International Sakharov Environmental Institute,
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MOLECULAR AND CELLULAR RADIOBIOLOGY

The course of lectures

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The publication systemize scientific knowledge on the molecular and cellular aspects of the effects of ionizing radiation on biological systems. The problems of cell survival under irradiation and the forms of cell death, non-targeted effects of the action of ionizing radiation in modern interpretation and the mechanisms of radiation-induced carcinogenesis are given consideration. The materials of the textbook prepared and tested by scientists, experts in the field of radiobiology of the International State Ecological Institute named after A.D. Sakharov BSU.

It is intended for students, master course students, PhD students and university teachers of biological, biomedical and environmental specialties of higher educational establishments, as well as for researchers and practitioners working in the field of molecular and cellular radiobiology and radiation medicine.

Table –15. Figure – 69. References – 100 title.

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INTRODUCTION

The development of radiobiology as a science is connected with the latest achievements in nuclear physics and molecular biology. Consequently, when training professional personnel, it is necessary to provide students with high-quality and comprehensive knowledge in all areas of the discipline. Meeting the needs of the national economy and its innovative development by qualified specialists is due to the need to solve the following set of practical problems and tasks:

1. The study of the biological effects of ionizing and non-ionizing radiation on biological objects, including humans, under present-day conditions of industrial and post-industrial society.

2. The study of the peculiarities of the action on living organisms of low doses of ionizing radiation, as well as of chronic irradiation, which arose as a result of the disaster at the Chernobyl Nuclear Power Plant.

3. Determination of molecular and biochemical mechanisms of radiation injury and cell death, the effect of radiation on DNA, membranes, cell organelles in order to control the body's radiation reactions during radiotherapy, as well as to minimize side effects in medical radiology.

4. The study of the effects of the combined effects of ionizing radiation on the body and other environmental factors and to establish the mechanism for the development of environmentally induced diseases.

In addressing this issue, an important role belongs to the support of the educational process with educational materials for the training of specialists in the field of molecular and cellular radiobiology. That is what led to the creation of this textbook, which corresponds to the curriculum for students' training for the specialty 1-33 81 02 Radiobiology.

The textbook goes into details and comprehensively presents information on the physical basis of the interaction of ionizing radiation with the matter, radiation-chemical interactions, the theoretical basis of radiobiology, the molecular aspects of the biological effect of ionizing radiation, and the manifestation of radiation damage at the cell level. The interpretation of the concept of radio sensitivity as the most important concept of general radiobiology is presented. The issues of tissue radiosensitivity, as well as the reactions of various body systems are given consideration. The section «Cell death and survival» presents current information about apoptosis, its causes and its role in physiological and pathological processes. The section is dedicated to cell death and survival. A large section is devoted to the quantitative laws of the biological effect of ionizing radiation on the body and radiation reactions of the systems and individual organs and tissues of the body. The data on the effect of radionuclides on the body and protection are summarized.

The authors' team, consisting of leading scientists and specialists in the field of radiobiology, believes that the tested materials placed in the textbook will con-

tribute to obtaining theoretical knowledge and practical skills for working in organizations and at enterprises of the Republic of Belarus and abroad.

Part 1. PHYSICS AND CHEMISTRY OF RADIATION INTERACTION WITH MATTER

1.1. General Characteristics of the Mechanisms of Interaction of Ionizing Radiation with Matter

The processes occurring when ionizing radiation (IR) goes through the matter have practical significance both for Nuclear Physics and the areas of science and technology concerning it. Without good knowledge, it is impossible to realize nuclear particle detection methods or, for example, calculate the thickness of biological radiation shielding from nuclear radiation for a particle accelerator or a nuclear power facility.

The greatest practical interest is the energy range of $1 \cdot 10^{-3}$...10 MeV. The energies of particles passing through the matter in this entire area can be called high, meaning that they are large compared to the average ionization potential in matter. The pattern of the passage of high energy particles through matter is extremely complex. The particles interact with electrons located on different shells, are scattered by the Coulomb fields of the nuclei, and also cause various nuclear reactions at sufficiently high energies. In addition, at sufficiently high particle energies, various secondary effects inevitably arise. For example, a beam of high-energy electrons generates a powerful stream of secondary γ-quanta in the matter, which must be considered when calculating radiation protection. However, this does not mean that the processes of passing through the matter are completely incalculable. A number of the most important quantities characterizing these processes can be calculated fairly accurately or at least estimated. The following reasons contribute to this:

• First, with the passage of charged particles and γ-quanta through matter, well-studied electromagnetic interactions play a major role. The role of nuclear interactions in most cases is not significant because of the short-range nuclear forces, and also because the electrons in the substance are much larger than the nuclei.

• The second simplification arises due to the fact that the energy of passing particles significantly exceeds the binding energy of electrons in an atom and these electrons can be considered free.

According to the mechanism of ionization of matter, IR can be divided into two groups:

• direct ionizing radiation;

• indirect ionizing radiation.

Thus, direct ionizing radiation can be divided into:

• heavy charged particles with a mass greater or equal to the proton mass (p, d, α, fragments of nuclear fission, and etc.);

• light charged particles with electrons and positrons with a mass less than 200 electron masses (e^-, e^+, μ^+).

Indirect ionizing radiation include:

• γ-quanta;
• X-rays;
• neutrons.

1.2. The Interaction of Electromagnetic Ionizing Radiation with Matter

1.2.1. Nuclear Interactions of γ-Quanta

The methods of nuclear interaction of γ-quanta are rarely used (the use of radioactive isotopes as indicators).

Nuclear Reactions. γ-quanta of very high energy (above 6 MeV) can interact with the nucleus causing excitation of nucleons. This can lead to the ejection of a particle, usually a neutron and to the transformation of an atom into another nuclide (reaction γ, n).

Nuclear Resonance Scattering. In some situations, the γ-quantum can be absorbed by the nucleus without subsequent particle emission. The core remains in this excited state for a short but immeasurable period of time. Subsequent emission of the γ-quantum restores the stability of the nucleus. An atom that has been exposed to the γ-quantum remains the same without any transformations.

***Bragg Scattering (Diffraction*).** γ-quanta of low energy can be scattered by the crystal matrice without energy loss. X-ray diffraction can be effectively used to study the molecular structure; however, this phenomenon is of no importance for the method of the labeled atoms.

1.2.2. Photoelectric Effect

Photoelectric Effect means that the energy of the incident quantum is completely absorbed by the substance; as a result, free electrons appear that have a certain kinetic energy, the value of which is equal to the energy of the radiation quantum minus the work of releasing of the given electron from the atom. A free electron, associating with one of the neutral atoms, generates a negative ion.

The probability of the photoelectric effect depends on the energy of the incident quantum and the atomic number of the absorbing medium. The photo effect is characteristic only for long-wave X-ray radiation. Its probability depends on the atomic number and is proportional to Z^3 (fig. 1).

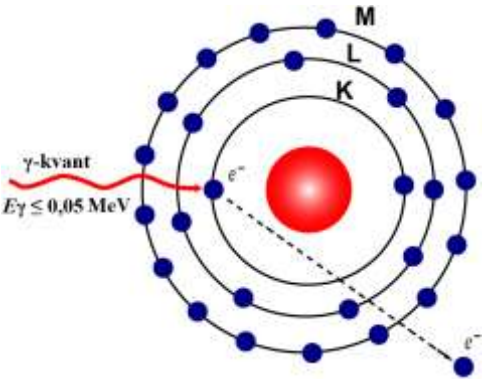


Fig. 1. Photoelectric Effect

With the increase of the radiation energy, the probability of the photoelectric effect decreases ; for radiation with the energy that is much higher than the intra-atomic binding energies (> 1 MeV), its contribution to the interaction can be neglected. The main role belongs at this to another way of energy exchange − *Compton Effect*.

1.2.3. Compton Effect

In the Compton Effect, elastic scattering of the incident photons of radiation occurs on free (or weakly bound) electrons; only part of the photon energy is transferred to them. The rest of the energy is carried away by new photons resulting from this interaction (fig. 2). Then, the secondary photon can again undergo the Compton Effect, etc.

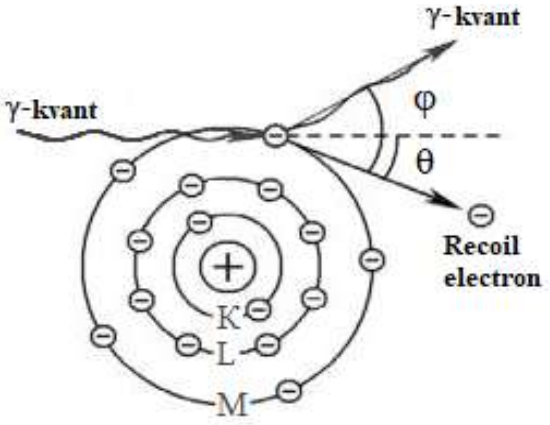


Fig. 2. Compton Effect

Thus, the Compton recoil electrons, despite the fact that their occurrence is due to the monoenergetic beam of incident γ-quanta, have an extended energy spectrum. When such electrons interact with an absorbing matter; naturally, considerable ionization occurs. Moreover, the scattered γ-quantum of lesser energy can undergo some several collisions of the same nature before it loses all its energy when interacting with the matter.

The incident photon knocks out the orbital electron of the atom of the irradiated matter. Part of the photon energy is transmitted in the form of kinetic energy to the electron. The resulting secondary photon has less energy and another direction.

The average energy of recoil electrons increases with increasing energy of the incident radiation. The proportion of energy absorbed by Compton electrons in the total amount of absorbed energy increases with increasing hardness of radiation. If the source of short-wave radiation, which is dominated by the Compton Effect, is calibrated using a standard ionization chamber, then the results of these measurements can be used to obtain data on energy absorption by various materials. Under the action of soft X-rays, when the photoelectric effect prevails, this cannot be done without appropriate corrections (it is not simple to introduce them), since serious errors in estimating the absorbed radiation doses can occur.

