected with the course of chemical reactions in different cell substrates. To develop these primary reactions the so-called kinetic parameters are characteristic: time, temperature, and conditions for formation of radicals and other active products. These processes clearly demand physicochemical interpretation.

In recent years attempts were made to apply the target-theory concepts about substances determining heredity (nuclear elements) to the cell membrane. In this case it is assumed that in the cell, as in an open system, a steady-state equilibrium exists, during which the amount of substances entering the cell is equal to the amount of substances consumed by it, i.e., entering into some reaction. In this equilibrium the most important role belongs to the cell membrane. On increasing its permeability, the possibility of admitting substances is increased and the equilibrium is put on a higher level, which is unusual for cells. According to this concept, an increase

in permeability occurs when electrons strike hypothetical targets on the surface of hypothetical cell membranes. This is now an ultra-speculative assumption. Even from the formal point of view, in this case the complete arbitrariness reigns in relation to determining target size, since the flow velocity of the substance is indeterminate. In addition, this completely disagrees with the known fact that permeability is a property which changes considerably later in radiation lesions. The bases for such assumptions are not some kind of new facts or methods, but the same statistical principles of death and survival. Any statistical process in vitro, for e.g. variations in yield of products during radiolysis, can be described from the target theory point of view.

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RADIOCHEMICAL PROCESSES IN THE BIOSUBSTRATES

General Principles

Under the effect of ionizing radiations, ionization events occur in biochemical as well as in inorganic components of the cells and tissues, during which atoms and molecules are stimulated along with ions. As a result of these primary events chemical bonds are broken and the active radicals

$$RH \xrightarrow{h\nu} RH^{*} \; R\cdot + H\cdot$$

occur.

In most cases the radicals have very high chemical activity. These fragments of molecules, having an uneven number of unpaired electrons, are characterized by a magnetic moment. They possess a free valence but do not have an electrical charge, which facilitates their entrance into reactions. Because of the high reactivity of the active radicals, their life time is insignificant and inversely proportional to their chemical activity. The radicals by virtue of their chemical activity can initiate the reactions which develop after the direct event of irradiation with high quantum yields.

Not every radical, however, is sufficiently effective to become a center of development for the chemical reaction. Large energy is necessary for this, but not all radicals possess it; in addition a corresponding partner is necessary for the reaction to occur, i.e., neutral molecules which can react with this radical. Such low-activity radicals can exist for a long time in different substrates after irradiation. Their concentration many times exceeds the number of active radicals, therefore it is easier to detect them than the active radicals, for instance, by the paramagnetic resonance method [3]. Such radicals, which are formed in the aqueous phases during irradiation, include the radical HO_2, to which a major role in indiect action is attribtued in radiobiology. This radical reacts only with inorganic compounds and with organic compounds [1].

Induction of oxidizing reactions in air occurs considerably more frequently. In the development of these reactions an important role belongs to peroxide radicals forming in organic substrates, which yield the peroxide compounds [2]

$$R\cdot + O_2 \rightarrow ROO\cdot$$
$$ROO\cdot + RH \rightarrow ROOH + R\cdot$$
$$R\cdot + O_2 \rightarrow ROO\cdot \text{ etc.}$$

The hydroperoxides are particularly important. The peroxide radicals and peroxides joining the organic molecules, form active peroxides of lipids, nucleic acids, pyrimidine bases, etc. These primary products obtained on irradiation are most interesting, since they can induce long-occurring reactions with high ionic yields. These are autocatalytic reactions catalyzed by end products. Considerably more important are the chain reactions whose catalysis is caused by intermediate products. Such reactions can enhance the primary effect of

radiation, which is of low energy, and even with lethal doses touches only a very small portion (10^{-10}) of the chemical bonds of the cells.

For reactions to occur by the chain mechanism, the formation of radicals possessing high energy is necessary, therefore an initial expenditure of energy is required to form them. Under normal conditions at low temperatures the probability is small that such radicals will be formed as a result of thermal collisions and effect of background radiation. On certain substrates this process proceeds slowly under normal conditions owing to weakening of peripheral bonds (effect of alleviation according to the terminology of N. N. Semenov). To realize the reaction, the formed radical must not only be coupled to the molecule of its partner, but must possess energy sufficient to break the bond in order to form a similar radical which would be able to react with another molecule and to bring about the chain process. If the energy of the initial activation in such reactions should be supplied from without, the subsequent development of the reaction and the formation of the intermediate active compounds occurs through the internal energy of the chemical bonds.

The theoretical bases for the formation and development of such reactions, as is known, were developed by N. N. Semenov [2]. Such a reaction, in which a new radical is regenerated for each one lost, could develop for an infinitely long time until the reaction material is exhausted. Such chain reactions, however, usually die out, since the intermediate radicals and peroxides forming during the reaction are, by virture of various contingencies, expended for other purposes (they recombine, perish, reacting with extraneous molecules). As the reaction develops, the number of radicals decreases and the reaction rate slows down, since the chains are breaking down. The

kinetics of development of these reactions proceeds according to an exponential fading curve and suggests the kinetics of development of monomolecular reactions.

Such reactions can unconditionally occur under radiation effects. For instance, long-occurring reactions develop this way for polymerization and depolymerization of nucleic acids in tests in vitro on irradiation in solutions [4-6]. These reactions, however, do not ensure an appreciable enhancing effect, and the general course of the kinetics for their development does not correspond to the development of various manifestations under lethal radiation doses, which develop not with an inhibition but with acceleration. In specific mechanisms, however, such reactions can be valuable.

Much higher ionic yields are obtained in chain-type reactions during which, on reaction of the radical with the substrate, it links up with the molecule and as a result of splitting two or three radicals are regenerated for each radical which reacted. In this case the number of radicals will continually grow and the reaction rate will increase. These are so-called reactions with branching chains. Such reactions develop in time according to an A^x self-acceleration type. Their yield is very high. Explosive reactions, as is known, develop this way. Oxidative reactions exist, however, which develop as typical chain reactions, but considerably more slowly. Examples are the oxidation of raw rubber, cured rubber, lacquers and oils, and hydrocarbons. The peroxide radicals and peroxides play a basic role in accomplishing these reactions. Peroxides play a major role in branching of chains in oxidative branched chain reactions. Hydroperoxides, forming from radicals, can dissociate and once more form radicals. The latter break the hydrogen atoms away from the

oxidizable organic substances and again form radicals R

$$POOH \quad RO\cdot + OH\cdot$$

$$ROOR \quad RO\cdot + RO\cdot$$

Since very little energy is expended when forming radicals, the development of the reaction is facilitated. The development of branched chain reactions is facilitated when the bonds in the peripheral atoms are weak. One of the factors weakening the bonds in compounds is the presence of double bonds. Therefore, the oxidative chain reactions induced by irradiation form most readily on substrates rich in double bonds (hydrocarbons, fats, and rubbers). Such reactions can occur spontaneously on these substrates at temperatures not exceeding 40°C [7]. These reactions generally develop very slowly despite the fact that, based on kinetics they belong to typical branched reactions characteristic of explosive reactions. These reactions are inhibited by steric hindrance and the presence of inhibitors. The kinetics of these reactions, which are of much practical interest, are well studied. These slow reactions are called degenerated-chain reactions [2].

A characteristic feature of the development of such reactions is their temporal nature. At first, when there are few radicals and many of them perish unproductively as a result of recombination or reaction with foreign substances, the reaction occurs extremely slowly at an almost constant rate. The amount of products forming during this is very small, therefore it is difficult to detect such a reaction. This is the so-called induction period of the reaction during which the system is close to equilibrium, i.e., it is found in a steady state. Using biologic terminology this period can be

called the incubation period. After radicals and peroxides gather, as a result of the slow process, the reaction begins to change from steady state to nonsteady state and rapidly self-accelerates. When the number of radicals increases as a result of irradiation, the change of the reaction from steady state to nonsteady state is accelerated. To demonstrate that the reaction is a branched-chain type, numerous criteria were assumed, the basic ones being:

1) the kinetic curves for the change in the amount of end and intermediate reaction products. Accumulation of end products occurs continuously (with respect to the sigmoid curve). The amount of intermediate products (active radicals and peroxides) depends only on the reaction rate at a given moment. Therefore, during the first period of development of the branched chain reaction their amount does not noticeably increase, on passing the period of self-acceleration is grows, and at the maximum reaction rate attains maximum magnitude. When the reaction is retarded because of a decrease in the number of reactive molecules, the number of radicals and oxides is reduced. At the end of the reaction the quantity of end products is maximum and that of intermediate products approaches zero (Fig. 2);

2) the general course of development of the reaction is subjected to the law A^x;

3) the activation energy (temperature coefficient) of these reactions is higher than that of normal reactions (20,000-40,000);

4) a very high ionic yield (10^3-10^7);

5) not governed by Arrenhius' law which is expressed in that the dependence of the velocity logarithm on the inverse absolute temperature is nonlinear and the activation energy, so to speak, increases with increasing temperature;

6) suppression of the reaction by different inhibitors inactivating the intermediate radicals and peroxides;

7) kinetics and presence of chemoluminescence accompanying the radical and peroxide oxidizing reactions.

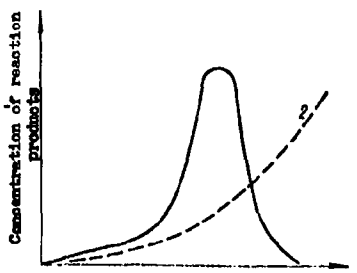

Fig. 2. Kinetics for accumulating intermediate 1) and endl 2) products during branched chain reactions.

A characteristic feature of oxidative reactions developing by this mechanism is that they are sharply retarded when the oxygen content drops below a certain limit (lower oxygen limit) and when the oxygen partial pressure increases above a certain threshold, they develop only in a certain range of the optimum [8]. Theoretically this phenomenon is substantiated by N. N. Semenov.

As was already pointed out, because of the weakness of peripheral bonds it is easier for oxidative chain reactions with branched chains to form in the lipids than in other substrates. In addition to the numerous data about the occurrence of chain reactions in commercial fats, there are data about the spontaneous formation of branched chain reactions in fats of biological origin [9] and about the marked acceleration of these reactions when irradiated with ionizing radiations (butter [10], squalene [11], sunflower oil and hepatic fat [27]).

Lipids play a major role in the formation of the fundamental structural elements of cells. Their resistance to oxidation is ensured by the presence in the lipids of antioxidants which impede oxidation reactions and protect the structural lipids from numerous radicals and peroxides forming in the cells during natural metabolic oxidative processes. Similar antioxidant systems exist in other cell substrates. Therefore, to understand the possibility of the occurrence of chain reactions in cells of the organisms on irradiation, the kinetics of oxidation and radiation oxidation of a substrate in the presence of an inhibitor-antioxidant is of interest. The kinetics of oxidation for such systems was repeatedly studied on different oxidizable organic compounds [13, 14]. These investigations showed that in the presence of an antioxidant, the development of branched chain reactions is strongly retarded, but nevertheless the process occurred, since we can detect the conversion products. In this case weak inhibitors operate. Under strong inhibitors the reaction is completely retarded and we could not detect peroxides or any other reaction products. In both cases, however, a reaction always occurs between molecules of the inhibitor and the radicals being formed, the inhibitor consumption is governed by the linear law. In the presence of a sufficient amount of inhibitor the reaction occurs slowly in the steady state (incubation period). This steady-state regime is maintained until some portion of the antioxidant is completely destroyed. On further reduction of the antioxidant concentration, the reaction rapidly changes to a nonsteady autocatalytic regime and begins to develop rapidly. In a number of investigations it was demonstrated that for the reaction to change from a steady regime to a nonsteady, it is sufficient to lower the concentration of

the antioxidant by several per cents.

Analogous processes occur in the lipids of organisms (Fig. 3). Their antioxidants under normal conditions are continuously but slowly consumed, thus maintaining a steady regime of oxidation of lipids at a very low level. It is impossible to relegate the antioxidants contained in lipids to the group of strong inhibitors, because lipid extracts from animal organs always contain a small quantity of lipoperoxides [15, 16], which attests to the presence of the reaction. This developmental process of reaction and destruction of antioxidants in the steady state occurs at a constant rate, since to replace the spent inhibitor new portions of it are taken up from the external environment also at a constant rate, and the concentration of antioxidants remains constant the whole time. The change to a nonsteady autocatalytic state can occur if this equilibrium course is disrupted by the appearance of numerous radicals as a result of which the antioxidant is destroyed at a higher rate, and its concentration will no longer be compensated by uptake from without. Such disturbance of equilibrium occurs under the effect of ionizing radiations, and at critical reduction of the antioxidant concentration the destruction reaction begins to develop rapidly.

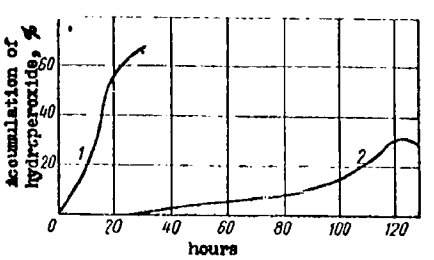

Fig. 3. Oxidation of isopropylbenzene without inhibitor (1) and with inhibitor (2).

L. I. Zhuravlev, who determined the content of antioxidants and peroxides in lipid fractions of the liver γ-irradiated rats, experimentally proved that such a change from steady to nonsteady state occurs when the amount of natural antioxidants is decreased by 10-15%.

The induction of branched chain reactions requires a large initial energy, therefore the development of the reaction is slow. Acceleration of the reaction is more easily accomplished when the reaction developes very slowly. Induction of chain reactions in lipids is facilitated since chain reactions of oxidation are continuously occurring in them and only in the steady state. Experiments on oxidation of paraffins [17] is of primary interest to understand the mechanisms for inducing chain reactions in biological systems. Paraffins are resistant to oxidation, and large doses of γ-rays are required to oxidize. It was shown that if we induce development of a slow oxidation reaction by increasing the temperature, then upon subsequent irradiation doses of 70 r are sufficient to provoke a vigorous oxidation reaction.

It is probable that in the high-polymers of cells, chain reactions can develop upon irradiation. Physicochemical observations of living cells and tissues show that immediately after irradiation, changes in viscosity and hydrophilism are observed in the cells. In the biological systems [18] polymerization occurs through peroxide radicals and peroxides as a result of the formation of oxygen cross-links between macromolecules. Similar reactions are observed when nucleic acids and proteins are irradiated.

Statistical regularities are characteristic for the emergence and development of chain reactions at initial stages. Each radical formed is far from becoming the center of reaction (it can be

destroyed upon recombination or react with substances not participating in the reaction). On the other hand, as a result of destruction of intermediate products, the bond which has already formed can be broken. This probability of breaking the bond is decreased, of course, as the number of bonds increases. For small doses the probability of forming centers decreases, and sporadic bonds can be formed for hundreds of ionization events. This statistical character in the formation of a chain process allows us to explain, why lesions do not develop in all cells of a cell population when irradiated and many ionization events occur.

We can explain all the principles of hitting a target from the point of view of the probability of forming a reaction, and these kinetic dependences can be described by the same formulas by which the target theory operates.

When evaluating the role of the radiochemical reaction, just as when evaluating the primary reaction in radiation injury, it is first of all necessary to take into account the possibility of the quantum yield of the reaction and its time dependence.

Oxidative radiochemical reactions occur both in water and in waterless solutions. In the primary intensifying mechanisms, those which develop by the chain mechanism are of import, regardless of whether induction occurs through the radicals of the solvent water, directly. Such reactions can occur in fats, aldehydes, carbohydrates, nitrogen-containing organic substances, and in nucleic acids. In all cases, along with the radicals, a basic role belongs to the peroxides as intermediate products. According to the data of N. N. Semenov they are factors for chain branching

$$ROOH \rightarrow PO\cdot + OH\cdot$$

$$PO - PH \rightarrow ROH + R\cdot$$

$$R + O_2 \rightarrow POO \text{ etc.}$$

It is known that in the presence of oxygen, reducing reactions can be induced along with oxidizing reactions. True, this occurs considerably less often. In most cases the reducing agent is the hydrogen radical. The rate of certain reactions in the biochemical components is completely independent of the oxygen concentration and the antioxidants present. For instance, radiation destruction of ribonuclease and carboxypeptidase even in water solutions do not depend on the concentration of oxygen and cysteine [19, 21]. Radiation disintegration of phosphorus-containing compounds with the formation of phosphorus ethers [20] is also independent of the content of oxygen and protective substances. Data are known about the reduction of certain dyes in the presence of oxygen. On the basis of the quantitative principles we can assume that radiation induced nonoxidizing reactions also play a part in primary mechanisms. The specific nature of these reactions, however, has not yet been disclosed. There are bases to assume that the significance of these reactions increases under the effect of radiations with a large ionization density. In this case their yield is increased.

Indirect Action

The chemical effect on various substances in weak solutions under irradiation is basically determined by the active radicals H, OH, HO_2 and hydrogen peroxide forming in the aqueous phase [21].

These radiochemical concepts about the indirect induction of reactions through the solvent were later applied to radiobiology [22].

Such a point of view opposes the target theory, and is widely recognized. According to this so-called theory of indirect action the biological effect depends on the formation of radicals in the aqueous phase of the cells and tissues, i.e., induction of the reactions in organic substances is accomplished solely by the indirect method. The basis for such an assumption is the generally known fact that live tissues contain much water (up to 60-90%).

