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# RADIOBIOLOGY
# (SELECTED CHAPTERS)

By

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# UNEDITED ROUGH DRAFT TRANSLATION

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English Pages: 118

SOURCE: Radiobiologiya, Biologicheskoye Deystviye Ioniziruyushchikh Izlucheniy, Gosudarstvennoye Izdatel'stvo Literatury v Oblasti Atomnoy Nauki i Tekhniki, Moskva (Atomizdat), 1961, pp. 20-120.

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## CHAPTER II

## CHEMICAL EFFECT OF RADIATION

### Radiation Chemistry of Water

Water is the main component of all living organisms, in whom metabolic processes proceed intensely. The human organism contains about 65% water, and a number of his organs contain up to 80% water. Therefore, the attention of radiobiologists is attracted to what occurs in water and aqueous solutions under the effect of ionizing radiation. This is the subject of the radiation chemistry of water.

Let us examine what can theoretically take place on irradiation of pure water. The water molecules will be ionized. An electrically neutral water molecule will lose an electron and be converted to a positive ion. We can write this reaction as:

$$\text{ionizing radiation} \rightarrow H_2O \rightarrow H_2O^+ + \text{electron } (e^-).$$

The knocked-out molecule, being incorporated into another neutral molecule of water changes it to a negative ion:

$$H_2O + e^- \rightarrow H_2O^-.$$

Ions of this type, whose formation is possible only under the action of ionizing radiation, are extremely unstable. This is what distinguishes ions $H_2O^+$ and $H_2O^-$ from ions $H^+$ and $OH^-$ which are formed by electrolytic dissociation of water molecules. Free radicals are the product of the transformation of $H_2O^+$ and $H_2O$. Radicals are formed upon splitting water ions:

$$H_2O^+ \rightarrow H^+ \; OH\cdot \text{ and } H_2O^- \rightarrow H\cdot + OH^-$$

(the period by the chemical symbol means that the given atom or group of atoms are free radicals).

Therefore the free radicals H· and OH· originate under the effect of ionizing radiation on pure water. H· is atomic hydrogen. The hydrogen atom can exist in this state only a very short time, of the order of $10^{-5}$ or $10^{-6}$ sec. During this period, two atoms of hydrogen either combine, thus forming a hydrogen molecule, or the free radicals H· and OH·, formed on splitting of the water molecule, combine, thus creating a water molecule again, or H· loses an electron, giving it to another atom and is converted to the ion $H^+$, or, finally, H·, if some substance is dissolved in the water, can be attached to it. OH· is just as unstable. In this aggregate of atoms, one valency of oxygen is combined with hydrogen, and the other remains unoccupied. Substitution of a free valency in the OH radical is necessary for conversion to a stable compound. It is necessary that the unpaired electron become paired and that an even pair of electrons be in the molecule formed.

Figure 4 shows schematically the formation of the OH radical upon irradiation of water. The water molecule is on the left. On the outer shell of the oxygen atom entering into this molecule there are eight hydrogen atoms, two of which are combined with the oxygen atom. The water molecule is stable in this form. Ionizing radiation knocks out one electron. This event is shown by the wavy and straight arrows. A hydrogen ion is released and a free radical, electrically neutral, forms, since the number of electrons in it equals the number of protons in the nucleus of the oxygen and hydrogen atom. The free radical is very active chemically. The following reactions are most probable in the presence of dissolved oxygen in the water:

$$\text{Ionizing radiation} \rightarrow H_2O + O_2 \rightarrow OH\cdot + HO_2\cdot \text{ or } H\cdot + O_2 \rightarrow HO_2\cdot.$$

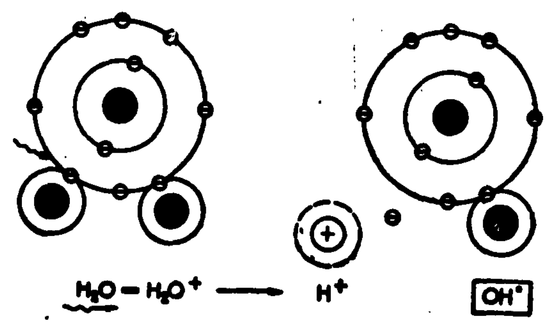



Fig. 4. Diagram of the free radical OH. In the water molecule, one atom of oxygen is combined with two atoms of hydrogen. On ionization one electron is split off from the molecule and the ion $H_2O^+$ is decomposed to a proton and the radical OH.

Thus, decomposition of water in which oxygen is dissolved proceeds with the formation of the $HO_2$ radical. Oxygen as a rule is dissolved in the fluids of an organism, therefore the formation of the $HO_2$ radical is, evidently, the first step in the conversion of energy of ionizing radiation to energy of chemical reactions. However, there is no direct proof of the formation of the $HO_2$ radical. It is assumed that the radical $HO_2$, on combining with another such radical, is converted to hydrogen peroxide and oxygen:

$$HO_2\cdot + HO_2\cdot \rightarrow H_2O_2 + O_2.$$

In the presence of a dissolved compound in the water, the radical $HO_2$ or, as it is called, hydroperoxide, by virtue of its oxidizing properties will remove an electron from the other compound, thus being converted to the ion $HO_2^-$, and the latter in all solutions except those strongly alkali, changes to hydrogen peroxide:

$$HO_2^- + H^+ \rightarrow H_2O_2$$

Hence follows that upon irradiation of water in which oxygen is dissolved, hydrogen peroxide should be formed. This actually occurs upon X- or $\gamma$-irradiation of such water. The hydrogen peroxide thus formed can be qualitatively determined by chemical methods.

Hydrogen peroxide is also formed in pure water without oxygen dissolved in it, but only when irradiated with $\alpha$-particles or protons, i.e., irradiation with great density of ionization. In this case OH radicals are formed in the immediate vicinity of one another, therefore the reaction of combining these radicals is most probable:

$$OH\cdot + OH\cdot \rightarrow H_2O_2.$$

Decomposition of water by $\alpha$-particles and protons leads to the formation of hydrogen peroxide and hydrogen.

The formation of hydrogen peroxide is possible in an irradiated organism, but this is difficult to prove since the cells of an organism have a very active enzyme, catalase, which breaks down $H_2O_2$ immediately into water and oxygen.

Figure 5 schematically shows reactions when water absorbs the energy of ionizing radiation. It is possible to detect ions in water experimentally by mass-spectrographic investigation of water vapors.

The formation of the OH radical is confirmed in the liquid phase of water by an indirect method.

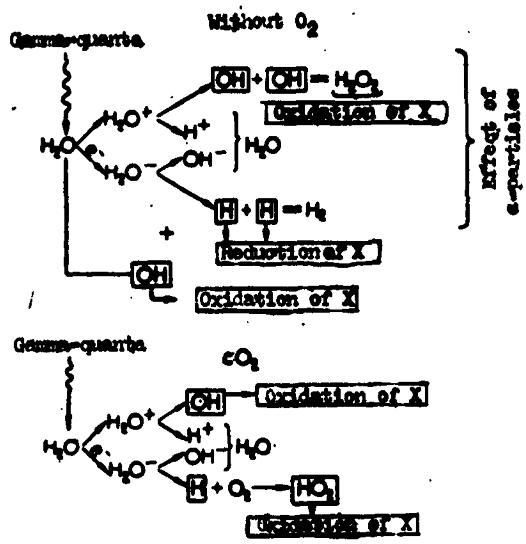



Fig. 5. Diagram of the effect of radiation on water. The diagram shows the effect of gamma quanta and alpha particles on pure water (without $O_2$) and on water in which oxygen is dissolved. X is the dissolved substance.

The formation of free radicals completes the physicochemical stage of the action of ionizing radiation on the absorbing medium. In the next stage, a chemical stage, the free radicals react with the substances dissolved in the water. This will primarily be an oxidation reaction, but reduction reactions are also possible. In chemistry, oxidation reactions proceeding with the attachment of oxygen, the removal of hydrogen, or the removal of an electron are called oxidation reactions. Reduction reactions, on the other hand, proceed with the removal of oxygen, attachment of hydrogen or an electron.

The chemical reactions occurring here are of interest to radiation chemists and biologists. The former are attracted to the study of such reactions relative to the possibility of using ionizing radiation as a powerful factor for changing a substance in order to impart properties to it that are beneficial to man. Many successes have been achieved in this direction which permit us to consider that, in the near future ionizing radiation will occupy a place among such factors affecting the course of chemical processes as temperature, pressure and catalysis.

Biologists investigating chemical reactions observable in vitro, seek a means to analyze the complex processes which take place in the irradiated cell and organism.

An example of oxidation as a result of irradiation of an inorganic substance is the oxidation of bivalent iron to trivalent. This reaction can be presented in the following form:

$$4Fe^{++} + 2H_2O + O_2 \rightarrow 4Fe^{+++} + 4OH^- .$$

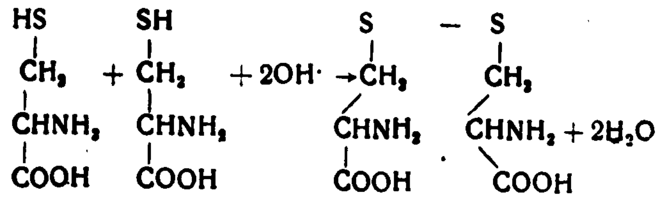
Free radicals oxidize iron. On oxidation of weak solutions of ferrous sulfate in 0.8 N of sulfuric acid, the amount of iron sulfate formed is directly dependent on the dose, so this reaction can be used for chemical dosimetry. For this purpose we can resort to other chemical reactions where the yield is in direct relation to the dose. In a medium containing organic substances, the formation of their free radicals, which are longer lived than water radicals, is possible. According to the theory of B. N. Tarusov, free radicals of fatty acids play an important role in the biological action of radiation. The method of electron paramagnetic resonance is widely used to detect relatively long-lived free radicals. Soviet scientists L. A. Blyumenfel'd and A. E. Kalmanson successfully used this method for studying radiochemical effects in biological objects.

## Irradiation of Biogenous Substances in Vitro

The endeavor of radiobiologists to learn what chemical changes occur in plants and animals after ionization made it necessary to study these changes in biogenous substances in vitro under maximally simplified conditions. The most diverse substances serve as objects of such a study. Most attention was devoted to an investigation of the action of ionizing radiation on the components of protein, the amino acids, and also on the simple and conjugated proteins, including proteins of the nuclei of cells and their constituents, the nucleic acids. Studies explaining the effect of radiation on enzymes and viruses pertain to this.

The amino acids are constituents of proteins. The proteins include more than 20 amino acids which differ in structure. The simplest amino acid, glycine, has the formula $CH_2NH_2COOH$. The $NH_2$ group conditions the basic properties of amino acid and the COOH group, the acid properties. The most common action of radiation on amino acids is the removal of ammonia ($NH_3$) from them (deamination), however such changes in amino acids are observed at doses considerably exceeding those which cause death of the whole organism.

Only in one amino acid are substantial changes observed at comparatively small doses of radiation. This is the sulfur-containing amino acid, cysteine; it contains the so-called sulfhydryl group (– SH). Under the effect of two free OH radicals, the two sulfhydryl groups, belonging to two molecules of cysteine, are oxidized (give up hydrogen), and the bond is closed between the two sulfur atoms, thus forming a single molecule of a new amino acid, cystine. This reaction can be written as:



At large doses other chemical transformations of the sulfhydryl group can occur, resulting in the formation of more oxidized products. The fact of the easy oxidation of the sulfhydryl group upon irradiation has attracted the attention of radiobiologists.

How proteins are modified upon in vitro irradiation. Proteins are the most important constituent of each living organism; their properties determine the development of the vital activity of an organism. There would be no life without proteins with their unceasing renewal, decomposition, and synthesis, without their continuous metabolism.

Approximately half of man's dry weight (without water), or about 15% of body weight, belongs to the protein portion, of which $^1/_3$ is in the muscles. There is a great multitude of proteins in the human organism, their purpose is very diverse: for example, such a protein as the collagen of skin, tendon, and bone is adapted to bear a support function, the hemoglobin of the red corpuscles for transferring oxygen from the lungs to the tissues, muscle proteins for performing work, etc. The general plan of the structure in the great diversity of proteins is similar. They all consist of a chain of amino acids joined together by an amino and carboxyl group. This combining is accomplished with the release of water particles and bears the name of the peptide bond.

The diversity of proteins in structure and function is provided by a set of amino acids in the protein, by the sequence of their bonding with one another, by the form of the protein molecule, i.e., by how the polypeptide chains, of which the protein molecule consists, are arranged in space. The molecule itself can have a different size. Protein molecules belong to macromolecules consisting of thousands of atoms. The most studied protein, a hormone of the pancreas, insulin, which regulates sugar metabolism in an organism, is built up from 51 amino acid residues; amino acid residues number 400 in the egg protein, albumen.

It is natural, of course, to assume that the proteins are the point of application of the effect of ionizing radiation on living organisms, especially if we take into account that the proteins include enzymes, the catalysts that affects the rate of biochemical reactions in an organism. Great efforts were expended to establish what changes proteins undergo when irradiated in vitro and to answer the problem whether such changes occur on irradiation of the whole organism.

At low concentrations of protein in an aqueous solution and relatively low doses of irradiation, chemical changes in protein will be caused by the products of the splitting of water, free radicals. The indexes of the irradiation effect are first of all the changes in the physicochemical properties of protein, its molecular weight, conductivity, optical properties, etc. These indexes testify that irradiation of protein in vitro leads to its denaturation, a coarse example of which is the coagulation of egg white on boiling. But denaturation of protein, as much as it can be judged by the indexes mentioned, ensues only at large doses, tens and hundreds of times exceeding those which cause death of an animal. For instance, one of the blood proteins, serum albumin, is denatured and precipitated at a dose of 72,000 r, whereas a 0.04% solution of egg albumen changes its optical properties at a dose of 4,300 r.

An exception relative to radiosensitivity are certain protein-enzymes whose activity is associated with the presence of free sulfhydryl groups (– SH) in their molecules. When it was revealed that the amino acid cysteine is easily oxidized to cystine by ionizing radiation, attention was turned to these enzymes. They are frequently called sulfhydryl enzymes. Most of them catalyze oxidative processes in an organism, i.e., have a relation to processes of cell respiration.

The sulfhydryl enzymes include myosin, the protein responsible for muscle contraction, which has the function of an enzyme.

Enzymes can be separated from an organism in a pure form so that they do not lose their activity, and this activity can be measured before and after irradiation. Measurements are made by taking into account the conversion of the substance, or as they say, the substrate of a given enzyme.

It turned out that myosin freshly liberated from muscle loses its enzymatic function in a diluted aqueous solution, in other words, it is inactivated by 10% at a dose of only 10 r; another enzyme, phosphoglyceraldehyde dehydrogenase, loses 21% activity at a dose of 100 r. It is interesting that this inactivation, caused by the effect of the OH radical, is fully reversible in the first case and by 62% in the second case, if after irradiation a substance containing the sulfhydryl groups is added to the enzyme. Such a substance is, for example, glutathione whose composition contains the amino acid cysteine. It is possible that before the enzyme is inactivated, its quality, specificity, is changed or the rate of lysis or synthesis of the active substrate of a given enzyme is inhibited, but these problems are still little studied.

On the basis of experiments on irradiating sulfhydryl enzymes in vitro, the American biochemist Barron hypothesized that under the effect of ionizing irradiation on the whole organism, the intracellular enzymes, whose sulfhydryl groups are oxidized, are the first to suffer. In his opinion, this is the beginning of disorders in metabolism leading in the end to the development of radiation sickness.

However, although rather many facts are satisfactorily explained by Barron's theory, data have been accumulated in recent years which contradict it. Thus, it was not possible to detect by present methods the fall in the amount of the sulfhydryl groups in blood and organs immediately after irradiation. Enzymes, inactivated by irradiation in vitro, remain active on irradiation of the whole organism where the free radicals will react not only with the enzymes, but also with other cell substances and the extracellular fluid. These substances serve to protect the enzymes from the effect of rays.

It could be suggested, as Barron did, that the organism has unique SH compounds which are represented by a small number of molecules, but which have considerable importance for the normal course of metabolism. Oxidation of the sulfhydryl groups in such molecules can remain unnoted against a general background of unchanged sulfhydryl groups, but will nevertheless be the first link in the chain of subsequent disorders in metabolic reactions. But such a suggestion is still not substantiated by experimental proof.

The facts contradicting the theory of Barron prevent its acceptance as a universal theory of the biological action of ionizing radiation. Nonetheless, this theory had a positive effect on the development of radiobiology in recent years. Many investigators, striving to confirm or refute this theory, set up and continue to set up numerous experiments which enrich radiobiology with many new facts, especially for protection against radiation damage (see below).

Changes in nucleic acids upon in vitro irradiation. Along with proteins and enzymes, the changes induced in nucleic acids by ionizing radiation are also persistantly studied.

These compounds are the components of complex proteins—nucleoproteins, which are contained mainly in the cell nuclei. On enzymolysis or by chemical reactions such a complex protein is decomposed to a simple protein, consisting only of amino acids, and nucleic acid, which in turn represents a very large number of "blocks" of four types which are called nucleotides. Each nucleotide consists of a nitrogenous base—a compound containing nitrogen and having basic properties, carbohydrate, and phosphoric acid. Only four types of nitrogenous bases are found in nucleic acid. Two of them are purine derivatives (adenine and guanine) and two are pyrimidine derivatives (thymine and cytosine) (Fig. 6). The difference in "blocks" is that the nitrogenous bases entering into them are different. All nucleic acids are divided into two groups. One of the grounds for such divisions is that different carbohydrates are found in the composition of nucleic acids. One group of nucleic acids contains sugar, deoxyribose, and the nucleic acid of this is called deoxyribonucleic acid (DNA); the other group contains a sugar, ribose, and the nucleic acid is called ribonucleic acid (RNA). This, of course, is not the only difference. The DNA molecule is larger than that of RNA. DNA has a molecular weight of the order of several millions ($6 \cdot 10^6$ to $8 \cdot 10^6$), whereas RNA has a weight of several hundreds of thousands. DNA is contained in the cell almost exclusively in the nucleus, and RNA in the nucleus and cytoplasm. The physiological role of the nucleic acids is dissimilar, although it still cannot be considered fully explained.

RNA and DNA can be separated chemically from the cells of an organism. The DNA molecule has a stranded structure. The length of the

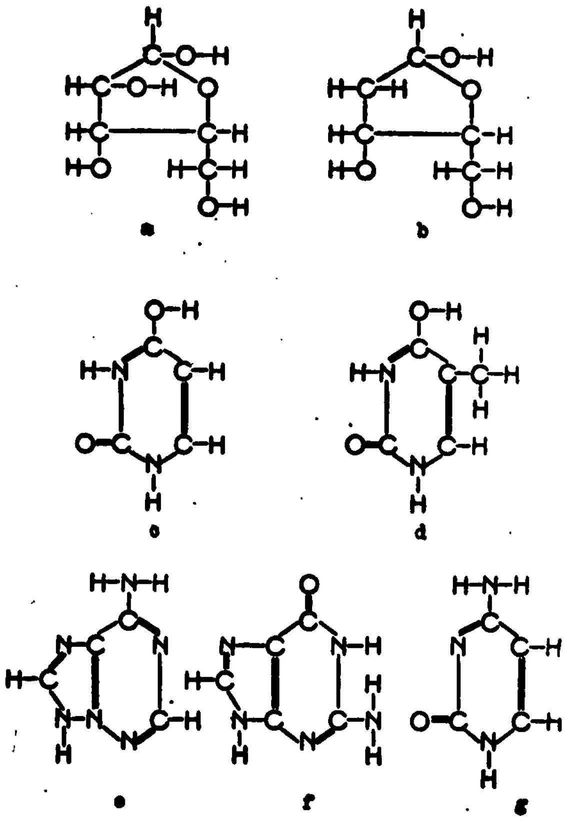


Fig. 6. Chemical structure of carbohydrates and nitrogenous bases participating in ribonucleic* and deoxyribonucleic acids: a) d-ribose; b) d-2-deoxyribose; c) uracil; d) thymine; e) adenine; f) guanine; g) cytosine.

* Ribonucleic acid also contains a nitrogenous base, uracil, but has no thymine.

molecule is thousands of times longer than its width. On the basis of the data of x-ray structural analysis and other investigations it was suggested that the DNA molecule consists of two strands wound helically around one axis like two strands of electrical wire. Each strand consists of phosphate groups and deoxyribose alternating along it. A side branch consisting of a nitrogenous base ramifies from the deoxyribose. It is bonded through a hydrogen atom with another nitrogenous base belonging to a parallel helical strand. The nitrogenous bases serve, so to say, as cross braces between them. They form pairs. Each pair consists of purine and pyrimidine; thus, adenine is bonded with thymine, and guanine with cytosine. The alternation of these pairs in the molecule can be very diverse. And this, evidently, explains the specific properties of each given molecule of DNA.