In water and biological tissues, the absorption of radiation with a photon energy of more than 300 keV mainly occurs due to the Compton Effect.

As a result of several successive Compton interactions, the quantum energy decreases so much that it can already be completely absorbed as a result of the photoelectric effect. If the energy of an incident quantum exceeds 1.022 MeV, a third type of interaction becomes possible, i.e., the effect of the pair formation.

1.2.4. Formation of Electron-Positron Pairs

This type of interaction of radiation with matter is characterized by the possibility of converting a high-energy γ-quantum (> 1 MeV is equivalent to the rest mass of one electron and one positron) into a *pair of charged particles – an electron and positron,* which are ejected from the place of their occurrence with different energy. This process is caused by the interaction of the γ-quantum with some atomic nucleus*; an electron-positron pair* is formed in its field. The likelihood of such a process is proportional to Z^2 ; therefore, for heavy elements it is more than for the light ones. The resulting electron and positron, arising from this, spend their energy mainly on ionization. After stopping, the positron annihilates, throwing two γ-quanta with the energy of 0.51 MeV in opposite directions (fig. 3).

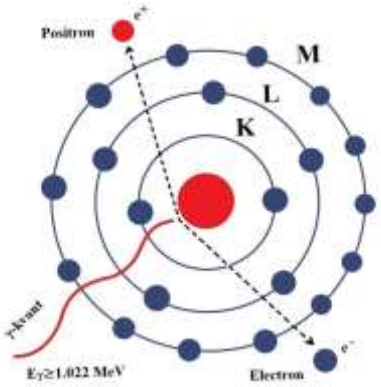


Fig. 3. Formation of Electron-Positron Pairs

Consequently, depending on the energy of the incident electromagnetic radiation, one or another type of its interaction with matter prevails (fig. 4). In most cases, when biological objects are irradiated, the energy of the used electromagnetic radiation is in the range of 0.2–2 MeV; therefore, the Compton Effect is most likely.

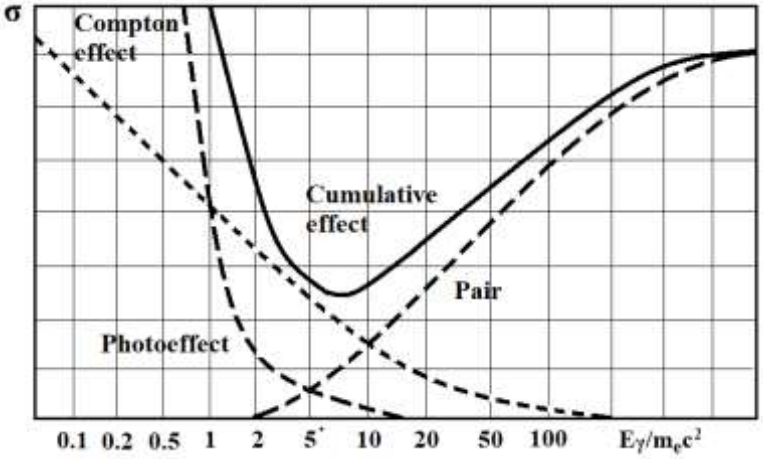


Fig. 4. The relative role of the three types of interaction of γ-radiation with matter

With increasing the energy of γ-quanta, the formation of pairs plays an increasingly important role in the mechanism of absorption of γ-radiation. It is especially large in absorbers with a high atomic number. The photoelectric effect and the Compton Effect play a smaller role with energy increase; starting

from 3 MeV, the effect of the formation of pairs makes compensation for the decrease of absorption.

1.2.5. Patterns of Absorption of γ-Radiation

Linear absorption coefficient ($\mu_л$). Absorption of γ-radiation occurs exponentially (the thicker the absorber, the greater the absorption is), so this type of radiation does not have a strictly defined range. It is usually calculated per centimeter (1 / cm). The linear absorption coefficient depends on the energy of γ-quanta and the material of the absorber (table 1).

Table 1

Linear absorption coefficients in some absorbers

The energy of incident beam, MeV	Linear absorption coefficient, cm^{-1}			
	H_2O	aluminum	iron	lead
1,0	0,071	0,168	0,44	0,79
1,5	0,057	0,136	0,40	0,590
2,0	0,050	0,117	0,33	0,504

Mass absorption coefficient ($\mu_м$). This value is equal to the linear absorption coefficient divided by the density of the absorber. The advantage of this value is that it does not depend on the nature of the absorber. Mass absorption coefficients (cm^2/g or cm^2/mg) are approximately equal in the media considered earlier.

Sometimes the following is used

• atomic absorption coefficient (μ_a), which takes into account the actual number of atoms in the absorbing material and is equal to the fraction of energy absorbed per absorber atom;

• electronic absorption coefficient ($\mu_э$), which is the fraction of radiation absorbed by the electron absorber. It is applied for γ-quanta of low energy, which interact mainly with orbital electrons.

The thickness of the semi-absorption layer is the value that is defined as the thickness of the layer of material of the absorber, which halves the intensity of the incident radiation. It is used in calculating the protection required to reduce the intensity of the γ-radiation to the required levels.

1.3. The Interaction of Corpuscular Ionizing Radiation with Matter

1.3.1. The Types of Interaction of α-Particles with Matter

The mechanism of energy transfer in the object from all charged particles is the same. When passing through matter, a charged particle loses its en-

ergy, causing ionization and excitation of atoms until the total energy supply decreases so much that the particle loses its ionizing ability. Depending on the charge, during the particle transit in matter, it is attracted or repelled from positively charged nuclei, experiencing an electrostatic interaction. The larger the mass of a particle, the less it deviates from the original direction. Therefore, the trajectory of protons and heavier nuclear particles is almost rectilinear, and the trajectory of electrons is strongly broken due to scattering on orbital electrons and atomic nuclei.

The interaction of charged particles with a substance, elastic and inelastic interactions are defined.

In the case of *elastic interaction*, the total kinetic energy of the particles before the interaction is equal to the total kinetic energy after their interaction. The consequence of this interaction is the change in the direction of the movement of the particles.

Inelastic interaction is a process in which a part of the kinetic energy of particles is spent on ionization and excitation of atoms, excitation of nuclei, nuclear fission or bremsstrahlung. In this case, the total kinetic energy of the particles before the interaction will be equal to the total kinetic energy of the particles after the interaction plus the energy spent on ionization and excitation of atoms, excitation and fission of nuclei or bremsstrahlung.

Both types of interaction are characteristic of α-particles: inelastic interaction of α-particles with orbital electrons (the result is ionization and atomic excitation); elastic scattering of α-particles on atomic nuclei. The strong electrostatic field surrounding the α-particle has a significant effect on the orbital electrons of atoms lying near the path of the particle. In many cases, electrons located in external orbits can be completely detached from the atom. In other cases, electrons from internal orbits can move to orbits farther from nuclei. In this interaction with the orbital electrons, the kinetic energy of the α-particle is scattered.

Excitation is an interaction when orbital electrons receive energy from an α-particle passing through, but do not leave their atoms. Then, the electrons are again transferred to their orbits and emit the excess energy in the form of photons of visible or close to the region − scintillation. The amount of energy transferred in this process is usually small.

Ionization is the disruption of an orbital electron by an α-particle from an atom with which it interacts, the loss of a negatively charged electron leaves the atom in the form of a positively charged ion. Making a pair of ions with an electron and a positive atom is called an ionization process. The formation of each pair of ions requires an average cost of about 34 eV of the kinetic energy of an α-particle: an α-particle with an energy of 6.8 MeV forms until its energy is completely consumed, about $2*10^5$ ion pairs. Therefore,

ionization is the most important process of energy transfer of an α-particle to the matter interacting with it. The tracks of the α-particles are almost straight.

Specific ionization is the number of ion pairs formed per unit path (cm) in an α-particle track (or other ionizing particle) in the air at normal pressure, that is, *the intensity of ionization*. Fig. 5 shows the curve of the change in the specific energy loss of the α-particle along its path in the air. It is called *the Bragg curve.*

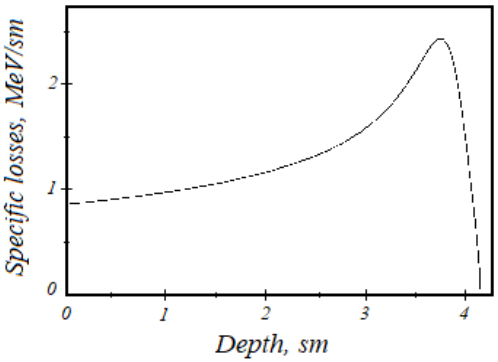


Fig. 5. The curve of distribution of the energy of α-radiation in the air

The specific ionization of the α-particle beam sharply increases at the end of their path. This happens because as a result of many collisions, the α-particles lose most of their kinetic energy, and their speed decreases. Due to the reduced speed, they remain longer near the molecules along their path; thus, the probability of α-particles interacting with these molecules significantly increases. When the value of specific ionization reaches a maximum, it sharply decreases to zero. At this point, the α-particles, which have lost their kinetic energy, capture two electrons and become neutral atoms of helium-4. Since the energy of the α-radiation emitted by a given radioactive source is discrete,

α-particles fly a strictly defined distance in the air (table 2). The run of the α-particles in medium other than gas will be significantly less due to the higher density of liquids and solids. In practice, they are very small and are expressed in micrometers (μm).

Table 2

Linear paths of α-particles with the energy of 7 MeV in some absorbers, mkm

Linear paths of α-particles with an energy of 7 MeV in some absorbers, mkm					
air	Water (tissue)	aluminum	mica	copper	lead
57 000	74	34	29	14	2

Since the measures of these ranges are very small, the term *equivalent thickness* is used; the thickness (in cm) of the absorber (which is equivalent to 1 cm of the air by absorption of α-radiation) multiplied by the matter density (in g/cm^3 or if multiplied by 1000, in mg/cm^2). Equivalent thickness is measured in g/cm^2 (table 3).