The theory of indirect action in radiobiology completely disregards the possibility of direct induction of radiochemical reactions in organic components of the organisms without taking into account that the formation of radicals and peroxides occurs in organic substances and in some of them the yields of the radicals is much higher than in water [24, 25].

In water solutions the predominance of direct or indirect action is determined by the ratio of the volumes of the solvent and solute. On the basis of numerous investigations it is considered that the predominance of indirect action is observed when the ratio between the volume of the solvent and solute is less than 1.5 (0.1 for low-molecular-weight substances). When this ratio is increased the direct action begins to vigorously increase. In biological systems this ratio is considerably higher than 1.5 [23, 26]. In addition, it should be noted that indirect action is observed in weak molecular solutions, where each molecule is surrounded by the solvent, and therefore does not contact other molecules, i.e., the possibility is precluded of the interaction of the dissolved molecules, and propagation of the reactions and energy migration do not occur. In biological systems the water is distributed unevenly; there are structures forming phases consisting of many thousands of molecules,

where both energy migration and propagation of radiochemical processes are possible [27].

We cannot disregard that a certain portion of the water is bound, therefore energy migration to the organic molecules can occur and this increases the volume of the organic phase [28].

The water and peroxide radicals forming in the aqueous phase of the cells, of course, participate radiochemical reactions developing in organic substances. We cannot, however, attribute an exclusive role to this factor. Under the physicochemical conditions of cells we can speak only about the ratio of the direct and indirect actions.

At present there is rather much evidence indicating chemical reactions can be induced in different biochemical components of organisms directly by irradiation in the absence of water. For instance, the yields of peroxide-active products in squalene when irradiated with equal doses are two orders higher than in water. It is necessary to take into account that the formation of radicals in the organic phases can occur after direct irradiation as a result of the development of aftereffect reactions. This is impossible in water. In the aqueous phase recombination is easier, and the hydrogen peroxide is destroyed quite actively with respect to the kinetics of the chain reaction, therefore its steady-state concentration is very low [26, 27]. Hence, we cannot indicate that only indirect action occurs in biological systems. The formation of radicals in the aqueous phase and in the organic phase to the same extent can induce reactions in organic substance of cells. The predominance of some will depend on the physicochemical factors determining the kinetic principles for developing and inducing reactions. This depends not only on the ratio of volumes, but on the temperature and

ionic yields of the reactions. Thus, on the basis of available data we can say that, when higher organisms are affected, direct induction in organic substrates prevails. In many cases, both water radicals and those of the organic phase induce the same reactions. There are characteristic examples, however, showing that under direct and indirect actions the reactions occur differently. For instance, on radiolysis of acetic acid in weak solutions, H, HO, and succinic acid are formed, whereas in more concentrated solutions CO, CO_2, CH_4, C, and H are formed [26, 28].

Investigations of the effect of water content on the radiosensitivity are usually cited as the strongest argument in favor of the indirect-action theory. Inactivation of viruses, spores of lower organisms, bacteria, and seeds in the dry state requires considerably larger doses of radiation than in a swollen state. In certain tests the difference reached three orders.

Many investigations have been carried out which showed that on dilution of cellular and viral suspensions, their radiosensitivity increased, and that dealth of cells, for instance paramecia, in low concentration in an aqueous medium depends on the presence of hydrogen peroxide which formed during irradiation [29]. The dilution effect is also cited as proof of the indirect action. At the same irradiation dose the amount of inactivated enzyme in a wide range solution [30]. For instance, when two weak solutions whose concentrations differ 60-fold are irradiated, their inactivation was identical. This test actually shows that enzyme inactivation is determined by the products of the radiolysis of water, since the probability of electrons hitting molecules changes, whereas the probability of the formation of products of radiolysis remains

constant. Despite the fact that the concentration of enzyme changes almost by two orders, the total volume of molecules of the enzyme remains negligible with respect to the mass of water. This dilution effect only confirms the general radiochemical law that indirect action is predominant in weak homogeneous aqueous solutions.

The main argument for proof of the monopolistic action of radiation on living organisms through water is that as the amount of water content in seeds of plants and spores gradually increases, their radiosensitivity increased [31, 32]. These convincing tests, however, should not be taken as proof. The increase in water is always accompanied by an increase of metabolic processes and as these increase the sensitivity generally increases, since various radicals and peroxides in the cell increase, thus facilitating the induction of radiochemical reactions.

There is no direct relation between increase of water content in seeds and radiosensitivity. For instance, in tests with wheat seeds it was established [33] that when the amount of water drops from 10.5% to 3%, their radiosensitivity does not decrease but increases. With slight increase in water content of bean seeds their radiosensitivity increased, on a further increase in water within a certain range it did not change, but upon still further increase of water, it decreased. We can explain such a relationship only by the change in the rate of the so-called metabolic processes which increase when the water is increased and which decline when there is too much water (water paralysis).

Model tests on polymerization in aqueous solutions are often resorted to as proof of the indirect action of the reaction. Actually, polymerization of water-soluble monomers (vinylpyrrolidonacrylate)

explicitly depends on water radicals, and polymerization of nucleic acids is accelerated in the presence of hydrogen peroxide. Here, however, the radicals forming from the molecules of the monomer are important, therefore the role of water radicals in weak solutions decreases when the concentration increases. For instance, on polymerization of methacrylic acid the rate of polymerization in a rather wide range depends on the concentrations of the monomer.

As proof of the primary role of indirect action, we point to the fact that in aqueous solutions the antioxidants (cysteine, β-mercaptoethylamine) retard polymerization of methaacrylate induced radiation and caused basically by water radicals, and at the same time when introduced into an organism they attenuate irradiation injury. We cannot make conclusions from this, however. These substances, being typical antioxidants, generally tie up any oxidizing radicals and peroxides, thus inhibiting oxidative reactions, including the oxidizing radical OH· and hydrogen peroxide. This fact indicates only that oxidizing reactions are important in a number of primary processes induced by radiation.

One of the arguments often cited to support the exceptional value of indirect action is the possibility of explaining the attenuation of radiation lesions by the drop in oxygen partial pressure during irradiation. In accordance with this concept, on radiolysis of water in the presence of oxygen the radical HO_2 is formed

$$H - O_2 \rightarrow HO_2 \qquad (1)$$

Upon a decrease in oxygen concentration, its formation is markedly retarded and as a result the biologic action is attenuated. Such a reaction, is obviously, possible and during its course a certain

number of HO_2 radicals can be formed, however, as current investigations show this radical is formed by the reaction

$$\left.\begin{aligned} OH + OH &\rightarrow H_2O_2 \\ H_2O_2 + HO_2 &\rightarrow H_2O \end{aligned}\right\} \qquad (2)$$

The yield of this radical induced by γ-radiation is very low, it is considerably higher (by more than one order) when induced by radiations with a high ionization density [34, 35]. At the same time it is well known that the dependence of the survival of irradiated organisms of the oxygen pressure is demonstrated only upon radiations with a low ionization density, and is certainly not observed upon radiation with a high density. In addition, the reactivity of this radical to the induction of reactions in the organic phase is very doubtful. This radical, as many radiochemists point out, is completely ineffective with respect to organic compounds, and its action can be demonstrated through the formation of hydrogen peroxide when these radicals recombine.

In addition, and this is very important, an increase in survival when the oxygen pressure is lowered yields a marked threshold effect, and it is quite impossible to explain this from the point of view of the indirect-action theory, since the formation of HO_2 radicals with respect to reaction (1) should be proportional to the oxygen concentration.

Indirect induction of course is important in the radiobiological action, however the ratio of direct and indirect induction in biological systems can be strongly modified. This ratio depends on the percentage of water, the reactivity of water and organic radicals and peroxides, and on the possibility of forming chain and

autocatalytic reactions with high ionic yields in the substrate under consideration.

Indirect action is mainly characteristic for cell suspension of bacteria and viruses, where there is much water per cell. In this indirect action the role of the stable product of radiolysis, hydrogen peroxide, is important. Indirect action is demonstrated more strongly, the lower the reactivity of the cell substrates.

Thus, when the ratio of the water-organic substance shifts toward an increase in the volume of the organic substance, the organic substance forms continuous phases, the cells contact each other and reactivity increases (for instance, as a result of the high level of metabolic reactions, a lower activity and smaller amount of natural inhibitors of the reactions), the direct action prevails. The ratio between direct and indirect induction is determined by physicochemical conditions. Therefore, the proof of the primary role of indirect action obtained on mock-ups and cell suspensions where the optimum conditions for indirect action are created, does not have a general, fundamental significance, and we cannot apply it to tissues of higher organisms.

The relative significance of direct and indirect action can markedly vary even in the cells of a single species of lower organisms owing to the reactivity of the compounds entering into the composition of the cells. In cells of higher multicellular organisms, direct action should predominate direct induction of reactions for the majority of the basic cell substrates (lipids, nucleoproteins, nucleic acids); indirect action is characteristic for other substrates with low radiation reactivity (proteins).

The predominance of direct action in cells of higher organisms is confirmed by the numerous facts of the detection of active peroxide compounds in the organic phases.

In a number of cases an immediate differentiation of direct and indirect action and their relationships is possible. In particular this dependence can be demonstrated on lower organisms which endure a wide range of oxygen-pressure changes without injury. As is known, when the oxygen pressure drops, survival or organisms irradiated by ionizing radiations is increased. This phenomenon, true, by stretching the imagination, can be explained from the point of view of the theory of indirect action (but only qualitatively). It has been established that on increasing the oxygen pressure above a certain limit, the survival of yeast increases, and radiosensitivity decreases. This phenomenon is impossible to explain from the point of view of the indirect-action theory. The number of oxidizing radicals, in particular HO_2, continuously increases with an increase in oxygen pressure. At the same time, it is well known that a decline in reactivity when the oxygen pressure is increased occurs as a result of the development of oxidative branched chain reactions. It was established that for yeast of a diploid race in a water culture, an increase in oxygen pressure has a very weak effect (increase of survival) and the median lethal dose (LD_{50}) here is equal to 40 kr. In an air culture on agar, an increase in oxygen pressure above 1 atm considerably increases survival and LD_{50} = 120 kr. Hence, in a water culture indirect action predominates and the over-all radiosensitivity thus increases: in an air culture, on the other hand direct action predominates and the radiosensitivity decreases (Fig. 4). For the haploid breed of the same species, the radiosensitivity is much

higher (LD_{50} = 4 kr) and the change (increases) in survival when the oxygen pressure is increased is identical both in the air and in the water cultures, i.e., the water content has no effect on the extent of damage and the effect is dependent on direct action. The physicochemical conditions in the haploid cell are favorable for the development of radiochemical reactions (Fig. 5).

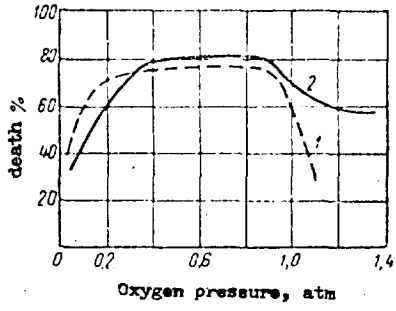

Fig. 4. Destruction of yeast Sacharimyuc vini (diploid vs. oxygen pressure; 1) air culture; 2) water culture.

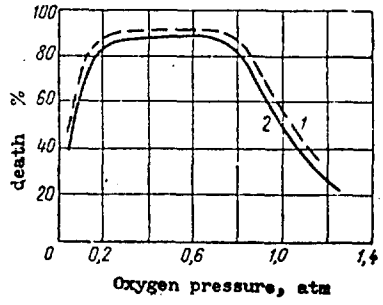

Fig. 5. Destruction of yeast Sacharimyuc vini (haploid vs. oxygen pressure: 1) air culture; 2) water culture.

As was already noted, the appearance of energy-producing, very active radicals and peroxides which can become centers for the formation of reactions is required for the development of radiochemical reactions of the autocatalytic and chain type in irradiated biological substrates. The products of the radiolysis of water can be such active products. Here, however, the active products forming in the organic substrates are important [36].

Detection of the radicals and peroxides in the biological substrates is an indicator for such radiation-induced reaction. In recent years, attempts were made to demonstrate such products, primarily free radicals, using their characteristic feature, the presence of a magnetic moment. The formation of free radicals in biochemical components and even in living cells was recorded by the

paramagnetic resonance method [37-42]. Attempts to detect radicals directly in the aqueous phase at normal temperatures were not successful. However, the presence of radicals H· and OH in ice at low temperatures after irradiation was proved. The difficulty in determining free active radicals is because their life span is short (of the order 10^{-13} sec), therefore their steady-state concentration is very low and is outside the limits of sensitivity for the EPR method, by means of which we can detect the concentration of a radical of no more than 10^{-12} M. On freezing, the processes of recombination and chemical interaction are greatly retarded and the concentration of radicals is increased.

A considerable increase of free radicals (100 and more) is detected upon irradiation of amino acids and native proteins in dry form [37]. It is much more difficult to detect the appearance of radicals in the organic phases by this method in the presence of water, since the signal is attenuated, and therefore it is necessary that the sensitivity of the device be very high. With an increase of the sensitivity of the EPR method, it was possible to detect the appearance of radicals in both water mediums and in living cells, however, the results were not too distinct and therefore most studies are carried out on dehydrated tissues of organisms.

It was of interest to investigate frozen yeast cells which when thawed were completely revived [41]. The cells were nurtued on deuterium water because it was possible to shift its signal which usually keeps the basic signals in the background. This made it possible to detect more distinctly the signals from organis substances. These tests proved that upon irradiation radicals appear both in the water phase, and in the organic phase. When the well-

known protective substance β-mercaptoethylamine, which has antioxidizing properties, was introduced into the culture during irradiation, the radicals in the organic phase reacted with it and their number decreased, the number of radicals, however, remained the same in the water phase. This testifies to the high reactivity of the organic radicals and indicates that during irradiation oxidizing radicals are formed in the organic phases.

Subsequent investigations established that radicals forming in amino acids and proteins during irradiation are manifested in proteins exposed to different denaturing factors under large doses of radiation which induce denaturing. These radicals are very stable. We were able to detect the appearance of radicals after irradiation in living objects, for instance in normal and desiccated embroyos. A study of the nature of the signals showed that they originate from the proteins also. (The tests were conducted at high irradiation doses.)

In tests on desiccated organs of rats subjected to irradiation with normal doses (1000 r), such radical formation was not detected [43]. In all organs, except the spleen, an increase in the number of radicals was not observed during 48 hours after irradiation. In the spleen immediately after irradiation the number of radicals decreased considerably and after 48 hours was restored. This means that the steady-state concentration of active radicals is so small that it is impossible to detect it by means of contemporary devices which record the presence of radicals only when their concentration exceeds 10^{15} per 1 g. The concentration of radicals drops in proportion to their reactivity. Therefore, we can, as a rule, detect the inactive or slightly active radicals. This is very nicely

confirmed by the cited data obtained on spleen tests. A decrease in the number of radicals present before irradiation shows that these radicals energetically enter the reaction and their life span is shortened. This is understandable from the point of view of the data which indicate that radiation mainly accelerates those reactions which develop at a low steady-state stationary level. What was observed in the spleen obviously occurs in other cells, but under the given test conditions it went unnoticed, and completely correlates with the fact that the spleen is one of the most radiosensitive organs.

In this respect, the investigation of radical formation in amino acids at very low temperatures is significant [38]. On irradiation at low temperatures, another type of radical appears, in addition to the slightly active radicals detected earlier, which gives signals only at low temperatures. These are more active radicals; their steady-state concentration at normal temperature is very low. Attempts to detect by using the EPR method, radicals in lipids, where irradiation induces intensive oxidizing chain-type reactions and where radicals are definitely present, which also can be detected by other methods, did not yield results, since their steady-state concentration is very low owing to high reactivity and short life span.

Attempts were made to determine the presence of radicals upon irradiation, having used for this purpose substances which react with radicals and bind them.

Besides the low steady-state concentration of active radicals in living cells, a big obstacle in detecting them in living biological system by the EPR method is the large quantity of water, in

whose presence considerable damping of the signals occur. Therefore, the EPR method has so far yielded very little information about the balance of radicals in living, functioning cells and their change when irradiated. More sensitive methods are necessary for this than those available to us.

All methods directed at eliminating the effect of water (mechanical homogenization and desiccation) automatically distort the actual picture, since any mechanical action and desiccation per se cause the formation of radicals, and the interpretation of the obtained data is inevitably arbitrary. In this respect measurements at very low temperatures are promising, but for the present there are very few of them. We must use other methods to evaluate the compositional change of the radicals in living systems. As is know, the polymerization process is a radical reaction; during this the basic role belongs to the oxidizing peroxide radicals. If we introduce monomers into a system containing radicals, the polymerization process will develop in proportion to the number of radicals, and based on the degree of polymerization we will be able to evaluate their quantity. If there are chains of a polymer of another compound in the system, then as a result of radical reaction polymerization will occur along the chain of the existing polymer and a stable bond will develop between them. This process is called grafting.