Figure 7 shows a planar model of a short segment of the DNA chain. Figure 8 shows the scheme of a spatial model of DNA. The DNA molecule is a polymer similar to nylon, but, of course, appreciably more complex.

In recent years nucleic acids of the RNA and DNA type have attracted the attention not only of radiobiologists, but also of scientists of quite different specialties, even mathematicians and philosophers. The reason for this is that the more familiar scientists become acquainted with the properties of nucleic acids, the clearer becomes the pathway of the synthesis of protein in the cell. As is assumed, nucleic acids of the RNA type are their own kind of matrix on which amino acids collect and bind in regular order, forming protein molecules specific for each cell. One of the proofs of the involvement of RNA in the synthesis of protein is that there is much of it in cells intensely synthesizing proteins (gland cells), for example, salivary, gastric, pancreatic glands, and little of it in biologically active cells (e.g., muscle cells), but which do not synthesize proteins.

The process of cell division is associated with DNA. This acid has a direct relation with the transfer of hereditary properties, being the main constituent of chromosomes (Chapter VI).

What happens to DNA when its solutions are irradiated in vitro? Here we most easily note the diminuation of the viscosity of the solutions, the more so, the greater the irradiation dose. If we recall that DNA is a polymer, then the decrease in viscosity can be explained by depolymerization, i.e., breakage of the DNA strands with the formation of shorter fragments. According to Alexander and Stacey, breakage can be detected when both strands, bonded together, are broken (Fig. 9) for which the expenditure of greater energy is needed than for breaking one of the two strands. When only one strand is broken, the changes in the DNA molecule remain hidden and can be revealed only by treating irradiated DNA with a solution of urea, which breaks the hydrogen bonds (Fig. 9). Alexander and Stacey established this with DNA extracted from herring-sperm heads, but it is possible that DNA from other sources is depolymerized by a different pathway. Chemical changes in the DNA molecule on irradiation of its solutions in vitro are possible according to new investigations. These changes occur in the nitrogenous bases which are released from the DNA molecule and are partially decomposed. Irradiation can lead not only to splitting of complex molecules, but also to "crosslinking" of individual molecules into larger ones, or to the formation of additional bonds inside the largest molecule.

Soviet investigators found that a decrease of the relative viscosity of nucleoprotein (A. G. Pasynskiy) and breakages of its strands (A. M. Kuzin and Ye. V. Burilova) take place at comparatively small doses, 100-400 r. An artificially prepared complex of DNA and serum albumin is also depolymerized at lower doses than each of the components.

In spite of numerous investigations, there still remains unresolved the problem: to what extent can the changes observed in DNA on irradiation in vitro help the understanding of what takes place with this important substance in the cell? The complexity here is that DNA

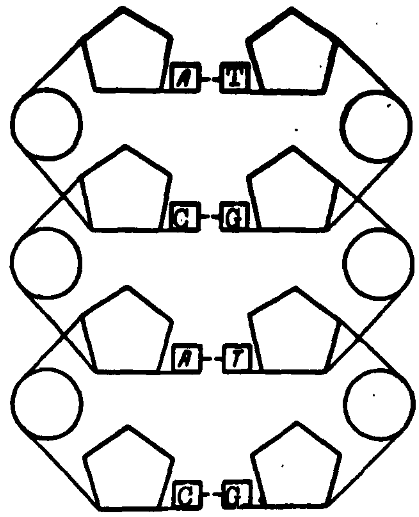



Fig. 7. Scheme of the structure of a DNA fragment. The pentagon is deoxyribose, circle is phosphoric acid, A-adenine, T-thymine, C-cytosine, G-guanine; dashed lines are the hydrogen bonds.

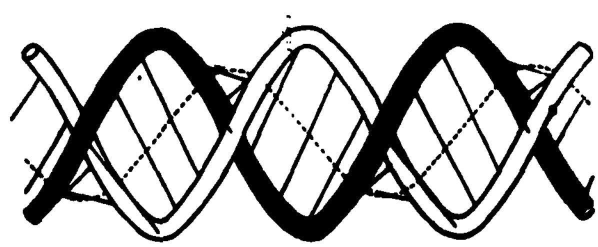


Fig. 8. Postulated structure of the DNA molecule. Each molecule consists of two helices interwoven around a single axis. The helices are connected with one another by hydrogen bridges (straight lines). The broken line drawn through the center of these bridges also has the shape of a helix (after Watson and Crick).

is encircled in the cell and bonded with its other components, which can influence the intensity and character of the changes of DNA.

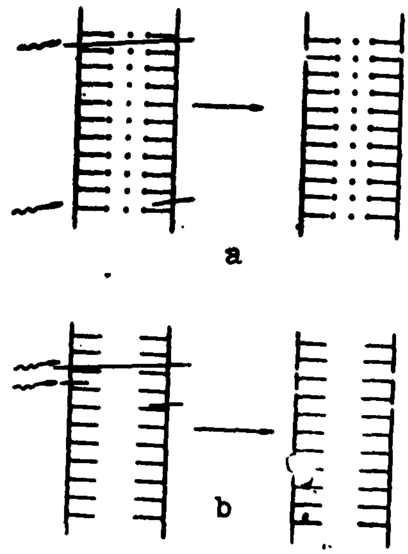


Fig. 9. Diagram of the breakage (depolymerization) of the DNA molecule by ionizing radiation. The break becomes evident when both strands of the DNA molecule are affected. When only one chain is affected, the break becomes hidden. Breaks of this kind appear if, in addition to irradiation, the DNA molecule is affected by urea which destroys all hydrogen bonds between the nitrogenous bases of the two strands (lower picture). a) double strand, 160 ev for breakage; b) single strand, 10-20 ev for breakage.

Influence of the aftereffect. When DNA is irradiated in vitro we observed that a decrease in viscosity continues to increase also after irradiation. The same is noted upon inactivation of the enzymes pepsin and trypsin which split proteins in the digestive tract. Inactivation of these enzymes occurs not only at the time of irradiation, but also afterwards. The same was observed for other biological objects. The influence of the aftereffect occupies the mind of radiobiologists and incites them to search for an explanation of its mechanism, because this influence in a whole organism is primary in the development of radiation damage.

Now then, whatever components of cells (proteins, enzymes, nucleic acids, vitamins, hormones, etc.) are subjected to irradiation in vitro, they all change in one way or another: the chemical bonds are broken, individual chemical groups are oxidized or, more rarely, reduced, the biological active characteristic of a given substance is lost. As a rule, such changes in biogenous substances in vitro are detected at large doses considerably exceeding those lethal for cells and the whole organism.

## Direct and Indirect Effect of Ionizing Radiation

So far we have been concerned with alterations of substances of a biological origin arising as a result of reactions with free radicals—the products of splitting the water molecule. We can say that here the water molecule is affected directly by ionizing radiation; it has only an indirect reaction to chemical changes of dissolved substances.*

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* New opinions on the type of bond between water and cell components will probably find reflection in the future when assaying the role of water in the biological effect of radiation. The following extracts from the recently published book of Szent-Györgyi, Bioenergetics, (Moscow, Fizmatgiz, 1960) indicate these new views: "A study of water in relation to its biological structure and processes of electron excitation opens an absorbing and much-promising field of investigations, which, possibly, will lead us far in understanding normal and pathological processes of life...water is a single system with structural elements in which electronic-excitation states, which are improbable without it, become possible." "Biological functions can actually be included in the formation and disruption of the water structure; water is an inseparable part of living matter, and not simply its medium; water structures and their interaction with electron excitations are intimately associated with the very essence of the "living state." "Living matter is, so to speak, a system of water and organic material which compose, like the gears in a watch, one indivisible unit—a system."

There is another possibility: ionization occurs directly in the irradiated object, be it protein, enzyme, nucleic acid, or something else. Then we are dealing not with the indirect but the direct effect of irradiation. Of course, if the protein, enzyme, nucleic acid are irradiated in the dry form, we have no doubt as to whether the effect was direct or indirect. Here it can be only direct since there is no mediator—water. But what proves that the action of radiation on water is accomplished through mediators—free radicals? These proofs were obtained by the British biochemist Dale and other investigators. Dale gave the name "dilution effect" to one of them.

Among the enzymes splitting the bonds between the carboxyl and amino groups in the polypeptide chain is the enzyme carboxypeptidase. Aqueous solutions of the enzyme were prepared in different concentrations and irradiated with a single dose. With direct action, the probability of an ionizing particle striking a molecule of the enzyme should increase with an increase in the concentration of the solution, because the number of these molecules increases in a determined volume of solution. With indirect action, the number of altered molecules will remain one and the same regardless of the concentration of the solution since irradiation with the same dose leads to the formation of an identical number of free radicals capable of reacting only with a determined number of enzyme molecules.

It turned out that within a wide range of variations in enzyme concentrations the number of inactivated molecules remains the same, and the per cent of inactivated molecules relative to all dissolved enzyme molecules in the irradiated solution decreases with an increase in concentration. Under the direct effect, the per cent of inactivated molecules would be the same. Deviations from this regularity are found only in greatly diluted solutions (less than $10^{-5}$ f of enzyme per 1 ml) and, conversely, in very concentrated solutions. In the first case this is because the radicals have difficulty finding the object of their action and they combine with one another, in the second case the probability of an ionizing particle directly striking a molecule of the enzyme increases. Figure 10 shows Dale's data on the relationship of the direct and indirect effect of irradiation in solutions of carboxypeptidase of different concentrations.

Another proof is the temperature effect, i.e., the temperature dependence of the radiation effect on the object. Table 3 gives data on irradiation of two vitamins with the same dose of beta-particles: ascorbic acid (vitamin C) and nicotinamide (vitamin PP). The per cent of inactivated ascorbic acid and nicotinamide is considerably lower if a frozen solution is irradiated. It is natural to assume that in such a solution the mobility of the free radicals is constrained and they cannot "get to" the vitamin molecules. If the same molecules here are destroyed, then, evidently, it is in consequence of the direct striking of an ionizing particle. The same is found for other objects. Seeds and pollen of plants irradiated at -170°C are less damaged than at room temperature.

Dale pointed out still another fact which can be considered proof of the indirect action of radiation. If we irradiated a solution containing not only a highly pure enzyme, but also certain other substances, for example, glucose, thiourea, cysteine, etc., the enzyme will lose its activity to a lesser degree than when it is irradiated in a pure form, i.e., the added substances, so to speak protect it from the radiation effect. Such a result of the experiment can be explained by the free radicals reacting with the added substances, and therefore fewer remain for the enzyme share.

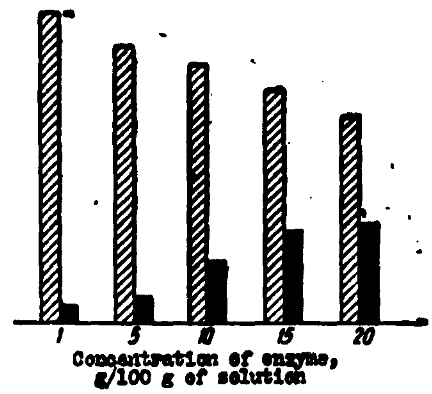


Fig. 10. Direct and indirect effect of radiation on a solution of the enzyme carboxypeptidase. At a low concentration of the enzyme, indirect action prevails (hatched column). At large concentrations the probability of an ionizing particle directly striking a molecule of the enzyme is increased (black columns).

TABLE 3

Radiation Effect on Ascorbic Acid and Nicotinamide at Different Concentrations

| Vitamin concentration in water | Dose, arbitrary units | Temperature, °C | Loss of activity, % |
|---|---|---|---|
| Ascorbic acid 1.3 · $10^{-3}$% solution . . . . . . . . . . . . . . . | 1 | −35 | 8 |
| Same . . . . . . . . . . . . . . . . | 1 | +18 | 37 |
| Nicotinamide (vitamin PP) . . . . | 4 | −35 | 12 |
| Same . . . . . . . . . . . . . . . | 4 | +18 | 79 |

The American scientist Hutchinson irradiated yeast in a dry and wet state and compared the doses leading to inactivation of 63% of molecules of the enzymes invertase and alcohol dehydrogenase from these yeasts. He was convinced that, first, for inactivation of enzyme molecules in a cell very large doses, of the order of millions of rad, are needed and, second, that in a wet medium inactivation of enzymes ensues at doses several times less than in dry preparations.

These proofs seem conclusive enough to make the conclusion: the radiation effect will be indirect in aqueous solutions of biological substances. But we cannot impart an absolute value to them. This follows, in particular, from the experiments of Alexander with irradiation of artificial polymers in a solid state. It was shown that when they are irradiated, a temperature dependence and protective effect can be observed. The medium in which irradiation of the polymer is carried out will be an influence. For example, the viscosity of polystyrene sulfonate is lowered more on irradiation in an atmosphere of air than in a vacuum. It is evident that we must assume the presence of various mechanisms of the effect of irradiation conditions on a substance in a solid state and in solution.

There are grounds to assume that when radiation energy is absorbed in some part of a macromolecule, changes need not occur in its structure at this site; the energy can be transferred along the molecule until it is concentrated at a "weak" site—at this point the molecule is broken or chemically changed. It is even possible that the absorbed radiation energy is transmitted from one molecule to another. This could explain the protective effect of certain additions to artificial polymers when irradiated in the solid state. The energy is distributed between the polymer and the additive. Under certain conditions the reverse is possible: the additive increases the irradiation sensitivity of the polymer. We cannot preclude that the transfer of energy and its concentration in certain portions of the macromolecule occurs also in biological polymers of an organism—proteins and nucleic acids. This is indicated in particular by the fact that the resistance of enzymes irradiated in a dry state in vitro is increased by the addition of certain substances. For example, the resistance of the enzyme invertase, which splits sucrose into glucose and fructose, is doubled when cysteine is added to it; on the other hand table salt reduces the resistance of this enzyme.

The possibility of the energy transfer from one molecule to another is apparent from the following experiment. So-called hyaluronic acid can be extracted from certain tissues, for example, from the umbilical cord. This is a mucopolysaccharide composed of substances related to glucose. Hyaluronic acid plays the role of the intercellular cement substance in an organism. In the organism is an enzyme splitting hyaluronic acid—hyaluronidase. If this enzyme together with the substrate, hyaluronic acid, is irradiated in the dry state, a calculation shows that one ionization at any place of this complex of macromolecules is sufficient to inactivate the enzyme. Binding of the enzyme with its substrate evidently facilitates the transmission of energy from one macromolecule to another. Thus, in vitro inactivation of biogenous substances by ionizing radiation occurs through the cleavage products of water—free radicals,—if their aqueous solutions are irradiated, and directly upon irradiation in the dry state.

## Target Theory

The concept of the direct effect of radiation originated long before Weiss developed the above-cited theory of activated water. The concept of the direct effect of radiation underlaid the general theory of the biological action of irradiation, the so-called "target" theory. Originally this theory won itself many supporters among radiobiologists. The seeming possibility to assay quantitatively the effects observed on irradiation of biological objects, if the mathematical apparatus of the theory of probability was used, was an attractive point; but in the light of new experimental data, which contradict the main premises of this theory, it became evident that it cannot be adopted by radiobiologists although many attempts have been made and are now being made, especially in the West, to broaden the theory and to enrich its mathematical apparatus for an analysis of these new experimental facts. However, most radiobiologists now agree that the applicability of the target theory is limited to rather narrow confines. It is justified where radiobiologists deal with nonliving substrates or with the simplest manifestations of life.

Nevertheless, what are the main premises of the target theory?

The development of the theory began many years ago with an analysis of the dose-effect curves. On irradiation of a number of objects, these curves had a simple shape, easily amenable to mathematical analysis (Fig. 11). Inactivation of irradiated objects was observed at very small doses, then the per cent of inactivation rapidly grew, and a further increase in dose led to a slower inactivation of the remaining objects. If we calculate the fraction of inactivated objects per unit dose, we would find that this fraction remains constant. This type of regularity is characteristic for many natural processes. For example, disintegration of radioactive atoms takes place so that the fraction of decay atoms of a certain radioactive substance per unit time remains constant.

An analysis of the curves from the position of the theory of probability led to the conclusion that inactivation occurs by the direct hit of an ionizing particle on the irradiated object. One "hit," a single ionization, suffices for inactivation.

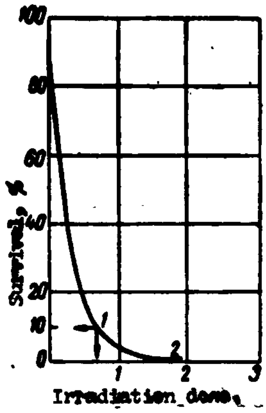


Fig. 11. Death (inactivation) of organisms as a function of dose, if, for this purpose, a single ionization is sufficient ("one-hit" mechanism). 1) 90% inactivated organisms? 2) 99% inactivated organisms.

A decrease in the effectiveness of further irradiation as the per cent of inactivated objects increases has a simple explanation: many hits strike an already damaged object and are ineffectual, like pellets which struck an already winged bird.

The British physicist Crowther formulated the concept of one-hit inactivation of a biological object or cell necrosis. Another British physicist Lea developed this concept of Crowther into the target theory. Inactivation of an irradiated object, cell death or other biological effects are demonstrated only when the hit fits the target, the sensitive volume in the cell. If an ionizing particle intersects this volume and excites a single ionization in it, this must lead to a biological effect.

The smaller the dose causing a biological effect, the larger the "sensitive volume," i.e., the target. In order to strike a large target in the shooting range it is necessary to use fewer bullets than for a small target.

Originally the target concept was not connected with specific structural formations of a cell. A target was considered the sensitive volume, which when hit led to inactivation of the object, calculated from a comparison of the radiation dose and biological effect. But it is easy to answer the question as to what really is a target if we deal with simple biological objects, for example, if we irradiate the enzyme crystalline trypsin in the dry state. Then for the dimensions of the target we can take the linear size of the molecule and calculate the molecular weight of trypsin from the results of analyzing the inactivation curves of the enzyme as a function of dose.

The linear dimensions can be found from the formula

$$R(\mu^3) = 0{,}7/\rho D_{63}$$

(where $\rho$ is density, $D_{63}$ is the dose causing 63% inactivation) and the molecular weight from the relationship

$$M_B = \frac{0{,}72 \cdot 10^6}{D_{63}(rad)}.$$

The triumph of the target theory was when the size of particles of certain simple viruses determined by means of ionizing radiation coincided with the results of the direct measurements of the size of these particles in an electron microscope, and the molecular weights of a number of substances, calculated from the target size, were close to the molecular weights found by other methods.

The claim of the theory that the sizes of the target turned out to be the same regardless of whether irradiation was with gamma quanta, densely ionizing alpha-particles, or heavy ions was valid for such objects as small viruses and enzymes. A considerable portion of radiation energy with a great density of ionization was spent "in vain" because, of the many pairs of ions formed in the target one was sufficient to affect it. The notions of the target theory and its mathematical apparatus can be used in a comparatively limited field for determining the size and molecular weight of certain simple biological systems (viruses, bacterial spores). The target theory also serves as a means for deciphering the internal structure of certain simple biological objects.

Beyond these limits the target theory in its classical form is impotent for explaining experimental facts.

First of all the exponential inactivation curve (Fig. 11) turns out to be not a rule, but an exception. For most biological objects the S-shaped curves shown in Fig. 12 are more typical. Such curves in the target theory are explained by the fact that several sensitive volumes can be in an irradiated object, or it is deemed necessary for inactivation that several ionizations occur in one sensitive volume. Here the departure from the concept of a one-hit mechanism is evident and two, three, and more hits are permitted.

It was erroneous to attempt to connect a target with actual or hypothetical cell structure, to explain the genetic effect of ionizing radiation from the position of the target theory (see below).

At the same time it turned out that the shape of the dose-effect curve is not something constant, it changes under various conditions of irradiation, for example, at a different concentration of the hydrogen ions or in relation to temperature, and this means that the target size is not stable.

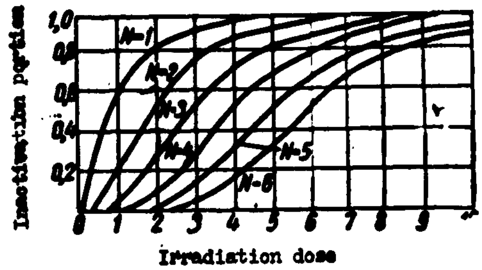


Fig. 12. Death of organisms plotted against dose in the "multihit" mechanism. N) number of "hits" needed to cause death (inactivation) of the organisms.

But a decisive defeat was inflicted on the target theory by the proof of the predominance of the indirect effect of radiation over the direct effect in living organisms. The very important regularity in radiobiology, the so-called "oxygen effect," did not fit into the frameworks of the target theory.