Table 3

Equivalent thickness of some of the most commonly used absorbers of α-particles with an energy of 7 MeV, mg / cm^2

Equivalent thickness of some of the most commonly used absorbers of α-particles with an energy of 7 MeV, mg / cm^2					
air	mica	aluminum	copper	silver	gold
1,2	1,4	1,62	2,26	2,86	3,96

1.3.2. Interaction of β-Radiation with Matter

Like α-particles, β-particles dissipate their energy mainly in the processes of ionization and excitation of the atoms which they interact with. The type of interaction of light particles, in which the direction of their movement rather than energy is practically changed, is sometimes called *elastic scattering*, unlike *inelastic scattering* (drag), which is observed when a very high energy electron passes near the nucleus. At the same time, there is one more type of energy loss – during deceleration of high-energy β-particles in the Coulomb field of atomic nuclei, electromagnetic radiation is emitted with the wavelengths corresponding to x-ray. This process is called *bremsstrahlung*. Consequently, during the passage of high-energy electrons through matter, *the formation of electromagnetic radiation* also occurs.

β-track is very winding. For β-particles, specific ionization decreases rapidly as their energy increases. At the end of the path of a β-particle, when its energy decreases to several keV, the ionization and, consequently, the energy loss per unit path increases.

The β-particle range is expressed in the equivalent thickness. Most often aluminum is used as an absorber.

1.3.3. Interaction of Neutrons with Matter

Unlike charged particles, neutrons do not have an electric charge, which allows them to freely penetrate deep into atoms, and reaching nuclei, they are either absorbed by them or scattered on them.

Elastic neutron scattering

As a result of the elastic neutron scattering (n, n) on the nucleus, the energy of the primary neutron E_n is distributed between the scattered neutron and the recoil nucleus (table 4).

Table 4

Relative fractions of neutron energy δ_E, transferred to the nuclei of some elements during elastic scattering

Element	H	C	O	Pb
Mass number, A	1	12	16	208
δ_E	0,5	0,142	0,111	0,0096

As a result of the elastic neutron scattering, *highly ionizing protons* are formed. When neutrons are absorbed, the atomic nuclei become unstable and in decaying, generate protons; α-particles and photons of γ-radiation are also capable of producing ionization. In such *nuclear reactions*, radioactive isotopes of elements can form and cause the induced radioactivity, which also can cause ionization. The substance and the recoil nuclei that occur during nuclear transformations are ionized.

With elastic scattering on nuclei of carbon, nitrogen, oxygen and other elements that make up tissues, a neutron loses only 10–15 % of energy, and when colliding with hydrogen nuclei – protons – that are almost equal in mass with them, the neutron energy decreases on average twice being transferred to the recoil proton (fig. 5). Therefore, substances containing a large number of hydrogen atoms (water, paraffin) are used *to protect against neutron radiation* because neutrons quickly waste their energy in them and slow down.

In this case, part of the neutron energy is transferred to the recoil proton as kinetic. The neutron scattering deviates from the previous direction and has less energy. Thus, in neutron irradiation, the final biological effect is associated with ionization producing indirectly by secondary particles or photons. Consequently, the predominant contribution of one or another kind of nuclear interaction of neutrons depends on their energy, as well as on the composition of the irradiated substance.

Neutron Inelastic Interaction

It occurs as a reaction of inelastic neutron scattering (n, n'γ), all kinds of nuclear reactions: (n, γ); (n, p); (n,α); (n, α); (n, nρ); (n, 2n) and others, as

well as the induced fission reactions of atomic nuclei (n, *f*). Nuclear reactions under the action of neutrons can take place as direct ones or through a compound nucleus. Depending on the energy balance, reactions can be exoenergic (passing at any neutron energy), for example:

Radiation capture reaction (n,γ);

$^{3}_{2}$He (n, p) – $^{3}_{1}$ H (Q ≈ 0,77 MeV);

$^{6}_{3}$ Li (n, α) – $^{3}_{1}$H (Q ≈ 4,78 MeV);

$^{10}_{5}$ B (n, α) – $^{7}_{3}$ Li (Q ≈ 2,78 MeV);

nuclear fission reactions (n, *f*) with isotopes $^{235}_{92}$U and $^{239}_{94}$ Ри or others.

or threshold: reactions (n, 2 n) at En > 8 МэВ and others.

fission reactions u^{-238} (En ≥ 1,5 MeV).

1.4. Ionization Potential, Linear Energy Transfer (LET)

The same amount of energy can be transferred to a biological object when irradiated with various types of ionizing particles. The absorbed energy is spent on the excitation and ionization of atoms and molecules. The final radiobiological effect is based on the physicochemical transformations of excited and ionized molecular structures. The biological effect of ionizing radiation is associated not only with the amount of the absorbed energy, but also largely depends on the nature of the spatial micro distribution of the absorbed energy. Absorption of the same dose of radiation leads to different effects.

The energy transferred by a charged particle per unit of its range in a substance is called ***linear energy transfer*** (LET).

The value of the energy loss per unit of the range (linear energy transfer – LET) is inversely proportional to the kinetic energy of the particle and is related to the density of the distribution of ionization events along the particle track. LET is measured in keV per 1 μm of water. Most of the energy of ionizing radiation is absorbed by reducing the energy of an ionizing particle or photon quantum.

This is the criterion of «quality» of radiation, the effectiveness of its biological action. In mathematical expressions, LET is denoted by the symbol *L*:

$$L = \frac{\text{energy transferred by a particle to a substance,keV}}{\text{distance traveled by particle,mkm}}.$$

The concept of LET was introduced by R. Zirklem in 1954. The unit of LET is 1 keV/μm of tissue (1 keV/μm = 62 J/m).

Typical levels of LET for the most common types of radiation are the following: α-radiation of ^{60}Co and X-rays with the wavelength of ~ 20 nm (250 keV) have LET of about 0.3 and 2 keV/μm, respectively, neutrons with the energy of 14 MeV − 12, and heavy charged nuclear particles − from 100 to 2000 keV/μm. However, such a division is rather arbitrary, since LET is as-

sociated not only with the physical nature of radiation but also depends on the speed of a particle's flight.

In modern powerful accelerators, heavy particles accelerate to such high energies that their speed approaches the speed of light. In this case, LET of all particles is reduced to the minimum value, which is typical to rarely ionizing light particles (for example, electrons) with the energy of 1 MeV. Therefore, at a very high speed of the movement, fast protons and electrons have the same LET since they have the equal charge.

Depending on the value of LET, all types of ionizing radiation are divided into *rarely and densely ionizing types.*

• Usually all types of radiation are referred to *rarely ionizing radiation* (regardless of their physical nature), with LET <10 keV/μm,

• *Densely ionizing types of radiation* are those for which the LET exceeds this value. Neutrons are classified as densely ionizing radiation, since the recoil protons formed by them strongly ionize matter. However, their occurrence is at great depth due to the high penetrating power of neutrons.

The boundary between them is the conventionally accepted value of LET = 10 keV/μm. As the velocity of charged particles decreases, LET increases.

So, all types of ionizing radiation directly or indirectly cause either excitation or ionization of atoms or molecules of biosystems. However, when objects are irradiated with different types of ionizing radiation at equal doses, quantitative and sometimes qualitatively different biological effects occur, which is associated with the spatial distribution of the energy released during the interaction in the irradiated microvolume − with LET.

LET of charged particles increases with a decrease in their speed; therefore, at the end of the range the energy output by any charged particle is maximum. This feature of the interaction of heavy nuclear particles is used in the tumor treatment as it allows concentrating the significant amount of energy at the depth of the affected tissue with minimal scattering in healthy tissues along the beam.

It has been established that LET is proportional to the square of the charge: the α-particle, which is formed during radioactive decay and has a charge of + 2, causes the appearance of ions four times more often. In air, α-particles, depending on the initial energy, form 40 000−100 000 ion pairs and β-particles − 30–300. The mean range of particles increases with increase of their energy. At present, the relationship between these parameters for each particle is precisely defined.

The value of LET in keV/μm depends on the density of the substance. When LET is divided into a substance density p, we obtain the value *L/ p*, which does not depend on the density. This value is also called LET I, or the braking power of the substance, which is measured in MeV / cm^2g^{-1}. As follows from the definition, the LET value characterizes the distribution of the energy transferred to the substance along the particle track. Knowing LET, it

is easy to determine the average number of ions formed per unit of a particle track. An average of 34–36 eV is spent on one pair of ions. If to divide LET into energy to form one pair of ions, we obtain the linear ionization density (LID):

LID = LTE / 34 (number of ion pairs per μm range).

The higher the LET value, the more energy the particle leaves per unit of the range and the more densely the ions generated by it are distributed along the track.

LID is a quantitative value of the ionizing ability of ionizing radiation and is equal to the number of ion pairs created by a particle (quantum) per the unit of the range in a substance, and LID depends on many factors such as speed, mass and charge of the particle, the energy of quanta, properties of matter, etc. To avoid uncertainty because of the properties of the substance, and to characterize only the properties of ionizing radiation, the LID is determined in the standard substance − dry air (table 5).

Table 5

Average values of LID in the air

The type of the radiation	LID (pairs of ions* cm^{-1})
α-particles	40000
β-particles	400
X-rays and γ-quanta	5
protons	10000

It is obvious that in other substances with the different density, the values of the LID will be different. The ratio of LID measured under standard conditions (in air) and in human tissues is of practical interest. It should be noted that it is more reasonable to predict the development of the reaction in biological tissues, realizing the degree of its ionization, but not the air. It is empirically found that the LID, which is contained in human tissues, is about 800 times higher than the LID measured in air: $LID_{tissue} \geq 800\ LIP_{air}$. The greatest damaging effect on living tissues is caused by radiation, which creates a large LID since each pair of ions is a destroyed biomolecule.

Ionization density is increased at the end of the particle track. With an equal particle velocity, the level of ionization is proportional to the square of the particle charge; also, with equal energy, the ionization density increases with a larger particle mass.

1.4.1. Penetrating Ability of Ionizing Radiation

The depth of penetration of ionizing radiation depends, on the one hand, on the nature of the radiation, the charge of its constituent particles and the

energy; on the other hand it also depends on the composition and density of the irradiated substance. Ionizing electromagnetic radiation has a high penetrating power.