Since cell protoplasm consists of higher-polymer molecules, it should be expected that such grafting will occur when monomers are introduced into the cells and radicals are present. Based on the magnitude of the resulting polymerization, we can evaluate the number of radicals in the cells in the norm and on irradiation. Therefore monomers soluble in water which easily penetrate living

cells were selected. These are derivatives of vinylpyrrolidone and acrylnitrite. At a 2% concentration in the culture medium, these compounds have no toxic effect on the living yeast cells, which normally grow and propagate. The radicals forming in the cells cause polymerization of these polymers and their grafting to the biopolymers. As investigations on yeast showed, the polymerization process develops very slowly in normal cells. By determining the chain length of graft polymers and their total number, it is easy to calculate the number of free radicals present in the cells.

This method has advantages over others: it enables us to account for the presence of free radicals in the living undamaged cell, to take into account the active radicals which can enter into the reaction and, in addition, which is very substantially cumulative. The accumulation of the effect occurs after a certain time interval, which allows us to demonstrate active radicals whose one-time steady-state concentration is very low and is impossible, for instance, to determine by the EPR method in tests with live cells radiated by γ-rays.

We attempted to determine the total number of radicals under irradiation by introducing a water-soluble vinylpyrrolidone monomer into the living cells of sprouts of winter wheat seeds Triticum vulgare [44]. The monomer easily penetrated the cells, and at 2-4% concentration did not have a toxic effect on them. Polymerization and grafting of the vinylpyrrolidone monomer to the biopolymers occurred in the cells in the presence of radicals. The amount of graft polymer was determined by the increase in weight of the mass, and from this, the number of radicals [45, 46]. It was found that the number of radicals sharply increases after irradiation with their

increased number appearing in the cells both in the presence and absence of oxygen. In the presence of oxygen, with a 1° temperature rise grafting increases (Fig. 6), and in the absence of oxygen it decreases with an increase in temperature (see Table).

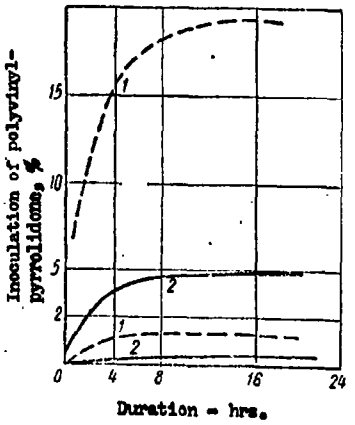

Fig. 6. Radiation grafting of polyvinylpyrrolidone on wheat seeds at 30°C. 1) no oxygen; 2) with oxygen.

Weight Increment of Seeds $(P_2 - P_1)/P_0$, %*

Without radiation at temp. (°C)			With radiation at temp. (°C)		
30	50	70	30	50	70
In presence of oxygen					
0,3	9,0	12,2	2,6	17	21,2
In absence of oxygen					
1,0	7,8	6,7	21,5	13,6	7,1

*P_0) initial weight; P_1) weight of non-radiated seeds; P_2) weight of irradiated seeds.

Data on radical formation in cells during irradiation show that intensive radical formation occurs after direct irradiation, and this confirms the development of chain reactions during which such radical formation is possible.

A positive temperature coefficient in the presence of oxygen shows that during primary activation, importance is attached to the peroxide radicals and peroxides whose number increase with temperature rise; they are grafting centers. The negative temperature coefficient in the absence of oxygen indicates that grafting depends on radicals of a nonoxidizing character. A temperature increase accelerates their recombination, and this lessens the rate of grafting reaction. Thus, in primary activation the ionizing radiation induce nonoxidizing reactions along with oxidizing reactions.

Investigations of the chemoluminescence of living cells provide considerable information about the formation of radicals and their nature. Chemoluminescence, as is known, accompanies oxidizing reactions of an exothermic character, i.e., mainly chain-type reactions which develop by the radical mechanism.

It was previously established [47, 48] that normal tissues of higher organisms radiate extremely weak light fluxes (10-100 kv/sec) in the green and blue region of the spectrum. This radiation is caused, as analysis has shown, by chemoluminesence occurring during a slowly developing process of autoxidation of structural lipids. In the norm this process in cells of the liver, spleen, muscle and the brain is maintained at a very low steady level. Upon irradiation, emission increases and is established a higher steady level, and after several days increases again. This increase, as the kinetics of chemoluminescence point out, occurs after irradiation, which indicates radical formation already in the process of developing an exothermic chain reaction.

Peroxides are of great interest from the point of view of understanding primary reactions. They are formed from the peroxide

radicals and, in turn, the hydroperoxides can be broken down forming radicals. In addition to the radicals, the peroxides are important as intermediate products in the development and initiation of chain reactions. The peroxides are a factor for branching the chains, therefore their detection confirms the development of oxidative reactions with high quantum yields. Peroxides have an important role in promoting and fostering chains in oxidizing chain reactions which spontaneously develop in lipids exposed to irradiation, and they have a similar role in the polymerization processes. Therefore, the paper of V. Horgan and J. Philpot caused much interest. After using various oxidizing-reducing reagents, they detected lipoperoxide in butanol extracts from tissues of irradiated animals, which was not identified with a single known peroxide compound. Later many investigators detected lipoperoxides following irradiation. The kinetics of their formation were studied. As a result, it was established that in the lipids of irradiated rats and mice the lipoperoxides appear very rapidly. As a rule, they exist in a small stable quantity. Their quantity increases immediately after irradiation. The ionic yield of this initial reaction is, unconditionally, about 300 [49, 50]. The formation of peroxides does not noticeably increase later and is retained for a certain time at a stable level [51]. Upon introducing antioxidants before irradiation, the yield of these primary peroxides decreases [52]. The presence of peroxides immediately after irradiation was detected in the skin and various organs of rats by Philpots' method [53] and in spleen mitochondria of irradiated rats [55]. The only work in which, as was reported, it was not possible to detect an increase in peroxides after irradiation in the carcass of rats and in their skin [54, 56] was more than once

repeated with positive results. It is characteristic that lipoperoxides are vigorously formed in animal organs under small lethal doses; under large doses (20,000 r) this is not observed. Investigations of the peroxide forming-reactions lipids in vitro demonstrated the total listed lipids [16] and squalene [11], and that an identical picture is observed in the tests in vitro and in vivo [57]. The detection of lipid peroxides during the direct act of irradiation indicates the development and induction of initial chain reactions with high quantum yields. Presently there are reports about the detection of peroxides of nucleic acids forming during irradiation.

On analysis of the possibility of inducing radiochemical reactions in cells and tissues, it is necessary to take into account that these systems are not homogeneous, multiphase, and their radiochemical behavior in vitro often will not correspond to the radiochemical transformation in mixtures, where the different components are inadequate with respect to their radiosensitivity.

In the statistical distribution of the ionization events, ionized molecules and excited states appear in different biosubstrates. These primary physical events will often cause chemical conversions and the development of reactions in the same substrate. In other cases the excitation energy can be transferred to other molecules and cause (or enhance) the development of reactions in other substances of the mixture [58]. Such a transfer is mostly accomplished by the direct impact of molecules. As a rule, this transfer is possible from molecules possessing a higher excitation potential to molecules with a lower potential. If $E_A > E_B$, the energy transfer is possible only from A to B, but is not the other way.

Molecules B, having received the energy, can undergo a chemical conversion or liberate this energy and return to an unexcited state. A particular case of this transfer is the protective effect when substance B protects substance A and makes it impossible for radiochemical reactions to occur there. For instance, in the mixture toluene-cyclohexane (potential is equal to 8.8 ev) — benzene (potential is equal to 9.4 ev), radiolysis of the tuolene primarily occurs. In a lipid system with inhibitors-antioxidants the excitation energy of the lipid molecules transfers to the antioxidant (cysteamine, β-mercaptoalanime, and tocopherol) and to a certain extent is deexcited, thereby protecting the lipids from oxidation. This same mechanism in reverse can sensitize the development of the reaction in certain biochemical substrates necessary for the organism. It is very probable that a certain amount of energy from excited molecules of bound and free water can be transferred over chains of hydrogen bonds to the organic substances (without the formation of water radicals), and this obviously explains the catalytic effect of water when all of it is in the bound state. A sharp decrease in radiosensitivity when removing the water occurs only when the last traces of water are removed. Such transfers are possible from molecules of one substance to those of another when the distance between them is no more than 5 A.

Assumptions have been repeatedly made that in biological systems the excitation energy can be transferred over several molecular radii to a place where the chemical event can occur without losses, i.e., energy migration can occur. This assumption is postulated by supporters of the modernized target theory who, resting on the possibility of energy transfer over large distances, attempted to

eliminate the incongruities arising in the target theory when evaluating the sensitive volume. As is known, the transfer of excitation energy is possible to distances of up to 50 A. It was proved that the energy migration over protein cross-links is possible according to the resonance mechanism. This mechanism, judging by certain conclusive papers, is characteristic for the photosynthesis process [59]. There are, however, no direct experiments which would confirm the possibility for energy migration of ionization events in biologic systems under the effect of ionizing radiations because these assumptions do not have any concrete basis.

For the time being we should refer with care to the attempts of arbitrary interpretation of every distant action as energy migration. We cannot forget that during chemical processes, distant action can be accomplished in heterogeneous systems and is accomplished more often at the expense of other mechanisms. Many processes at a distance are caused by diffusion of active products, peroxides, and radicals having a long life. A similar distant action is observed, for instance, when under conditions of direct action the hydrogen peroxide molecules diffuse to organic molecules. Such a method can diffusely propagate the toxic molecules formed during irradiation. Distant action can occur as a result of propagation over some sort of organic phase, for instance along high-polymer chains of a chain-type chemical reaction. In this case, advancing along the structural elements, like along a wick, these reactions are propagated to other substrates and induce other reactions there by their radicals and peroxides.

The assumption was hypothesized earlier that primary activation can occur as a result of secondary radiation (ultraviolet) occurring

when the bonds break in the ionization event. This point of view has not been confirmed. First, the yield of this luminescence proved very small and, second, the fact that the effect of ultraviolet can be removed on irradiation with the visible spectrum contradicts the participation of secondary (ultraviolet) radiation in inducing primary reactions. Such reactivation does not occur under the effect of ionizing radiations.

It was hypothesized that activation of the initial reactions developing under irradiation is accomplished by the iron of chromoproteins. During irradiation bivalent iron is converted to trivalent iron in the cytochromes, therefore transfer of oxygen is disrupted and enzymatic activity of iron-containing enzymes decreases. This point of view does not correspond to well-known facts: iron-containing oxidative enzymes are very radioresistant, in the same way as the act of breathing. Iron, however, and very probably trace elements, are important in the activation of initial oxidizing reactions. Cyanides, suppressing the catalytic effect of heavy elements, exert a protective action when administered before irradition. In the presence of iron and other metals, cyanides do not exert antioxidizing actions. For instance, adding bivalent iron to oleic acid considerably accelerates its rate of oxidation and makes this system more sensitive to irradiation. The addition of iron-binding cyanides retards this reaction, but not completely, and only by that magnitude which increases in the presence of iron.

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INHIBITION OF THE PRIMARY REACTIONS IN BIOSUBSTRATES

Of great importance in understanding the nature and role of primary reactions in radiation damage were the investigations which showed that, with certain physicochemical effects on living organisms the development of radiation injury can be reduced considerably during irradiation. This direct inhibition of initial reactions at an early stage of their development is good information about their mechanisms.

Of greatest interest are the data on the inhibition of the injury reaction by changing the oxygen pressure, the so-called oxygen effect and by using certain chemical compounds, so-called chemical protection.

Oxygen Effect

It has been established that when the oxygen partial pressure is lowered during the action of ionizing radiations having a low ionization density (X-rays, γ-rays), the radiosensitivity of organisms is reduced considerably. Such a protective effect when the oxygen pressure is reduced is a general biological phenomenon — it is observed in viruses, bacteria, and higher and lower animals. A reduction of oxygen pressure during irradiation reduces mortality,

the per cent of hereditary mutations, decreases the amount of chromosome breaks, and other manifestations of a pathological nature [1].

It was hypothesized that the attenuation of the radiation effect when the oxygen pressure is reduced is connected with a slowing-down of respiration and metabolism, and thus, as is known, radiosensitivity is decreased. On the other hand, it was proposed that a reduction of oxygen pressure attenuates the rate of development of oxidizing radiochemical reactions caused by irradiation in any biochemical cell component, i.e., this reaction is not connected with metabolic oxidative processes.

In most cases these two possibilities cannot be discerned, since metabolism is slowed down when the oxygen pressure is reduced. Certain experimental data, however, indicate that a change in radiosensitivity is not connected with a change in metabolism. For instance, on a reduction of oxygen pressure a protective effect is observed in both aerobic and anaerobic bacteria [2].

The effect of oxygen pressure on the number of chromosome aberrations of irradiated rootlets of horse beans were studied both under normal and inhibited respiration [3]. Respiration was reversibly suppressed by the ammonium salt of nitrosophenylhydroxylamine and its retardation was monitored by a manometer. As a result, a typical oxygen-effect curve was obtained during suppression of respiration.

From these data it follows that a reduction of oxygen pressure renders a direct inhibiting effect on the initial oxidation reaction induced by radiation in any substrate.

In higher animals, respiration is sometimes indirectly connected with the oxygen effect. When breathing is inhibited the oxygen

partial pressure in the tissues is reduced. It was established that substances (morphine, tryptamine) suppressing the respiratory center reduce the radiosensitivity of animals. In rabbits, injected with such substances, the oxygen pressure in the tissues is reduced to values where protection occurs when the oxygen pressure of the inspired air is reduced [4-10].

Reduction of the oxygen pressure does not create absolute protection of organisms and mammals, but under the most optimal conditions it can reduce the effect of irradiation (magnitude of the dose) by approximately 50%.

The effectiveness of protection upon an increase in oxygen pressure is connected with the ionization density. Maximum reduction of radiosensitivity is attained at low ionization densities, and the protection effect decreases with increasing ionization density. At a high ionization density, for instance when irradiating with α-particles, the oxygen effect is completely absent. An increase in survival upon reduction of oxygen pressure indicates that oxidizing reactions play some role in the deleterious effect, and the incompleteness of the effect shows that these reactions are not unique.

As is known, attempts were made to explain the oxygen effect from the point of view of the indirect-action theory. According to this theory, which is shared by many investigators, in the presence of oxygen the radical HO_2 ($2HO-O \rightarrow 2HO_2$) is formed in addition to radicals H and OH during radiolysis; when the oxygen concentration is reduced, the amount of this radical decreases and the oxidizing capability of water is increased. Current papers on radiochemistry pay much attention to this radical, however, as was established, its role in radiobiology is greatly exaggerated. This radical is formed

in the presence of air in a small quantity under the action of radiations having low ionization density, and its amount increases considerably as ionization density increases. Its yield under the action of heavy particles is approximately 10 times more than that when irradiating with X- and γ-rays, i.e., a picture is observed which is inverse to that which occurs under the oxygen effect, which is completely absent under the effect of heavy particles on organisms when there are many HO_2 radicals, and which is demonstrated at a low ionization density when there are few of these radicals. The data from a study of the binding of radicals HO_2 in cells by organic antioxidants contradict this concept. These antioxidants react with radicals of the organic phase and do not react with radicals of the water phase.

To understand the mechanism of the initial oxidizing reactions induced by radiations, the kinetic principles of the rates of injury reactions in the biological objects are of interest. While making a quantitative study of the survival of organisms after irradiation under various oxygen pressures, many investigators noted that the protective effect is not proportional to oxygen pressure, but a threshold pattern (all-or-nothing) is observed. They focused attention on this phenomenon when studying the survival of mice X- irradiated in an atmosphere with an increased oxygen content [5]. It was established that when the oxygen concentration is increased from 30 to 80% the survival of mice did not change, but when the pressure was reduced from 8 to 6% a sharp increase was observed. This kinetic pattern is characteristic for many cases and for different organisms (for bacteria [6], bean rootlets (Vicia faba) [3, 7], granary weevils [11], etc). This phenomenon (Fig. 7) was not explained from

the point of view of the indirect-action theory. Formation of the hydrogen peroxide and radicals was studied during irradiation with γ- and X-rays; no threshold effect was observed when the oxygen pressure was changed during irradiation, and the amount of these products increased continuously with an increase of oxygen pressure.

There are reactions, however, where their rate changes irregularly depending on oxygen pressure. As N. N. Semenov and his students pointed out, on increasing the oxygen pressure the rate of such reactions does not change up to a certain limit, then an abrupt inhibition sets in. The latter occurs both when the oxygen pressure is reduced and when it is increased above a certain limit. According to N. N. Semenov's terminology, these are upper and lower oxygen limits between which an oxidizing chain reaction with branched chains develops. Such an irregular dependence of the reaction rate on oxygen pressure is explained by the fact that when oxygen pressure is either increased or lowered, conditions develop under which rupture of the chains as a result of triple collision begins to prevail over their formation. The presence of upper and lower limits in oxidizing reactions, which were observed on various substrates, is an indication that the reaction develops by the chain mechanism with branched chains.

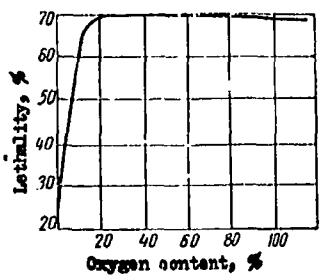

Fig. 7. Mortality of granary weevils vs. oxygen content when irradiating dose is 5000 r.