However, rejection of the target theory as a general theory of the biological action of ionizing radiation does not mean rejection of the notion of the possibility of the direct effect of radiation and of the recognition that structures and system, more or less sensitive to irradiation, can be in cells and organisms.

## Role of Oxygen in Irradiation

An important external factor on which depends the radiosensitivity of biological objects is the oxygen pressure in them. The essence of the oxygen effect is that, on irradiation of biological objects in the absence of oxygen or at its low pressure, the effect of irradiation, all else being equal, will be less expressed than at a normal oxygen pressure. In other words, biological objects become less sensitive to irradiation when oxygen is lacking. In this manner radiosensitivity can be reduced by a factor of 2 or 3. Numerous experiments on bacteria, insects, animals, and plants led to the conclusion that radiosensitivity of living organisms increased with a decrease of oxygen concentration in the medium. For example, if bacteria (Escherichia coli) are irradiated in a nitrogen atmosphere, its resistivity will be appreciably higher than on irradiation in an oxygen atmosphere. The oxygen effect is manifested only if there is no oxygen at the time of irradiation. Cultivation of microbes in an anoxic medium before irradiation or their cultivation in such a medium after irradiation had little effect on the survival of the microbes (Fig. 13).

The same was found for animals. If the oxygen pressure in air breathed by rats is lowered from 21% (normal) to 5% and they are irradiated at this time, they will remain alive at a lethal dose whereas all control animals will die.

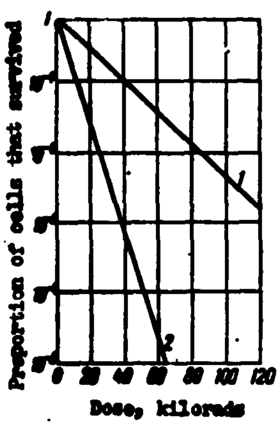


Fig. 13. Significance of oxygen for inactivation of Escherichia coli: 1) Irradiation in nitrogen atmosphere; 2) irradiation in oxygen atmosphere.

The oxygen effect was not noted when isolated cells e.g., lymphocytes (mononuclear white corpuscles), are irradiated. But this effect was detected even in simpler systems. Thus, oxidation of bivalent iron to trivalent proceeds only in the presence of dissolved oxygen.

The oxygen effect is manifested in the most diverse indexes of the effect of ionizing radiation on living objects: survival, growth inhibition, and also in morphological and biochemical changes in cells. Labeled thymidine helped in the investigations of the inhibition of DNA dynthesis in tumor cells cultivated in vitro and irradiated with different doses of X-rays. In the presence of oxygen, DNA is inhibited three times more than when the cells are irradiated in a nitrogen atmosphere. The increase in radiosensitivity with an increase in oxygen pressure has its limit: an oxygen concentration above 20% has no effect on sensitivity. But this effect is sharply reduced or even disappears completely on irradiation with alpha-particles or protons, i.e., irradiation with a high density of ionization.

What is the mechanism of the oxygen effect?

Probably, the closest to the truth will be the postulation that the direction of the initial chemical reactions occurring as a result of ionization in water depends on the presence of oxygen. It was earlier indicated that the radical $HO_2$ is formed in water in which oxygen is dissolved. This postulation is in conformity with the absence of the oxygen effect on irradiation with alpha-particles and protons, since the radical $HO_2$ in this case is formed in an anoxic medium. It is also understandable why it is important for the effect that the lack of oxygen was felt at the time of irradiation but not before or after it. The radicals are formed and disappear, participating in the primary chemical reactions for only $10^{-5}$ to $10^{-6}$ sec. The universality of the oxygen effect, its presence even in nonliving matter, is satisfactorily explained; the primary physicochemical reactions evidently are rather similar regardless of the object irradiated.

However, this is not the only explanation of the mechanism of the oxygen effect. There have been attempts to associate the oxygen effect with the direction of biochemical processes in an irradiated cell, with the change in activity of enzymes during irradiation. The explanation of the mechanism of the oxygen effect is also complicated by the fact that under certain conditions this effect is observed when dry enzymes (e.g., the enzyme trypsin) are irradiated, where only a direct effect was possible.

The discovery of the oxygen effect has acquired great importance in radiobiology. A study of this phenomenon will serve to explain the basic problem of radiation chemistry and radiobiology, viz., the mechanism of the chemical action of radiation.

A number of practical conclusions follow from this discovery. For instance, when irradiating malignant tumors it is expedient to provide an adequate oxygen supply to the tumor cells so as to increase their sensitivity and to achieve death of the tumor cells at reduced irradiation doses. Foreign doctors have developed a number of methods for achieving this purpose. One of them is to irradiate patients in a caisson where high oxygen pressure is created. On the other hand, by decreasing the oxygen pressure in tissues at the time of irradiation it is possible to reduce the sensitivity of an organism to it. This was the basis of investigations for substances protecting against the damaging effect of ionization (Chapter V).

## CHAPTER III

## RADIATION EFFECT ON CELLS

A cell is the elementary structural unit of living matter. At the same time a cell is a very complex formation constructed of billions of molecules. In a unicellular organism (bacteria, amebae, infusoria) the cell functions as a whole in continuous unity with the medium. In multicellular plant and animal organisms an individual cell is a negligible part of the whole. Its existence and activity are determined by other cells, other components of the organism, the coordination of their work, the normal course of regulatory processes, and the interrelation of the organism as a whole with the environment. A cell isolated from an organism dies. Only under special artificial conditions, in a tissue culture, can certain cells live and and multiply.

### Cell Structure

In multicellular organisms, the cells of different tissues and organs or even of one organ perform dissimilar functions and correspondingly have a different internal structure, size, and outline. Nevertheless typical features of their structure can be distinguished.

Figure 14 shows a diagram of a cell. This diagram depicts individual structural elements inherent to a cell. First of all the nucleus and protoplasm are distinguished in a cell. In an undividing cell the nucleus is uniformly colored with basic stains. Only one or more nucleoli are distinguished in it by color. The main structural material of the nucleus is nucleoprotein, i.e., a conjugated protein consisting of protein proper and deoxyribonucleic acid. Ribonucleic acid is found in the nucleolus. The nucleus is separated from the cytoplasm by a membrane.

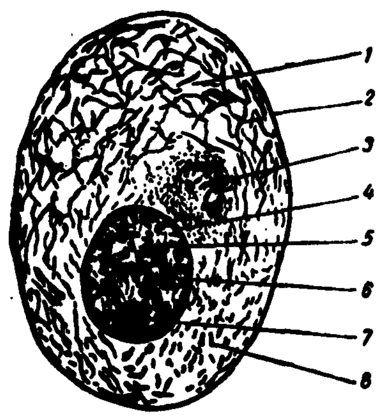


Fig. 14. Structural diagram of an animal cell: 1) cytoplasm; 2) plasma membrane; 3) centrioles, 4) nuclear membrane; 5) nucleus; 6) chromatin; 7) nucleolus; 8) mitochondria.

The cytoplasm has a complex structure. A number of structural formations can be distinguished in it against the general background. In animal cells these include mitochondria (in plant cells, chondriosomes). These structures are "chemical workshops" of the cell. In the mitochondria are concentrated the enzymes which effect the processes of oxidative splitting of nutrient substances and other metabolic processes. The mitochondria can be separated from the cell cytoplasm and collected separately from other portions of the cell by the method of differential centrifugation. In principle, this method is to centrifuge a mixture obtained after separation of the nuclei from cells. Individual structural elements have different size and weight and therefore on rotating the centrifuge slowly the heavy particles precipitate onto the test-tube bottom and on repeated, more rapid rotation the light particles are collected on the bottom. In addition to mitochondria, other particles even smaller in size and rich in RNA, the microsomes, are separated by the same method from the cytoplasm. Special minute formations—centrioles or centrospheres, which are related to the process of cell division, can be distinguished sometimes in certain cells close to the nucleus.

Modern techniques enable us to look into a microscope and photograph not only killed, fixed, and stained cells, but also live and active ones. Figure 15 shows a photomicrograph of a cell from a human tissue culture; the nucleus, nucleolus, cytoplasm, and mitochondria are seen in it.

Fig. 15. Photomicrograph of a human cell in a tissue culture (M-mitochondria, K-nucleus, N-nucleolus).

## Cell Division

Investigations of the effect of rays on cells were begun at the end of the last century, almost immediately after the discovery of X-rays and the production of radium. In accord with the then predominant trend in biology, these investigations were mainly carried out by morphological methods. The scientist was armed with a microscope and by means of it attempted to reveal what changes occur in an irradiated cell.

It was soon noted that irradiation inhibits cell division and at large doses causes cell necrosis. Changes in irradiated cells were detected in the microscope by fixing and staining cells in various phases of divisions.

At the present time the entire process of cell division can be recorded on movie film and studied at greater convenience than was previously possible. The picture of cell division as it evolves before the spectator of the movie, produces an ineffacable impression. The astonishing sequence, harmony, and beauty of the magnificient process which was generated at the dawn of the origin of life on earth and which has continuously created life since then, is revealed.

The readers are forced to be satisfied with a dry description of the successive stages of cell division. With certain exceptions this will be a process of indirect division, or, as it is also called, mitosis (Fig. 16).

In the "resting" cell, when it is still impossible to note in it with a microscope or by microfilming, signs of future division, vigorous preparation for it is actually going on, which is demonstrated mainly by the increase in the intensity of synthetic processes and their predominance over processes of decomposition. The cell accumulates material for division. The nucleus contains stable DNA, prior to division it doubles by the synthesis of new DNA molecules.

The study of DNA synthesis was greatly abetted by the new method of labeling this compound by means of radiohydrogen ($H^3$)—tritium (beta-radiator with very low energy beta-particles). It is possible chemically to incorporate tritium into a thymidine molecule so that the tritium atom replaces the ordinary hydrogen in the thymine ring—one of the nitrogenous compounds participating in DNA. Thymidine is a compound of this nitrogenous base with deoxyribose. Thymine is found only in DNA, therefore when thymidine is administered to a bacteria culture medium or to an animal, labeling develops only in the newly synthesized DNA molecules. This label is detected by the radioautograph method. The essence of the method is that tissue sections or cell suspensions contact the photographic emulsion. After exposure, sometimes lasting a month or more, the emulsion is developed and darkening is seen where the beta-particles of tritium were absorbed. A preparation is stained. By comparing the radioautograph and the stained preparation, by accurately superposing them, we can establish from what cell structures the darkening originated, i.e., where the thymine is located. In the dividing cell the labeled thymine is detected in the chromosomes. Labeling helps to reveal how synthesis of new DNA molecules proceeds, how these molecules are distributed between daughter cells, and what is the mechanism of chromosome doubling, which is important in the transmission of hereditary properties.

The first sign of coming cell division is the change in its shape: the cell becomes round, the nucleus appears larger than in the "resting" cell (Fig. 17).

In the cell the mechanism responsible for accomplishing the division process, the so-called mitotic apparatus, begins to be built from

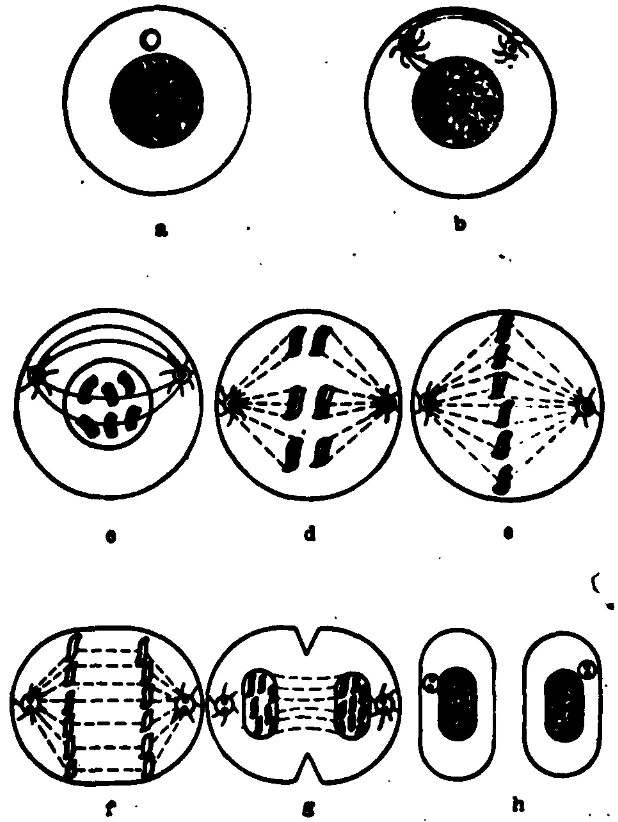


Fig. 16. Diagram of cell division (mitosis). a) resting stage; early prophase (centriole is divided, chromosomes appear); c) late prophase (chromosomes are seen, six are shown in the diagram); d) late phase (nuclear membraneis dissolved, spindle figure is visible); e) metaphase (chromosomes are arranged along the equator perpendicular to the strands of the spindle); f) anaphase (longitudinal splitting of the chromosomes becomes visible; the halves depart to the opposite poles of the cell; g) telephase (nuclear membranes are formed, the chromosomes elongate, constriction of the cytoplasm begins); h) daughter cells (resting stage).

the protein and RNA. The centriole divides, both halves diverge to opposite sides, and around them appear radically diverging fibers, like rays from a star. In the end the centrioles are arranged at the cell poles, the strands extending from them now join both centrioles, forming the spindle figure. The centrioles with their strands are reminiscent of a network of meridians on a map of the hemispheres. While the spindle figure is being built in the cytoplasm, everything is activated in the nucleus also: its homogenicity disappears, at first thin, long double strands become visible and then these strands shorten and thicken, becoming coiled into balls. The nuclear membrane liquefies and the ball, so to speak, swims into the cytoplasm. The strands in the ball are continuously in motion. It becomes distinctly evident how, as a result of "rearrangement," the fibers are arranged in the plane of the equator of the cell, strictly in the middle between the centrioles perpendicular to the strands extending from them. These fibers extending along the equator and separated from each other are the chromosomes. Their number is characteristic for each species: the human cell has 46 chromosomes, mice 40, corn 20, broad beans 12. The shape of different chromosomes is different: they resemble a hairpin, others a fish hook, others a horseshoe. It is not difficult to make out that each chromosome consists of two which are joined together at one point. This point the old biologists called the centromere. Here are attached the strands that together with the centrioles compose the spindle figure. This terminates the first stage of the process and the building of the mitotic apparatus. The preparatory first stages last three times longer than the subsequent stages. Then the most critical moment arrives. The centromeres divide and each of the pairs of the now completely separated chromosomes is rapidly "pulled asunder" by the spindle fibers or "glides" along them in opposite directions—to the cell poles.

The further phase of division is a picture demonstrable from the end to the beginning: the spindle disappears, the chromosomes become indistinguishable, and the bulk of the newly formed nuclei is again homogeneous.

Fig. 17. Cycle of cell division of broad bean (Vicia faba): a) Early prophase; b) metaphase (side view); c) late prophase; d) anaphase; e) metaphase; f) telephase.

The mitochondria in the cytoplasm double and are aligned between the daughter cells, the cytoplasm constricts, and the cells again seem at rest until the time for a new cycle of division arrives. Thus, from one mother cell arise two daughter cells, each with an identical set of chromosomes exactly corresponding to the mother cell.

Division in bacteria lasts only 10 min, in mammals from 45 min to an hour, and in plants many hours.

Cell division in different organisms can proceed with great deviations from the described typical picture. Division occurring in a fertilized egg differs substantially from the mitotic process which ensures the resemblance of the newly formed cell with the original cell. Following the combining of two sexual cells, ovum and sperm, each having a half set of chromosomes, there occurs on subsequent division an exchange (recombination) of the maternal and paternal chromosomal substances. The maternal and paternal characteristics and their combination are transmitted in this way.

Division preceding the formation of mature sexual cells is accomplished in a special manner. As a result of complex transformations in the sexual cell (ovum or sperm) a single set of chromosomes remains instead of a double set, which is characteristic of all other cells of an organism.

In higher organisms that have attained maturity, the most specialized cells with a complex structure and especially responsible function, for example, nerve cells, never divide. This in no way means that a continuous renewal of the cell components does not take place in these cells. In contradistinction to nerve cells, the cells of a number of tissues and organs in a mature organism are unceasingly replaced by new cells generated in the division process. This pertains in particular to the cells of the mucous membrane, cornea, skin, and blood cells. The life span of white blood corpuscles is reckoned in days. Their decomposition proceeds continuously in a healthy organism, and at the same rate the losses are filled by cells newly formed in the hematopoietic organs. And, finally, there are cells which, although they don't divide in the mature organism, do not lose this ability altogether and begin to divide if there is an external or internal stimulus.

## Radiation Effect on Cell Division

Inhibition of mitosis. Henri Becquerel in 1901 reported on a phenomenon detected in his laboratory: the prolonged effect of radium rays inhibits germination of mustard seeds. Other investigators continued and enlarged Becquerel's experiments.

In our country one of the first scientists who made a great contribution to the development of radiobiological problems was Ye. S. London.

Worked proceeded successfully, and in 1906 the French scientists Bergonié and Tribondeau, summing up their investigations carried out mainly on white rat testes and the investigation of other authors on different objects, enunciated the well-known principle: "The radiosensitivity of cells is proportional to their degree of reproductive activity and inversely proportional to the degree of differentiation."

Rapidly multiplying, frequently dividing cells, for example, of an embryonic type, are especially sensitive to irradiation, whereas strictly specialized cells adapted to perform some complex function, are considerably less sensitive. Actually, the greatest sensitivity to ionizing radiation in animal organisms is had by the hematopoietic organs (marrow, lymph glands), thymus, intestinal mucosa, ovaries, testes, etc., i.e., those tissues and organs in which cell divisions continue vigorously during the entire lifetime of the organism. A dose of only 5 r kills the cell in mouse testes from which the sperm would have been formed.

The Bergonié-Tribondeau law was the theoretical basis for the development of roentgeno- and radiotherapy of malignant tumors. Tumor cells are among those which frequently divide: they are usually less differentiated than cells of the tissue where the tumor develops, therefore in many cases the tumor is more sensitive to irradiation than the surrounding normal tissue.

However, since the beginning of the century many facts have been accumulated which do not fit into the principle of Bergonié and Tribondeau. Nerve cells, for example, are an important exception. These cells, as was already noted, do not divide, but nevertheless they are rather sensitive to irradiation. This is demonstrated by disorder of the functions of the nervous system in an irradiated organism, which was convincingly shown by Soviet scientists (A. V. Lebedinskiy, M. N. Livanov, N. N. Lifshits, P. D. Gorizontov. et al.).

We can point out another example of the explicit nonconformity of the observed result of irradiation to the Bergonie-Tribondeau law. One of the varieties of white blood corpuscles, lymphocytes, does not divide but has a high sensitivity to irradiation.

The dose is a decisive factor in the response reaction of a cell. Sufficiently large doses cause necrosis of all cells either during or soon after irradiation. At small doses, cell death is postponed for some time interval, but is nevertheless inevitable. Within certain dose levels a portion of the irradiated cells dies and in others a temporary cessation of division is observed, which is expressed in the decrease or complete disappearance of mitotic divisions or inhibition of the division process if it had already started. Doses leading to the temporary suppression of mitosis can be of the order of 1000 r for yeasts, 50 r for many plants and animal cells, and 10,000 r for the eggs of the sea urchin. Such a radiation effect will be manifested in a delay of growth. Favorite objects of these investigations are flower pollen, root cells of the broad bean and onion, cells of eye cornea, malignant tumors, and other cells dividing mitotically.

Figure 18 shows the results of the experiments of Fritz-Niggli with irradiation of the root tips of the broad bean with different doses of high-energy X-rays (31 Mev). A dose of 250 r reduced by half the number of mitotic divisions in 4 hr, a dose of 500 r completely inhibited cell division by the 12th hr after irradiation. By this time the number of mitotic divisions in plant rootlets irradiated with a smaller dose even exceeded the original number. This, evidently, occurred because the cells whose division was previously blocked at the same time had time to recover and began cell division at the proper time.*

This example serves as an illustration that a cell has the capacity to eliminate damage inflicted on it and again start up the complex mechanism of division. This capacity is not unlimited and if it is exhausted the cell will die. The cells are most sensitive during the period immediately preceding division and during its initial phase. The rate of building the mitotic apparatus in this case is slowed down. Cells that irradiation caught unaware when the mitotic apparatus was already built, complete the process of division and usually do not lose the capacity for it later.

Chromosomes breaks. Observations of the changes in cells after the effect of ionizing radiation are not limited only to counting mitotic divisions or comparing the sizes of roots and shoots of

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* Certain radiobiologists (in the Soviet Union N. V. Timofeyev-Resovskiy and L. P. Breslavets) propose that ionizing radiation (beta-particles, X- and gamma-rays) under certain conditions can stimulate growth by enhanced cell multiplication. This effect was primarily investigated on plants. L. P. Breslavets insists that presowing treatment of grain or vegetable seeds with X- or gamma-rays can noticeably increase the yield. Such experiments have been set up on a limited commercial scale. However, on the whole, the problem of the mechanism of the stimulating effect of radiation is still not solved.