The penetrating ability of ionizing radiation is the rate of energy loss in a substance. It is inversely proportional to the ionizing ability (LID): the more ions a particle generates per the unit of the range in a substance, the faster it loses the energy. α-particles have a large penetrating ability: even in the air, their range is a few centimeters. More dense substances (fabric, wood, paper) completely retain α-particles with a layer thickness of 0.1 mm.

The penetrating ability of β-particles is about 100 times more; they pass several meters in the air, and in solid media - a few mm.

X-rays and γ-quanta, which have a small LID, slowly lose energy and, therefore, penetrate deeply even into dense media (soil, concrete, etc.). The specific values of the depth of penetration of γ- and X-ray quanta in the same substance depend on their energy. With an increase in the energy of quanta, the penetrating ability also increases. Hard γ-quanta (with high energy) can surpass a concrete layer of several meters.

Table 6 shows the values of μ for four substances (air and water, but the values of μ for biological tissues are close to its value for water, iron and lead) and the dependence of this coefficient on the radiation energy: the smaller μ, the weaker the absorption and the greater the penetrating power of electromagnetic radiation. Thus, it can be calculated that to attenuate an X-ray beam with the energy of 250 keV by 100 times, 7–8 mm of lead is sufficient, which has a high absorptive capacity, and, therefore, is used as a shield to protect against the harmful effects of radiation. Lighter metals (Al, Cr, Fe) are used as filters, cutting off a very soft component of X-rays, strongly absorbed in the substance (large μ) causing radiation burn of the skin. The value of LET is the most important radiobiological characteristic of radiation, an indicator of its biological effectiveness, or, as it is sometimes said, «quality»; the physical nature of the particles or quanta does not affect the specifics of the biological action. For example, with equal LET, an equally effective suppression of cell proliferation is observed both as a result of x-ray irradiation and under the action of α-particles.

The same amount of energy can be transferred to a biological object when irradiated with various types of ionizing particles. Since the number of excitations and ionizations is determined by the magnitude of the absorbed radiation dose, different types of ionizing radiation can be expected to lead to the same biological effect provided that the object absorbs the same radiation dose. However, this is not factually accurate.

Table 6

The dependence of the linear absorption coefficient of γ-radiation of various substances μ, on its energy (μ, cm^{-1})

The energy of γ-radialtion, MeV	The linear absorption coefficient of γ-radiation of various substances μ, on its energy (μ, cm^{-1})			
	in air (·10)	in water	in iron	in lead
0,1	1,98	0,172	2,8	59,9
0,25	1,46	0,126	0,82	6,3
0,5	1,11	0,096	0,65	1,67
1,0	0,81	0,070	0,45	0,75
2,0	0,57	0,050	0,33	0,51
3,0	0,46	0,039	0,28	0,46
5,0	0,36	0,030	0,24	0,48
10,0	0,26	0,022	0.23	0,62

Consequently, the integral absorbed dose cannot be used to compare the effectiveness of various types of radiation. Similarly, the magnitude of the energy of ionizing particles does not coincide with the degree of the final biological effect.

In order to predict a possible cause of the different efficiency of radiation with high and low values of LET, the following situations can be considered:

• α-particles with an energy of 4 MeV, transfer 130 keV per 1 μm of the range to the substance, which corresponds to approximately 3800 ionizations per 1 μm. With such a high ionization density on the scale of a protein molecule, a particle can produce several successive acts of ionization and excitation.

• The electrons with the energy of 0.5 MeV have a LET value of 0.2 keV/μm. Such electrons form about 6 pairs of ions per 1 μm range; that is, the probability of ionization within a protein molecule with a thickness of about 0.003 μm is very small.

When cells are irradiated with ionizing radiation, the value of the absorbed dose shows only the average amount of the energy transferred to the irradiated system. The density of ionization in the cell can be judged by the value of the LET. If a moving particle produces the types of ionization that are significantly distant from each other, the probability of the occurrence of several ions within the macromolecule, subcellular organelle, or the cell as a whole is relatively small. On the contrary, when ionization acts follow continuously along a particle track, many ions can be expected to appear within a single subcellular structure, for example, two ionizations in complementary

regions of a double-stranded DNA molecule. The biological consequences of a lesion (as a result of ionization) of both strands of this unique molecular structure are sure to be much more significant for the cell than the destruction of any part of one DNA helix while maintaining the integrity of the complementary chain. With an increase in the linear ionization density, the probability of just such a «two-strand breakage» increases. Accordingly, densely ionizing particles (with high LET) should affect significantly more efficiently not only DNA but also the associated cellular functions compared to rarely ionizing ones.

1.4.2. Relative Biological Effectiveness (RBE) of Various Types of Radiation. RBE And LET Relationship

For a comparative quantitative characterization of the biological effects of various types of radiation, their *relative biological effectiveness (RBE)* is determined. It should be noted that the RBE is mainly determined by LET and is in a certain relationship with it. RBE is a relative ability compared to X-rays with an equal absorbed dose to cause radiation damage of a certain degree.

For various biological objects, a comparison of the effectiveness of various types of ionizing particles was made. In experiments on mammals the lethal effect of radiation and various long-term effects as the appearance of radiation cataracts and malignant tumors and a decrease in life expectancy was a criterion of efficiency. When cells were irradiated with the same radiation doses by different types of ionizing particles, the number of irradiation-induced chromosomal aberrations and mutations was calculated. These and other experiments allowed to quantify the effectiveness of various types of ionizing radiation and introduce coefficients that for each specific biological system show the effectiveness of this type of radiation compared to the selected standard radiation.

The coefficient of relative biological efficiency. A quantitative value of the RBE radiation is the RBE coefficient (C_{RBE}), which is the ratio of the dose of the given and the standard radiation; they have the equal biological effect under otherwise equal conditions (uniform distribution of the absorbed dose in the body, fractional exposure, dose rate, etc.).

C_{RBE} is determined from the ratio:

$$C_{RBE} = \frac{\text{biological effectiveness of the investigated radiation}}{\text{biological efficiency of x-rays with an energy of about 200 keV}}. \quad (1)$$

As follows from the definition, X-ray radiation with quantum energy of 200 keV, which forms approximately 100 ion pairs per 1 μm path in water, is chosen as the standard. For such radiation, RBE is taken as a unit. For each investigating system, the RBE coefficient is found by comparing the effects of the standard and the investigating radiation applied in the same dose. It should

be considered that the value of the RBE may vary depending on whether the same dose rate is absorbed once or fractionally. It is desirable that the compared types of radiation had the same kinetics of action on the selected test system.

For calculations of various sanitary standards, the relative values of RBE, which are the average results of experiments on different systems, are taken. These values are given in table 7.

Table 7

Quality factor for some α-particles with a specific LET

LET_{α}, keV/μm	< 3,5	7	23	53	> 175
C, coefficient of quality	1	2	5	10	20

The dependence of RBE on LET radiation has the form (fig. 6):

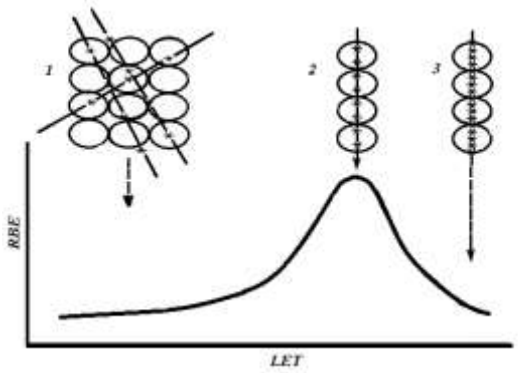


Fig. 6. The dependence of the relative biological efficiency of radiation from linear energy transfer

As follows from fig. 7, for LET values above 90–100 KeV/μm, the curve of RBE dependence on LET goes through a maximum and decreases. Perhaps, this may be due to the fact that at LET values = 100 KeV/μm, a critical number of ionizations occurs in the cell, which is sufficient for its death.

Further increase in ionization density is inefficient. In addition, each subsequent particle of ionizing radiation loses its energy in a dead cell. Consequently, the energy of ionizing radiation is spent with no purpose, and its specific efficiency, despite the high values of LET, decreases.

Thus, after the optimal value of LET (maximum of affected targets per unit dose), the effect of «overkill» occurs.

The relation of RBE with LET of ionizing radiation in practice turns out to be much more complex.

The absolute value of the RBE is not constant but depends on the degree of the damage and on the radiation dose. With increasing radiation dose RBE decreases.

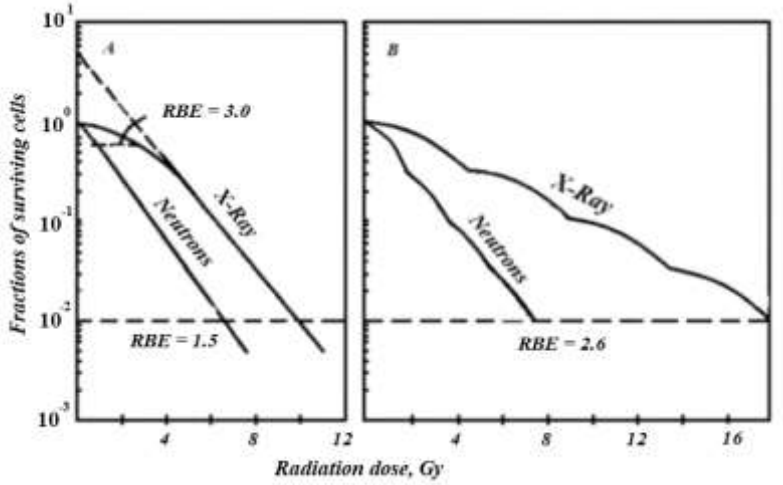


Fig. 7. The dependence of RBE on the dose with single (A) and fractionated irradiation (B)

Thus, the same biological effect can be achieved with fractionated irradiation in smaller total doses of densely ionizing irradiation (compared with the total dose of rarely ionizing irradiation) than with a single irradiation (fig. 7). This phenomenon is used in radiation therapy of tumors using dense ionizing radiation characterized by a large LET.