In living organisms it is usually difficult to verify the presence of the upper oxygen limit, since when the oxygen pressure is increased the organisms die even without irradiation. Such a test, however, can be conducted on certain organisms which, without disturbing vital activity, endure the increase of oxygen pressure even up to several atmospheres. It has been demonstrated on yeast of the diploid race Sacharamyus vini and on agar cultures that survival of yeast after irradiation under different oxygen pressures increases when the pressure is reduced below 100 mm Hg, as well as when the pressure is increased up to 1 atm and higher [12, 13]. The protective effect is the some under a reduction or increase of pressure (Fig. 8).

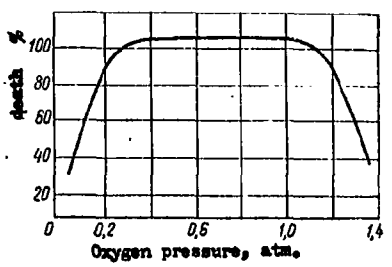

Fig. 8. Death of yeast under irradiation vs. oxygen pressure.

An analogous phenomenon was observed upon irradiating yeast in an atmosphere of pure oxygen under normal pressure [8].

The protective effect of the haploid race is considerably higher than that of the diploid.

The protective effect of reduced oxygen pressure is difficult to detect in higher animals, since it is disguised by the toxic effect. In a certain range, however, under increased oxygen pressure, when toxicity is still low, we can detect a small reduction in the radiosensitivity of mice.

The irregular dependence of the death of the object on oxygen pressure, which was observed in the tests described above, suggests dependences characteristic for the occurrence of oxidizing reactions with branched chains. The presence of two limits allows us to conclude that the oxygen effect is connected with inhibition of the chain oxidizing reaction induced by radiations in organic phases of cells, and that these reactions develop with branched chains. The presence of the threshold effect is clearly detected when the oxygen pressure in higher and lower animals is reduced, however for lower animals this is not always characteristic in the presence of a large amount of water. Often in these cases the curve of increasing survival or oxygen pressure decreases proceeds smoothly, which is explained by the predominance of indirect action during which death of the organisms is determined by reactions of another type. Obviously we can note that indirect action will prevail when the radiosensitivity of cells is low, i.e., conditions for inducing chain reactions are unfavorable. In this case indirect action increases radiosensitivity. The kinetics of the development of the oxygen effect relative to the pressure allows us to differentiate these two mechanisms. On the basis of this kinetics we can conclude: in tissues of multicellular organisms (from mollusks and higher) direct radiochemical action on organic biosubstrates prevails.

The presence and universality of the oxygen effect under the action of ionizing radiations having low ionization density clearly indicates that oxidizing reactions are induced in cells by radiations.

The threshold character of the dependence of survival of the objects on the oxygen pressure indicates that these reactions

develop by a chain mechanism with branched chains. In individual cases the oxygen effect is absent under these conditions. This was established particularly on phages, where a dependence of survival on hydrogen pressure was observed. The presence of the oxygen effect was noted on certain biochemical systems in tests in vitro. These exceptions indicate that under the effect of radiations nonoxidative as well as oxidative reactions can be induced in certain substrates.

The absence or marked weakening of the oxygen effect under the action of radiation having a high ionization density shows that under these conditions nonoxidative reaction or reactions in which oxidation occurs as a result of utilizing bound oxygen, are strongly accelerated. If these reactions develop more quickly and induce such a volume of damage that death of the organism ensues, they will be determinants and the oxidative reactions will be hidden.

Chemical Protection

The effect of chemical protection is highly significant for understanding the nature of the primary reactions during radiation reactions.

It is known that many chemical compounds when introduced into protozoan cultures and into higher animals immediately before irradiation can significantly reduce the number of deaths and other manifestations of damage. When these substances are introduced, primary reactions developing under irradiation are inhibited [14-16]. Basically, such inhibition (retardation) is attained due to the fact that the protective compounds rapidly react with the active radicals and other active products forming in the biosubstrate upon irradiation. Such compounds can also retard the development of reactions

by binding the intermediate products of complex reactions. Finally, these products, influencing the physiological mechanisms, can suppress respiration and thereby cause anoxia (reduction of oxygen concentration in the tissues).

Recently numerous compounds capable of reducing damage when introduced before irradiation have been described. Compounds containing sulfhydryl groups and sulfur have a significant protective effect. Cysteine, glutathione, 2-mercaptoethylamine (MEA), and others are effective in tests in vitro, protecting the proteins from radiation denaturation, nucleic acids from oxidative polymerization during irradiation, lipids from oxidation, etc. These compounds reduce the mortality of protozoans and higher animals. MEA is the most effective in this group. All the fundamental investigations devoted to an explanation of the mechanism of prophylactic effect were carried out with the group of these compounds.

At present other types of compounds are known (which may or may not contain sulfur) — amines, steroids, certain antibiotics (aureomycin, penicillin, tetracycline), quinones, flavones — which also have a protective effect [15]. Attempts were made to explain the protective effect of substances containing sulfhydryl groups by the fact that the primary mechanism of the radiation effect on cells is the inactivation of -SH groups (which are important in oxidative enzymes) by the OH radical that forms on radiolysis of water. This point of view has not been confirmed. The -SH groups are resistant to irradiation, and at first no changes in their content in irradiated organisms for several hours after irradiation the content of the reduced glutathione did not noticeable change [18]. Sulfhydryl enzymes (succinic oxidase, succinic dehydrogenase and cytochrome oxidase) in various organs of higher animals proved most resistant

to irradiation. In addition in the protective effect of substances containing -SH groups, no peculiarities were detected which would qualitatively differentiate the effect of this group from the effect of other protective substances (thiourea, sodium azide, sodium thiosulfate, tryptamine, sugars, etc). Therefore, attempts were made to find some effect common to all the prophylactic substances. Wide generalizations were made on the basis of model tests. The process of radiation polymerization of polymethacrylic acid and other high-polymer compounds, which develops in aqueous solutions and is accomplished mainly by indirect induction by products of water radiolysis, is inhibited by many prophylactic (protective) substances. A certain correlation exists between the inhibiting action and the biological effect of protection. This parallelism in the effect on the system, in which direct action clearly prevails, gave grounds to assume that all prophylactic protective substances possessed a capacity to bind active radicals of water forming during irradiation in the aqueous phase of the cells. Thus, this substantiated the primary role of the products of the radiolysis of water in the biological effect [17].

Actually, under the conditions for the course of reactions in aqueous solutions, when the conditions for the prevalance of indirect action are created, the mechanism of the protective action of the substances is demonstrated in the neutralization of active products of radiolysis of water since all the protective substances belong to readily oxidized compounds.

Lately it was hypothesized that the basic property uniting all the known protective substances is their antioxidizing capaicty [20-23].

All the prophylactically reacting substances are, as a rule,

inhibitors that retard the development of oxidizing reactions, in particular chain oxidizing reactions. Their antioxidizing capacity appears with respect to reactions occurring in organic substances without water and in aqueous organic systems; they can retard oxidizing reactions which are developing both by direct and indirect mechanisms. Certain substances which directly create an antioxidizing effect, causing anoxia by reacting on the respiratory center, are exceptions.

It is interesting to note that the antioxidants widely used in industry for protection against spoilage of nutritive and industrial fats, petroleum products, rubber, and other materials, belong in those groups of compounds which include the biological protective substances [25]. A very good correlation is observed between the capacity of chemical compounds to protect organisms against the effect of irradiation and their capacity to retard development of chain oxidation reactions in vegetable fats [24].

F. Yu. Rachinskiy and N. M. Mozzhukhin [20], after testing numerous organic substances for their capacity to retard the oxidation reaction in lard, established that each substance which shows a protective effect in the biological experiment is an inhibitor of oxidizing reactions (antioxidant). But not every antioxidant can be used as a protective substance. Protective substances possess reducing properties and therefore retard oxidizing reactions. A difference exists, however, between the concept "antioxidant" and "reducing agent." The antioxidizing effect of a substance depends upon its capacity to bind the intermediate products of oxidizing reactions which are complex, and in organic substrates they very often occur by the chain mechanism. The intermediate products can

differ in nature and have different redox potentials. Therefore reducing agents do not always reveal antioxidizing properties. Cases are described where oxidizing agents retard the oxidizing reaction. There are many examples where one and the same substance in substrates similar in properties in one case retards the oxidation reaction, and in another activates it. Alanine, for instance, retards the oxidation reaction in oleic acid and activates it in linoleic acid [25]. Pyrogallic acid retards the oxidation of benzaldehyde, but accelerates oxidation of toluene. These investigations point out that the correlation between the capacity of compounds to retard (inhibit) the chain reactions of oxidation in lipids and their capacity to protect the organisms against irradiation prove to be more complete than on models where the indirect action prevails.

It has been established that the effect of chemical protection is observed on dried biological objects (spores). For instance, a reduction of oxygen pressure on irradiation of bacterial spores carefully dried at low pressure has the same protective effect as on normal living organisms.

A significant protective effect, not inferior to that of thio compounds, was demonstrated by amino compounds, particularly by tryptamine and its derivatives. The latter proved very effective. Under X-irradiation it reduced the effect of the dose by 50%. These compounds, however, have a peculiarity which differentiates them from other protective substances. They provide a good protective effect on higher organisms and weak effect on lower organisms. Tryptamines apparently have antioxidizing properties, but weaker than those of thio compounds.

The stronger protective effect on higher organisms indicates a physiological protection. Tryptamine inhibits the action of the respiratory center and thus reduces the oxygen pressure in tissues of higher animals. Here protection also leads to an antioxidizing effect, but only after a reduction of the oxygen pressure. Still stronger is the protective effect of morphine. It also protects only higher animals by acting on the respiratory center and offers no protection at all to lower organisms.

Certain investigators are generally inclined to consider the effect of any prophylactically protecting substance as the effect of the substance lowering the oxygen concentration in tissues to limits where the oxygen effect occurs, i.e., the entire problem of protection against irradiation reduces to the single factor of anoxia [26, 32]. This assumption is based, first, on facts which show that when protection is attained by a reduction of the oxygen concentration, the addition of protective substances proves to be ineffective [27-29]. This is not proof at all of the uniqueness of their particular mechanism of action, but only indicates that lack of oxygen and the action of the antioxidants which actively scavenge radicals and peroxides which formed as active intermediate products, tend to retard the complex oxidizing reaction. If this reaction is retarded by a deficiency of oxidative material, then the addition of radicals and peroxides of binding substances will not be effective, since in the retarded reaction there is nothing to bind: there are neither peroxides nor radicals there. Second, for proof that prophylactic protective compounds lower the amount of oxygen in tissue of higher organisms, certain investigators determined the oxygen pressure in tissues by the polarographic method, using an inserted platinum

electrode. For instance, based on the oxygen wave this method established the drop in oxygen pressure in rabbit tissues under radiation protection by tryptamine [4].

We can agree with these data if we take into account that the oxidizing properties of tryptamine are very doubtful. Certain investigators determined by this method the drop in oxygen pressure when large doses of cysteine, cysteamine, and other antioxidants were introduced into the organism [31]. The oxygen pressure can be determined polarographically on the platinum electrode, but this determination is indirect. The oxygen pressure is determined the effect of its concentration on the electrical reaction of hydrogen peroxide formation at the electrode. The antioxidants affect the rate of this process and lower its electromechanical potential. Therefore these data do not completely characterize the oxygen content, and indicate only to the presence of protective substances. Of course, between protective action of anoxia and the action of antioxidants there is a relation – inhibition of one and the same reaction. It should be noted here that a reduction of oxygen pressure guarantees maximum protection. This, of course, is explained by the fact that a reduction of oxygen in the tissues is accomplished easily and is not accompanied by secondary phenomena. The effect of the same prophylactic protective compounds is weakened by a number of phenomena. The protective substances in most cases have a toxic action, therefore it is impossible to use them in a high concentration. Their permeability into cells is very often insignificant, in any case they can compete with gaseous substances. The protective substances can be used up on various secondary reactions and can be oxidized by numerous substances available in the cells.

Presently known prophylactic substances and an oxia act on one and the same reaction. For instance, on irradiation of mice in a state of anoxia the protective action of cysteine is not demonstrated [32]. On a reduction of oxygen pressure cysteamine – the best protective preparation – does not ensure additional protection for bacteria [28]. In the absence of oxygen cysteine also does not ensure protection against irradiation of a suspension of rabbit thymocytes, etc. Certain indefinite results obtained previously are apparently explained by the fact that tests with animals are not always set up under conditions of a maximum level of protection by anoxia.

Under optimum conditions, however, protection by chemical agents and oxygen reduction is nevertheless incomplete. When the ionization density is increased, the protective effect is decreased, and when radiating with a high ionization density (α-Particles, protons) it completely disappears. Under optimum protective conditions (low ionization density) the best protective agents and anoxia cannot remove more than 50% of the dose. In addition, protective substances have a beneficial effect only for a very short period at the primary stages and during development of initial reactions. Many categorically affirm that the protective agents are effective only during irradiation, i.e., they react and find the radicals and peroxides induced directly by radiation, and after irradiation are completely ineffective. This is not so. They can have a beneficial effect after irradiation; however, it is significantly weaker. Their effectiveness is rapidly reduced in time owing to development of initial and competing reactions. This complies with the mechanism for inhibiting chain reactions in general. The inhibitors, as a rule, have a maximum effectiveness in the initial period of development of the

reactions, and then their effectiveness rapidly decreases. There are other explanations, however, for the decrease of efficancy under the biological action of radiation [33, 34].

The protective agents, in particular those containing the -SH group, when systematically administered after irradiation do not weaken, but enhances radiation lesion. This effect for cysteine was detected on mice under external and internal irradiation.

When 2-mercaptoethylamine and cysteine are introduced into irradiated mice in nontoxic doses their mortality increased when these preparations were introduced postirradiation. The low effect of oxygen protection, protection by chemical substances, and the negative effect of prophylactic protective substances when introduced postirradiation can be explained only by the fact that among the initial radiochemical reactions are those of a nonoxidizing character developing independently. Under the effect of ionizing radiations with a high ionization density, the antioxidants do not completely guarantee the effect, although here the oxidizing radicals and peroxides are formed in a larger quantity than under the effect of radiations with a low ionization density. Attempts were made to establish a relation between the redox potential of chemical systems and their protective capacity.

The redox potential was determined homogenates of animal organs injected with protective substances, as well as in model tests. These investigations did not reveal a correlation between the values of the redox potential and the protective effect. In living, functioning cells of organisms there is, as is known, a steady-state equilibrium, since these are open systems found in continuous exchange with the external environment. The existence of oxidation-reduction

steady-state equilibria among others is ensured by the entire structural mechanism of the cells. During the destruction of cells the oxidation-reduction dynamic (steady-state) equilibria in homogenates are disturbed, the values of potentials drop, and thermodynamic equilibrium is established which is completely inappropriate to that existing in the living cells.

A correlation exists, however, between the redox potential and the effect of protective agents. Only for this purpose it is necessary to determine the values of these potentials under conditions of steady-state equilibrium normal for cells. On measuring the redox potential in cricket hemolymph by microelectrodes it was established that the introduction of various protective substances and the creation of anoxia shifts the potential to the positive side [29]. Here the protective effect depends linearly on the value of the potential. Besides the general chemical properties determining the capacity of a compound to bind radicals and peroxides, and thus render a protective effect, of importance for its protective effect is the complex of additive properties of physicochemical nature which make it possible to accomplish this protective effect in complex organisms and cells. The capacity of protective substances to easily penetrate cells is of importance. The chemical potential of these compounds cannot be too high, since as it moves it will react too rapidly with different substances encountered along its path, for instance with oxygen, and therefore be expended before it penetrates the cells. An example is the low protective effectiveness and even its complete absence in strong reducers (hydroquinone and others). These substances should not be absorbed, since this reduces their capacity to penetrate cells. These characteristics can explain the absence

of effectiveness in a number of substances which are good acceptors of radicals and peroxides. It is because of this that we sometimes observe anomalies in the protective effect. For instance, while studying radiation hemolysis of erythrocytes it was established [26] that at a concentration of 10^{-2}M thiourea has a good protective effect, when the concentration is reduced to 10^{-3}M its protective effect is weakened, and when the concentration is reduced to 2×10^{-3}M it already activates the radiation hemolysis. This phenomenon is obviously connected with the principles of sorption on cellular surfaces.

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PRIMARY AFTEREFFECT REACTIONS

Principles of Development

As a result of the radiation effects in biochemical components of cells the following processes occur in sequence: 1) the absorption of radiation energy by the substance; 2) the formation of active radicals and ions — the conversion of radiation energy to chemical energy and 3) development of initial radiochemical reactions after direct irradiation.

The first and second processes are photochemical reactions. The quantity and quality of radicals forming here and their energy determine the possibility of the formation of intermediate active products which can initiate reactions with high quantum yields [1, 2]. Irradiation of organisms at low temperatures makes it possible to distinguish these processes clearly. For example, when studying the temperature dependence of the mortality of digitalis polen in a range of low temperatures, a value of 1.1 was obtained for the temperature coefficient, which distinctly indicates a photochemical process which determines further development of initial reactions [3].