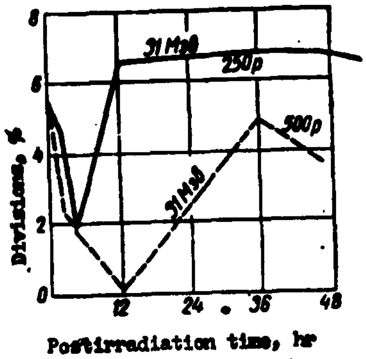


Fig. 18. Inhibition of cell division of the root tips of broad beans at different periods after high-energy X-irradiation (after Fritz-Niggli).

irradiated and normal plants. The investigations go deeper. Cytologists* and geneticists* excel in detecting, describing, and understanding the significance of coarse, fine, and quite hidden breakdowns in the cellular mechanism, biochemists discover the pathways for decoding the chemical processes which lead to the breakdowns seen by the cytologists.

Cytologists long ago noted that in addition to the over-all effect on a cell, radiation causes local damages which are detectable when the cell divides. These are chromosome breaks. By break we actually mean disruption of the wholeness of the chromosome, the formation of fragments of it, which become visible during cell division phases when the chromosomes in general are distinguishable. But irradiation in this case should precede the start of cell division, i.e., occur at the time

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* Cytology, the science of cells; genetics, the science of heredity; biochemistry, the study of the chemical composition and chemical processes in living organisms.

of greatest sensitivity of the cell. At precisely this time there occurs a splitting of the chromosomes into strands (chromatids) which later produce two identical chromosomes.

There are many variants of damages caused by chromosome breakage. This is understandable if we allow that the chromosome can be broken at one or two places before it splits into chromatids, or a chromatid is broken, or breaks occurs in two chromosome arranged in rows, etc.

Moreover, the cell "attempts" to eliminate the breaks. In the most favorable case the fragments of one and the same chromosome fuse and damage become unnoticeable, in another case fusion may occur, but only after ejection of a portion of the chromosomal material and it becomes shorter, and in still another case individual fragments belonging to different chromosomes fuse. This process proceeds with the expenditure of energy.

During cell division these structural changes in the nucleus are manifested as separately lying fragments of nuclear material, as chromosome cross-overs, by the formation of bridges with the chromosomes diverging toward the poles of the cell, the occurrence of a micronucleus along with the two daughter cells, and in other ways (Fig. 19). Cytologists carefully study these damages. In addition, the conditions affecting the occurrence of chromosome breakage are investigated in detail.

It was first of all established that the number of breaks depends on the dose. In general, breaks happen more frequently, the higher the dose. The dose-power dependence is characteristic for other biological reactions as well as for chromosome damages. At one and the same dose, rays of low intensity will be less effective than concentrated rays. Evidently, both in other cases and in the case of chromosome breaks, at low radiation powers the cell has time to "restore" the damage, whereas at large powers there is not sufficient time for this.

The environment also affects the number of breaks. The importance of oxygen is especially evident. For example, irradiation of the microspores of the Tradescantia flowers in a nitrogen atmosphere reduced by a third the number of chromosome damages (translocations).

On the other hand, anoxia after irradiation increases the number of damaged chromosomes.

What is the mechanism of chromosome breakage? It is easiest to imagine that an ionizing particle pierces the chromosome like an arrow and the chromosome breaks along its passage. For this purpose, as is assumed, it is necessary that 20 pairs of ions be generated in the chromosome. The formation of such a number of ion pairs is most probable during the passage of densely ionizing particles (alpha-particles, protons, and electrons at the end of the path). The diameter of a nucleus is about 10 microns, the diameter of the "bullet," alpha particles, is a million times smaller. It would seem that an alpha-particle has as many chances to strike the nucleus in the chromosome as a pellet of a blindly fired cartridge has of hitting the heart of a bear.

But experiments show that a single particle striking the nucleus is sufficient to break the chromosome. This means that we are not dealing here with a direct hit. This is indicated by the presence of the oxygen effect. Apparently, chromosome breaks, with all its aftereffects, is the result of a change in the course of chemical processes in the cell. This is brought about by ionization not only in the chromosome itself, but also in its surroundings. Possibly the synthesis of deoxyribonucleic acid is inhibited (see below).

Fig. 19. Photomicrograph and diagram of the changes in mitotic cycle (see Fig. 17) of broad bean cells after X-irradiation: a) metaphase with breakage of one chromosome; b) anaphase with "a bridge and chromosome fragment;" c) telephase with chromosome fragment which did not enter into the daughter nuclei; d) resting period with micronucleus.

## Role of the Nucleus and Cytoplasm

Up until now we have been concerned with nuclear damages. But what are the grounds to assume that the entire matter amounts to damage in the nucleus and the cytoplasm is not affected? The answer to this question is frequently given in accordance with how the researcher generally evaluates the role of the nucleus in the cell. Some investigators assert, "The nucleus is the coordinating and controlling center of the cell and the carrier of its genetic plan," meaning by this that in the nucleus is coded the program which determines the construction of subsequent generations of cells. Others do not agree with ascribing only to the nucleus such executive functions, considering that the cytoplasm is not just the executor of "plans" proceeding from the nucleus.

Many attempts have been made to solve this problem experimentally with respect to the action of ionizing radiation. Here are some of them. Thirty years ago the American scientist Zirkle set up a very beautiful experiment on fern spores. The diameter of the spores of this plant is 38 microns, the nucleus in them has a diameter of 10 microns, but it lies not in the middle of the cell but close to one edge, near the membrane. Nature marked the place where it lies by a spot on the membrane. Zirkle irradiated the spores with polonium alpha-particles whose path is 30 microns. If the spores were arranged with the nuclear end toward the radiation source the alpha-particles reached the nucleus, if they were arranged with the opposite end toward the source the particles did not reach the nucleus and bombarded only the cytoplasm. Cell division was inhibited in both cases, but on irradiation of just the cytoplasm the dose required for this was 20 times greater than on irradiation of the nucleus.

Similar investigations were recently repeated by another investigator on wasp eggs, where the nucleus is also arranged eccentrically. Here the difference was even more striking. A million alpha-particles had to penetrate the cytoplasm in order to cause the same effect which one particle striking the nucleus produced.

The works of the Soviet scientist B. L. Astaurov, which were published in 1947, made a very great impression on biologists. He was able to "construct" the egg of a bombyx so that all cytoplasm in it was irradiated with a large dose of X-rays but the nucleus was not irradiated. This egg developed up to the release of the butterfly and no deviations from normal were noted. From this the conclusion was made of the preeminent value of the nucleus in the biological effect of radiation. American and British scientists later set up similar experiments on amebas. The nucleus of an irradiated ameba was transplanted to an unirradiated ameba whose own nucleus had been preliminarily removed. Then the ameba "became ill" and died in three days. The experiment was set up in reverse: the nucleus was removed from an irradiated ameba and a nucleus taken from a healthy ameba was "inserted" in its place. This ameba died not after three days, but after three weeks (Fig. 20).

Such experiments at first seem very simple, but actually they demand great skill on the part of the experimenter. A nucleus with a diameter of 10 microns* had to be removed from a cell visible only in a microscope and transferred undamaged to another cell—this is a matter requiring truly exceptional skill.

The technique of irradiating cells has reached high perfection. At the First International Conference on the Peaceful Use of Atomic Energy (Geneva, 1955), Zirkle and Bloom reported on their works of irradiating cells of mouse embryo, human uterine cancer, newt cardiac muscle cultivated in vitro, with narrow beams of protons and ultraviolet

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* To engineers and workers calculating and dealing with the micron accuracy of ball bearings, a diameter of 10 microns does not seem so small, but steel is nowhere near as brittle as the nucleoprotein in a nucleus.

rays. Eighty per cent of protons with an energy of 1.6 Mev struck a circle with a diameter of only 2.5 microns and 96%, a circle with a diameter of 5 microns, which made it possible to act on individual sections of the chromosomes at different stages of cell division. This process was filmed and then analyzed and the changes in the chromosomes described. The effect of irradiating individual sections of the chromosomes was noted when only several tens of protons struck them. But several hundreds of thousands of protons were insufficient to reveal any apparent effect upon irradiation of the cytoplasm.

The British radiobiologist Gray collected in the literature all "testimonies" of the "guilt" of the nucleus in cell damage by radiation and on the involvement of the cytoplasm in this.

It was asserted in 13 of the 16 testimonies that cell damage by ionizing radiation is determined by the radiosensitive nucleus. It would seem that the "condemnation" is clear: the nucleus is guilty. But there is still another experiment carried out by Duryee on frog and salamander ova. He extracted the nucleus from the eggs. There was no noticeable changes in them even at an irradiation dose of 30,000 r. Radiosensitivity was restored in the nucleus when it was returned to the cell, to the surroundings of the cytoplasm. Then all changes typical of irradiation were detected in the cytoplasm.

Another experiment of Duryee is even more indicative. He extracted 0.0001 ml of cytoplasm from a cell irradiated four days previously with X-rays and injected it into unirradiated cell close to the nucleus. In two hours all characteristic signs of radiation injury, even to chromosome breakage, was noted in it. This could be explained most simply by the fact that in the irradiated cytoplasm toxic substances are formed which act appropriately when injected into an unirradiated cell. But what this substance is is still unknown.

Fig. 20. Diagram of the operation for transplanting the nucleus of chromosomes. Left: nucleus from irradiated ameba (dark) is transplanted to unirradiated ameba. It lives about three days after this. Right: nucleus from unirradiated ameba is transplanted to irradiated ameba. After transplantation of nucleus the irradiated ameba continues to live about three weeks.

The biochemists have attempted to apply their skill to explain the role of cytoplasm and the nucleus. The Swedish scientists Euler and Kahn isolated the cell nuclei of calf thymus and irradiated them. They did not find alterations in the properties of the nucleoprotein extracted from these nuclei. But if whole cells were irradiated, typical changes, like a drop in viscosity, were detected in the nucleoprotein from the nucleus. This means that the cytoplasm participated in these changes of the nucleus.

A. L. Shabadash established by the fine method he developed that on irradiation the RNA changes in various structures of the cytoplasm.

A. M. Kuzin in a report at the International Symposium on Radiobiology in October 1960 in Moscow (on the basis of experiments with irradiation of plant leaves and observations of the changes in the number of mitotic divisions at the growth points) hypothesized that "disorders in the processes of oxidative metabolism in cytoplasm resulting in the formation of substances which penetrate the nuclei of cells play an essential role in the radiation inhibition of division ..." These substances inhibit DNA synthesis at the period the cell is in the resting stage.

The importance of cytoplasm in the restoration processes of cells is substantial. This is seen from experiments. One variety of amebas was irradiated. As could be expected the cell division of this unicellular organism was inhibited, but it returned to a normal rate after cytoplasm taken from an unirradiated cell was injected into them.

Therefore the truest answer to the problem raised at the beginning of this section as to whether the nucleus or the cytoplasm is responsible for cell damage and what is the significance of the cytoplasm microstructure, the mitochondria and microsomes, will be the following: the cell reacts to irradiation as a whole; individual structural formations in it can be more sensitive to irradiation, other less sensitive, but this difference is revealed only when the whole cell is irradiated. Disruption of division is a demonstration of changes in the vital activity of the cell as a whole, which evidently is the result of distortions in the normal course of biochemical processes in the cell. The events in a cell can be schematically represented as:

Physical processes → radiochemical processes → reactions of metabolic disorders → changes visible in the microscope.

We deal with something more complicated in a multicellular organism where the individual cell is only a part of a more highly organized whole.

# CHAPTER IV

## RADIATION EFFECT ON THE WHOLE ORGANISM

A multicellular organism, even the simplest, is not just a crowd of cells. The cells composing it differ in shape and function. They are gathered into tissues and organs, fulfilling in turn definite assignments in the whole organism. Special cell systems in animals and such highly specialized organs as the brain and endocrine glands unite and coordinate the activity of all parts of an organism. A considerable proportion of the cells of an organism are in contact with the external environment, not directly, but through the agency of the internal environment of the organism, the intercellular fluid and blood. And if radiobiologists still find it difficult to give a full answer to the mechanism of action of radiation on unicellular organisms in spite of tens of years of intense work, it is even more difficult to do this for the complex organism of animal and man composed of an enormous number of cells. Moreover his reaction to irradiation is not the average of the reaction of individual cells.

The organism responds as a whole to the effect of radiation even on a small portion of the body, although there is no sensation of pain like that which compels withdrawal of the fingers when touching a hot iron. The reactions of an organism to radiation are multiple and they are determined both by the characteristic of the acting factor, radiation, and by the properties of the organism itself. One of these properties is dissimilar radiosensitivity.

## Radiosensitivity of Animals and Plants

The wide range of radiosensitivities found in nature is amazing! The growth of one species of fungus can be inhibited with a dose of only 0.01 r, whereas flies irradiated with a dose of 80,000 r fly about, eat, and behave as usual. Infusoria are killed by a dose considerably higher than 300,000 r. But even this is not the limit. The alga Chlorella, the probable future oxygen supplier of cosmonauts, becomes "ill" but is not killed from doses of millions of roentgens.

Recently, associates at a laboratory in Los Angeles (USA) noted that the water surrounding a submerged nuclear reactor, became turbid. An enormous number of bacteria of the species Pseudomonas were detected in a drop of the water under a microscope. This was under conditions where the dose in the water was 10 million r during 8 hr. The bacteria multiplied, feeding on the ion-exchange resins of the water filters. The bacteria Pseudomonas is a record-holder with respect to radioresistance in the living world.

No less astonishing are the rapidity and wide limits in which the radiosensitivity of cells can change. For example, irradiation of a plant rootlet 1 cm from its tip with a dose of 200,000 r causes a negligible temporary inhibition of growth, whereas a few days earlier these cells were at the very tip of the root and a dose of only 30 r was sufficient to noticeably inhibit their growth. During a 24-hr period the resistance of the cells increased a thousandfold.

Table 4 shows data on the dose of X-rays which kill half the irradiated animals over an observation period of 30 days. This dose is called the "50% lethal dose" ($LD_{50}$). The very fact that at a certain dose half the animals perish and the other half remain alive indicates different radiosensitivity even of animals of the same species, in other words, the individual variations of radiosensitivity.

On the basis of studying accidents and assaying the damaging effect of atomic bombs at Hiroshima and Nagasaki, $LD_{50(30)}$ for man is about 300-400 r.

TABLE 4

Irradiation Dose Causing Death of One-Half of Animals in 30 Days ($LD_{50(30)}$)

| Species | Dose, r | Species | Dose, r |
|---|---|---|---|
| Guinea pig | 300—350 | Rats | 450—600 |
| Pig | 275—335 | Monkeys | 500 |
| Dog | 325—400 | Rabbits | 900—1000 |
| Mice | 350—500 | Ameba | 100 000 |
| | | Chicken | 1000 |

As a rule mammals are more sensitive to irradiation than birds, fishes, amphibians, mollusks and unicellular organisms.

The differences in radiosensitivity of various species in the plant world are also very great. This is rather accurately checked when plants are irradiated on so-called gamma-fields. Plants are set out on them in a circle at different radii. In the center of the circle is a gamma-radiator, usually $Co^{60}$. Seeds of different plants also have different radiosensitivities. For instance, flax seed is especially resistant.

What is the secret of such a great difference in reactions to irradiation? The desire to reveal this secret is dictated not only by the thirst for knowledge of scientists, not only by man's eternal drive to penetrate deeper into the secrets of nature, but also by completely real, practical demands. It suffices to say that to reduce man's radiosensitivity means to reduce the thickness and weight of nuclear reactor shielding, and this would immediately be a giant step forward in the use of atomic energy in transport and in other fields of the national economy. But the secret has not been revealed although there has been no want of attempts. We could assume, and not without basis, that the entire matter lies in the intensity of metabolism. Actually, if frogs are X-irradiated with a dose of 3000 to 60,000 r at a temperature of 23°, and then they are kept at 5°, from 80 to 90% of the frogs will live longer than 3-4 months, while all frogs which after irradiation remained at room temperature, die 3-6 weeks after irradiation. If frogs which were in a cold room from 3 to 4.5 months after irradiation are transferred to a warm room, they become ill and die. Marmots and susliks irradiated during winter hibernation do not become ill, but one has only to awaken and feed them and the disease appears. It seemed that the matter was clear: a low temperature lowers metabolism and then radiosensitivity is low, high temperature increases metabolism and the radiosensitivity is increased. But in mice and rats and even moreso in birds, the metabolic rate is considerably higher than in man, however, the radiosensitivity is lower.

Cells cultivated in vitro have less variability in radiosensitivity than the species of animals from whom these cells were taken.

The cause for the difference in radiosensitivity was sought in the characteristics of the chemical composition of the body and metabolism of different animal species. Certain geneticists attempted to find a relationship between the number of chromosomes in cells of different animal species and their radiosensitivity, but no regularity was found. In all probability the cause for the difference in radiosensitivity is not one, but many.

We already mentioned above the presence of individual radiosensitivity of animals of a single species even when they are selected by sex, age, weight, and similar maintenance conditions were provided for them before and after irradiation.

These differences are even more apparent when all conditions of selecting the animals are not observed. For example, very young and very old animals suffer from irradiation more than mature animals. The causes are different: in the young animals the number of dividing cells is great and the divisions rate is high, in the old animals the restoration processes proceed slowly and with difficulty. Therefore, the young and the old animals are less resistant to irradiation.

## Irradiation Conditions and Sensitivity

The data in Table 4 pertains to a single irradiation of the entire body with X-rays at dose powers of the order of several tens of roentgens per minute. This must be stipulated because it makes a difference whether the entire body or only a part of it is irradiated and whether the entire dose is given immediately or as separate portions, and in the latter case, at what power and at what intervals, or whether irradiation will be continuous over a long time but at very small powers.

Knowledge of all this is acutely needed by radiologists treating patients with malignant tumors, by hygienists establishing maximal permissible doses of radiation, by therapeutists making clinical observations of people handling radiation sources.

Much of the data cannot be obtained directly from observations of people; the data has to be obtained in experiments on animals with calculation, as much as possible, of all the differences between man and laboratory animals: rats, dogs, monkeys, so the data obtained can be applied to man.

Shielding of even a small portion of the body during irradiation increases the radioresistance, especially when the section of the body shielded corresponds to the position of radiosensitive tissue or organ. This can also be an organ which is per se resistant to irradiation, but important for the subsequent course and outcome of the disease. If we irradiate a whole rat weighing 200 g with a lethal dose but shield its adrenal glands weighing 20-25 mg, the rat will survive, whereas the control will die.

On the other hand, it was shown in works carried out by many investigators that if we protect the body of an animal with a lead shield in which openings are made for those portions of the body we wish to irradiate, we can reveal the relative importance of various organs exposed to the irradiation field by the organism's reaction to this factor as a whole. Local action on the entire abdomen or part of it with equal doses and with an equal irradiated mass of the body leads to greater over-all damage than irradiation of other body sections.

The radiologist can irradiate tumors with several thousands of roentgens, distributing them over several positions, but total-body irradiation with this same dose and with the same order would inevitably lead to death. The hygienist establishes the maximum permissible dose for hands when working with radioactive substances at several times greater than for irradiation of the whole body, and he has grounds for this.

Here we are dealing not only with the fact that cells of different tissues and organs differ sharply in radiosensitivity. Still more important is how much of the recuperative powers of the organism are retained at some order of irradiation. The dose power is of importance here.

## Natural Radiation Background

All life on earth is constantly subjected to the effect of natural ionizing radiation. Life on earth arose under such conditions, evolved from more simple forms to more complex and continues to do so and will continue if the advocates of turning the cold war to a hot one do not inflict substantial damage on it.

Man under natural conditions is irradiated from both external and internal sources (Fig. 21). This natural background is composed of the radiations of radioactive substances scattered in the earth and of radon contained in the air, from cosmic rays, and also the radiations of radioactive substances found within the organism. The human body has several radioactive substances (potassium-40, carbon-14, uranium, radium, etc.); potassium* stands first in radioactivity, then radium and the products of its decay.

All radiations composing the natural background are not stable. The intensity of cosmic rays increases with height and approach to the poles. Gamma-radiation of the earth is higher where ancient rocks, granites, outcrop and lower where these rocks are covered by a thick stratum of sedimentary rocks. Irradiation in a reinforced-concrete house is slightly higher than in a wooden house. But the limits of variation of the natural background are comparatively small.