1.5. Dosimetry Values Used in Radiobiology

The practice of monitoring of occupational exposure is based on the modern system of dosimetric quantities and international experience in the safe development of radiation-hazardous technologies. As we improve our knowledge of the effects of ionizing radiation, the radiation safety system and the practice of monitoring occupational exposure change. Regularly published ICRE reports and the ICRP Recommendations allow considering a modern system of dosimetric values consisting of three large sections:

- **basic physical values,** which are the measure of the effect of ionizing radiation on a substance;
- **standardized values** representing the measure of the damage from radiation exposure on a person;

• **operational values**, which are directly determined in the measurements of values and intended for estimating standardized values during radiation monitoring.

Basic physical values are the measure of the physical effects of ionizing radiation on matter. They also characterize the *radiation* source, the actual *radiation* and radiation fields arising when the *radiation* passes through matter. Physical dosimetric values are not directly used to describe *the exposure* (exposure of radiation to a human). *The exposure* is characterized by standardized dosimetric values; subordinate basic physical values are used in defining them. The measurement of standardized values in the control of radiation is almost impossible. In assessing the compliance of radiation conditions with regulatory requirements, operational values are used with the values that are close to the values of the corresponding standardized values under certain irradiation conditions. The most important property of the operational values is that they can be directly measured by radiation monitoring.

1.5.1 Basic Physical Values

The basic physical values that characterize radiation sources, radiation fields, and the interaction of radiation with matter constitute a section of dosimetric values, which has remained unchanged for a long time. Following the introduction into practice of the International System of Units (SI), the units of measurement of basic physical values change, but their definitions remain unchanged.

The phenomenon of radioactivity was discovered in 1896. Since then, a substance containing radioactive isotopes (radionuclides) has been called radioactive. Such a substance is considered as a radionuclide source of ionizing radiation. The main characteristic of a radionuclide source is its ***activity*** (A), which is a measure of the radioactivity of the amount of a radionuclide that is currently in the certain energy state. The expected number of nuclei of a radionuclide that have undergone spontaneous nuclear transformations per unit time is proportional to the total number of nuclei N of this radionuclide:

$$A = -\frac{dN}{dt} = \lambda N, \qquad (2)$$

where dN is the expected number of spontaneous nuclear transformations from the given energy state occurring over a period of time dt; λ is the constant of radioactive decay characterizing the probability of decay of the nucleus of an atom of the given nuclide per unit time. Unit of activity is Becquerel (Bq). In the source with an activity of 1 Bq, on average, one spontaneous nuclear transformation occurs per second (1 Bq = 1 dis./s). The previously used off-system unit of activity is curie (Ci), which is $3.7 \cdot 10^{10}$Bq.

Important characteristics of the radiation flux during its transfer in the medium from the source to the irradiated object are fluence and the flux density of particles (quanta) of radiation:

• *particle fluence* (F) is the ratio of the number of particles dN penetrating into the elementary sphere to the area of the central section dS of this sphere; the unit of fluence of particles or quanta freq./cm^2;

• *particle flux density* φ is the fluence per unit of time.

Energy is the most important characteristic of ionizing radiation. In nuclear physics, the off-system unit of energy is electron volt (eV) is used. 1 eV = $1.6020 \cdot 10^{-19}$ J.

Initially, the development of dosimetry was determined by the need to protect natural radioactive substances from exposure to X-ray and γ-radiation in the medical use of ionizing radiation. The ionization of the medium under the influence of these radiations was the first physical effect, which was compared with the biological effect of radiation. To assess the field of photon radiation in the air, the value of the exposure dose is used. Exposure dose is a measure of the ionization effect of photon radiation, determined by air ionization in electron equilibrium conditions. The directly measured physical value in determining the exposure dose of photon radiation is the total electric charge of ions of the same sign formed in the air during the exposure time. For photons with the energies of less than 3 MeV, air is a good sample of muscle tissue in assessing the ionization effect. The exposure dose is defined as the concentration of ions of the same sign in the air and is equal to the ratio of the total charge of all ions of the same sign created by radiation in air when the secondary electrons and positrons generated in the elementary volume are decelerated to the mass of air in this volume. The unit of exposure dose is one pendant per kilogram (C/kg). Non-systemic unit of exposure dose is X-ray. One X-ray is equal to $2{,}58 \cdot 10^{-4}$ C/kg.

With the discovery of the neutron and nuclear fission, new powerful sources of radiation appeared; they are neutron fluxes, accelerated electrons, positrons, and heavy charged particles. The need to protect against the effects of various radiation types led to the creation of a universal energy concept applicable to all types of ionizing radiation and to all media.

The absorbed dose of radiation, D, was introduced as a basic dosimetric value, which is a measure of the energy transferred by ionizing radiation to a substance:

$$D = \frac{d\bar{\varepsilon}}{dm}, \qquad (3)$$

where $d\bar{\varepsilon}$ is the average energy transferred by ionizing radiation to a substance in the elementary volume; dm is the mass of the substance in this volume. The absorbed dose defines the concentration of the radiation energy

transferred to the substance. The unit of the absorbed dose is gray (Gy), 1Gy = 1 J/kg. The previously used off-system unit is rad, which is 0.01 Gy.

To assess the impact on the environment with indirectly ionizing radiation, the concept of kerma is used. **Kerma** (K) is the ratio of the sum of the initial kinetic energies $d\varepsilon_k$ to all charged ionizing particles formed under the influence of indirectly ionizing radiation in an elementary volume of a substance to the mass dm of a substance in this volume:

$$K = \frac{d\varepsilon_k}{dm}. \quad (4)$$

The unit of kerma, gray (Gy), is the same as the unit of absorbed dose. A single absorbed dose (1 gray) is equal to kerma with the sum of the initial kinetic energies of all charged ionizing particles formed under the influence of indirectly ionizing radiation in a substance weighing 1 kg is equal to 1 J.

Kerma is determined by the kinetic energy of secondary charged particles including the part that is then consumed on bremsstrahlung. Kerma and the absorbed dose of photon radiation are equal to each other to the extent that equilibrium of charged particles is achieved, and the bremsstrahlung of secondary electrons and positrons can be neglected, as well as the attenuation of the flux of primary photons along the path of the secondary electrons. Consequently, the kerma value for photons under the conditions of electronic equilibrium coincides with the absorbed dose with an error determined by the fraction of energy of the secondary charged particles, which is spent on bremsstrahlung. For the photon energies of radionuclide sources ($E_\gamma \leq 3$ MeV), the value of kerma in air can exceed the value of the absorbed dose in air by no more than 1 %. In biological tissue, kerma decreases with depth due to attenuation of primary radiation. Thus, the maximum kerma of photon radiation is observed on the surface of the human body.

Neutron kerma is the same as the absorbed dose from secondary charged particles under conditions of their equilibrium. For a volume of matter with a sufficiently large mass, which is surrounded by the same substance (an organ inside the human body) and if the equilibrium conditions of charged particles are met, kerma is usually practical coincides with the absorbed dose from secondary charged particles (hereinafter, the word “practically” means that the statement is true if the energy losses of the secondary charged particles for the formation of bremsstrahlung can be disregarded). For thin layers of matter at the interface of various media (skin at the interface between the air and the human body), these dosimetry characteristics differ. For neutrons in equilibrium of charged particles, the absorbed dose can practically be represented as the sum of the kerma and the absorbed dose from the secondary γ-radiation. Therefore, kerma per unit neutron fluence is less than the absorbed dose per unit fluence. This difference is especially important in the region of intermediate energies, where the contribution to the absorbed dose from secondary γ-radiation is significant.

The dimension of the absorbed dose and kerma is different from the dimension of the exposure dose. These values have a different nature. The kerma of photon radiation in air is considered as the energy equivalent of the exposure dose. Since one X-ray corresponds to the formation of $2{,}08 \cdot 10^9$ ion pairs in 1 cm^3 of air, taking the energy of formation of an ion pair in air equal to 34 eV, we obtain the ratio: 1 P corresponds to the photon kerma in air, which is approximately $8{,}8 \cdot 10^{-3}$ Gr.

An important characteristic of ionizing radiation, showing how radiation transmits its energy to matter, is *the linear energy transfer* – the energy transmitted by the ionizing particle to matter in a given neighborhood of its trajectory per unit of the length of the trajectory. As a rule, in radiation safety, *linear energy transfer* (LET or L) of radiation means the complete transfer of energy in water:

$$L = \left(\frac{d\varepsilon_{cp}}{dl}\right), \quad (5)$$

where dl is the path traveled by a charged particle in a substance; the average energy lost by a particle in interactions. As will be shown below, taking this radiation characteristic into account makes it possible to describe the biological effect of various radiations, for example, consisting of photons and alpha particles.

1.5.2. Standardized Values

Standardized dosimetry values characterize human exposure, i. e. exposure to ionizing radiation. Their definition has the task of ensuring the radiation safety of a person. The basis of radiation safety is the radiation biology of humans and animals, which is based on the data from radiobiological experiments and long-term epidemiological studies of the effects of radiation in the groups of exposed people. The biological effects of irradiation are largely determined by the properties of the irradiated object. Therefore, radiobiological experiments on animals are intended to study the general laws of radiation damage, and the actual (experimental) basis of radiation safety is long-term monitoring of the groups of exposed people. At the beginning of the twentieth century, radiologists were the observed group. After World War II, residents of Hiroshima and Nagasaki who suffered from the military use of nuclear weapons, as well as victims of radiation accidents, patients exposed to therapeutic radiation, and professional workers in the nuclear industry and industry were supervised. The purpose of those studies was to identify patterns of action of ionizing radiation in area of low doses of chronic radiation, which are typical of normal use of radiation sources. The result of such studies is the following: the development of scientific concepts to limit the harmful effects of ionizing radiation on humans without unreasonable limiting the practical use

of sources. With the change of concepts, the basic standardized values also changed:

- since the beginning of the 30s of XX century and before World War II – the exposure dose;
- after the Second World War until the end of the 70s – the dose equivalent;
- in the 80s – the effective dose equivalent;
- from the 90s of the last century to the present – the effective equivalent dose.