A characteristic feature of the development of further reactions is the presence of high temperature coefficients (activation energy), therefore for many biological objects temperature characteristic can yield values of the order of 20,000-30,000 kcal. By virtue of this the rate of such primary reactions should severely drop with a drop in temperature.

In most cases a change in temperature during short-time irradiations does not effect the subsequent development of injury. For example, on irradiation of tadpoles of Rana cubitschiana the development of morphological indexes of radiation lesion (pyknosis, homogenization and fragmentation) does not at all depend on temperature within limits from 0 to 20°C in which irradiation was carried out [4, 6]. But if the irradiated tadpoles are kept at different temperatures, this very strongly affects the rate and degree of their damage on subsequent days. Upon a rise of temperature from 0 to 20° the temperature coefficient becomes equal to 2. Upon a drop in temperature there usually occurs a delay in the manifestations of radiation lesions, however with a subsequent rise of temperature the signs of injury again begin to rapidly develop [5, 7]. This demonstrates that the reaction can practically stop, its active intermediate products are retained for a long time at a low temperature and can again initiate the reaction.

One of the clearest papers in this direction was the investigation conducted on frogs [8]. If frogs are kept at a reduced temperature for a long time after irradiation, the development of reactions is stopped for several weeks. When the temperature was raised, radiation lesion developed at the same rate and intensity as in frogs irradiated and maintained at their normal temperature (25°C).

Analogous data were obtained on salamander eggs [9]. This means that as a result of the first photochemical reaction in cells and substrates of organisms, initial active products can form which trigger initial reactions with a high activation energy. In some cases, for instance irradiation of ascarid eggs kept in to the cold, not only is a delay of the phenomenon of radiation lesion observed, but also a subsequent increase of survival, i.e., a partial destruction of the active products or their washing out from the cells [5]. These facts do not contradict what was already stated, but show that in this case there occurs not a recovery of some structures (this is impossible at low temperatures), but a decrease in the amount of active products. Thus, on the basis of numerous investigations we can conclude that the magnitude of the temperature coefficient for the development of radiation lesion must be more than two and sometimes it even reaches 6-7. This, of course, is the total. When the temperature increases the rate of the metabolic reactions increases, however, it is significantly slower. The manifestation of the destructive effect caused by the development of initial reactions, or the effect of products formed during these reactions is possible only during metabolism.

A characteristic feature of the development postirradiation lesions is its phase quality and presence of an incubation period, during which the initial reactions develop at a very low, almost stationary level. Medicine has long differentiated three phases of acute radiation lesion: 1) initial reactions, 2) false sense of well-being, and 3) acute development of lesion. This system approximately characterizes the kinetics for the development of radiochemical aftereffect reactions, arising postirradiation in cells of not only

higher but, as current investigations show, in those of lower organisms.

It is usually shortly after irradiation, minutes or hours depending on the dose and rate of irradiation, that changes of a biochemical, physiological, and physciochemical character are detected in the organism. Immediately after irradiation the swelling capacity of cells increases and their volume somewhat increases [10]. Shortly after irradiation leucocytosis develops and an excited state of the central nervous system and in increase of the bioelectrical activity are observed. The state of hyperactivity is noted in various isolated cells. Numerous biochemical investigations show that shortly after irradiation we can note in the tissues activation of certain enzyme systems (protease, hydrolase, lipase) and sometimes — the opposite effect: their temporary inhibition, increased decomposition of the phosphorus compounds, disturbance of intermediate protein and carbohydrate metabolism, nucleic metabolism, etc. Under small doses these changes increase for a certain time, reach a maximum, and again return to normal. Under a high dose these changes increase more quickly, under higher doses this process becomes irreversible and leads to death of the organism [1, 2].

These changes are, of course, a reflection of the development of rapidly forming initial reactions with high ionic yields following the immediate effect of irradiation. Many investigators observed such reversible reactions at minimum lethal doses on different lower organisms (the simplest mollusks, hydra, and plants). After this period, if the doses are not too great, the organism returns to normal, and during this subsequent period it is difficult to detect in the cells any changes of a biochemical or physiological character

connected with irradiation. This is the so-called period of false sense of well-being. The changes are demonstrated when the organism dies after a long time interval (tens of days). The duration of this period in lower organisms is prolonged when the temperature is reduced; on the other hand, when the temperature is increased it becomes less. After the period of false well-being, signs of damage begin to develop rapidly.

The transition from the period of false well-being, when all the indices are normal, to the terminal period is characterized by a rapid increment of biochemical and physiological changes.

The general picture of the changes for various indices after irradiation indicates that in the first approximation the general aftereffect reaction is composed of two reactions with different time characteristics superposed on each other. Analysis of these changes shows that the two peaks of biochemical and physiological changes in the reaction of radiation aftereffects are associated with two different initial radiochemical reactions induced by radiation.

These characteristics of the reaction of radiation aftereffects are taken as the phase quality of the development of radiation lesion (Fig. 9).

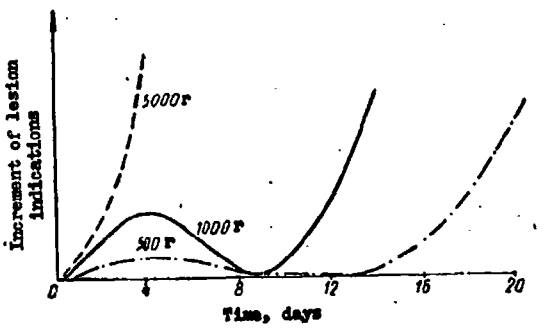


Fig. 9. Time-dose dependence of the character of increment of lesion indications in mamuals.

Kinetic analysis shows that in this case two reactions occur, having different mechanisms which develop independently each other. A very clear analysis of these two reactions was carried out on hydra [11]. After irradiation in small and medium doses the body contracts during the first 20 hours. Gradually, the length of the hydra decreases and reaches minimum. This reaction develops in time as a dying reaction with an exponential character of development. When the doses are large the specimen dies during the development of this reaction; at small and medium doses this reaction dies out after a certain time and the condition of the hydra becomes normal: it straightens out (Fig. 10).

The temperature coefficient of this reaction within 5-25°C proved to be equal to two. For 2-4 days the hydra behaves normally, then its body again begins to contract. This process of contraction now occurs by the self-accelerating type. The temperature coefficient of this second contractile reaction turns out to be equal to 3.5. This means that the initial radiochemical reaction outwardly yielding the same effect of contraction differ in nature. The peculiarities of the curves of increment of contraction demonstrate the principle difference of these two reactions.

The presence of two similar maxima was detected by studying the time-death dependences of yeast [12]. Here the dependence of the magnitude of these maxima on the dose was indicated (Fig. 11). With increase of dose the number of cells dying at the first maximum increases, and at very large doses they all perish and the second maximum is not formed. With decrease of dose (within limits of absolute lethal doses) the number of cells perishing at the second maximum, which ensues appreciably later, increases, and at minimum

lethal doses they all die. This character of the time-death distribution after radiation is explained only by the fact that of the two reactions forming as a result of irradiation, the first causes a smaller ionic yield, but is formed in time more quickly and with a small induction period. The second reaction has a long induction period, but develops with a high ionic yield. Therefore, the volume of lesion, in which irreversible pathological conditions are already developing, is attained in the first reaction under large doses, and not in the second. Since the first reaction is formed in time earlier than with large doses, it causes death of all organisms, and the second reaction does not have time to develop.

The probability of death at the second maximum will increase with decrease of dose, and the probability of death at the first maximum will increase with increase of dose. If we take into account the dependence of cell death on dose at the first maximum, we obtain an exponential curve; if we take the same dependence into account at the second maximum in the smaller dose range, we obtain a sigmoidal curve.

Analogous dependences were obtained for higher animals [13]. Data obtained by B. N. Rayevskiy [14], who took into account the dose-death time for mice, testify to the presence of two radiochemical processes (reactions competing with each other). One reaction has a high ionic yield, whose rate increases exponentially with dose and reaches saturation at a 5 kr dose, and the other has a lower ionic yield, which predominates at higher doses. As a result, the summation of these effects reach a plateau (3.5 days), which depends on the time parameters of these reactions. This constant magnitude can be changed not only by whole-body irradiation, but also by local

irradiation of individual systems and organs, since the reactions develop at different rates [15].

On the basis of statistical processing of data on death of mice at more moderate doses, Mewissen [16] also concluded that death by γ-irradiation is caused by two independently occurring processes separated by a time interval. The time-death distribution of mice at several maxima was also observed by N. V. Luchnik [13], who differentiated five peaks of death for γ-irradiated mice (Fig. 1,2). These peaks are significant, but they clearly characterize secondary processes. In the dose range with which he worked the second reaction was dominant and the first was reduced; however, in this case we can discriminate two main rises.

It was noted that prophylactic substances act differently on the death distribution at different peaks, however, these data are incomplete.

Thus, the few investigations of the quantitative character of the time-death distribution of organisms confirm the inconsistency of the assumption about one mechanism or one reaction responsible for the biological effect of ionizing radiations. In the initial event of irradiation many reactions occur in very diverse biological substrates. If these reactions do not form by the autocatalytic or chain mechanism, in which they involve hundreds and thousands of molecules into the reaction for a single initial event and thus ensure development of the aftereffect reaction, then they fade out, causing insignificant chemical changes which, however, can influence certain biological functions. When acute lesion and cell necrosis is used as the criterion, two competing action s are clearly manifested which develop independently of each other.

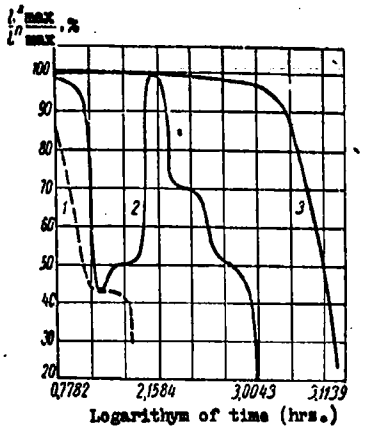
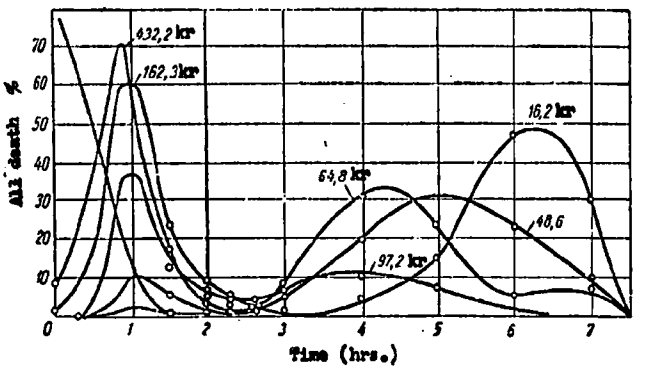


Fig. 10. Change in time of contraction of body of hydra after irradiation depending in the dose: 1) dose 40 kr; 2) dose 15 kr; 3) dose 3 kr.



Time distribution destruction of yeast cells after irradiation in various doses.

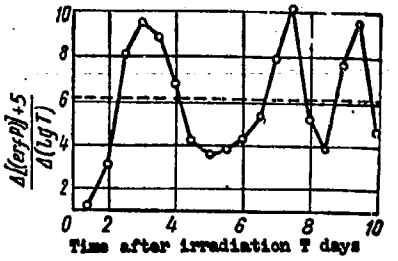


Fig. 12. Peaks of lithality for mice irradiated by γ-rays in 1000 r dose.

The general character of the development of signs of radiation lesion in time with small and medium doses of irradiation are similar to the kinetics for development of chain reactions. The external manifestations (biological reactions in time) are the result of complex, diverse processes, but they reflect the kinetic principles of initial reactions which produced them. The degree of development of these initial reactions and their significance in cell death depend on dose and rate (ionization density).

For the most part, reaction 1 (Fig. 13) with a long incubation period develops with the minimum absolutely lethal doses. This reaction has the highest quantum yield but it forms slower than the others; its incubation (induction) period depends on temperature and dose. This is clearly an oxidizing reaction. Investigations of the survival rate of diploid yeast at this peak showed that the incidence of death greatly decreased when the oxygen pressure and the effect of antioxidants was reduced. The reaction clearly indicates a chain reaction with degenerate branching. The following kinetic characteristics testify to this: 1) incubation (induction) period; 1) high (3.5) temperature coefficient and 3) the presence of oxygen limits (survival rate increases on reducing the oxygen

pressure and on increasing it above 1 atm and, what is particularity characteristic, the threshold effect is observed, as demonstrated in yeast).

Reaction 2, corresponding to the average maximum in Fig. 4 and to the firstin Fig. 5, is also an oxidizing reaction, however, its behavior with respect to a change of oxygen pressure is different. Inhibition of yeast cell death on reducing the oxygen pressure occurs only when the pressure is decreased. This confirms the oxidizing characteristic of the reaction. We cannot, however, relate it to a type of chain reaction with degenerate branching. The closer temperature coefficient also precludes this possibility. This reaction probably develops by a type of unbranched reactions, but considerably faster and with a smaller yield. Competitive relations between these two reactions, which apparently develop simultaneously in different substrates with different rates, are clearly expressed in the test of G. G. Polikarpov with hydra and A. D. Kolontarov with yeast (see Figs. 9-13).

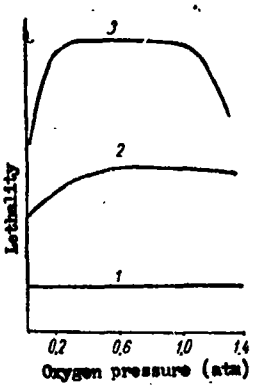


Fig. 13. Effect of oxygen pressure on destruction of yeast in 3 peaks (dose 60-300 r).

At average lethal doses, reaction 2 causes the biological reaction of contraction, but does not lead to destruction. The organism perishes at later time, during reaction 3. At more powerful doses (40 kr for hydra and 100 kr for yeast) the specimens perish during development of reaction 2, and reaction 3 cannot develop. Upon decreasing the dose to the minimum lethal dose, the development of reaction 2 does not even reach the limit at which the biological response is manifested, and only a remote contraction to the body is observed accompanied by death (reaction 3). At still higher doses, reaction 1 appears, which develops with the greatest rate and with the least quantum yield (see Fig. 10).

The death rate at the maximum of reaction 1 is completely independent of oxygen concentration (see Fig. 13). This reaction is clearly of nonoxidative character, and the protective substances, antioxidants, do not reduce death at this maximum.

Thus, under the action of ionizing radiations, in different biosubstrates all three reactions develop, from which the aftereffect reaction is added. These reactions, developing in parallel with different time parameters, produce in the complex biological system a well-defined response.

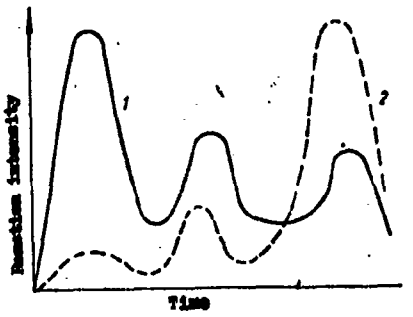


Fig. 14. Destruction distribution of organisms vs. ionization density in time: 1) α-rays; 2) γ-rays.

In the competition of these reactions their intensity and quantum yield are important. The probability that a decisive event (death, mutation, etc.) will develop as a result of one of these reactions will depend on ionization density. Scatter is obtained within the time limits for the development of each reaction as a result of statistical fluctuations in development and their yields. Therefore, the aftereffect reaction (biological effects) will be composed of three statistical distributions overlapping each other, but whose maxima will be distributed in sequence on the time axis (Fig. 14).

At greater ionization densities, the intensity of reaction 1 can attain a limit at which all the cells will die at the first maximum from nonoxidizing reactions. This is observed under the action of radiation with a high ionization density (α-particles, protons, etc.). Reaction 1 in this case will be decisive although reactions 2 and 3 will also develop; in the case under consideration the oxygen effect and chemical protection by antioxidants should be absent. At smaller ionization densities reaction 1 can cause partial destruction, but during reactions 2 and 3 only the cells remaining after reaction 1 can perish. At very low ionization densities, reactions 1 and 2 will produce very low yields and reaction 3 will be decisive.

Substrates of Initial Reactions

Many investigations are devoted to a study of radiochemical reactions in individual components of cells and tissues. They were conducted on nucleic acids owing to the importance of these compounds in biological processes and in hereditary mechanisms, on different proteins, lipids, hydrocarbons and enzyme systems in tests

in vitro. These investigations show that in various cell components the most diverse reactions can occur (polymerization and depolymerization in nucleic acids in proteins, denaturation, oxidation, and reduction). These investigations give an idea about the possibilities for developing chemical conversions in biosubstrates under the action of radiation. Unfortunately, among these investigations are very few in which the kinetics of these reactions were studied relative to the kinetic parameters and conditions actually existing in the cells. In many cases the course of the reactions was examined under conditions in which indirect action through the products of the radiolysis of water was accomplished. For instance, the study of the radiochemical reactions in the aforementioned substances was carried out with great dilutions in aqueous solutions, and as tests on solutions of enzymes and nucleotides indicated, the inactivation dose was reduced. These tests, however, do not explain the causes of high sensitivity of most organisms. These results were obtained under conditions not existing in cells when indirect action was disregarded.