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* Native potassium is a mixture of three isotopes: $K^{39}$, $K^{40}$, and $K^{41}$. Of these, only $K^{40}$ is active. Out of 100,000 potassium atoms, 12 are radioactive. The mature human organism contains 140 g of native potassium, of which 17 g are radioactive.

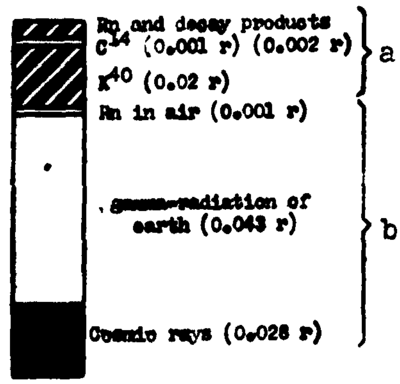


Fig. 21. Natural background of irradiation of human sexual organs based on data of British scientists (total for year, 0.095 r): a) internal irradiation; b) external irradiation.

Under natural conditions, man, during his life, is irradiated with a total dose of the order of 5-7 r. Only in certain locales on the earth, for example in the state of Kerala in southern India, because of the rich content of thorium oxide in the sands, are inhabitants from generation to generation during the life span of a single person subjected to irradiation with a total dose of about 50-60 r.

Man has adapted to a dose of 5-7 r, but contemporary science does not have indisputable proof that it has a harmful effect of an organism.*

Are the inhabitants of Kerala different with respect to health, life span, and other signs from inhabitants of other states of India where the natural background corresponds to that existing on the

* Certain scientists, however, consider that randomly occurring deviations in an organism in the properties transmittable by inheritance partially owe their origin to irradiation of the sexual glands under natural conditions (Chapter VI).

greater part of the earth? The answer to this question should be given a special examination by the World Health Organization in the state of Kerala and adjacent states.

The results of this kind of examination can help solve the problem as to how great a risk it is for an individual, for all mankind, and for future generations if irradiation exceeds the usual dose of 5-7 r during man's life span.

Sources exceeding the natural background for the entire population of the earth at the present time are radioactive fallout from nuclear tests*, for a considerable proportion of the population, from radioscopy and X-raying, and for a rather large and ever increasing group of people, from the requirements of their occupation dealing with radiation.

Ionizing radiation renders only a damaging effect on an organism so that the ideal solution of the problem for mankind would be to maintain the natural level of irradiation and prevent any possibility of its increase. It is not without reason that the leading scientists of the world persistantly struggle for a ban of nuclear tests, for elimination of the danger of nuclear war. Doctors are narrowing the use of radioscopy, limiting their cases to where the use of the device for proper diagnosis by means of X-rays outweighs the possible damage from irradiation. This explains why during the past decade the maximum irradiation doses permitted when working with radiation sources and radioactive substances, established by health codes, have been repeatedly reexamined from the aspect of lowering them.

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* In this book we will not analyze in detail the problem of radioactive fallout from nuclear tests. For this see the book: "Soviet Scientists on the Danger of Testing Nuclear Weapons" (Moscow, Atomizdat, 1959) and A. M. Kuzin's "How Nuclear Explosions Treaten Mankind" (Moscow, Izd. AMN SSSR, 1959).

## Small Doses of Irradiation

Although the avoidance of increasing the natural background of irradiation is the ideal to which we must strive, the problem of the chronic effect of small doses on an organism remains one of the most urgent problems at the present time in radiobiology. But what can we consider a "small dose" in chronic effects? Evidently we must consider as "small doses" those which are commensurable with the natural background, exceeding it only by one or two orders, i.e., by tens or a hundred times.

A study of the effect of small doses within such limits meets with very great difficulties. First of all, because it is not easy to find an index which would be specific for ionizing radiation. During life the human organism faces many pathogenic factors. Millions of people breathe not the ideally pure and healthy air of the alpine meadows and pine forests, but the more or less dust-filled air of large cities. Millions of people drink water which is far inferior to spring water; millions smoke and over the period of many years are daily poisoned by nicotine. Is it possible against such a background to distinguish some kind of effect of small doses of ionizing radiation? American and British scientists have attempted to do this by carrying out a comparative questionnaire investigation of roentgenologists and doctors of other specialties. They were interested in average life span, morbidity of various groups of doctors, number of children, number of those stillborn, with congenital defects, etc. A statistical treatment of the collected data, however, did not make completely reliable conclusions.

At the Second International Conference on the Peaceful Use of Atomic Energy (Geneva, 1958), A. V. Lebedinskiy, Yu. G. Grigor'yev, and G. G. Demirchoglyan in their report summarized the results of their numerous experiments. They irradiated animals with relatively small doses, not spread over a period of time, but at one time or over a short interval. They used very fine methods of investigating the reaction of the nervous system to radiation (changes in the biocurrents of the brain and retina, reflex motor reaction, etc.). The authors arrived at the conclusion that their data "testify to the participation of the nervous system in the reactions of an organism, occurring as a result of the effect of radiation in small doses." The hypothesis was made that the synaptic structures are disrupted, i.e., the mechanism which transmits the nerve impulse from one nerve cell to another.

The detected changes in the activity of the nervous system are apparantly nonspecific and can occur under other harmful effects on an organism.

When carrying out chronic experiments, animals are kept during their entire life under conditions of continuous irradiation and investigators attempt to reveal how they differ from control animals which are subjected only to natural irradiation. The criteria of the effect of radiation are the life span, frequency of blood diseases, and occurrence of tumors, fertility, quality of offspring, etc.

Figure 22 shows the results of experiments carried out on rats and mice. Shortening of life was evidenced at weekly doses of gamma-rays above 10 r, i.e., approximately 6000-10,000 times above the natural background. This cannot be called a small dose.

The data obtained for mice cannot be directly applied to man. The value of these data is that they approximate the possible after-effects of chronic irradiation and even though the shape of the curve for man can be quite different than that in Fig. 22, further investigations should show how different.

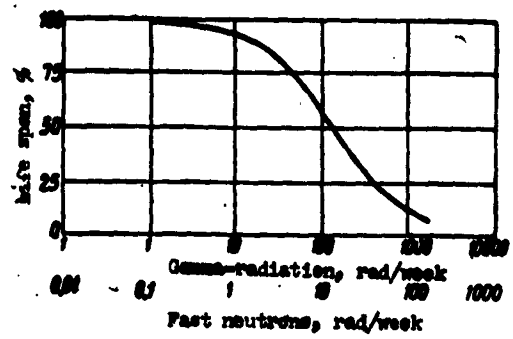


Fig. 22. Reduction of average life span of rats and mice (norm 100%) induced by chronic irradiation with gamma-rays or fast neutrons. When irradiated with gamma-rays doses up to 10 rad/week, the life span of mice and rats remains within the limits of the norm. Fast neutrons are more effective than gamma-rays by a factor of 10 with respect to this index.

The problem of the effect of small doses of radiation being emitted by radioactive substances entering inside an organism has acquired particular importance in connection with contamination of the earth's surface by radioactive fallout. In contrast with general external irradiation by gamma-rays, we are primarily concerned here with the nonuniform irradiation of cells by beta-particles and highly effective alpha-particles. But in general this leads not so much to a qualitatively different reaction of the organism to this kind of chronic radiation action as to a quantitative difference and to special characteristics in the localization of injuries to the organism (e.g., in the arrangement of tumors induced by radiation).

Thus, when maintaining the position that the effect of ionizing radiation, no matter how little it exceeds the background, is harmful, it is necessary to acknowledge that the existing methods cannot reveal with adequate reliability the apparent damage to the health of persons irradiated for a long period with doses 10-100 times exceeding the natural background. This of course refers to adults and does not take into account the genetic aftereffects (transmittable by heredity) (Chapter VI).

## Effect of Average and Large Doses of Ionizing Radiation on the Human Organism

If the effect of small doses of radiation remains latent and it is partially or completely neutralized by the recuperative powers of an organism, then the effect of large doses is violent. At doses of the order of 100,000 r, death of the animals for all practical purposes ensues during irradiation. Mice die in 6 hr at a dose of 64,000 r. At doses from 1000 to 1600 r, mice live 3.5 days, with smaller doses the life span rapidly increases, and, finally, at doses below 100 r, mice completely survive.

It is fully evident that death of animals within different ranges of doses is due to damages of a different nature. Fulminant death from irradiation is caused by destruction of vitally important substances,—this death is called molecular. Death during the first two days is preceded by explicit signs of severe lesion of the central nervous system and death on the fourth day, by lesions of the G.I. tract? at later periods, from the ninth to the 18th day, the immediate cause of death is lesion of the hematopoietic organs, and during subsequent days, infectious complications are the cause. Of course this is only a scheme. But hidden behind this is the fact that time is necessary for development of injuries of various systems of an organism? to what extent and when these injuries appear depends mainly on the dose.

In the human organism the diversity of demonstrations of radiation injury are observed at doses of the order of 400-1000 r, i.e., at doses which do not kill on the first days postirradiation. At such doses sickness can last weeks.

Acute radiation sickness. Cases of acute radiation sickness in persons who were victims of accidents have been studied most carefully. Few such cases have been described, but those that were, were described in great detail. The irradiation dose of the victims was always more or less known. Of course, Japanese doctors, who have observed and treated thousands of radiation sickness patients and who are still dealing with its aftereffects, have gained great experience under extremely sad circumstances.

But here we will relate how radiation sickness proceeded in six scientific workers who were victims of a nuclear reactor accident in Yugoslavia. The study was made by a group of French and Yugoslavian doctors and scientists.*

This occurred on October 15, 1958 at the Institute of Nuclear Energy near Belgrade. Six persons were setting up an experiment on an experimental nuclear reactor. The experiment consisted of measuring the flux of neutrons generated during the spontaneous fission of natural uranium and after introducing a neutron source in the form of a beryllium-radium mixture. As a consequence of an increase in the level of heavy water in the reactor when the beryllium-radium mixture was introduced, the reaction went out of control. As a result radiation of neutrons and gamma-quanta occurred. Gamma-quanta were emitted

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* A detailed account of the pathophysiology, clinical features, and treatment of acute, chronic radiation sickness in people can be found in the book "Radiation Medicine," edited by A. I. Burnazyan and A. V. Lebedinskiy (Moscow, Atomizdat, 1960).

during the decay of the short-lived fission products and also during the nuclear reaction proceeding with capture of neutrons and emission of gamma-quanta, and by the decay of radioactive substances formed by this reaction. At the very beginning it was supposed that the victims were irradiated with doses bordering on lethal or even exceeding them. All six persons were flown to Paris the next day and placed in the Radiopathology Department of the Curie Institute.

The basis for estimating the received irradiation doses were the physical measurements of the induced radioactivity* in the metal objects surrounding the reactor and in the victims. The activity of the radioactive sodium formed in the body of the victims on absorption of neutrons was measured by a special instrument.

The total irradiation doses with consideration of the relative biological effectiveness of fast and slow neutrons were approximately: for one victim C, 1000-1200 r; for three victims M., G., and D., 700-1000 r; for X, 600-800 r; and for B, 300-500 r.**

During the first hours after the accident the victims complained of general and muscular weakness, anorexia, nausea, vomiting, hidrosis, and unpleasant sensations in the hands. During the first days, erubescence and blepharitis were noted. Then the latent period of the disease lasting three weeks ensued. During this period the well-being of all the victims, except C., was satisfactory. There was a noticeable deterioration in the over-all condition of C. during this period. His body temperature rose on the 4th and 15th day. All victims lost

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* Induced radioactivity is that generated by neutron bombardment.

** In April 1960 the greatest specialists in the field of dosimetry in the USA reproduced a situation of reactor failure and on phantoms (mockups of people) measured with the latest instruments the doses received by the victims. The dose values proved to be lower than those cited in this book, but the clinical damage conformed more to the earlier found doses.

0.3-2.3 kg of weight. Their blood composition changed. Then the latent period was replaced by a sharp deterioration in over-all condition. With the exception of B., all suffered from fever. Appetite was lost, nausea appeared, there were profuse night sweats, the amount of excreted urine decreased. The hair on all the victims' heads fell out: in C. starting with the 14th day, in B. with the 20th. The hair on the beard also fell out on the men (there was one women among the victims, D.). Hair growth was restored only after three months. The changes in the blood were typical for radiation sickness. The blood picture was normal in all before irradiation. On the first day the number of white corpuscles (leukocytes) increased from 5000-7000 in the norm to 9000-10,000 in 1 $mm^3$ of blood; during the latent period the number of blood cells gradually decreased. Least of all was the decrease in the number of red blood corpuscles (erythrocytes) and most of all, the number of lymphocytes. The number of lumphocytes dropped to a minimum during the first five days, then it fluctuated around the low level. The content of blood platelets and juvenile forms of erythrocytes also dropped. The blood composition indicated an almost complete destruction of the bone marrow at the climax of the disease in those that suffered the most. Their number of leukocytes dropped to 50 in 1 $mm^3$; only in B. was their number 900 per 1 $mm^3$. Anemia developed. Bleeding of the nose and gingivae was frequent, punctiform extravasations appeared on the legs. Intestinal and pulmonary hemorrhage was observed in C., who received the greatest dose. Hematemesis and hemoptysis gradually increased in him. Disorders in the activity of the G.I. tract were enhanced in all the victims at the end of the critical period: inflammatory processes developed in the mouth and pharynx; various pains in the abdomen were manifested, the motoricity of the intestines was disturbed. Affection of the internal organs of

C. increased in spite of all the measures taken: obstruction of the intestine and gaster occurred, the kidneys stopped excreting urine, and on the 32nd day, C. died. An autopsy revealed extravasation throughout the entire intestine, in the lungs, slight hemorrhage in the kidneys and bladder, severe damage of the testes.

An investigation of the sperm of the five men on the second week showed a drop in the number of spermatazoa and their morphological and functional alterations. In subsequent analyses no spermatazoa at all were found in two men and very few were present in the others.

In view of the evident threat of death from the disease for the four out of the five remaining alive, the previously developed experimental method of treatment with bone marrow transplantation was used (for greater details see the next chapter). The method proved highly effective and all victims, except C., were saved.

Soviet doctors A. K. Gus'kova and G. D. Baysoglov presented a report at the First International Conference on the Peaceful Use of Atomic Energy (Geneva, 1955) in which they reported on two cases of acute radiation sickness after a single irradiation with gamma-rays and neutrons: with a dose of 300 r for one victim and a dose of 450 r for the other.

The case history of these patients can be schematically represented as follows:

| Period of disease | Symptoms | |
|---|---|---|
| | first victim (300 r) | second victim (450 r) |
| First, primary reaction | Loss of appetite, general debility, dizziness, nausea, vomiting during first days. Changes in the blood: leukocytosis, lymphopenia. Suppression of hematopoiesis in bone marrow; duration of first period, three days. | |

(Continued on next page)

| Period of disease | Symptoms | |
|---|---|---|
| | first victim (300 r) | second victim (450 r) |
| Second, latent course of disease, relatively good sense of well-being | Duration 21 days | Duration 16 days, Trichorrhea from 13th day |
| | General debility, at times dizziness and attacks of nausea. Drop in number of leukocytes versus the norm. Few lymphocytes. | |
| Third, climax of disease | Duration 20 days<br>Temperature to 38°C<br>Pulse, 74-92 per min | Duration 40 days<br>Temperature to 40°C<br>Pulse 100-110 per min |
| | General debility, clouding of consciousness, anorexia, sharp drop in the number of leukocytes, moderate drop in the number of erythrocytes and of the hemoglobin. Minute extravasations on separate sections of skin. | |
| Fourth, reconvalescence | Improvement in the general condition, drop of temperature to normal, disappearance of hemorrhagic symptoms, improvement in blood composition. | |

Workability returned to the one less affected three months after the start of the disease and to the second, in four months.

A description of radiation sickness reported and codified in medical terminology cannot, of course, give a concept of the sufferings of the patients and their experiences. Radiation sickness affects all tissues and organs, it makes the organism defenseless against infection. Once having had radiation sickness many complications can set in at remote periods.

Upon repeated irradiation with doses, not causing acute radiation sickness, but considerably greater than the maximal permissible, chronic radiation sickness can develop, the most characteristic symptoms of which are the change in the blood composition (drop in the number of white blood corpuscles, anemia) and a number of symptoms from the side of the nervous system.

Radiation burns. External irradiation with beta-particles and soft X-rays in sufficiently large doses leads to skin burns which heal with vastly greater difficulty than burns from thermal or ultraviolet (solar) rays, or don't heal at all. The trail-blazers in the use of X-rays in medicine, the first roentgenologists, on the basis of their bitter experience, became convinced that daily irradiation of the hands caused a number of morbid phenomena: the skin on the dorsal surface of the hand becomes dry to the touch, spotty, the nails crumble, warts appear, and after a certain time morbid ulcers not ammenable to treatment appear on the altered skin, and skin cancer develops.

One of the first radiobiologists, Bergonier, whose name was mentioned in Chapter III, underwent 14 operations to remove canger-affected fingers, hand, forearm, and arm.

Thus, the doctors and biologists, the pioneers of roentgenology and radiobiology, unwittingly became aware from their own experiments of the cancerogenic action of ionizing radiation, i.e., that it can be the cause of the development of cancerous tumors or malignant blood disease, leukemia; victims of leukemia in particular were Maria and Irene Curie, Enrico Fermi, etc.

## Cancer, Leukemia*, and Ionizing Radiation

Since the time of the first victims of X-rays, much has changed: now we have learned to protect ourselves from X-rays and other types

* Leukemia, a blood disease, is manifested by a sharp increase in the number of white corpuscles in the blood, which are produced in the healthy organism in the bone marrow, spleen, and lymphatic nodes. Cells, from which the leukocytes are formed, regenerate malignantly, thus leading to their unlimited multiplication. Leukemia can occur acutely or chronically. Moreover, several forms of leukemia are distinguished depending on the number of which leukocyte increased in the blood. According to the statistical data, the morbidity of leukemia has increased in recent years in various countries for unknown reasons.

of radiation, but the problem of the cancerogenic action of radiation in nevertheless still not eliminated. The attention of investigators is riveted to this problem and they study it from various aspects.

How does a normal cell degenerate to a cancer cell if it is not affected by radiation? Is the mechanism of this degeneration the same as that under the effect of ultraviolet rays and chemical substances, or something different? Is the cancerogenic action of different types of radiation the same? What minimum dose is still dangerous with respect to the cancerogenic effect? Does it depend on the irradiation conditions—single, fractional, chronic? What is the cancerogenic effect of various radioactive isotopes deposited in an organism? Where, in what organs are these deposits most dangerous? In what dose? Strenuous studies of these and other problems are being carried out throughout the world. Here are several examples characterizing different approaches to a solution of the problem.

One of them is statistical. According to the latest British data, the frequency of leukemia in roentgenologists as compared with doctors of other specialties is higher only in those who began their professional activity before 1920, i.e., when shielding of the apparatus was poor. But the British doctors are convinced that patients can suffer from X-rays. There is a rheumatic affliction called progressive curvature of the vertebral column. One of the methods of treating it is by X-irradiation of the vertebral column. Fourteen thousand case histories were examined and 38 cases of leukemia were found. Although a small proportion of the spondylitis patients treated with X-rays were sick with leukemia, this proportion is ten time greater than in the control and increases in proportion to the total dose of irradiation of the vertebrae, starting from a dose less than 250 rad to a dose higher than 2750 rad.

The relation between the irradiation dose and the frequency of leukemia was also established from the statistical data collected during the decade following the explosion of the atomic bomb at Hiroshima (Table 5).

TABLE 5

Number of Cases of Leukemia Among Hiroshima Victims, August 6, 1945

| Distance from hypocenter, m | Dise, rad | Number of victims | Number of leukemia cases |
|---|---|---|---|
| Less than 1000 | 1300 | 1 241 | 15 |
| 1000—1499 | 500 | 8 810 | 33 |
| 1500—1999 | 50 | 20 133 | 8 |
| More than 2000 | 2—0 | 65 655 | 12 |

The data of Table 5 are shown graphically in Fig. 23. An increase in the frequency of leukemia among those who were near the hypocenter during the explosion is quite evident. The British scientist Brown, who was much interested in the problem of leukemia, arrived at the conclusion that total irradiation doses of 30-50 r doubles the probability of this disease. However, there are still no reliable data that prolonged irradiation with small doses can have an effect of increasing the morbidity rate of leukemia.

In experiments on mice (this is the only animal whose frequency of leukemia cases can be increased by irradiation) it was established that fast neutrons are more damaging in this respect than gamma-rays, and that shielding of part of the body sharply reduces the probability of the formation of leukemia.

There is no lack in the statistical data of the increased frequency of tumors induced by ionizing radiation. Children are especially sensitive in this respect. In recent years it was found while processing the data of malignant tumors that, several years before the occurrence of tumors some of the children were treated for various reasons by X-irradiation of separate sections of the body at doses from 200 to 750 r. Even more impressive was the discovery that children up to 10 years of age developed cancer as a consequence of irradiating them with quite small doses, of the order of 2 r, while still in utero, when the pelvis of the gravida was X-rayed for diagnostic purposes.