With the study of the biological effects of radiation and the development of nuclear energy and industry, the concepts of radiation standardization of occupational exposure have developed. Until the end of the 70s of the XX century radiation standardization was based on the concept of preventing deterministic effects of radiation, which was based on the hypothesis of a threshold impact of radiation. During this period there was the rapid development of atomic science and technology, mainly in the defense sphere, which occurred in the conditions of a lack of knowledge about the biological effects of radiation and some shortcomings of radiation technologies, which led to significant doses of radiation. The development of nuclear energy, as well as other areas of the commercial use of ionizing radiation sources, has required new approaches to ensuring radiation safety that allow optimizing radiation protection in the context of improving technologies of source handling and the increase in the number of professional workers. In the late 70s, standardization was based on the concept of limiting the probability of premature death due to the occurrence of stochastic effects of radiation, which was based on the hypothesis of the no-threshold effect of radiation. Since the 90s, this system has been replaced by the concept of limiting damage due to stochastic effects of radiation, which was formulated in the 1990 Recommendations of the ICRP.

ICRP describes damage as a complex concept combining the probability and severity of the effect and the time of its manifestation, and the level of the damage can be expressed as the number of years of a full life that are lost as a result of a premature illness or death caused by exposure to ionizing radiation. When determining the damage resulting from exposure, the following shall be taken into account:

1) the probability of premature death as a result of deadly cancer for the entire life expectancy or a severe genetic disorder, which leads to premature death of the offsprings of the exposed individuals in the first two generations;

2) the contribution to the damage from non-fatal (curable) cases of cancer as the realization of the stochastic effects of radiation;

3) the duration of the lost years of a full life as a result of the implementation of various stochastic effects.

Radiation Quality. Radiobiological studies have shown that at low-doses, the same radiobiological effect of irradiation of an organ or tissue can

be observed at different absorbed doses if ionizing radiation of a different nature acts on the organ or tissue. The concept of relative biological radiation efficiency (RBE) was introduced to describe these differences. Numerous investigations have shown that when irradiating the same biological objects, RBE depends on:

- the specific effect,
- the exposure conditions,
- the type of radiation, its energy and intensity.

For the same biological effect, for example, the survival of the certain amount of irradiated cells, RBE depends on LET and is close for different types of radiation with equal LET. As a rule, the higher the LET of particles is, the higher its biological effectiveness. The dependence of RBE on LET radiation turned out to be different for different biological effects. The latter actually made it impossible to use RBE directly in radiation safety. As for the chronic exposure ith small doses, the ICRP in the 1990 Recommendations suggests using two values derived from RBE, a **weighting coefficient for W_R radiation and the average radiation quality coefficient** $\overline{Q}$. The area of applicability of these values is characterized in table 8.

Table 8

Values characterizing the quality of radiation

The size and the area of its use		Properties	The method of determining
RBE	Radiobiology	It characterizes the exposure depending on its properties and the properties of the biological object and the studied biological effect.	It is defined in a radiobiological experiment
WR	Radiation Safety (Exposure Limits)	It characterizes the effect of a radiation source on a person depending on the properties of radiation on the human body (external radiation) or arising from the nuclear transformation of radioactive nuclei inside the human body (internal radiation)	WR It is established on the basis of a generalization of RBE values for stochastic effects and in vitro transformation of mammalian cells
$\overline{Q}$	Radiation Safety (Radiation Monitoring)	It characterizes the transfer of radiation energy of biological tissue depending on the distribution of the absorbed dose by LET at the point of interaction of radiation with matter	The dependence Q (L) is determined on the basis of coordination with the established values of WR

Radiation weighting factor is used in determining the standardized value of the equivalent dose to an organ or tissue. The established ICRP-dependence of W_R on the energy and type of radiation is the result of a generalization of the available radiobiological data on RBE radiation in relation to the occurrence of radiogenic cancers of different localization in mammals and malignant transformation of mammalian cells *in vitro*. Weighting coefficients refer to external radiation incident on the surface of the body and, in the case of internal radiation, to radiation emitted during the nuclear conversion of radionuclides entering the body. For photons (x-rays and γ-radiation) $W_R=1$, for other emissions $WR\geq1$. In contrast to the RBE values, which are determined only for a specific biological effect, the irradiated object and the irradiation conditions, the established values of the weighting radiation coefficient cannot be correlated with any specific effect of human irradiation. Being a generalization of a large amount of experimental data, the values of W_R characterize the probability of a certain standard stochastic effect occurring under the influence of radiation of various natures on a standard person under the conditions of chronic exposure at low doses. The weighting coefficient of radiation is equal to the ratio of the dose of x-ray or γ-radiation to the dose of this radiation when the probabilities of the standard stochastic effect are equal when the standard person is exposed.

The ICCRP uses the average radiation quality factor in determining the operational values of external exposure for dose equivalents that are subordinate to normalized values. To ensure compliance between operational and standardized values, the dependence of the radiation quality factor Q (L) was established:

$$Q(L)=\begin{cases} 1 \quad \text{if } L\leq 10\frac{\text{keV}}{\mu\text{m}}, \\ 0{,}32\cdot L-2{,}2 \text{ if } 10\leq L<100\frac{\text{keV}}{\mu\text{m}}, \\ 300/\sqrt{L} \text{ if } L\geq 100\frac{\text{keV}}{\mu\text{m}}. \end{cases} \qquad (6)$$

Thus, the WR values were established for all penetrating types of radiation (neutrons and gamma rays) and the equality is

$$\overline{Q}=\frac{1}{D_R}\int_0^\infty Q(L)D_R(L)dL=W_R, \qquad (7)$$

where $D_R(L)dL$ is the absorbed dose of radiation R at the point of interaction of radiation with matter, due to the particles with LET in the interval (L, L + dL).

The equivalent dose. As a measure of the damage when irradiating an individual tissue or human organ, the 1990 recommendations of the ICRP introduced a special value – *the equivalent dose of an organ or tissue exposure* – equal to the absorbed dose in an organ or tissue multiplied by the corre-

sponding radiation weighting factor WR. The equivalent dose is a functional that leads to irradiation of human organs and tissues with any radiation to an equivalent radiation damage equivalent to standard sparsely ionizing radiation:

$$H_T = \sum_R D_{T,R} \cdot W_R. \quad (8)$$

The equivalent dose unit is Sievert (Sv). The value of $D_{T,R}$ in the expression for the equivalent dose, the absorbed dose of radiation of the form R in an organ or tissue T, is equal to the absorbed dose that is averaged over the mass of tissue or organ of the human body:

$$D_{T,R} = \frac{\varepsilon_{T,R}}{m_\gamma}, \quad (9)$$

where m_t is the mass of an organ or tissue; $\varepsilon_{T,R}$ is the radiation energy R that is transferred to the mass of an organ or tissue. The unit of the absorbed dose in an organ or tissue is gray (Gy). The damage to a human health is caused at the moment when radiation passes through the body, although it is expected that the realization of the damage in the form of a disease (radiation effect) at normal dose levels, which are typical to the exposure to professional workers, and it is an unlikely event that can happen throughout the rest of a person's life.

Different organs of the human body are shielded differently by other parts of the human body, which leads to a significant difference between the equivalent doses of their exposure. That is why the indication of the irradiated organ is essential in determining the equivalent dose in organ. This value must be distinguished from the "equivalent dose", which was used until recently in the Russian-language scientific and regulatory literature. The Russian term "equivalent dose" refers to a value that is equal to the product of the absorbed dose and the radiation quality factor and is an incorrect translation of the English term for dose equivalent.

The expected equivalent dose of an organ or tissue. An important quantity introduced into the practice of radiation safety by the 1990 recommendations of the ICRP is the expected equivalent dose of the internal exposure of an organ or tissue, NT (τ). This value is an analogue of the equivalent dose of external radiation when an individual tissue or individual organ is irradiated with internal radiation sources. Unfortunately, in the translation of this term, adopted in Russian literature, the meaning of completeness of action (exposure) and the inevitability of its consequences contained in the original English term is lost: *committed equivalent dose* is literally "inevitable equivalent dose". The "inevitability" of the effects of internal exposure means the following. The intake of a radioactive substance in the body leads to the irradiation of organs and tissues for a long time. In contrast to external exposure,

a dose of internal exposure to an organ or tissue has been formed for a long time after the radioactive substance enters the body. It is almost impossible to control this process after the penetration of a radioactive substance into the body. Using the laws of the biokinetics of radionuclides, the magnitude of the dose rate in individual organs of the body of a conditional person at different points in time can only be predicted. These features of internal exposure make it possible to consider the radioactive substance intake into the body as an event, which is inevitably followed by irradiation of organs and tissues and, as a result, possible damage. The expected equivalent dose is defined as the time integral of the equivalent dose rate in an organ or tissue, which is formed for some time τ after the radioactive substance enters the body of a standard person:

$$H_T(\tau) = \int_{t_0}^{t_0+\tau} H_T(t)dt, \quad (10)$$

where t_0 is the moment of the intake, and $H_T(\tau)$ is the equivalent dose rate in the organ or tissue T at time t. The value of τ corresponds to the expected remaining life expectancy of a person (fig. 8). To standardize dosimetric calculations, it was assumed that τ = 50 years for adults over twenty years and τ=(70- t_0) for children and people under twenty years. The unit of expected equivalent dose is Sievert (Sv).