Numerous biochemical investigations have been devoted to a study of the changes in biochemical components in radiation damage; it was not possible, however, to demonstrate the initial radiochemical reactions. Among the changes detected during development of radiation damage, it is impossible to differentiate the initial phenomenon from the secondary even in early periods. Attempts to postulate some selective effect ended in failure. The biochemical methods were insufficiently sensitive to show those insignificant initial chemical changes which occur in irradiated cells. It is necessary to also take into account that during the first moments of the development

of initial reactions, the secondary reactions begin to overlap them. Many investigators did not take the biochemical changes into account during irradiation and use of superlethal doses. This test setup is purposeless, since the development of not only initial but secondary reactions is accelerated. In addition, there are serious reasons to assume that under large doses, not only quantitative but qualitative differences develop. Nevertheless, from data of the study of radiochemical action in tests in vitro on irradiated organisms, by an appropriate manner we can obtain knowledge about the nature of initial reactions.

The earliest point of view based on biochemical determinations was the theory of Neuberg who asserted that the fundamental substratum on which ionizating radiations have in effect are proteins, since under the effect of radiation the proteolytic enzymes are activated. This theory did not receive due development although there was a grain of truth in it, and we should refer to certain of its aspects. The basis for rejection of the theory was data which showed that under the effect of ionizating radiation, proteolytic enzymes are not more radiosensitivity than other enzymes systems in addition, numerous investigations of proteins irradiated in vitro indicated that they are highly resistant to ionizing radiation. To coagulate and denature proteins by ionizing radiation, large doses are required, and even with these doses the protein molecules prove very stable and scarcely undergo decomposition. Numerous attempts to detect in irradiated cells of various organisms, changes which could be considered as denaturation were not successful, even with large doses.

Lately, however, investigations have appeared which allow us to assume that the reaction of proteolysis, which is characteristic for

the development of autolytic processes, must play a role in the initial processes. An increase of swelling of cells and their organoids, as was noted, is a very early physicochemical indication of radiation lesion when cells are irradiated even with small doses. Soon after irradiation we can observe an insignificant increase in cell volume (erythrocytes) in rats, mice, and rabbits [10]. The size of the nucleus in the cells of beansprouts soon after irradiation begins to increase, and after 40-45 hours its diameter is twice that of the nucleus of unirradiated cells.

These data show that in irradiated cells, reactions develop causing an increase of hydrophilism. Usually the increase of hydrophilism in different pathological processes is due to depolymerization of protein, the basic component of protoplasm.

On studying the electrical conductivity of rat organs after irradiation it was detected at various times [17], that the fundamental electrical parameters (capacitance, resistance) and frequency response, which are very good indicators of preservation of biological structures and cell death, retain normal values long after irradiation, i.e., during the incubation period no noticeable lesion of the basic cellular structures occur. If even at early stages, however, organs that are normal in all respects are held for several hours in the incubator at 40°C, then the differences between organs of unirradiated and irradiated animals is immediately detected. Changes of conductivity (decrease of the resistance and capacitance), characterizing the process of cellular decomposition, occur considerably quicker than in tissues of unirradiated animals as a result the development of autolytic processes in tissues of irradiated animals. When determining the increase of free nitrogen in such

tissues, i.e., a direct determination of autolysis, Yu. B. Kueryashpe and V. V. Lamsadze [18] showed that apparently completely normal liver tissues of irradiated rats and autolized considerally faster than tissues of control unirradiated rats. With respect to the increase of free nitrogen in blood of rats γ-irradiated, on incubation for several hours the autolytic process actively develops which leads to complete autolysis of blood within 8 hours [19, 20]. No indications of autolysis are observed in blood of unirradiated animals during this time. The effect of increasing autolytic processes in blood of irradiated animals appears at very early periods (20 minutes after irradiation in a dose of 600 r), and it can be detected under doses considerably lower than lethal [21].

Very interesting data were obtained by V. N. Benevolenskiy [22, 23] who studied the breakdown of liver extract from normal rats subjected to thermal denaturation under the effect of native extracts of liver from irradiated animals at different periods after irradiation. The appearance under the effect of these extracts of hemolytically acting substances, which are a mixture of unsaturated fatty acids, served as the criterion for breakdown [1]. Extracts from a normal rat liver do not cause breakdown or appearance of hemolytics. Extracts from the liver of irradiated rats soon after irradiation demonstrate an increased capacity to breakdown protein complexes with fatty acids, which in the free state are hemolytically very active. This capacity increases with an increase in time after irradiation [24].

Apparently a reaction soon develops during irradiation which loosen the bonds of protein-lipid complexes, and thus leads to breakdown of these complexes which are important in the construction

of structural cell elements. The protein separated from the complex becomes accessible to the effect of proteolytic enzymes (Fig. 15, and 16).

This assumption is confirmed by investigations of the conductivity of biological tissues [25, 26]. Change in conductivity, as is known, is a method by which we can obtain data on the condition of the structural elements in cells. The presence of electrical polarization (capacitance) is associated with the presence of polarized structures, and these structures are constructed basically from lipoprotein complexes. Destruction of these structures is accompanied by a drop in capacitance and electrical polarization. In rat liver, as was previously shown [26], very soon after irradiation the electrical parameters decrease, i.e., a shift of the curve of the loss maximum, on the basis of which we were able to conclude about certain decomposition of electropolar elements. In freshly prepared homogenates of rat liver electropolar complexes are preserved for several hours, as a result of which their capacity to polarize an electrical current is also preserved, and a dependence of electrical conductivity on the frequency characteristic for living tissues is observed [25]. With the same measurements on liver homogenates of rats irradiated in a dose of 800 r, the ability of homogenates, even in the cold, to retain electrical properties characteristic for living cells dropped abruptly.

Homogenates prepared from rat liver 24 hours after irradiation did not completely demonstrate the ability to polarize an electrical current.

The process of autolysis occurred so rapidly that complete breakdown of the complexes forming the structures occurred during

preparation of homogenates. Parallel with this it was shown that in the upper layer of centrifugates of the liver homogenate of unirradiated animals there is a considerable amount of protein along with the lipids. Under these conditions electropolarity was absent in the upper layer of centrifugates of liver homogenates of irradiated animals; protein was very low, and all of it was found in the precipitate.

Many investigators noted indications of autolysis in different irradiated cells and organisms. Therefore, an autolytic reaction leading to complete decomposition of proteins deserves particular attention as a reaction connected with initial processes.

The process of autolysis is characterized usually as activation of proteolytic enzymes, however, the mechanism of this activation is not clear. Under conditions of radiation lesion, it clearly develops after decomposition of the lipoprotein complexes. This total process, as is known, is activated by all factors inhibiting oxidation processes. Autolysis is activated in cells and organs when the concentration of oxygen and the effect of antioxidants is reduced. Good activators of the autolytic process are compounds containing sulfhydrl groups (cysteine and glutathione). The process of autolysis is clearly opposite to oxidation. This is the chain of hydrolytic reactions leading to decomposition of protein and lipoprotein complexes. In normal cells this process is clearly delayed. It develops only at a very low level. An organism apparently has substances which under normal condition retard the development of this process. It was previously established that blood, in its protein fraction (globulin), has such substances which retard the development of autolysis. The behavior of these substances in animal blood (rats)

when irradiated was studied by R. Kh. Mkartychyan [20]. As he pointed out, the blood of irradiated animals very quickly after irradiation begins to lose the ability to maintain autolytic processes in homogenous organs.

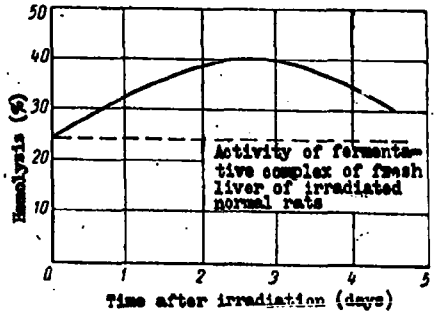


Fig. 15. Change of activity of fermentative complex causing formation of homolysis during radiation lesion.

The role of autolytic processes as initial processes is emphasized by the fact that compounds activating autolysis (cysteine, glutathione) when administered to animals after the direct event of irradiation impair their condition and increase mortality [27, 28]. The characteristic of protective agents, however, to have an activating effect on this reaction only after irradiation indicates that this reaction is not the first link in the chain of initial reactions.

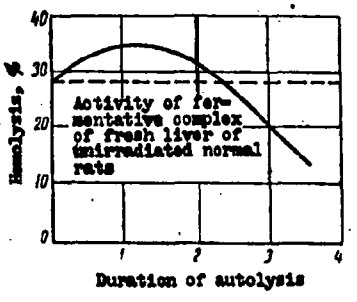


Fig. 16. Change of activity of fermentative complex causing formation of hemolysis during autolysis of liver of unirradiated animals.

As was indicated earlier, oxidizing reactions are induced under the effect of radiation in biochemical cell components. Among the substrates most unstable to oxidation are lipids. Fats possess a very high oxidation-reduction and negative potential. In them, particularly under the effect of irradiation and even without it, oxidizing reactions evolve and develop in the presence of air. These reactions are induced by peroxide radicals and their development by the chain mechanism is provided by peroxides which are formed in the intermediate links and which ensure branching of chains. The final products of these reactions are unsaturated fatty acids [24, 28].

In the last decade much evidence has been accumulated confirming the formation of products of these transformations in cells. This is an indication that radiochemical reactions develop in the cells of irradiation organisms. By using hemolysis of erythrocytes to demonstrate toxic products, it was established that in cells of the liver, spleen, and other organs after irradiation there was a continuous accumulation of agents homolytically acting erythrocytes [2]. These are unsaturated fatty acids. A characteristic peculiarity of the development of these toxic agents in contract to other biochemical changes was that the increase in concentration of these substances after irradiation occurs with self-acceleration (Fig. 17). The appearance of the hemolytic agent was detected by other authors. These unsaturated fatty acids are identical to acids possessing hemolytic effects which are formed during the usual autolysis of tissues [29-31]. Although the formation of these fatty acids can be detected in different organisms, the liver is the most significant object, since under treatment in the cold in the norm there are no unsaturated fatty acids in it. The appearance of fatty acids in the liver

cells of irradiated animals is obviously connected with those thread-like formations which were detected in the homogenates of this organ when they were examined under an electron microscope [29]. These thread-like formations have a lipid nature. This was established by their solubility in organic solvents. Their thickness is equal to 50-90 A, length 500-800 A. The threads thicken as the aftereffect process develops, by longitudinal cross-linking. Detection of lipid peroxides in butanol extracts from organs of irradiated animals is a very interesting fact, indicating that after irradiation oxidizing reactions develop in biolipids, since lipoperoxides are usually intermediate products of complex oxidizing reactions in lipids occurring by the chain mechanism. In lipids extracted from organs of animals oxidation branched chain reactions are easily induced when irradiated. Thus, by slow freezing of normal dog liver and extraction in the cold in vacuo, it was possible to obtain an almost oxygen-free butanol fraction. Under the effect of radiation a typical branching chain-type reaction with a well-expressed incubation period develops in this fraction at 40°C for 15-18 days. The final products of this reaction were unsaturated fatty acids. As was established, these unsaturated acids have a hemolytic effect and by their action they are identical to the hemolytic factors detected by A. S. Mochalinaya in organs of irradiated animals. The intermediate products are peroxides identical to those detected in the organs of animals after irradiation. Their quantity at the beginning slowly increases, then rapidly reached maximum, and again decreased in accordance with the kinetics of the formation of intermediate compounds in chain branching reactions. We studied the effect of different prophylactic substances on the kinetics of this reaction in

lipids of liver, sunflower oil, and pure oleic acid. It was shown that all the prophylactic substances are inhibitors of this reaction.

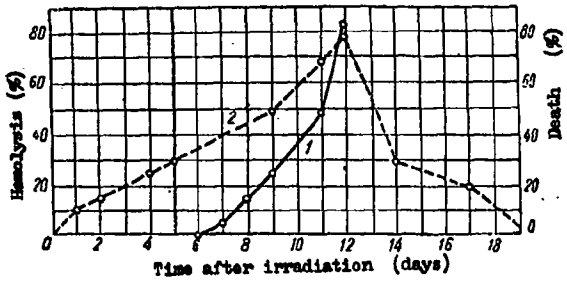


Fig. 17. Hemolytic activity for liver of irradiated rats (dose 1000 r): 1) destruction of animals; 2) hemolysis.

When cod-liver oil is γ-irradiated in vitro in the presence of air, a typical oxidation chain reaction with branching chains occurs for a day forms [32, 33]. The products of this reaction, fatty acids, have a hemolytic effect. Under the effect of γ-radiation on lipid (squalene), peroxide compounds develop. The high ionic yields allowed us to conclude that the chain reaction in this case is induced by radiation. The short investigative period did not allow us to completely elicit the kinetics of this reaction or to establish what product was the detected peroxides, intermediate or end.

In many lipids chain branching reactions form spontaneously in the air, but they develop very slowly. Under the effect of ionizing radiations the development of these reactions is greatly accelerated (for instance, in butter). Of interest are the data in which the active products of oxidation reactions in the lipids of organisms were detected after irradiation and their change in time after irradiation was indicated.

Upon a change in the number of lipoperoxides after irradiation, the nature of this accumulation in rat liver is the same as in tests in vitro, and its kinetics resemble those of the accumulation of intermediate products in chain branching reactions. In the liver of rats immediately after irradiation with a dose of 1,000 r, there is an increase in the amount of lipoperoxide compounds, and their amount declines on introducing 2-mercaptoethylamine. A similar increase in lipoperoxides was detected in various rat organs after irradiation with a dose of 1,000 r. Such a reduction in the number of peroxides was observed under very large doses (20,000 r). This indicates that the optimum doses for the development of this type of process corresponds to small lethal doses.

To determine peroxides, a reaction for reducing iodine, titanium and tin is used. A very reliable investigation of the change in the amount of peroxides in rat liver during the entire period of radiation lesion under lethal and sublethal doses showed [34] that the kinetics for peroxide accumulations liver lipids corresponds to those of the accumulation of intermediate products in chain branching reactions (Fig. 18). Immediately after irradiation the peroxide level becomes higher. Subsequent increase of peroxides after two days is observed only under absolutely lethal doses; with sublethal doses, the peroxide level begins to fall after an initial rise. These data over a wide dose range (200-10,000 r) indicate at least two chain reactions with different time parameters developing obviously in different lipid components.

The presence of peroxides in animal fats when irradiating with a dose of 1,000 r was previously detected by Ts. Bak.

Lipoperoxides were detected in animal tissues by a reaction with thiobarbituric acid. This method for determining is not direct, since thiobarbituric acid does not react with organic peroxides, but reveals oxidized products of unsaturated fatty acids (aldehydes and ketones). Therefore, it was a positive thiobarbituric reaction in homogenates of irradiated animals that was detected only after long incubation.

The presence of lipoperoxides developing after irradiation in organisms and their identity with lipoperoxides forming on oxidation and radiation oxidation in vitro, as well as the kinetic characteristics of lipid oxidation allow us to conclude that in living organisms one of the most important initial reactions is the oxidizing chain reaction with branching chains induced by radiation. The various lipids composing cells belong to readily oxidized substances. Nevertheless, the main structural elements of cells are constructed from them. As was indicated, lipids in cells and particularly structural lipids were protected from oxidation by antioxidants which by binding the intermediate products of simultaneous chain reactions, inhibit their development. The presence of the latter is easy to detect by its capacity to greatly inhibit the oxidation reactions developing in tests in vitro. The addition of ether and alcohol extracts from different animal organs to oxidized oleic acid in air retards its oxidation. We can judge the amount of antioxidants by the degree of retardation. According to A. I. Zhuravlev, antioxidants are contained in all animal organs [35-37]. Especially rich in them are lipids of brain tissue. They reduced the radiosensitivity of lipid formations in cells in general to ionizing radiations, act as a buffer, and bind the radiation-induced radicals and peroxides.

This, however, the antioxidants are consumed and their amount decreases. If the uptake of antioxidants from without does not compensate their consumption, the oxidation reaction in the presence of an antioxidant cannot be retarded at a normal steady-state level, and its rate begins to increase. As was noted, to transfer the reaction from a steady-state to a nonsteady-state condition in which its self-acceleration begins, it suffices to reduce the amount of antioxidant by only several per cents. A study of the kinetics of the accumulation of lipoperoxides in irradiated rat liver established [39] that simultaneously with an increase in the amount of peroxides and the transition of the reaction to a nonsteady-state condition, the amount of antioxidants continuously decreases, thus leading to death of animals (Fig. 19). Under nonlethal doses, restoration of the antioxidant is observed after a certain decrease. A postirradiation decrease in the amount of antioxidants during development of the aftereffect reaction was observed by A. I. Zhuravlev on rats and mice.