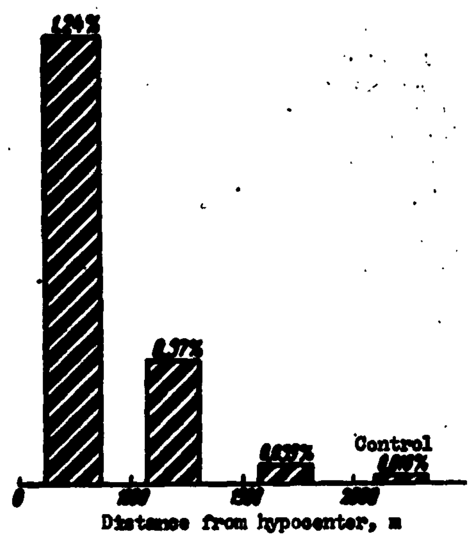


Fig. 23. Incidence of leukemia in persons located various distances from the hypocenter of the atomic explosion in Hiroshima.

The literature contains sufficient evidence of the occurrence of tumors when high amounts of radium are deposited in human bone and uranium dust and radon decay products are deposited in the lungs. These cases also pertain mainly to the time when little was known about the cancerogenic action of radioactive substances. For example, until 1932 certain American doctors were very enthusiastic about treating patients having podagra, syphillis, tuberculosis, schizophrenia, etc. with intravenous infusions of salts of radium and other radioactive substances. One of the doctors, as he himself reported in a scientific journal, made more than 7000 infusions of radium and obtained, according to his words, good results. After a quarter of a century information began to be collected concerning what happened to these patients. Those that were still alive were carefully and manifestly examined. The investigators attempted to find out how much radium remained in their bones, whether there were any changes and of what nature in the bones, what was the frequency of osteoncus of these persons, what was the nature of these tumors and where were they localized. Osteopathies were more severe, the greater the amount of radium in the organism. Malignant tumors, osteosarcoma, had developed in 15 of the 78 persons examined.

The stimulus for such an investigation was the desire to gather data on remote aftereffects of chronic irradiation of bones with alpha-particles of radium. Most of such data have been obtained in experiments on animals. Naturally if it is possible they must be checked in the human being. Such a possibility was afforded. These data, moreover, are needed for a proper evaluation of the deposition in bones not only of radium but also other radioactive substances, e.g., plutonium and strontium-90 ($Sr^{90}$). Of all the fission products the danger of this isotope* entering an organism in food and water is the greatest. It is well-known that the "strontium danger" created by testing nuclear weapons causes unrest in all corners of the world. This "strontium danger" is that the deposition of strontium in bones of adults and children threatens an increase in the frequency of bone cancer and leukemia. We are dealing with tens of thousands of persons

* Isotopes are varieties of an atom of one and the same element which differ in atomic weight.

who can be affected with tumors or leukemia by strontium entering the organism with contaminated food or water.

No one doubts the cancerogenic action of ionizing radiation. But there is a difference in the quantitative evaluation of this radiation effect. Certain American scientists tend to underestimate this danger, as if to justify the continuance of nuclear tests. Other scientists evaluate it rather soberly and objectively.

As regards the answer to the question of how the cells of an irradiated organism degenerate to cancer cells, it still has not been found in spite of all research endeavors, and later, more or less substantiated postulations still need verification.

One of these postulations is that the effect on a cell of sublethal irradiation doses cause alterations in the number and structure of the chromosomes, thus inducing malignant degeneration; another hypothesis "shifts the blame" to the mitochondria, where respiratory enzymes are concentrated. According to this theory, radiation injuries the respiratory enzymes, therefore cell metabolism proceeds over the improper pathway, and the cells "become disobedient" and begin to grow unchecked. Apparently, all occurrences of tumors over a rather long period of time are the result of the unfavorable reaction of an organism as a whole to the radiation effect, in which the nervous system plays an essential role.

## Effect of Radiation on Embryo and Fetus

Earlier investigations showed that in utero irradiation of the fetus led to the development of tumors several years after birth. But this is not the only possible effect on the embryo and fetus. During the developmental period, living organisms are in general quite sensitive to ionizing radiation. This conclusion was reached at the beginning of the century by the first radiobiologists who studied embryos of frogs, pregnant cats, rabbits, and guinea pigs; since then investigations have continued in this direction. Mice and rats are the favorite objects for these investigations.

The initial developmental stages of the mammalian fertilized egg are very similar, a difference being in the duration of the individual stages of the process. In man the first division of the ovum is accomplished 28 hr after fertilization, and 6.5 days later the dividing egg attaches to the uterine wall. The embryonic stage is completed by the 36th day; the fetus, which grows and develops until the end of intra-uterine life, is formed.

In mice and rats the same developmental stages occur more rapidly. Irradiation at different periods of embryogenesis and fetation leads to dissimilar consequences.

According to Fritz-Niggli the following can occur: a) the embryo does not develop further and is resorbed; b) the embryo continues to develop further and dies somewhat later; c) the development of the embryo is inhibited; d) development terminates and the child is born, but with such defects that it is inviable; e) the fetus develops and is born normal.

The stage preceding attachment of the ovum to the uterine mucosa, is the most sensitive. This was shown in experiments on mice that were irradiated with the same dose at different periods after fertilization. Irradiation during anlage of the organs leads to the development of various terata. For example, irradiation of rats during the 9-11 day period after fertilization with a dose of 25 r causes teratism of the organ of sight, like the absence of one eye. A dose of 100 r on the 9th day after fertilization causes in 90% of the cases various defects of the eyes and other organs (central nervous system, internal organs, skeleton).

Observations of people have been limited to individual cases of irradiation of pregnant women which was done owing to the need for radiation therapy of cancer.

After the bomb explosion in Hiroshima and Nagasaki these observations were supplemented, to the great sorrow of all peoples of good will, by the victimized pregnant women. The Japanese doctor Iamasaki studied the effect of radiation on children born of women irradiated during pregnancy. They investigated 98 women of which 30 had radiation sickness in an explicit form, the others suffered to a lesser degree or were not irradiated at all and served as the control.

The death rate of children born of irradiated mothers was 43%; of mothers without explicit signs of radiation damage, 9%; and in the controls, 6%. The fetus died and abortion occurred more frequently in the first group than in the second and third groups (Fig. 24). Various degrees of mental retardation and mental deficiency were observed in 4 of the 16 children of irradiated mothers by 1954. The other children were smaller in growth than those of the same age, their head dimensions were smaller. The same results in general were obtained by other investigators.

From these data we can make the conclusion that irradiation of a pregnant woman with doses of the order of several tens of roentgens can lead to fetal death before birth or to death of the newborn and to numerous physical and mental terata in children. Roentgenologists are presently resorting to radioscopy and X-raying of the abdomen and pelvis of pregnant women with greater care owing to the possible danger to the offspring.

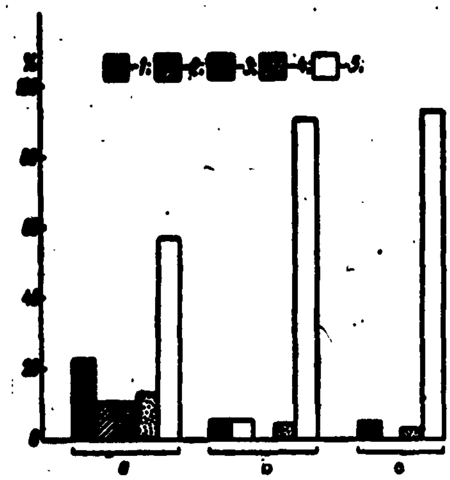

Fig. 24. Death rate of embryos, fetuses, and children irradiated in utero during the atomic explosion in Nagasaki. State of health of children that survived: a) mothers within a radius of 0-2000 with radiation sickness; b) mothers within the same zone without evident signs of radiation sickness; c) mothers within a radius of 4000-5000 m from explosion hypocenter (control); 1) fetal death; 2) death rate of newborn; 3) death rate of children; 4) mental retardation; 5) norm.

## Metabolic Disorders Induced by Ionizing Radiation*

In Chapter 3 we hypothesized that disorders of biochemical processes precede the apparent morphological alteration in irradiated cells. Just as in the whole organism, changes in the normal course of metabolism precede evident signs of radiation damage. Considerable

* By metabolism we mean an organism's absorption of the constituents of food, water, oxygen from the air and chemical changes which food products undergo in the organism, assimilation of these products by the organism for building its tissues and organs and for performing work, and, finally, the excretion from the organism of the end products of metabolism not needed and harmful to it.

endeavors and efforts have gone into studying these changes. The search for early and characteristic changes in human metabolism should lead in the end to the development of reliable methods of early diagnosis of radiation injury even before its clinical signs are analyzed. We can also expect that investigations of metabolic disorders will help to deepen our knowledge of the mechanism of development of radiation sickness, which is a necessary condition for the development of effective methods of treating it. Of greatest interest are the changes in metabolism associated with the formation of ions and excited molecules which occur during irradiation and immediately afterwards.

Nucleic acid metabolism. Disorders in nucleic acid metabolism and especially of deoxyribonucleic acid (DNA) are among the earliest, general, and relatively specific metabolic changes.

In Chapters II and III we were concerned about the structure of nucleic acids, their physiological role, and about the DNA contained in the cells and the RNA in the cytoplasm and nucleolus, and the changes in DNA on irradiation in vitro (depolymerization).

Investigations showed that the DNA content in such radiosensitive organs as bone marrow abruptly drops after irradiation; the DNA content in other organs also drops. This could be expected if we take into account that cell division is inhibited, some cells die, and, therefore, their number decreases at some period after irradiation.

Very curious data have been obtained on the qualitative changes of DNA in cells. As is known, bacteria "become accustomed" to antibiotics: they acquire the capacity to neutralize the lethal effect of an antibiotic. This capacity is transmitted by heredity, i.e., all cells produced from bacteria resistant to the antibiotic are also resistant to it. The transmission by heredity of this acquired property is associated with DNA. This property of resistance can be transferred together with the DNA isolated from resistant forms to strains* sensitive to the antibiotic. Experiments of this type have been carried out in particular with pneumococci, bacteria causing pneumonia, with strains resistant and sensitive to streptomycin. Pneumococci resistant to streptomycin were irradiated with gamma-rays under various conditions (dry and moist, whole and dissolve, in the presence and in the absence of oxygen) and the investigators studied whether DNA from these cells loses the capacity to transmit the property of resistance to the strains sensitive to the antibiotic. It turned out that on irradiation with doses of the order of millions of rads this property disappears, evidently owing to structural damages in the DNA.

The fact of the inhibition of the rate of DNA synthesis induced by X-rays, which was first established in 1942 by the Swedist scientists Hevesy and Euler, excited great interest. This discovery was made by the method of labeled atoms, or, as it is also known, the isotope method.

The isotope method was used in the following manner for investigating DNA metabolism. It is known that phosphoric acid is part of the composition of DNA. If we inject an animal, i.e., a rat, with the salt of phosphoric acid where some of the atoms of the phosphorus are represented not by its natural isotope $P^{31}$, but the artificially produced radioactive isotopes $P^{32}$, this salt will behave in the organism like the usual salt except that the radioactive phosphorus will make itself known by its radiation.

The pathway of the salt of phosphoric acid which enters the

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* Strains of bacteria, these are bacteria which belong to one species but differ from one another by characteristic morphologic and physiologic characteristics like breeds of animals.

intestine with food is easily traced if the salt is radioactive. The phosphorus, absorbed as an inorganic salt or in the composition of organic compounds, enters the blood, spreads throughout the entire body, penetrates the intercellular fluid from the blood, and thence into the cells. Here, with participation of enzymes, the phosphorus becomes involved in all possible metabolic processes and enters the composition of organic compounds more quickly and in greater amount, the greater the number of molecules of the organic compound decomposing and similtaneously be formed in unit time. This is called the metabolic rate of a given compound. If we separate a radiated compound and determine its radioactivity by comparing it with radioactivity of the injected phosphorus, we can calculate what number of molecules of a given compound decomposed and formed during a given time interval (if the processes of synthesis and decomposition were balanced).

By analyzing organs and tissues at different periods after the injection of radiophosphorus, we can trace the changes in the radioactivity of the compound in time.

Of all the phosphorus-eontaining compounds of a cell, DNA metabolizes most slowly, especially in those organs where there are few dividing cells. Hevesy found that in the mucous membrane of the small intestine, 16% of the DNA molecules are labeled, in other words metabolized during a day, 7% in the spleen, about 1% in the liver, and even less in the muscles and brain.

In order to solve the problem whether ionizing radiation affects the metabolic rate of DNA, Hevesy and Euler set up the following experiment. Rats were injected with a malignant tumor, sarcoma. When the tumor reached noticeable dimensions it was X-irradiated in doses from 335 to 2000 r. Immediately after irradiation they injected radiophosphate ($Na_2HP^{32}O_4$) into the rats. The rats were killed at different periods, from 30 min to 6 hr after injection of radioactive phosphate, the DNA was extracted from the irradiated tumor, and its quantity and radioactivity were determined. Rats which also inoculated with a tumor served as the control. The same was done with the controls as with the experimental rats, only they were not irradiated. It turned out that the radioactivity of DNA extracted from the tumor of the control rats was 2.3-3.2 times greater than that of the experimental rats. In other words ionizing radiation noticeably inhibited DNA metabolism, and this could be detected during the first hours post-irradiation before the development of evident signs of radiation lesions.

These experiments served as an impetus for carrying out investigations over the past two decades of DNA and RNA metabolism in the irradiated organism. The experiments were set up with a great diversity of variants: on different animal species (mice, rats, dogs, monkeys), with different radiations (X- or gamma-rays, neutrons), with different doses, with extraction of DNA from various organs of young, adult, and old animals, etc.

In general all these experiments, if we disregard details, confirmed the fact established by Hevesy and Euler: ionizing radiations inhibits DNA synthesis in cells and not only tumor, but also normal cells. This inhibition is most evident in radiosensitive tissues and organs: mucosa of the intestines, bone marrow, spleen, etc. Such a type of radiation effect is characteristic for all living organisms. For example, M. N. Maisel detected a decrease of radiophosphorus in the DNA of yeast cells when these cells were irradiated with a dose of 15,000 kr. This same effect is also detected in plants. For instance, irradiation of the roots of broad beans with a dose of 140 r lowers DNA synthesis by 40% by the 12th hr after this in comparison with the control. This process was also studied in insects.

Strictly speaking, radiophosphorus makes it possible to detect only inhibition of a compound of phosphoric acid with another component of DNA, deoxyribose. And how does the metabolism of still another component of DNA, the nitrogenous bases, change? The answer to this question was obtained by injecting compounds of labeled radiocarbon ($C^{14}$), which goes into building the nitrogenous bases (adenine, guanine, thymine, cytosine), into organisms. The radioactivity caused by the incorporation of $C^{14}$ into the nitrogenous bases was determined in the DNA extracted from the organism. The data shown in Table 6 testifies to the inhibition of synthesis and incorporation of nitrogenous bases into the DNA, detected by means of the radioactive tracer ($C^{14}$). Experiments with labeled thymidine (thymidine-$H^3$ or thymidine-$C^{14}$) led to the same results.

TABLE 6

Effect of X-rays on Incorporation of $C^{14}$ into Nitrogenous Bases (Purines) of DNA

| Animal species and organ | Dose, r | Injected substance labeled with $C^{14}$ | % of inhibition in comparison with control |
|---|---|---|---|
| Young rat liver | 950 | Acetic acid | 46 |
| Rat intestinal mucosa | 500 | Glycine | 57 |
| | | » | 74 |
| Rabbit intestinal mucosa | 800 | » | 74 |
| Rabbit bone marrow | 800 | | |

Therefore, the inhibition of DNA synthesis in an irradiated organism is an unquestioned fact detectable in animals, plants, insects, and microbes. Cell division in an organ or tissue occurs more vigorously, the more rapidly inhibition of DNA metabolism appears after irradiation and the more strongly it is expressed. This effect is reversible: after several hours or days, if the organism does not die from radiation damage during this period, DNA synthesis is restored and even exceeds the norm at a certain period.

However interesting or significant this fact is in itself, just the statement of it cannot satisfy the investigator. Why does inhibition of DNA synthesis occur? Are enzyme systems affected here and if so, what ones? What relation exists between inhibitions of DNA synthesis and inhibition of mitosis? What is the cause here and what is the effect? Is inhibition of DNA synthesis the initial change of biochemical processes in cells or is it preceded by blocking, by radiation, of some other processes preceding DNA synthesis? What is the role of the inhibition of DNA synthesis in the evolvement of the entire picture of radiation damage? Can a damaged cell be again induced to synthesize DNA at a normal rate and how is this done? Can not a disorder in nucleic acid metabolism be detected in a whole animal and this used for diagnosing radiation damage?

The search for answers to all these questions is stimulated in a theoretical sense in that they can elucidate the course of the most important biochemical processes in a cell, and in a practical sense in that a deeper penetration into the essence of the phenomenon promises success in the search for effective methods of diagnosing and treating radiation lesions.

The pages of scientific journals and the programs of conferences are filled with accounts of investigations whose purpose is to find answers to the aforementioned questions; lively discussions revolve around these problems and there are still no conclusions accepted by all. Therefore, we can limit ourselves to just one work.

At the Second International Conference for Peaceful Use of Atomic Energy (Geneva, 1958) the Czech scientists Paricek, Arient, Dienstbier, and Skoda reported that a product of nucleic acid metabolism, deoxycytidine, is excreted in an increased amount in rat urine during the first day after irradiation. This substance contains carbon, deoxyribose, and cytidine. The amount of deoxycytidine in urine within certain limits is proportional to the dose. Soviet investigators (T. A. Fedorova, Ye. N. Romantsef, and Z. I. Zhulanova) confirmed this fact.

It is still not known whether it will be possible to use the given index for diagnosing radiation lesions in the human organism, especially with small irradiation doses.

As regards nucleic acid metabolism no one doubts the fact of the inhibition of DNA synthesis, all agree that this is somehow associated with inhibition of mitotic divisions in the cell, and all are in accord that disruption of this biochemical process is relatively specific for ionizing radiation and ensues during the early periods after it. There is no such unanimity relative to other types of metabolism.

Protein metabolism. The greatest efforts have been applied to an investigation of protein metabolism since the data indicates its disruption.

In experiments on animals this results from a negative balance of nitrogen, which is found during the postirradiation days. A negative nitrogen balance means that more nitrogen is excreted from the organism than is taken up into the organism with food. We could assume that the reason for this is the animal's refusal of food, but if a control healthy animal is given as much irradiated food as it can eat, their negative balance will be less evident. With a normal ration the amount of nitrogen in the food and in the excretions will be equal for a healthy animal. A negative balance is characteristic of any severe illness in which an enhanced decomposition of protein, not covering its synthesis, occurs in the organism.

Observations of people also indicate an enhanced decomposition of protein. At the First International Conference on the Peaceful Use of Atomic Energy (Geneva, 1955), American researchers told about 4 persons who were victims of a reactor accident. They were irradiated with small doses of gamma-rays and neutrons, therefore, there were no signs of radiation damage in them; only one person, irradiated with a dose of about 150 r had slightly evident signs. Scrupulous laboratory investigations by the latest methods showed that the excretion of amino acids in the urine sharply increased (10 times) in all victims regardless of the dose received in the first week after injury and especially by the 6th day (Fig. 25).

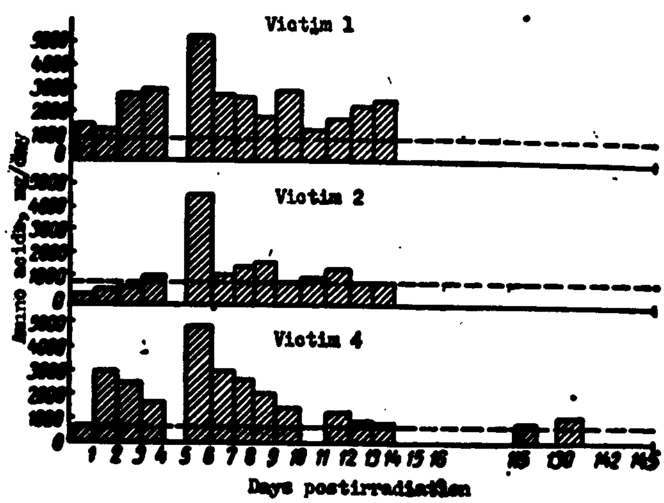


Fig. 25. Excretion of amino acids in the urine in those injured by gamma-rays and neutrons. We note an increased excretion beginning on the 1st day postirradiation with a maximum on the 6th day. The increased excretion is retained for a long time.