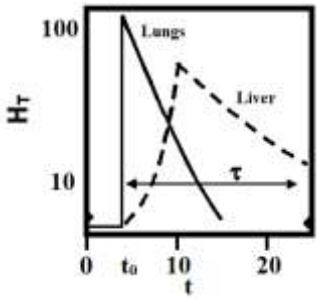


Fig. 8. Determination of the committed equivalent dose of internal exposure to an organ or tissue

For the purpose of ensuring radiation safety, during the time of causing damage to a person as a result of internal irradiation of his organs or tissues, they take the moment the radioactive substance enters the body; it is expected that the realization of damage in the form of one or another radiation effect can occur throughout the rest of a person's life. This leads to a uniform measure of different lengths of exposure. ***If the values of H_T and $H_T(\tau)$ are equal, the same consequences of external and internal exposures during the remaining life can be expected.***

The Effective Dose. For small doses, irradiation of various organs or tissues with different equivalent doses can lead to the same damage.

A measure of the damage caused to a person as a result of irradiation of the whole body or several organs and tissues is the effective equivalent dose or *the effective dose*, for short. The effective dose is defined as the functional that leads all possible cases of spatially inhomogeneous (external or internal) irradiation of tissues and organs of the body of a standard person to equivalent uniform damage of the whole body, i. e., the equal types of damages correspond to the exposure with equal effective doses.

In the case of external exposure, the effective dose of $E^{external}$ is defined as the sum of the products of equivalent doses of H_T and the corresponding weighting factors for tissues and organs W_T:

$$E^{exter} = \sum_T W_T \cdot H_T, \quad (11)$$

where H_T is the equivalent dose in T tissue of a standard person; W_T is the weighting coefficient for T tissue of a standard person.

The regulated numerical values of the weighting coefficients W_T were found to be approximately equal to the ratio of the equivalent dose of the uniform exposure to the entire body of a standard person and the equivalent dose H_T of an organ exposure T, when the same damage is expected due to the reduction in the duration of a person's full life as a result of stochastic effects caused by radiation.

In the case of internal exposure, the effective dose is determined similarly to the effective dose of external radiation and is called the expected effective dose $E(\tau)$:

$$E(\tau) = \sum_T W_T \cdot H_T(\tau). \quad (12)$$

To simplify the calculation of the effective dose in common standard practice of exposure conditions, the following ratios are used:

$$E^{exter} = \sum_K \Phi(\varepsilon)_R \cdot e(\varepsilon)_R^{exter}, \quad (13)$$

where $\varepsilon e(\mathcal{E})_R^{внеш}$ is the dose coefficient of radiation R, which is equal to the effective dose when a human body is irradiated with a radiation flux R with a unit fluence and the energy ε, Sv/(freq./ cm^2); F (ε) R is the radiation fluence R with energy ε, freq./cm^2 and

$$E(\tau) = \sum_{U,G} \Pi_{U,G} \cdot e(\tau)_{U,G}^{inter}, \quad (14)$$

Where $e(\mathcal{E})_R^{inter}$ is the dose coefficient of the radionuclide U equal to the expected effective dose when 1 Bq of the radionuclide U is ingested as a compound of type G, Sv/Bq; $P_{U,G}$ is the intake of the radionuclide U in the form of a compound of type G, Bq.

In the system of dosimetry quantities, the effective dose of external exposure and the expected effective dose of internal exposure are equivalent, i.e., the damages caused by sources of external and internal exposure are summarized. Therefore, the annual effective dose is equal to the sum of the effective dose of external exposure received per year and the expected effective dose of internal exposure due to the intake of radionuclides in the body over the same year. Unless otherwise specified, the effective dose E is the sum of the effective dose of external exposure and the expected effective dose of internal exposure:

$$E = E^{exter} + E(\tau). \quad (15)$$

As a standardized quantity, the effective dose is the result of the consistent development of ideas about the biological effect of ionizing radiation and the search for a measure of exposure to ionizing radiation that meets the goals of radiation safety in assessing and limiting radiogenic damage. The application of this value allows us to move from the measured physical characteristics of the field of ionizing radiation to potential damage as a measure of the effect of radiation on a person, the use of which creates the conditions for reducing a single cost denominator of the harm, costs and benefits of using sources of ionizing radiation. It is believed that the potential damage was caused to a person at the time of exposure or the ingestion of a radioactive substance, but its implementation in the form of a disease leading to a shortened life span is a random event and is postponed for an indefinite time comparable to a person's life span. The value of the potential damage is considered as "the mathematical expectation of the size of the undesirable consequences: the product of the probability and severity of the consequences of the event (premature death as a result of exposure)." The value of the potential damage can be represented as the product of the lifetime probability of death from radiogenic cancer by the average number of years of a full life that can be lost as a result of this event. The latter value is in strictly limited limits (10–30 years depending on the type of cancer and the organ that are irradiated) and does not depend on the radiation dose (table 3). The shorter the latent period of cancer development is, the more years of life can be lost and the greater the severity of this effect is.

On average, one stochastic effect (deadly cancer, serious hereditary effects, and non-fatal cancers that are as harmful as deadly cancers according to the consequences) leads to a 15-year reduction in the duration of a full life. The probability of any stochastic effect depends on the dose, the irradiated organ, and the age of the irradiated person. An analysis of the available data on the formation of stochastic effects shows the following: when irradiated with the effective dose of 1 mSv, the lifetime probability of any stochastic effect leading to premature death is $6 \cdot 10^{-5}$ and is the sum of the probability of the occurrence of potential damage in the form of radiogenic cancer ($5 \cdot 10^{-5}$ mSv^{-}

[1]) and genetic disease ((1·10^{-5} mSv^{-1}). Thus, in predicting the effects of exposure to an individual, rare events that have a discrete spectrum of sizes are dealt with. All of the above indicates that the use of an effective dose to assess individual damage is practically useless since the statistical uncertainties of such estimates are enormous.

The effective dose assigned to a large group of exposed people reflects the expected (in statistical meaning) damage that is associated with the exposure to the members of such a group. A special dosimetry value in the area of low-dose irradiation for assessing collective radiological damage is ***the collective effective dose S,*** which is equal for a team of N people to the sum of the individual effective radiation doses of members of this collective E_1, ... E_N:

$$S = \sum_{i=1}^{N} E_i. \qquad (16)$$

The unit of the collective effective dose is man-sievert (man Sv). As a rule, a collective dose correlates with some practical activity and the period of time during which this activity leads to the irradiation of a certain group of people. For example, when analyzing the consequences of radionuclide releases, the annual collective dose of the population of the AC observation zone is defined as the sum of the annual effective doses to the residents of the zone from radionuclides that are released into the environment as a result of AC during the calendar year. The annual effective dose is understood as the sum of the effective external dose per calendar year and the expected dose of internal exposure from the intake of radionuclides in the body during the same year.

The collective damage is defined as the shortening of the total period of the full life for the members of the considered group of people due to the possible occurrence of additional, in relation to the background level, radiogenic stochastic effects:

$$G = \Delta t \text{ x } R_E \text{ x } S, \qquad (17)$$

where R_E is the probability coefficient (radiogenic risk) of reducing the duration of the total (collective) period of full life by an average of 15 years to one stochastic effect (from deadly cancer, serious hereditary effects and non-fatal cancer, adapted according to the damage to the consequences of deadly cancer) and equal to

- $R_E = 5{,}6 \cdot 10^{-2}$ 1/ man Sv for occupational exposure;
- $R_E = 7{,}3 \cdot 10^{-2}$ 1/ man Sv for public exposure;

Δt is the expected (average) number of years of reducing the length of the full life period when any stochastic effect of radiation is realized and equal to 15 years.

The collective effective dose is a tool for assessing the expected damage from exposure to large groups of people. Irradiation with a collective effective dose of 1 man Sv corresponds to the expected damage, which is equal to 1 year loss of the total length of the full life period of the irradiated group of people.

1.5.3 Operational Values

As a rule, the standardized values, where the basic dose limits are defined, cannot be directly measured. For the assessment of the standardized values during radiation monitoring, operational values are used. Introduction to the practice of radiation monitoring of operational values is necessary, first of all, to unify the monitoring methods and determine the requirements for the response function of radiation monitoring devices.

The operational value is the value that is uniquely determined by the physical characteristics of the radiation field at the point, as close as possible under the standard irradiation conditions to the standardized value, and is intended for its conservative assessment during dosimetry monitoring. In general terms, the associationbetween the values used in radiation monitoring is presented in fig. 9.

In determining the operational values of external exposure, ***the dose equivalent*** H is used, which is equal to the absorbed dose at the point multiplied by the average quality factor for radiation that affects the tissue at the given point:

$$H = \overline{Q} \cdot D = \int_0^\infty Q(L)D(L)dL. \qquad (18)$$

The unit of dose equivalent is sievert (Sv).

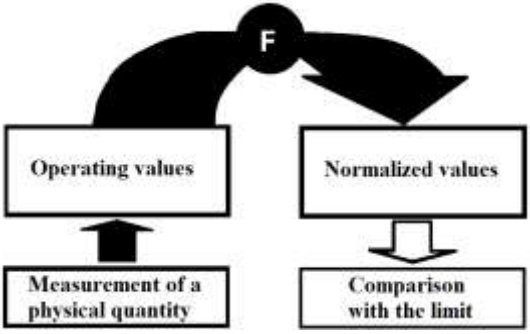


Fig. 9. The association between the values used in radiation monitoring

The interaction of radiation with the human body leads to a change in the radiation field. Operational values are determined so that the results of their measurement using appropriate dosimetry instruments take this effect into account.

The operational value of external exposure for individual control is considered to be *the individual dose equivalent* – $H_p(d)$ – the dose equivalent in soft biological tissue that is determined at a depth d (mm) below the point on the surface of a flat phantom or on an adult's body (fig. 10). The use of a phantom or a human body in this case allows you to directly ensure that the disturbance of the real radiation field by a person is taken into account.

The ambient dose equivalent (ambient dose)1 H*(d) was adopted as the operating value of external exposure to control the radiation environment. The operational values for monitoring the radiation environment are determined using the concepts of expansion and leveling in the description of the characteristics of the radiation field necessary to determine the characteristics of the dosimeters.