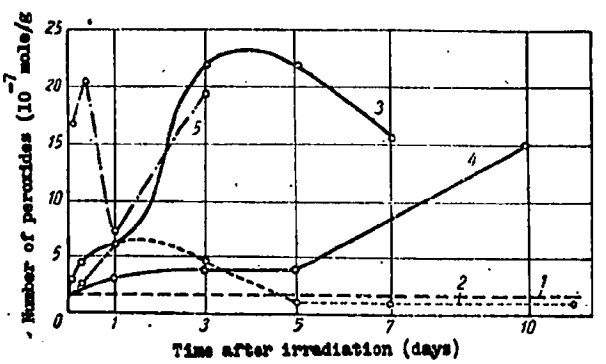


Fig. 18. Number of peroxides in liver of rats at different interval after irradiation: 1) control (unirradiated rats); 2) irradiation dose 200 r; 3) irradiation dose 800 r; 4) irradiation dose 800 r after introducing cysteine; 5) irradiation dose 10,000 r.

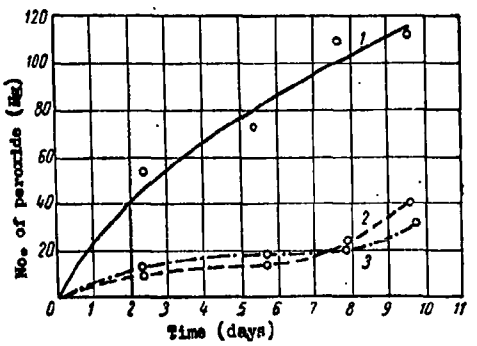


Fig. 19. Accumulation of peroxides in pure oleic acid in air when oxidized (1), when 1% ether extract from lyophilized rat liver (2) and when 1% alcohol extract from lyophilized rat liver (3).

Very interesting data on the development of the oxidation reaction in lipids of organs of living animals have been obtained by studying the superweak chemoluminescence of the liver of live rats [38]. Using a highly sensitive photomultiplier in quanti-metric conditions with deep freezing of the liver, it was disclosed that the liver of animals and other organs emit a superweak radiation in the visible range of the spectrum (blue-green of the order of 10-100 quanta/sec (10^{-13} erg/sec. $\times$ cm^3). Radiation was recorded in laparotomized animals. Analysis of different oxidation systems and fractionation of homogenates of liver showed that radiation develops in lipid fractions of cells when they are oxidized. As is known, chemoluminescence develops only during oxidation reactions, chiefly during reactions of chain oxidation, and during recombination of active radicals. Maximum scintillation (ractically 100%) is attained on interaction of antioxidants with the intermediate products of the oxidizing reactions. The natural antioxidants inactivate free radicals and inhibit the reaction

$$R + AH \rightarrow PH + A^* \rightarrow PH + A^{\cdot} + h\nu;$$

$$POO^{\cdot} + AH \rightarrow POOH + A^* \rightarrow POOH + A^{\cdot} + h\nu.$$

Under normal conditions this reaction occurs, but very slowly, and at a constant steady-state level. For steady-state equilibrium it is necessary to maintain the conditions of Prigozhin's equation: The Flow of Negative Entropy (the uptake of antioxidants and lipids into the structural elements of cells) should be equal to the flow of positive Entropy, which is approximately equal to the scintillation energy.

A similar steady-state condition exists in the norm, protecting the lipid compounds important for the life of the cells from

destruction. After irradiation the scintillation intensity for liver is increased, for a certain time stays at this increased level, and toward the sixth-seventh day after irradiation (dose 700 r, rats) significantly increases. This indicates that steady-state equilibrium shifts, and the number of radicals in the lipid systems continuously increases. Since in these conditions the flow of antioxidants from without cannot fulfill the deficiency of the antioxidant, the reaction begins to accelerate. The curves of radiation increase and accumulation of lipoperoxides coincide in time. In addition, these data agree with data obtained by A. I. Zhuravlev after studying the content of antioxidants in the liver lipids of irradiated animals. An increase in radiation dose is accompanied by a decrease in the amount of antioxidants in lipids. Directly confirming the role of radiochemical oxidation reactions in lipids are the data on the effect of protective substances. As a rule, all prophylactic substances (antioxidants) inhibit the chain reaction and lipids in tests in vitro, and this property is used for pretesting protective substances.

Many assumptions were expressed concerning the participation of nucleic acids and nucleoproteins in radiochemical initial reactions.

Under the effect of irradiation in vitro in aqueous solutions of these compounds, depolymerization phenomenona can develop. This was confirmed in certain investigations in vitro, in which it was established that depolymerization also occurs in tissues of irradiated animals. The significance of this reaction in the lethal effect is apparently not great. There are interesting studies which have established the development of peroxide compounds of nucleotides. At present, however, there is no information about the induction of kinetics of these reactions and their quantum yields. Apparently,

such radiochemical oxidation reactions can be induced in nucleotides. But the possibility of the formation of nonoxidizing reactions in this substate is of great interest.

Primary Toxins

Many investigators have repeatedly hypothesized that during the initial interaction between radiations and biological substrates, toxic substances develop in the cells which cause subsequent poisoning of organisms.

One of the basis for such an assumption was the analogy between the development of lesions by certain toxic substances and ionizing radiations. It was noted that when diphtheria toxin was injected into an organism, its damage suggested that of radiation: a long incubation period is observed and the hemorrhagic syndrome (capillary fragility) vigorously develop. These signs are characteristic for the development of lesions by other exotoxins. Damage by mustard gas and its derivatives occurs with similar characteristics. Mustard gas, in addition, causes hereditary mutation and this increases the similarity of its effect with the effect of ionizing radiations. All this gave grounds to combine the effect of these toxins with that of X-rays and radium into one group of damaging agents, the so-called protoplasmic toxins. Along with the similarity between the effect of different toxins and that of ionizing radiations, however, there are significant differences between them. The fact that the development of lesion and certain symptoms under the effect of protoplasmic toxins and radiations coincide is, of course, interesting. The toxic effect can appear in the course of radiation injury, but it still does not indicate the exclusive role of toxins in lesion.

In radiation lesion and during its development at later period, toxic substances, products of cell disintegrating, appear in the blood and in other body fluids which, as a rule, accompany any acute pathological process, but these toxic substances cannot be considered the initial toxins of radiation lesion.

Many investigations were conducted whose purpose was to detect the toxic products in radiation lesion [39-42]. The appearance of toxic substances was detected rather early. An hour after irradiation in the dose 600-200 r leukotoxins appeared in the blood of rats and porpoises; when the blood of irradiated animals was transfused into unirradiated animals, we observed in the latter shifts in the leukogram. Such toxic substances, however, were briefly present in the blood. After six hours the leukocytes disappeared, although radiation lesion progressed. It was assumed that the toxic substance characteristic of radiation lesion is histamine. Histamine-like substances appear during the process associated with cell destruction. The dynamics of histamine poisoning, however, are clearly different from the dynamics of radiation poisoning. For instance, there is no incubation period in histamine poisoning. Histamines, of course, are also present in the organism during the development of radiation lesion, but there are no grounds to consider them initial toxins. In a number of investigations attempts were made to detect the presence of toxic substances in irradiation by transfusing blood of irradiated animals into unirradiated animals. In a number of cases they cross-circulation was performed. The obtained results were rather contradictory. In some cases normal animals, into which the blood was transfused from the irradiated animals, demonstrated certain symptoms of radiation lesions, for instance,

alopecia. In most of these cases, it was impossible to detect any characteristic toxic effect of the irradiated organism on the unirradiated, [43]. It was noted, however, that the development of damage in irradiated partners occurred much more easily and their life span was longer than that of the control animals that received the same dose. Hence, washing-out of such products which appeared in the organism under irradiation has a beneficial effect on it.

Tests were conducted with separate irradiation of the nucleous and protoplasm in ova [9]. The irradiated cytoplasm, on contacting the unirradiated nucleus, affects it, and damage in the nucleus indications is noted which is of the same type as when the whose cell is irradiated.

The existence such agents in the cells after irradiation was demonstrated by the tests of A. N. Krontovskiy, who showed that the radiosensitivity decreases in cell cultures in vitro. If a chick embryo is irradiated with doses causing death after several days, and from this embryo take tissue, wash it, and cultivate in it vitro, then its development is completely normal. The existence of primary toxins were confirmed by tests which demonstrated that cell radiosensitivity increases when their concentration increases in cell suspensions. For instance, the radiosensitivity of erythrocytes increases, and radiation hemolysis occurrs more easily in them when their suspensions are more concentrated. On irradiating a colony of algae the lethal dose depends on the density of the colony, and on increasing the concentration, the dose is considerably reduced. Water in which protozoan are irradiated acquires toxic properties, especially when abundant organic substances exist in it. Irradiated plants affect unirradiated plants when grown together in a single

vessel or on a culture medium tobacco calluses and bean roots).

Hence, in the development of radiation lesion, as in other pathological processes, toxic substances appear because of metabolic disorders [44]. Among such substances, however, it was impossible to detect any substance which, when introduced into the organism, would cause a characteristic picture of radiation lesion. Sometimes, it is true, it was possible to induce individual symptoms of radiation sickness. It is difficult to decide whether such substances are toxic, having formed as a result of the development of initial reactions, or are secondary products of decomposition. The secondary effect is more probable in most cases. Among the products of initial reactions, however, there are substances which clearly have toxic properties.

Generally, the toxic substances are compounds having chemical activity with respect to the biochemical components of cells. Therefore, the compounds which are intermediate substances in complex reactions will have a toxic function in the primary reactions. Compounds which are end products of conversions can be such substances.

A number of investigators have detected lipoperoxides in irradiated organisms. As was already noted, peroxides are products of initial reactions [40]. Therefore it was hypothesized that the detected lipoperoxides are initial toxins. Some investigators attempted to prove this by testing on organisms the toxic effect of lipid substrates, whose peroxide concentration was increased by irradiation. This, for instance, was done with squalene. Lipoperoxides, as has been repeatedly demonstrated on the basis of the kinetics of their accumulation, are intermediate products in the oxidizing chain reactions developing in lipids. At the same time

end products are accumulated — mainly unsaturated and saturated fatty acids and their further decomposition products. The toxic effect is apparently produced by the unsaturated fatty acids. The intermediate products of chain reactions (peroxides) ensure development of the reaction and its branching. The introduction of additional peroxides into the oxidation reaction, of course, accelerates it. And since the oxidation reaction of lipids develops in the organism in a steady-state at a very low level, the peroxides should accelerate it, shift it from a steady state, and thus accelerate the processes of destruction. When we speak about initial toxins, it is assumed that in some event they appear, causing a new reaction qualitatively different from those in which they are formed. Peroxides are nevertheless necessary as a participant in the initial reaction of chain oxidation induced in the initial conversion of radiant energy chemical energy. They are formed and expended only for the development of this reaction. The removal of these peroxides from the fundamental reaction will weaken it and cause attenuation. At the same time the initial reaction is a toxic process, and we can speak about the toxic effect of peroxides only as a possibility of the quantitative increase of chains.

There are grounds to assume that end products of initial reactions can have a toxic effect. When vegetable oils are injected into mice, maximum toxicity is demonstrated when the oxidation reaction is retarded and the number of peroxides, as is known, decreases, whereas the number of end products reaches a maximum. Unsaturated fatty acids, the end products of chain oxidation of lipids, are toxic substances.

To take into account the possibility of a secondary toxic effect during the development of initial reactions of radiation lesion, the toxic effect of different products forming during chain oxidation of oleic acid, a basic component of lipids was studied in detail [39, 40]. Oxidized oleic acid has a secondary toxic effect. The greatest toxic effect is rendered by the end products, the products of oxidation (aldehydes and ketones), which can no longer induce the oxidation reaction in lipids and destroy other substrates, inducing them a different reaction. These oxidation products can enhance the processes of autolysis and this obviously is very important, since the process of autolysis is one of the most important links in the chain of initial reactions of radiation lesion. Aldehydes, ketones, and unsaturated fatty acids when introduced into an organism produced certain symptoms similar to those of radiation lesion. For instance, the change in the blood when poisoned by end products of oxidation conversions in lipids is similar to the change in the blood under irradiation. The cause of death of animals in this case is hemorrhage. The effect of these products was studied on two races of yeast — haploid and diploid. The diploid race, more resistant to irradiation, proved more resistant to the effect of oxidation products of lipids. The radiosensitivity of the haploid race is considerably higher. The haploid is more sensitive to lipotoxic compounds. Here the nature of the dose-death dependence is the same as in radiant.energy. For the haploid this dependence is described by an exponential curve, for the diploid, by a sigmoid.

The question of the role of toxins formed during development of initial reactions is of interest for understanding the mechanisms of the incidence of radiation-induced mutations. As is known, numerous

chemical substances exist which when introduced into the organism produce and activate mutation process. At one time the mutagenic effect was ascribed to hydrogen peroxide. Studied appeared in which it was confirmed that lower organisms produce numerous mutations when hydrogen proxide is introduced into the culture medium. This point of view, however, proved inconsistant. More tangible is the possibility of the manifestation of a mutagenic effect by decomposition products of organic molecules, originating during initial radiochemical reactions in various biochemical cell substrates [45-49].

The mutation process during irradiation as a whole explicitly depends on the development of reactions of a chemical and even chain nature. It is difficult to assume that such reactions could develop on some substrate where the genetic information is fixed forced. This would have to be accompanied by two great a rearrangement in the organism. The dependence of the yield of mutations on temperature and effect of protective agents is most probably connected with the development of initial reaction. During these reactions active mutagenic substances are formed, and each delay in the development of these initial reactions or their acceleration is reflected on the yield of mutagenic substances. Certain of these substances are clearly formed under radiation oxidation of lipids. It is known, for instances, that active mutagenic substances include the so-called epoxides. These products are obtained on oxidation of hydrocarbons and fats by hydroperoxides, wherein the characteristic bond CH-CH is formed. Such substances are formed during irradiation oxidation of oleic acids. In addition, the end products of lipid destruction, are aldehydes and ketones. These products also have a mutagenic effect. Their appearance was observed not only in tests in vitro,

but in lipids of rat liver in radiation sickness. Unconditionally, the question of the role of toxic substances formed during initial radiochemical processes in irradiated cells is very urgent. The appearance of radiomimetic substances was associated, apparently, with many manifestations of radiation lesion. Among these toxic agents a major role was played by the products of oxidation chain reactions in lipids induced by radiation. As was already noted, in initial mechanisms the most important role belongs to autolytic processes in which destruction of proteins occurs. These processes are antagonistic to oxidation processes and are activated by antioxidants. It is very probable that the incorporation of autolytic processes at early stages occurs under the effect of the toxic decomposition products of lipids which activate this process.

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RADIOSENSITIVITY

Radiosensitivity is determined basically by the conditions for the development of initial reactions. Where, by virtue of physico-chemical conditions, these reactions develop with a high quantum yield, the radiosensitivity is higher.

As was already noted, the basic initial reactions which are significant in the development of radiation damage are not reactions fundamentally new for the cell [1, 2]. These reactions destroy the cellular structures, developing at a very low rate, since in a steady-state they are suppressed by inhibiting substances present in the organism [3]. Under the effect of ionizing radiations, these reactions are activated, their steady-state is disrupted, and they begin to develop with self-acceleration. Therefore, radiosensitivity is associated with the reactivity of different biochemical components of cells, their stability, and the properties of natural inhibitor-antioxidants, antiautolytics, which stabilize the structural elements and protect them from destruction under natural conditions and under the effect of ionizing radiation, by inhibiting the development of radiochemical reactions [4,8-12].

In plant cells the role of radiation protection is performed by plant pigments — chlorophyll, carotene, and others. Pigmented plant cells as a rule have a sensitivity many times lower than nonpigmented cells. Photosynthesis of leaves is not disturbed even under large doses of ionizing radiations.[5-7]. At the same time, nonpigmented cells of rootlets have a rather high radiosensitivity (LD_{50} = 180 r).

Plant pigments can react with peroxide radicals and peroxides, both organic and inorganic. Their oxidation-reduction potential is

more negative than most biochemical compounds making up the cells, therefore, they react more energetically than other compounds, for instance lipids, with the intermediate active products of oxidation chain reactions and inhibit them [4]. Thus they are natural protective substances. Caroteinoids is a much stronger protective substance than chlorophyll (its oxidation-reduction potential is more negative than that of chlorophyll), therefore their presence protects chlorophyll both from oxidation under normal conditions of photosynthesis and under the effect of ionizing radiations.

On studying the kinetics of radiation oxidation of oleic acid, it was shown that the addition of chlorophyll retards the oxidation reaction by 10-12 times, and the amount of peroxides is correspondingly reduced [2]; here chlorophyll has a weak chemoluminescence in the red region of the spectrum. Carotene has a stronger effect, while the pigments themselves are oxidized.

In many lower organisms low radiosensitivity is determined by the presence of caroteinoids. Radiosensitivity of various races of bacteria Sarcin was connected with the presence of carotene in them. Carotene-containing races are very resistant ionizing radiations, and for some of them the semilethal dose reaches 10^6 r. The races having no carotene have a high radiosensitivity [8].

In cells of living organisms a significant role in their radiosensitivity is played by natural antioxidants, particularly by lipids which protect the structural tissue elements from oxidation. Especially important among them are tocopherol, vitamin E, and others. As a rule ether and alcohol extracts from animal organs when introduced into oleic acid oxidizing in air and other lipids, retard this oxidation [4]. The greatest amount of antioxidants is contained

in lipid extracts of liver and brain tissue; extracts from these organs are more active than those from muscles and connective tissues [11, 12].