Usually only traces of two or three amino acids are found in the urine, but in these cases 14 different amino acids were noted. The increased excretion of amino acids in the urine was retained for a long time. This was also detected by the French doctors in the severely injured Yugoslavians. The loss of amino acids by an organism depends apparently not only on the breakdown of protein. There should have been some kind of conformity with the dose because cell destruction increases together with the dose. In addition, loss of amino acids continues while the body weight is being restored, while the blood is being regenerated and while everything indicates that the organism is getting the better of the injury. It is still difficult to say what is going on here.

A decrease in the ability of an irradiated organism to produce antibodies* can be associated with disorder of the process of protein synthesis, which partially explains the drop in the resistance to infection which is so characteristic for radiation damage. This is expressed also by the change in the composition of blood plasma proteins, a decrease or complete disappearance of the so-called gamma-globulin content; these proteins participate in the complex mechanism of protecting an organism against infection. After irradiation the content in the blood serum of a special protein which is an important factor for natural immunity, also decreases. It has the property "to dissolve" bacteria, the so-called bacteriolytic property. When the content of this protein in the blood drops or is completely absent, the bacteria "flood" the blood. This is observed after irradiation. Bacteria, which penetrated the blood from the intestine, impermeable in

* Antibodies are specific substances of a protein nature which are formed in an organism in response to the entrance of foreign proteins, antigens.

the healthy organism for them, can scatter from the blood.

M. N. Maisel and his cohorts, using very diverse methods, including the isotope method, found that upon irradiation of yeast with a dose of 50,000 r the amount of free amino groups increases and continues with a further increase of dose. That this is a consequence of damage to protein molecules is indicated by the release of sulfhydryl groups from the protein.

N. M. Sisakyan, studying the effect of X-rays (5000-30,000 r) on rye shoots, at first observed an enhancement of the biosynthesis of proteins, sucrose, and nucleic acids. After a day, enhancement of the synthetic processes was replaced by suppression, and at large doses by total repression.

Fat metabolism. Numerous investigations into disorders and other aspects of metabolism, in addition to nucleic acids and protein, have been carried out on various biologic objects. The data on the metabolism effect and fatlike substances are most notable. It was established that synthesis of fatty acids from acetic acids in the rat organism X-irradiated with a lethal dose is not only not inhibited but, conversely, is stimulated. This also pertains to the synthesis of the multiatomic alcohol cholesterol, which is a structurally complex organic compound with 27 carbon atoms in the molecule. If $C^{14}$-labeled acetic acid is injected into a rat during the first postirradiation hours and then is killed after 4 hours and the cholesterol extracted from the liver or adrenal glands, then, as the author of this book and K. A. Tret'yakova showed, its radioactivity will be several times greater than that of the cholesterol in a control rat. Consequently, the rate of cholesterol synthesis after irradiation sharply increases, however its content did not change in the liver and even decreased in the adrenal glands. This means that not only synthesis, but also decomposition of cholesterol was accelerated.

The cause of the acceleration of fatty acid and cholesterol synthesis is still not clear. But changes in the metabolism of sterols are characteristic not only to the animal organism. M. N. Maisel, in a report at the First International Conference on the Peaceful Use of Atomic Energy (Geneva, 1955) reported that a substance similar to cholesterol, viz., ergosterol, is accumulated in yeasts on irradiation with 80,000 r. Its content increases by 200% as against the norm. At smaller doses the ergosterol content in yeasts also increased, but not so strongly.

Fatlike substances, phospholipids, are sensitive to ionizing radiation. Damage to them leads to changes in the structural organization of the cell and to disturbance of the permeability of cell walls.

Mineral metabolism. Disorder of mineral metabolism is certain. In the case of the victimized Yugoslavian scientific workers this was expressed in a change of the content of sodium, potassium, and chlorine in the blood plasma. In the experiments on rats carried out by the author of this book and by T. I. Ivanenko it was also found that during the first days postirradiation, sodium is retained in the organism and the excretion of potassium increases, which occurs apparently due to a change of the hormonal activity of the adrenal cortex.

On the cell level there is also found that irradiation with large doses changes the permeability of cell membranes for the potassium ions and leads to escape of the potassium from the cell.

The hundreds of works devoted to the biochemistry of radiation injury contain material characterizing disorder of other aspects of metabolism. It is strange, however, that deviations in such complex processes as oxidation of various cell substances (cellular respiration) and splitting of carbohydrates (sugar) with the formation of lactic acid, caused by the sequential action of a number of enzymes, were not detected in the early periods. This latter process is called glycolysis.*

Disturbance of oxidative phosphorylation. It became known comparatively recently that, although cell respiration is not inhibited, which can be judged by the consumption of oxygen by the surviving tissues of an irradiated animal, the use of the energy released on oxidation is not as efficient as in a normal organism. The essence of the matter is that the same end products, carbon dioxide and water, are formed on combustion of fat in the frying pan and on oxidation of fat in organism; in the first case all energy released on combustion is immediately converted to heat, whereas in the organism this energy is "stored" for accomplishing chemical reaction proceeding with the absorption of energy and for performing mechanical work, e.g., muscle contraction. Storage of "energy" occurs so that during the course of oxidation in a cell an inorganic compound of phosphorus, phosphate, is bound with an organic compound, a nucleotide, adenosine monophosphoric or adenosine diphosphoric acid, with the formation of adenosine triphosphoric acid. It is in this last compound that the oxidation energy is "stored" or, more correctly, is changed to a form convenient for utilization.

In the language of biochemists the binding of inorganic phosphorus which occurs during oxidation in a cell is called oxidative phosphorylation. In cells the enzymes responsible for oxidation and phosphorylation are concentrated in the mitochondria.

The Dutch scientist van Bekkum irradiated rats with doses of the order of 100-300 r, extracted the mitochondria from spleen or thymus

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* The process of photosynthesis which is accomplished in the chloroplasts of plant cells is resistant to irradiation.

cells after 4 hours, and checked their ability to oxidize and bind phosphorus. Mitochondria extracted from cells of the same organs of healthy rats and tested by the same method were the control. It turned out that irradiation little affected oxygen consumption, and appreciably inhibited binding of phosphorus. This was detected 30 min and 4 hr after irradiation when suppression of mitotic divisions could be noted in the cells but the cells themselves were still whole.

In order to remove doubt about the possible injury of mitochondria when they were extracted from the irradiated organisms and subsequently tested, experiments were set up on whole animals. The experimental setup was simple in principle: control and irradiated animals were injected intravenously with labeled inorganic phosphate ($NaH_2P^{32}O_4$) and 4 hr later adenosine triphosphoric acid was extracted from the spleen. On inhibition of oxidative phosphorylation less radioactive phosphorus should be incorporated into adenosine triphosphoric acid than in the control. And this is what actually occurred: adenosine triphosphoric acid from irradiated rats was less radioactive than from unirradiated ones.

But inhibition of phosphorylation concomitant with oxidation is not specific only for the radiation effect, although this effect like the effect of disorders of lipoid metabolism after irradiation, was general for animals and microorganism.

M. N. Maisel found that cell respiration in yeasts is resistant to irradiation, while phosphorylation associated with it is inhibited. As he surmises this results in the synthetic processes in suffering the most in the cell.

It is possible, as A. M. Kuzim proposed, that there is some kind of relation between the disturbance of DNA synthesis and inhibition of oxidative phosphorylation. The answer apparently will be obtained after the interrelation of biochemical processes in a normal cell become better known.

Summing up the results we can say that in spite of all the attempts of numerous investigators, the little chest with the secrets of the changes of biochemical processes induced by radiation hidden in it, is being opened with great difficulty. Nevertheless it is impossible to assert that a chain of processes leading to apparent changes in the cell and in the organs begins with inhibition of DNA synthesis, blocking of oxidative phosphorylation, acceleration of cholesterol metabolism, and synthesis of fatty acids.

It is possible that all these alterations during the course of biochemical processes are preceded by others which are still not revealed. Considerable significance has been imparted, for example, to an increase in the permeability of intracellular membranes which apparently separate the enzymes and their substrates in the cell. But an increase in the permeability of every possible membrane in cells and tissues of an organism also cannot be considered as a mechanical process of the "perforation" of membranes by an ionizing particle. The change of permeability is, in turn, the consequence of still unknown physiochemical and biochemical processes.

But no matter how sparse the results of investigations into disorders of biochemical processes, without them we could not move forward, striving for a deeper knowledge of the essence of the biological actions of radiation in order to eliminate it where it does harm and to use it where possible in the interest of man.

# Chemical Substances with an Effect Similar to Ionizing Radiation

Radiobiology has derived an undoubted advantage from the fact that poisoning with certain chemical substances are very similar to the picture of radiation injury with respect to their aftereffects. These substances have therefore received the name radiomimetic, i.e., imitating the action of ionizing radiation. These substances include the nitrogenous mustard gases:

$$R.N\begin{cases} CH_2CH_2Cl \\ CH_2CH_2Cl \end{cases}$$

Different groups of atoms can be joined to the nitrogen at the R site. This distinguishes the mustard gases from one another, but the group of atoms $(CH_2CH_2Cl)_2$ which enters into sulfur mustard gas used on the fields of combat in World War I has toxic properties.

Nitrogenous mustard gases and other radiomimetic substances are similar to ionizing radiation by their action. In particular these substances:

a) for a certain time inhibit cell division which is manifested in a decrease in the number of mitotic divisions; consequently they inhibit growth;

b) like ionizing radiation they cause breakage and rearrangement of chromosomes which can lead to cell death;

c) they damaged relatively more the frequently dividing cells, for example cells of the hematopoietic organs, therefore leukopenia and anemia develop on poisoning by radiomimetic substances;

d) they inhibit the formation of antibodies;

e) they cause loss and graying of hair where the skin is affected.

Poisoning with radiomimetic substances, like the action of ionizing radiation, leads to changes in an organism transmissible by heredity (Chapter VI). We can assume that radiomimetic substances change the structure and metabolism of nucleic acids.

The property to cause the formation of tumors which develops at remote periods after poisoning is also characteristic of these substances. M. N. Maisel and team confirmed the similarity of the effect of ionizing radiation and one of the nitrogenous mustard gases, mechlorethamine hydrochloride (embikhine) with the formula $CH_3N(CH_2CH_2Cl)_2HCl$ on yeast cells. Embikhine strongly suppressed multiplication and growth of cells and weakly acted on respiration and fermentation, inhibited oxidative phosphorylation, and, just like ionizing radiation, enhanced the accumulation of sterols in yeast cells.

It is very probable that the primary process from which cellular damage begins both on irradiation and under the effect of mustard gases is one and the same—inactivation of DNA, achieved apparently by different pathways.

However with all the similarities of affections from ionizing radiation and radiomimetic substances it is impossible to assert that they develop in the cell organism by virtue of the action of one and the same mechanism. A detailed study reveals differences in the character of the lesion, for example at the time of the appearance and in the type of breaks of chromosomes, in the clinical features of poisoning and of radiation sickness, etc. There is no doubt that the action of the purely physical factor, ionizing radiation and chemical substances of a certain structure, at least in the beginning, cause dissimilar changes in the cell, but in the end these changes inhibit cell division.

Not only radiobiologists are attracted to the possibility of reproducing lesions similar to those induced by ionizing radiation, by using radiomimetic substances. Specialists on the treatment of malignant tumors and those searching for chemical substances suitable for this purpose are also interested in this. If nitrogenous mustard gases suppress cell division, especially in the hematopoietic organs, then cannot they be used at least for treating leukemia and perhaps malignant tumors in general? L. F. Larionov has proposed using embikhine for treating certain forms of leukemia.

Tens and hundreds of various substances have been synthesized and tested to reveal with what characteristics of the structure of chemical compounds and with what atomic groups the radiomimetic action is associated. It was established that not only the grouping of atoms characteristic of mustard gases $(CH_2CH_2Cl)_2$ can render a radiomimetic effect, but also a number of other chemical groupings cause this same effect. At the same time several preparations were found which are now used in the treatment of malignant blood diseases. The effectiveness of these preparations still leaves much to be desired.

## CHAPTER V

## CHEMICAL PROTECTION AGAINST RADIATION AND PROBLEMS OF TREATING RADIATION SICKNESS

The theoretical investigations in the field of radiobiology have as their end purpose the discovery of methods and means to prevent radiation injury and to eliminate its aftereffects where they occur. In addition, while the radiation method of treating malignant tumors remains one of the effective methods, any progress in the field of radiobiology will foster improvement in the therapeutic methods of this severe disease.

### Prevention and Prophylaxis of Radiation Injury

It is well known that at the time, measures of engineering shielding, which the engineers themselves call biological, are practically the only method of preventing radiation lesion. By biological shielding the engineers evidently want to emphasize that for reactor operations there is no need to enclose the reactor in a thick steel shell, to surround it with a many-meter-thick concrete wall and to construct other complex and expensive structures to oblige the "delicate" nature of man which cannot tolerate ionizing radiation.

For a long time it seemed that this method of preventing radiation injury was the only practical and theoretical one possible and that is was impossible to consider that it would be possible to make a "vaccine" against radiation sickness like that against smallpox. Actually there is still no hope for this. But theoretical investigations in the field of radiobiology have led to the conclusion that the action of radiation can be reduced by chemical agents and "antitoxins" can be found.

Two facts here apparently have substantial significance: first, the discovery of oxygen effect, i.e., the decrease of damage on irradiation in an anoxic medium, and second, the presence of a similarity between the action of ionizing radiation and certain war gases (BOV). An effective antidote was found for one of the BOV during the Second World War. Reasoning by analogy, it is possible to test this antidote or a substance similar to it in structure against radiation damage.

Utilization of the oxygen effect. The search for chemical protective agents led to the discovery of many methods to increase the resistance of an organism to irradiation. A lack of oxygen in animal tissues can be caused by lowering the oxygen in the inspired air, by reducing the capacity of hemoglobin to bind the oxygen in the lungs and to transfer it to tissues, by lowering the blood supply of tissues and consequently the transport of oxygen to them, by creating conditions for enhanced oxygen consumption by tissues when its uptake does not cover expenditures, and by other methods.

Here are certain of the agents tested. A decrease in the capacity of hemoglobin to bind oxygen in the lungs is achieved, for example, by poisoning the animal with carbon monoxide. In this case the hemoglobin is partially bound with a carbon monoxide and loses its ability to transport oxygen to tissues. An experiment showed that mice with carboxyhemoglobinemia are more resistant to irradiation than normal ones when 60% of the hemoglobin is combined with carbon monoxide.

Another method of affecting the hemoglobin is the oxidation of its divalent iron to trivalent which is strongly bound with oxygen. This can be done by injecting sodium nitrite and a number of other substances into the animal. These experiments have also been set up, and again the intoxicated mice were more resistant to irradiation than healthy ones. For example, on injection into mice of sodium nitrite based on 100 mg per 1 kg of body weight 30 min before irradiation with 700 r, all mice survive whereas the controls died.

A hormone* of the adrenal gland, epinephrine, has the property to constrict the lumen of small vessels, therefore an injection of epinephrine prevents the supply of oxygen to tissues for a certain period. This probably can explain the protective action of epinephrine.

Many other agents and methods have been tested to increase radioresistance by lowering the oxygen concentration in tissues; this includes, for example, the injection of substances toxic to respiratory enzymes, easily oxidized substances like alcohol, glucose, etc. The prophylactic effect does not always pertain just to the oxygen effect. Apparently the oxygen effect does not explain the prophylactic action had by substances of the sex hormone type, for example hexestrol and

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* Hormones are substances dissimilar in structure produced by endocrine glands (thyroid, pancreas, adrenal, sexual, etc.). Hormones, passing into the blood and from there into tissues, regulate physiological and biochemical processes in an organism. For example, insulin and epinephrine regulate the blood sugar level.

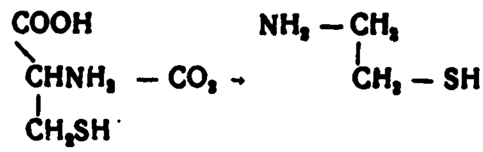
diethylstilbestrol, tested by N. I. Shapiro, N. I. Nuzhdin, and A. M. Kuzin, or the growth hormone tested by the author and also 5-hydroxytryptamine whose protective properties have been studied in greater detail recently.

Frequently it is not easy to analyze what causes a prophylactic effect since not all experimental facts fit into a single explanation. We still do not understand why a test substance is effective for one species of animals and ineffective for another, why, for example, it protects microbes and does not protect animals, etc. The desire to analyze why this is so has resulted in setting up numerous experiments with various substances and under different conditions. Researchers by affecting in one way or another the development of radiation damage, endeavor to explain how it arises and is developed in an organism. Of course not a single one of the named agents, poisoning with carbon monoxide, sodium nitrite or even the most harmless intoxication with ethyl alcohol, has practical value in the prophylaxis of radiation sickness. This does not belittle the theoretical value of the investigations that are carried out: they first of all confirmed that between the time of absorbing the energy of X- or gamma-rays and evident damage there occur biochemical processes which we can attempt to influence in order to normalize them.

Sulfur-containing protective substances. Another direction in the search for prophylactic agents was the testing of sulfur-containing compounds. The antidote against lewisite, so-called British anti-lewisite, found during the Second World War is one of them. This comparatively simple compound whose composition has two sulfhydryl groups (–SH) was ineffective against radiation when tested on mammals. A different result was obtained with the amino acid cysteine which was mentioned earlier. This amino acid, added to an aqueous solution of an enzyme after in vitro irradiation, partially or completely restored the activity lost by the enzyme.

Several groups of investigators almost simultaneously and independently began testing cysteine and related compounds in experiments on animals. The American scientist Patt and his team reported in 1949 that they injected cysteine into one group of mice after irradiation and in another just before irradiation, and mice X-irradiated with the same dose but not administered the preparation serve as the control. Cysteime proved to be completely ineffective when it was injected into the animals after irradiation, but this amino acid definitely protected animals when injected intravenously or subcutaneously immediately before irradiation. In order to kill 50% of the protected mice it was necessary to use a dose twice that used in the control. This effect of cysteine was varified in experiments in other species of animals, plants, microorganisms and also in cultures of normal and cancer cells. Cysteine, a constituent of proteins, is a substance not foreign to an organism, however the protective action of cysteine is manifested only when injected in relatively large amounts bordering on toxic.

In 1951 the Belgian scientist Bacq and his cohorts found that another substance close to cysteine in structure, cysteamine, has certain advantages over cysteine as a protective agent. Cysteamine is more easily prepared, it is more resistant than cysteine, and this preparation can be administered in smaller doses for obtaining the same effect. Cysteamine belongs to the group of so-called amines formed on splitting off carbon dioxide from amino acids. All amines have a strong physiological effect. The similarity between cysteine and cysteamine is seen from their formulas:



The results of one of the first experiments set up with cysteamine are shown graphically in Fig. 26. Mice immediately before irradiation were injected intraperitoneally with 3 mg of cysteamine in a neutral aqueous solution. Then they were irradiated with X-rays in doses of 700, 900, 1100, 1300 r. A dose of 700 r was sufficient to kill all control mice by the 8th day postirradiation. At this dose all protected mice remained alive, at a dose of 900 r some of the protected mice died, and at the larger doses all mice perished. Consequently, cysteamine increases by a factor of almost 2 the radioresistance at the minimal lethal dose.

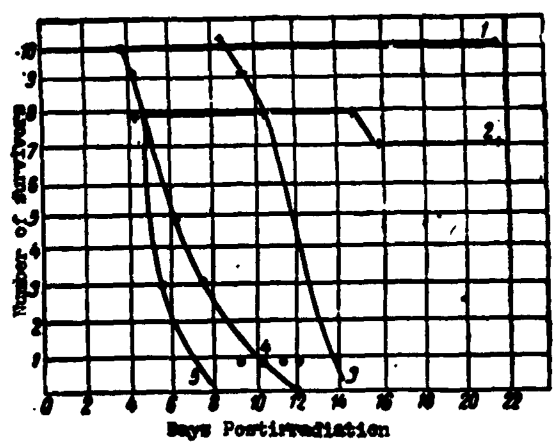


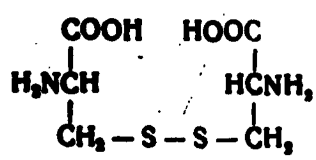

Fig. 26. Protective action of cysteamine on mice (each mouse was administered 3 mg of cysteamine before irradiation). Irradiation dose (r): 1) 700; 2) 900; 3) 1100; 4) 1300; 5) control 700.

The establishment of the fact of increasing radioresistance by chemical agents broke the resistance of skeptics who doubted that it was possible to change anything during the course of processes in organism if a lethal dose of ionizing radiation is absorbed in it.

Studies on the effect of protection develop in many laboratories of the world from the time of publication of the investigations of Patt, Bacq, etc. Researchers have sought and continue to seek agents more convenient and effective than cysteine and cysteamine, they checked the spectrum of their effect, and reveal whether the diverse aspects of radiation damage are removed by protective substances, and attempt to find the answer to the problem of what is the protective mechanism.