It was noted that after preliminary nonlethal irradiation the radioresistance of higher animals, mice, and rats increases by several per cents (10-20). After γ-irradiation in a dose of 200 r the amount of antioxidants and lipids of rat liver drops by 10-15%, however after this the number of antioxidants is restored for several days, and then increases, and after 10 or more days the general level of antioxidants and lipids becomes higher than before irradiation [9]. This is, of course, a biological adaptation reaction.

The radiosensitivity of organisms is determined by other systems inhibiting the formation of spontaneous destructive reactions. The natural substances inhibiting autolysis are very important. Activation of autolytic reactions, as was already noted, is very important in the initial mechanisms of radiation injury. In blood there is a substance bound with the globulin fraction which retards the development of autolysis. As has been demonstrated in tests on rats, the amount of this antiautolytic in blood decreases after irradiation.

In addition to inhibiting systems, interceptors of peroxides, and radicals, the radiosensitivity of organisms determines the removal of active peroxides and radicals to the external medium. We must take into account that organic peroxides and radicals forming under irradiation obviously have a comparatively long lifetime, measured in several seconds, with a large surface and small cell concentration. The toxic end products of the reaction, can diffuse from the cells into the medium. This factor is important for lower bacterial suspensions and cellular suspensions and less important

for the dense packing of cells in organs of higher multicellular organisms. This is confirmed by evidence that the radiosensitivity of cellular suspensions (erythrocytes, bacteria, protozoans) depend on density of their suspensions. The radiosensitivity, for instance of erythrocytes, to the hemolytic reaction greatly increases when their concentration is decreased. On changing the concentration from 0.1 to 1%, the radiosensitivity increases 2-3 times. Liquids, in which cells are suspended, after irradiation acquire a toxicity which is greater, the thicker the suspension. One of the most important factors on which the development and rate of initial reactions depends is the general level of metabolic reactions. Radiosensitivity increases with an increase in the rate of metabolic reactions. As is known, radiosensitivity of higher animals is lowered when they are in hibernation (marmots, bats) [10].

It is assumed that the radiosensitivity of higher cold-blooded animals is lower than warm-blooded animals. These data, however, are inaccurate. The radiosensitivity of cold-blooded animals increases with an increase of the temperature, and can attain values characteristic for warm-blooded animals. We cannot examine the increase of the radiosensitivity exclusively as an increase of metabolic reactions. Intensive metabolic reactions producing active intermediate products facilitate the development of initial reactions induced by irradiation. It is well known that the toxic effect of different toxins, especially protoplasmic, to which the radiomimetic substances are related, sharply increases with an increase of metabolism. However, retake the increase in radiosensitivity with rise of temperature cannot wholly be due to an increase of metabolism. In this case the dependence is more complex. We need

take into consideration that the radiochemical reactions induced by radiation in the biochemical components of tissues and cells have very high temperature coefficients.

The highest temperature coefficients are those of chain reactions developing in the lipids by the mechanism of branching reactions. The magnitude of the temperature coefficient in this case attains 4-9 in the temperature range of 20-40°C. This shows that on increasing the temperature of an irradiated organism from 10 to 35°C, the intensity of the initial reactions can be almost doubled.

Besides the general radiosensitivity of organisms on the whole, we can note the radiosensitivity of separate cell organoids and separate functions. Lately, much interest has been raised by the question of the radiosensitivity with respect to the yield of hereditary mutations induced by ionizing radiations. In the light of new investigations we must abandon the formal concept that this phenomenon is connected with a one-state destruction of genetic information in nuclic acids and as a result of the impingement of energy quantum upon the appropriate site of the gene under direct irradiation. As a consequence of such an understanding, the concept arose of the independence of genetic mutations on environmental conditions and physicochemical agents.

Investigations have appeared, however, which showed that mutagenic changes occur as a result of the development of processes after the direct event of irradiation, which clearly have the dimensionality of chemical reactions. Mutation radiosensitivity depends on the development of the aftereffect reaction. It is also associated with radiation-induction of oxidative radiochemical reactions developing by the mechanism of the chain branching process [13-17]. For

instance, it is characteristic that the incidence of chromosome aberrations in cells of spiderwort decreases with oxygen concentration. The curve for the dependence of oxygen pressure on incidence of aberrations has the same threshold characteristic as curves obtained for the survival of organisms. This dependence, as already pointed out, is characteristic for oxidative chain branching reaction (Fig. 20). Similar investigations conducted on fruit flies showed that irradiation of juvenile instars in nitrogen and an atmosphere CO decreases the number of mutants. This protective effect is manifested most strongly upon irradiating immature sperms, spermatides, and spermatocytes. Mutability and development of mutagenic changes in molds greatly decreases upon introducing cysteine, a well-known protective substance [14, 15, 17]. In certain works it was established that the number of mutations depends on the temperature under which the fruit flies are maintained after irradiation.

In cells of Ehrlich's ascites carcinoma, it was shown [13] that the chromosomal damage both under irradiation in vivo and in cell cultures depends on the oxygen pressure. A typical threshold curve has been obtained in the case under consideration. The protective chemical substances reduce the mutagenic effect which, for instance, was shown on the bean rootlets and corn [18]. The effect of radiation on the translocation of chromosomes in nuclei is attenuated if during irradiation the roots are placed in solutions of protective substances, antioxidants [19]. All this shows that mutagenic radiosensitivity was related with the occurrence of radiochemical oxidation reactions developing in time after irradiation. The effect of these initial reactions on the mutagenic effect was probably indirect, i.e., the destruction of hereditary information occurs as a

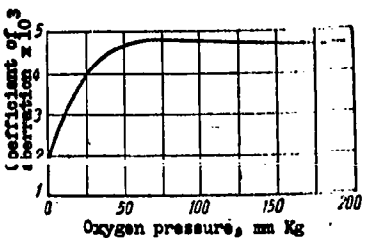

Fig. 20. Effect of oxygen pressure on the number chromosome aberrations during irradiation of Ehrlich ascite tumor cells.

result of the formation of toxic products possessing a radiomimetic effect. Radiosensitivity of organisms is evaluated in different ways. Such a lack of coordination is because many investigators did not at all take the time factor into account. Basically, destruction of organisms by medium and small lethal doses depends on the development of initial reactions of the autocatalytic and chain type in time after the direct event of irradiation. During direct irradiation only the initiation of these reactions occurs. These reactions mainly develop during the aftereffect period (incubation period), during which it is simply difficult to detect them. The arbitrariness of calculating organism death in time after irradiation yields extensive deviations in the data on radiosensitivity.

Evaluation of radiosensitivity of higher animals is always conducted with regard for the aftereffect whose duration is about 30 days.

In lower organisms this radiosensitivity is determined most often without taking the time factor into consideration during their slow death after irradiation.

At present it has been proved that for different groups of organisms, down to protozoans and microbes the aftereffect is characteristic. For instance, the semilethal dose for mollusks, when calculating their death soon after irradiation, is equal to 10^5 r, however, if this dose is taken into account for death 2-3 months postirradiation it will be equal to 10^3 r, i.e., it will be very close to the lethal dose for higher animals [1].

As many investigators showed, the presence of the aftereffect reaction was detected in many protozoans (yeast, infusoria, viruses).

The conditionality, however, in determining radiosensitivity was associated not only with an underestimate of the time factor. Initiation (formation) of initial reactions during irradiation and the subsequent kinetics of development of initial reactions depend on the conditions and regime of irradiation. As was already pointed out, the formation of chain reactions and their development at the first stages are defined by statistical principles, especially when the amount of energy is small, as is observed under the effect of radiations on biological objects.

The formation of chain reactions is determined by the probability that, with a given ionizing event sufficiently active radicals will form in some biosubstrate, which can be reaction centers. This probability is small and far from all ionization events will be reaction centers. When the reaction has started and the chain has developed, there is always the probability that this chain will be broken, forming a small number of links.

The dependence of survival and death in each cell is determined by the probability that in a given volume during the formation of ionizing events, a reaction will begin and this reaction will develop to such a volume (number) of links that during an irreversible change will occur in the cellular structures and normal function will become impossible.

The probability of forming a reaction and its continuation, of course, will depend on the concentration of radicals. On increasing the ionization density, the concentration of radicals increases and the probability of the development of chains increases, therefore radiations with a high linear ionization density are more effective and the radiosensitivity of organisms is higher.

A factor playing a major role in determining radiosensitivity is the capacity of cells for "repair." Repair, a decrease in radiosensitivity, occurs when, for instance, the dose of irradiation given is not continuous, but fractional, with time intervals between separate portions of radiation. To produce an effect which is attained for the same dose given continuously, it is necessary to increase the integral dose. In fractional irradiation the dose, so to speak, partially disappear and is not summed. The longer the interval between irradiations, the more it is necessary to increase the integral dose. This is because during these intervals, radiation-induced damage is repaired. For instance, a given semilethal dose on mice completely disappears if the second half of it is given after 7-8 days, i.e., complete repair will occur in this time interval [22].

Repair also explains the decline in damage when, after irradiation, the lower organisms are transferred to conditions where the metabolism is attenuated by cutting off food or by lowering the temperature [20].

To apply the term "repair" to these phenomenon is incorrect. During irradiation or immediately after it with normal low lethal doses, no disturbances can be recorded. And if they are, they are so insignificant that they do not have any effect on the vital activity of cells. The phenomenon of repair indicates not the repair of damages that have occurred, but the fact that under fractional irradiation or decline in metabolism the aftereffects are attenuated and this damage does not develop after a certain time interval. This is not repair but retardation (inhibition) of reactions developing during the aftereffects.

As was previously indicated, in the formation and development of complex reactions, especially of the chain type, or at early stages, a major role is played by statistical principles. The probability of the formation and development of reaction β depends on the amount and the reactivity of radicals and ions of peroxides, which are intermediate substances (promoters) of the reaction, and on their coefficient of regeneration and multiplication during its development. The probability of terminating reaction $1 - \beta$ depends on the extent of damage to the active intermediate reactions owing to their recombination with extraneous substances not participating in the process and their drift from the reaction zone. If $\beta > 1 - \beta$, the reaction will develop with acdeleration, if $\beta = 1 - \beta$ the reaction, for instance, oxidation in the presence of antioxidants, will occur at a constant rate, and if $\beta < 1 - \beta$ it will die out. This is the process which is called repair.

Conditions under which recombination of radicals, ions, and peroxides of toxic substances will be decreased or these conditions will promote their diffusion drift from the reaction zone will increase the probability $1 - \beta$ for terminating and attenuating the intensity of development of the initial reactions, and thus attenuate the extent of damage and reduce radiosensitivity.

The temperature is one of these factors. A dropin temperature lowers the reactivity, lengthens the lifespan of active intermediate reaction products, but has an insignificant effect on the diffusion rate. Thus, the possibility of diffusion drift of the active products from the reaction zone is increased. In a number of cases, a drop in temperature after irradiation reduces radiosensitivity for a while, attenuating subsequent development of aftereffects both in

higher, and in lower organisms. There are, however, exceptions which indicate that active products are stably bound with the substrate where the reaction occurs. A second active factor reducing injury is the decline in metabolism, obtained by lowering the temperature and cutting off the food supply. Such a reduction of radiosensitivity has been detected on transferring irradiated yeast cells to a non-nutrient medium [20]. This is completely understandable. Their inoculation on a non-nutrient medium stops reproduction and growth, i.e., the processes during which radicals and peroxides are formed in cells, increasing their general balance in the cells, and aid the development of initial reaction. Hence, the probability of termination is increased, since the products of the reaction, for instance, toxic products, enter into reaction with metabolic products and, not being partners, react with other molecules or diffuse from cells [21]. In diluted suspensions of yeast, recovery occurs considerably faster than in pressed yeast; in the presence of oxygen it is more intense than when its concentration is reduced. On increasing the temperature, this process is accelerated. The curves shown in Fig. 21 reflect the die out of initial reactions and decline in radiosensitivity associated with them. This die out is due to secondary chemical reactions, during which the peroxide radicals and peroxides which formed during initial reactions, are consumed. The diffusion processes obviously play a smaller role. In the case under consideration, the curves obtained at large doses when survival of organisms is increased, are indicative. With smaller doses the insignificant differences are because the limiting threshold (death-life) is easily attained under both conditions. For this purpose it is not necessary to postulate the presence of any other mechanisms [22].

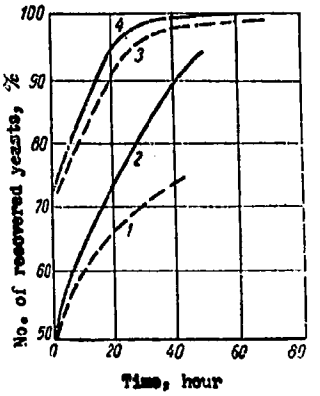

Fig. 21. Recovery of yeast (diploid) in time in noncultured medium vs. temperature when irradiated in dose of 60 and 40 kr: 1 and 3) 15°C; 2 and 4) 30°C.

To an equal extent retardation of the development of initial reactions should ensue under fractional irradiation. The probability of the development of reactions decreases with a general reduction in the number of initial centers. Therefore, discontinuation for a certain time of the external flow supplying the active initial centers in the initial stages before attainment of the critical threshold, after which the reaction can develop spontaneously, creates conditions for increasing the probability of termination.

Fractional irradiation is a special case of the general principle: on extending the dose in time, i.e., a reduction in rate, the effectiveness of the initial activation of chain reactions is weakened, since the steady-state concentration of active products is placed at a lower level. It is this that determines the higher sensitivity to radiations with a high ionization density.

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Conclusion

Data on the initial radiochemical reactions occurring in the biochemical substrates of cells are still very incomplete. Attempts to reduce the initial reactions and mechanisms to a single-valued solution (to attribute everything to some one factor) proved to be inconsistent. Formal physical presentations (target and hit theories) did not expose the real mechanisms underlying initial processes, and led only to the development of certain statistical principles.

In numerous papers on the effect of temperature and oxygen on the development of aftereffect reactions, it has been disclosed that the initial reactions are radiochemical, developing in time and after the immediate event of irradiation. Therefore, in the concept of the nature of initial processes of radiation lesion, the methods of chemical kinetics are importance. The first approached to the concept of physical and chemical mechanisms of primary reactions was the theory of indirect action. Without any doubt the water radicals take an active part in inducing the initial reactions in the organic phases of cells. In numerous papers, dedicated to the development of the theory of indirect action, however, the question was completely disregarded as to where, in what biochemical substrates are reactions induced, and what is their nature. In addition, the theory of indirect action completely ignored the possibility of the direct development of organic radicals in organic phases of cells. Arguments from the single idea of indirect or the direct action are worthless.

The initial activation occurs along both pathways. We are concerned about their relation. In most cases these two pathways apparently lead to activation of one and the same reactions.

A determining factor in the development of reactions with high ionic yields is not only the initial radical formation, but the reactivity of the corresponding substrate. The initial irradiation reactions are fundamentally not new reactions for the organism. This is mainly an activation of reactions occurring in cells of organisms in a steady-state conditions at a very low rate. Inhibition of initial reactions (oxygen effect, chemical protection, presence of active peroxide compounds, nature of the super-weak chemoluminescence) confirms the significance of oxidation reactions in initial effects. The main substrate of initial oxidation reactions are lipids, primarily structural. The kinetics of the change in peroxides, quantitative patterns of death in time, and kinetic characteristics of the oxygen effect – (threshold character)–all these are evidence that this reaction in lipids develops based on the principles of chain branching reactions and has high ionic yields.

Investigations show that this reaction develops according to the kinetics of chain branching reactions in the presence of inhibitors. Therefore, of importance in initial reactions is the nature of natural inhibitors of oxidation reactions – antioxidants. To a considerable extent the radiosensitivity of organisms is connected with their quantity and quality.

During development of this reaction, end products develop: lipoperoxides, unsaturated fatty acids, epoxides, aldehydes, and ketones which have a toxic effect, certain of which when introduced into the organism induce the appearance of toxic symptoms characteristic for radiation lesion.

The pattern of death in time and the temperature dependences allow us to conclude that, along with the indicated reaction, oxidation reactions occur with low quantum yields, but which develop at a high rate by reactions with nonbranching chains. A study of radical formation under irradiation in cells by the method of grafting polymers, the characteristics of the effect of protective compounds with an increase in ionization density, the absence of the oxidation effect with regard to mortality from particles with a high ionization density, and tests with individual biochemical components in vitro show that, along with the oxidation reaction nonoxidation initial reaction develop which are also important.

A study of the kinetic characteristics of the aftereffect reaction, of proteolytic reactions, and activation in irradiated cells of nonoxidation reactions by certain chemical substances shows that, basically, nonoxidation reactions induced by radiation are reactions entering the complex of autolytic reactions.

The major role in the formation of these reactions and the rate of their development is played by antioxidation factors, which, with respect to these reactions, play the same role as antioxidants with respect to oxidation reactions. These autolytic reactions develop with low ionic yields upon irradiation with low ionization density, and their yield increases greatly upon radiation with high ionization density. Since the differences in the rate of these reactions developing during initial stages are independent of each other, competition develops between them for the dominant role in attaining the biological effect. Domination of one reaction, however, does not preclude the possibility of the development of another. The absence of the oxidation effect and protective action of antioxidants

under high ionization densities is proof that under these conditions nonoxidation (autolytic) reactions are widely developed, and they play a primary role in the damage reactions.

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