To the vexation of researchers who retested hundreds of different compounds to reveal their protective ability, very few results have been obtained in the practical sense, which can be judged by the published data. True, it was revealed from these studies what chemical groups are important for protection, what should be the molecular structure, and a number of other problems important in a theoretical sense.

Cysteamine is not effective when administered perorally, but Bacq found that a compound close to it, cystamine




when administered perorally a half hour before irradiation produces a protective effect similar to cysteamine because cystamine is split into two molecules of cysteamine in an organism.

The Americans synthesized and tested another substance similar in structure to cysteamine with a long name of aminoethylisothiuronium bromide hydrobromide, abbreviated AET.* This substance is more stable than cysteamine, slightly less toxic for animals and more effective with any means of administration.

AET almost doubles the dose leading to 50% death of irradiated mice, namely from 692 to 1148 r for mice of a pure strain.

As was learned in a number of investigations, both cysteamines and AET manifest their protective action on various biological objects and not just on these. Alexander revealed that cysteamine and certain other substances on being added to a solution containing the polymer polymethacrylic acid protected from destruction by ionizing radiation.

Cysteamine and other substances reduce radiation damage in plants, microbes, infusoria, rats, guinea pigs, and monkeys. It was found for the intestinal bacteria Escherichia coli that cysteamine reduces the damaging action of gamma-rays by a factor of 12 (Fig. 27), whereas the absence of oxygen in the medium reduces it only by a factor of 3. L. F. Semenov found a protective action of cysteamine, AET, and certain other compounds on monkeys.

But whatever object was selected the effect of cysteamine, AET, and other substances occurred only when they were administered 5-15 min before irradiation. No matter how briefly was the irradiation and no matter how rapidly these substances were administered, as a rule they had no influence after irradiation on the outcome of damage although the time was measured in seconds.

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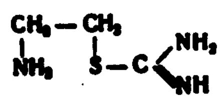
* Formula for AET:



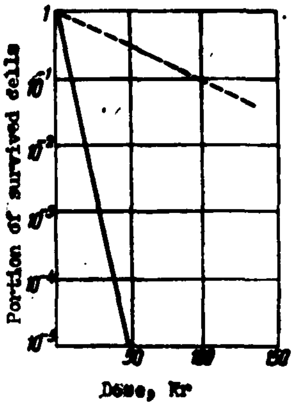



Fig. 27. Radiosensitivity of E. coli of the B/r strain to gamma-rays in the presence of cysteamine (dashed line) and without it (solid line).

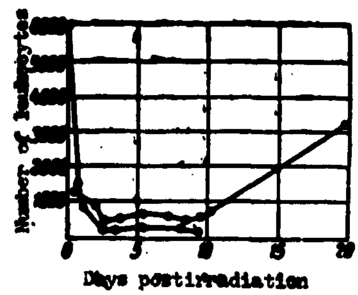



Fig. 28. Change in the number of leukocytes after irradiation of protected and unprotected mice. Originally the changes are the same, but after a certain time the number of leukocytes in the protected mice begins to be restored.

A check also showed that a protective effect can be detected only on irradiation with X- or gamma-rays; protection against neutrons which produce dense ionization, was very weak. A protective action of chemical substances is not demonstrated in the absence of oxygen.

What distinguishes the course of radiation injury in protected animals from control animals?

The differences are not great. This can be judged by such factors as the decrease in weight and change in the blood composition. In Fig. 28 we have compared data on the change in the number of leukocytes in mice irradiated with a lethal dose of 700 r with and without cysteamine protection.

Up to the moment of death of the control animals the number of leukocytes in the control and in the experiment were similarly small, but in the surviving animals it gradually increased as the animals recuperated; affection of the hematopoietic apparatus was reversible in the protective mice.

However, not all manifestations of the radiation effects disappear without a trace. In several laboratories, irradiated animals, mainly mice and rats, which did not perish owing to use of chemical protection, were left to live out their life, and observations were made of them as to whether they aged prematurely or whether the incidents of tumors and leukemia was greater in them than in unirradiated animals. Although no final conclusions have yet been made, it was nevertheless noted that the life span of these animals was somewhat less than that of the controls, that the cancerogenic action of radiation remains, and that protection apparently does not safeguard animals from the occurrence of deviations transmittable by heredity.

An important property of cysteamine, as was shown in experiments on rabbits, is the prevention of the development of cataracts occurring as a consequence of the effect of X-rays and beta-particles on the eyes.

Mechanism of protection by sulfur-containing substances. The universality of the protective action of cysteamine and AET has been shown as a result of a 10-year study. This was established on objects of a nonliving nature (polymers) and on most biological objects (plants, infusoria, bacteria, animals, cells in tissue cultures, etc.). It was proved that the protective substance must be present in the cells at the time of irradiation. It was found that preparations are ineffective on irradiation with neutrons, protons, or alpha-particles, and also when the investigated object is irradiated in an anoxic medium. All these facts are explained if we assume that underlying the protective action is some kind of general mechanism having a relation with the initial reactions following the absorption of radiation energy. Such a mechanism can be, as Bacq and Alexander consider, "scavenging" of free radicals, particularly the radical $HO_2$, by the protective substance. This radical is formed under the effect of X- or gamma-rays in a aqueous medium in the presence of dissolved oxygen in it. "Scavenging" of the $HO_2$ radical prevents it from acting on important biological substrates and at the same time, on all subsequent processes leading to radiation damage. Only those processes which need the direct effect of irradiation for their origination remain in force.

This theory, in which the protective action is considered as a purely physiochemical process of conquering free radicals, is not shared by all investigators, and even its creators are prepared to agree that it does not exhaust all possible mechanisms of protection in various biological objects.

In a report at the International Symposium of Radiobiology in October 1960 in Moscow, Bacq and Alexander expressed doubt that the main role in the radiation effect belongs to a radical $HO_2$ and that the effect of protection amounts to "scavenging" of precisely this radical. On the molecular level, in the opinion of these scientists, protection can be caused: a) by removal of absorbed energy from a biologically important molecule to the protective substance; b) restoration of the damaged molecule by means of the protective substance; c) by a temporary combining of the protective substance with a biologically important molecule which makes it more resistant to irradiation.

A number of scientists tend to think that the oxygen effect again goes into action on protection by cysteamine and substances similar to it. Oxygen is expended for oxidation of the protective agent, its concentration decreases in the cell at the time of irradiation, and its resistance increases. A number of facts fit into this theory, receiving an intelligent explanation in it. But certain agents having an unquestionable protective action do not belong to those that are easily oxidized, and therefore on penetration into the cell cannot lower the oxygen content there.

There is the hypothesis that at the moment of irradiation, cysteamine and similar substances "shelter" sensitive groups of enzymes from the effect of radicals and thus protects them from damage. But the objection to this is that the activity of enzymes in cells is not decreased after irradiation, that they are sufficiently protected by the normal cell constituents.

A variant of the theory of protection of enzymes is the recently expressed hypothesis that radiation converts atoms of metals in the cell to a form which can block enzymes, and cysteamine and other similar substances "sequester" these metals thus preventing them from poisoning enzymes.

Finally we note that protective agents have a strong effect on respiration, blood circulation, reduce body temperature, and change other important functions of an organism. It is possible that the pharmacologic effect of agents also has significance in the protection of higher animals.

To explain the mechanism of action, radioactive cysteamine and cystamine, by incorporating radiosulfur ($S^{35}$) into the molecule, and the fate (distribution, transformation, and excretion) of these substances in animals was studied. Cysteamine penetrated into all organs and tissues, but mostly into the liver, intestinal walls, and kidneys, and least of all into the sexual glands. A portion of it is excreted from the organism in an unchanged form; about 13-19% of the incorporated radioactive sulfur was retained in the organism more than

10 days.

Thus, it was proved that chemical substances can weaken by half the action of ionizing radiation (X- and gamma-rays). For many test agents this action can be associated with the oxygen effect, such an explanation is evidently insufficient for cysteamine-type substance. A deeper penetration into the mechanism of the protective effect promises that theoretical research in the field of prophylaxis of radiation damage by chemical agents will bear practical fruit. Bacq has repeatedly expressed the thought that under exceptional circumstances cystamine or cysteamine can be used before unavoidable irradiation to reduce its aftereffects.

## Recovery Processes in the Irradiated Organism and Problems of Therapy

In no matter what lies the initial mechanism of the protective action of cysteamine and other substances, attenuation of the irradiation effect is achieved by recovery processes in the protected animal, whereas in the unprotected animal they are completely suppressed or do not have time to be demonstrated before death of the animal.

Recovery processes have a decisive value in the outcome of radiation damage. But time is needed to accomplish them. This is indicated by experiments where animals are radiated at different time intervals with nonlethal doses. The greater the interval, the smaller is the residual damage from the first irradiation. By rigorously measuring the doses, it is possible to find a mathematical expression for the recovery process. It was also established in these experiments that the recovery process does not go to the end, that some portion of damage remains.

Depending on the conditions, the recovery processes can be demonstrated to a greater or lesser degree. This is especially apparent in experiments with irradiation of Escherichia coli. There the proportion of surviving cells is considerably increased if they are incubated after irradiation at a temperature of 18°C. A lower or higher temperature sharply reduces the number of surviving cells (Fig. 29). This effect is observed at any irradiation doses with X- or gamma-rays. Evidently the role of the medium temperature in the survivability of bacteria is that "destructive" processes actuated by radiation are inhibited at 18°C, whereas recovery processes, if they are slowed down, are to a lesser extent and, therefore, outweigh "destructive" processes.

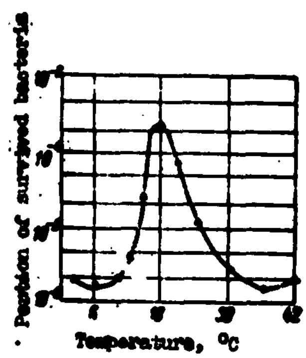



Fig. 29. Survivability of E. coli (B/r strain) after irradiation with a dose of 80,000 r versus incubation temperature. 18°C is the optimal temperature.

The matter is more complicated in warm-blooded animals and the significance of the temperature factor cannot be revealed for them, but the role of the recovery processes is easily established by simple experiments.

In Chapter III it was stated that it suffices to shield a portion of the body on total irradiation in order to increase noticeably the survivability of animals with lethal doses of irradiation. It suffices for this purpose to wrap a part of one haunch of a rat with lead foil which absorbs radiation; the same can be achieved by shielding the head, abdomen in the region of the liver or spleen, or even the tail. Shielding of parts of the body in total irradiation ensures the start of recovery.

A detailed study of the role of shielding for recovery after injury was begun in 1949 by Jacobson. He was interested in the spleen which is an important organ for hematopoiesis. The experiment was set up on mice. At first he made a simple operation: he opened the abdomen,exteriorized the spleen so that its blood supply and all nerve connections were completely retained. The spleen was enclosed in a lead sheath which protected it during total irradiation of the mouse with X-rays. The controls were mice whose spleen was not protected by lead during irradiation. Immediately after irradiation the sheath was removed, the spleen again was installed in its place in the abdomen and wound sutured. Then he observed the outcome of irradiation in the experimental and control animals, analyzing the blood from time to time. He learned that at a certain dose only 2% of the mice out of 176 survived by the 28th day in the control and 76% in the experiment. Although in mice whose spleen was shielded a drop in the number of leukocytes in the blood was also observed, it rather rapidly began to return to normal, whereas in the control mice it continued to remain low until their death (Fig. 30).

In later investigations and other investigators revealed new interesting facts concerning the role of the spleen in recovery processes. Here are some of them. If a lead shielded spleen is excised 1-5 min after irradiation, the survival rate of mice drops to that of the control. On excision of the spleen 1-6 hr after irradiation the per cent of surviving animals remains higher than in the control.

If after total irradiation of a mouse a spleen from an unirradiated mouse is transplanted in it, then the recovery processes will manifest themselves by an accelerated normalization of the blood picture. The transplanted spleen, as was subsequently learned, survives and becomes an accessory organ.

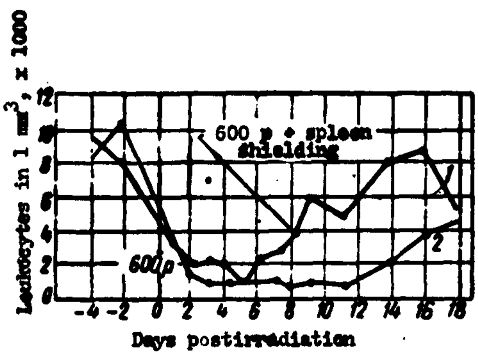



Fig. 30. Value of shielding the spleen during irradiation for restoration of the number of blood leukocytes: 1) change in the number of leukocytes in the mouse with a shielded spleen; 2) the same without shielding. In the first case restoration in the number of leukocytes proceeds more rapidly.

A great step forward in developing the problem were the experiments which showed that it is possible to transplant not a whole spleen but to prepare brei (homogenate) from it or bone marrow of an unirradiated mouse. Upon injection intravenously of this brei into a totally irradiated mouse immediately after irradiation or after a little while we observe what can justifiably be called a therapeutic effect of this preparation. A suspension of minced tissues of 12-day-old mouse embryo has the same therapeutic action. A therapeutic action was also rendered by bone marrow taken from animals of different species: rats, guinea pigs, as well as cells of the bone marrow cultivated in vitro for up to 3 weeks. Here we note that the portion of mice which received transplanted bone marrow taken from rats recovered from radiation sickness, but died owing to complications caused by the transplant of bone marrow of a different species of animals.

It is natural that the question immediately arose: with what is associated the therapeutic effect of bone marrow suspensions? We could assume that there is a substance in this suspension which can stimulate restitution of the hematopoietic organs of an irradiated animal. What is this substance? It was suspected that it is a conjugate protein consisting of DNA and a simple protein. Another postulation was that undamaged cells are retained in the bone marrow suspension and that their presence in the preparation explains the therapeutic effect. Presently the odds are in favor of the second hypothesis.

The advocates of the cell theory of the therapeutic effect based themselved mainly on the very skillful, fine, and beautiful experiments set up by Barnes and Loutit. They proved that in an irradiated organism, spleen or bone marrow cells taken from a healthy animal and transplanted there are retained and are not only retained, but multiply. The experiment was set up as follows. Mice of a certain strain were irradiated with a lethal dose of 950 r and then injected intravenously with a suspension of bone marrow extracted from the two femoral bones of young rats. After various periods the mice were killed and preparations made from their bone marrow, spleen, thymus, and lymph glands. After appropriate treatment the preparations were studied under a microscope. Cells fixed in the division process could be detected on them and the shapes of the chromosomes distinguished. In the cells belonging to mice, all chromosomes in the norm have the shape of a horseshoe, and some of the chromosomes in rat cells resembled crosses. It turned out that dividing cells, the shape of the chromosomes of which left no doubt that they belonged to the rat, were detected in the preparations of bone marrow and other tissues of the irradiated mouse after infusion of the suspension of rat bone marrow.

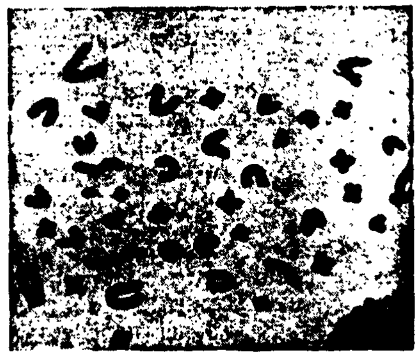


Fig. 31. Photomicrograph of chromosomes in the cells of the Wistar strain of rats. Taken in the metaphase of cell division. Characteristic crosslike chromosomes are seen here. Such chromosomes were detected in dividing cells from an irradiated mouse after transplantation of rat bone marrow.

It was also confirmed in another experiment that transplanted cells survive and multiply in an irradiated organism. This time spleen taken from another line of black mice was transplanted to white mice. A special feature of cells of this strain detectable upon their division was the presence among the 40 chromosomes of 1 exceptionally small one which immediately attracts the eye. In this case dividing cells with a small chromosome, i.e., belonging to mice whose spleen was taken for transplantation, were found in the preparation from tissues of the irradiated mouse. Thus it was established that during the first days and weeks after irradiation, dividing cells belong not to the host, but to cells of the donor which settled in the "living space", in the bone marrow and spleen, vacated owing to death of the natural cell. The natural cells of the irradiated animals begin to multiply only gradually, and more rapidly, the smaller the dose, and begin to displace the foreign cells which were beneficial from the irradiated animal.

Experiments of other investigators performed by different methods led to the same results. For example, it was found that if mice irradiated with a lethal dose are infused with bone marrow cells from a healthy rat then several days later many erythrocytes with properties characteristic of rat's erythrocytes appeared in the peripheral blood.

The most important conclusion from these experiments is that the irradiated organism loses for some time the ability to produce antibodies against foreign protein, as a result of which adaptation of the foreign cells becomes possible.

Therefore, it is possible to conduct successfully an operation of transplanting skin to an irradiated animal treated by foreign bone marrow or spleen. Transplanted skin takes hold. A similar transplantation to a healthy animal always terminates with rejection of the foreign tissue.

In experiments on mice administration of AET before irradiation and a bone marrow suspension from an unirradiated mouse after irradiation raised the lethal dose for 50% of the animals from 692 to 1863 r.

Until the autumn of 1958 the described work was carried out only on the experimental plane, but the unfortunate case with the Yugoslav scientific workers incited the French doctors to transfer the experiment to the clinic and to set it up on persons dying from irradiation. This was done when it became clear that the usual treatment did not guarantee recovery. It was a last resort—to test the administration transfusion of bone marrow was done on 5 of the victims.

Bone marrow was extracted by a syringe from various bone of volunteered donors who were under general anesthesia. The compatibility of blood groups and other properties of those from whom the bone marrow was taken and to whom it was transplanted was carefully checked beforehand. It was administered at the climax of the disease, on the 27-33rd day postirradiation, in an amount of 8.5-14 billion cells per 180-300 ml. After 2 days the condition of the patients improved, appetite was again demonstrated, the patients became more dynamic, and their body weight increased. Immediately after infusion of the bone marrow cells the number of leukocytes in the blood increased and other blood indexes improved (Fig. 32).

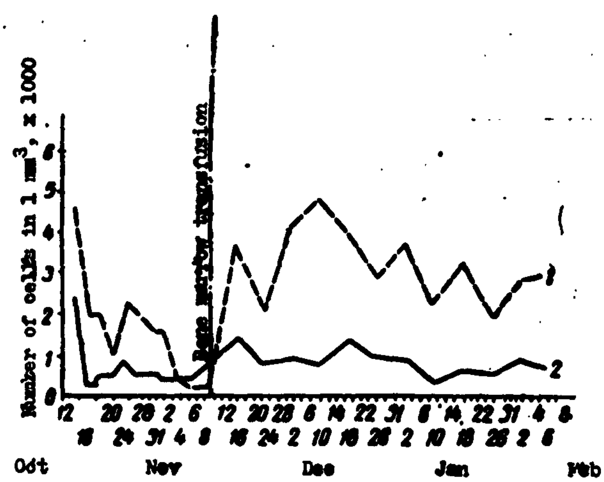



Fig. 32. Content of granulocytes and lymphocytes in the peripheral blood of victim T. after transfusion of donor bone marrow to him: 1) granulocytes; 2) lymphocytes.

By means of various sera it was established what the percentage of erythrocytes of host and donor equal in the peripheral blood at various times after infusion. We see from Table 7 that first foreign erythrocytes predominated and then they were replaced by host erythrocytes. Transplantation of bone marrow from the donor gave time for the accomplishment of recovery processes in the natural bone marrow. The new blood cells prevent the development of infection, hemorrhage, and anemia.

TABLE 7

Relationship of the Number of Donor and Natural Erythrocytes in Blood of Patient G. (Transfusion on 17 XI 1958).

| Date | Cells, % | | Date | Cells, % | |
|---|---|---|---|---|---|
| | Donor | Natural | | Donor | Natural |
| 28.XI 1958 r. | 12 | Not determined | 19.XII 1958 r. | 23 | 83 |
| | | | 26.XII | 14 | 82 |
| 5.XII | 25 | 80 | 17.I 1959 r. | 8 | 92 |
| 12.XII | 33 | 69 | 31.I | few | 92 |

Thus the experiment under the pressure of special circumstances found its way to the clinic. This of course does not mean that radiation sickness has already been conquered. This is far from true. But undoubtedly a step forward has been made in the search for rational methods of treating it.

The establishment of the fact per se that ionizing radiation suppresses the immune properties of an organism and makes possible transplantation of foreign tissue is important not only for radiobiology and the clinical aspects of radiation sickness, but also for other branches of medicine and biology.

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