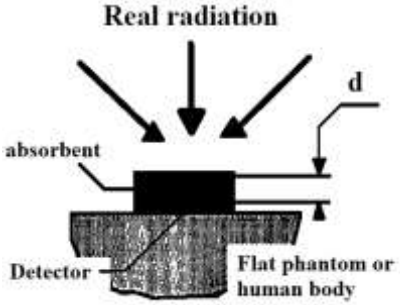


Fig. 10. The scheme for determining the individual dose equivalent

The device measuring H*(d) in a real radiation field should reproduce the dose equivalent value that would be created in the spherical phantom at a depth d (mm) from the surface in diameter parallel to the radiation direction if such a phantom were placed in the extended and a leveled radiation field (fig. 11), which is identical in composition, fluence, and energy distribution. The ambient dose equivalent is used to characterize the radiation field at a point that coincides with the center of such a spherical phantom. This value in relation to the real field characterizes a conservative estimate of the human radiation dose. The unit of the ambient dose equivalent is sievert (Sv).

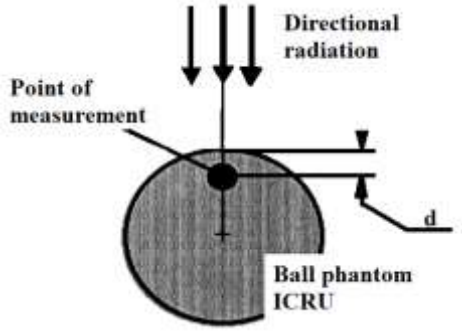


Fig. 11. The scheme for determining the ambient dose equivalent

In determining operational values, d is taken to be 10 mm to control the effective dose, 0.07 mm for the equivalent dose to the skin, and 3 mm for the dose equivalent to the lens of the eye (table 9).

When introducing into practice a modern radiation safety system, it is necessary to observe the consistency of indicators and units of measurement of dosimetry quantities. Particular attention should be paid to the interpretation of the measurement results of those values, which definitions have changed. First of all, this refers to the dose equivalent. The change that occurred after 1990 The change of the regulated ICRP dependence of the quality factor on LET, which happened after 1990, requires careful consideration when analyzing the data obtained using measuring instruments that helped to implement a different dependence of the quality factor on LET (proposed by the ICRP Recommendations of 1977).

Table 9

Basic Dosimetry Values and Their Ratios

Value	Unit		Ratio
	SI	Conventional	
Activity / A	Bq	Cu	1Cu = 3,7 ·10^{10} Bq
Radiation Energy R/ E_R	J	eV	1eV = 1,602·10^{-19} J
LET / L	J/m	keV/μm	1 keV/μm = 62 J/m
Exposure Dose / X	CL/kg	P	1P = 2,58·10^{-4} CL/kg
Kerma / K	Gr	rad	1 rad = 1·10^{-2} Gr
Absorbed Dose / D	Gr	rad	1 rad = 1·10^{-2} Gr
Equivalent Dose in an organ T / H_T	Sv	NP*	no
Effective Dose / E	Sv	NP*	no

Dose Equivalent / H	Sv	rem	1 rem = $1 \cdot 10^{-2}$ Sv

* Not applicable (NP), since this value was first introduced by the 1990 recommendations of the ICRP.

Part 2. THEORETICAL FUNDAMENTALS OF RADIOBIOLOGY

The change of ideas in radiobiology has occurred and is happening especially quickly since they are largely associated with the rapid progress of nuclear physics and molecular biology. In this case, ***two directions*** in the development of theoretical constructions can be outlined.

One of them aims at to establish general, mainly phenomenological, but necessarily *quantitative* patterns characterizing the initial links of radiation damage to the cell.

The other combines representations that establish a close connection in the whole variety of specific radiation reactions of biological objects; hence, the predominantly *qualitative* and descriptive nature of the hypotheses for this direction prevails.

The mentioned above theoretical directions have certain advantages and the limitations.

2.1. The Target Theory

To interpret the basic radiobiological paradox, it is necessary to correctly understand the discrepancy between the negligible amount of radiation energy absorbed by the cell and the caused extreme biological effect. Explaining it in the context of quantitative radiobiology, two principles were formulated that underlie *the target theory:*

First, *the principle of hits* characterizes the features of the active agent - discreteness of energy absorption.

Second, *the principle of the target* takes into account the peculiarity of the irradiated object (cell), its high heterogeneity in morphological and functional meaning, and, therefore, the difference in the response to the same hit.

In the late 20s, for the first time, to explain the radiobiological phenomena and create a fundamental theory of the biological effect of ionizing radiation, the theoretical concepts of quantum mechanics and nuclear physics were required as starting concepts.

F. Dessauer was one of the first to do this in his theory of "spot heating". Ionizing radiation has a low volume density; however, individual photons carry a huge supply of energy. Based on this, F. Dessauer suggested that when the system absorbs relatively small total energy (a lethal dose to a person causes the body to be heated by only 0.001 ° C); some discrete microvolumes absorb such large portions of energy that the effect of ionizing radiation can be compared to microlocal heating; as a result, there are deep structural changes and ultimately biological damage. The hypothesis was explained by the statistical distribution of "heat point" as the probabilistic nature of the manifestation of

the effect in individual objects. So, for the first time, the physical principle of hits was introduced into radiobiology.

Considering the fact that there are less significant structures and microvolumes in the cell, which are of more important for life, and the random distribution of "heat point", Dessauer, and later a number of other researchers (further development is associated with the work of J. Crowther, D. Lee, R. Zimmer, N. V. Timofeev-Ressovsky, V.I. Korogodin), came to the conclusion that the outcome of the cellular reaction depends on the probability of random hits of discrete portions of energy in precisely these vital microvolumes – ***targets.*** In this case, the quantitative laws of radiobiological reactions are realized only if a certain number of "hits" occur in the cell.

It should be noted that when analyzing the dependence of the effect on the dose, two specific features of the action of ionizing radiation are easily defined:

1. Most cellular reactions occur almost in the absence of a threshold, with an increase in effect with increasing the dose.

2. Survival curves reflect not so much the degree of manifestation of the effect in individual individuals (cells) with an increase in dose, but rather an increase in the number (fraction) of the affected units: an increase in the likelihood of a recorded reaction.

So, the lethal effect of ionizing radiation has a probabilistic nature due to the random distribution of elementary acts of the primary interaction with sensitive volumes of irradiated individuals.

The advantage of the described theoretical concepts about the mechanism of the lethal action of ionizing radiation is the simplicity of explaining the basic experimental data: the number of viable units decreases exponentially with the dose with increasing the dose. The authors explained the S-shape of the survival curves in linear coordinates by *the multi-impact process* of inactivation of the objects. At the same time, they believed that in order to inactivate an object, not one, but two or more hits in a single target or defeat of two or more targets, each should be hit, is necessary. However, this theory does not explain the numerous experimental facts of changes in the extrapolation number when using various modifying agents or changes in the living conditions of objects, which should not affect the number of targets, etc.

Along with the success of quantitative research, interesting results were obtained in the 40s in the analysis of the physicochemical nature of the processes occurring between the primary absorption of radiation energy and the final biological effect. The formation in the irradiated solution of highly active products of water radiolysis – free radicals – capable of diffusing over considerable distances and damage biological structures, was discovered. Radiobiology begins to operate with the ideas on the "indirect effect" of radiation mediated by the active products of water radiolysis. For this purpose, the physicochemical properties of the primary products of radiolysis of water and the nature of their interaction

with cell macromolecules were studied. These studies were carried out in collaboration with specialists in the field of radiation chemistry. The obtained data gave rise to the hypotheses about the possibility of attenuating radiation damage due to the introduction of free radical interceptors into the system, competing with biological structures for water radiolysis products.

2.2. The Hypothesis of Primary Radiotoxins and Chain Reactions

A significant contribution to the interpretation of the biophysical mechanisms of radiation damage was made by B. N. Tarusov's work. According to his theory, a few primary lesions initiate chain oxidation processes which multiple subcellular structures are involved in. The theory of the physicochemical mechanism for enhancing the initial radiation damage made it possible to explain many radiobiological phenomena such as the development of radiation damage processes over time, the influence of temperature, atmospheric gas composition, etc. The search for substrates, when oxidation-initiated oxidation processes are most likely to occur, starts.

In the mid-50s, Yu. B. Kudryashov found that the higher unsaturated fatty acids that make up cellular lipids have significant vulnerability to radiation exposure. He noted that lipid peroxidation products largely simulate the effect of radiation on a variety of biological objects and systems. Thus, the author showed the radiomimetic and radiosensitizing effect of the oxidation products of higher unsaturated fatty acids. The studies made it possible to assume that as a result of irradiation, the active participation of biomembrane lipids in the processes of peroxidation takes place, which subsequently leads to multiple lesions and cell death. B. N. Tarusov gave scientific reasoning of the thesis that normally oxidative processes in tissue lipids occur at a low level and are in a stationary mode. After irradiation, oxidation processes can change into an unsteady mode and involve various components of intracellular membrane structures causing the dynamics of radiation damage. For the development of these ideas, studies of the mechanisms of oxidative reactions conducted by N. N. Semenov, N. M. Emanuel and his school were of great importance. Further, a significant number of works carried out under the supervision of E. B. Burlakova, as well as other authors, were devoted to investigation of the mechanisms of lipid oxidation that is primarily induced by ionizing radiation.

Despite the desire of some researchers to attach exceptional importance to the one factor, the experimental data indicated the existence of alternative ways of realizing the protective effect even for the same radioprotector.

In the 70s, Yu. B. Kudryashov and E. N. Goncharenko found that various radioprotective agents, by the time of their maximum effectiveness, reduce the level of lipid peroxidation products (natural sensitizers of radiation damage) in animal tissues and increase the content of biogenic amines, which, along with thiols, are natural anti-radiation substances. Based on these data,

the authors suggested the hypothesis of an “endogenous background of radioresistance”.

These studies led to the accumulation of extensive factual material on general situations of radiation damage and their modifications, and made it possible to outline the ways to develop the basic laws of the origin of “starting”, “starting-up” physicochemical processes, and the mechanisms of attenuation or amplification of primary radiation reactions. As a result, scientific works devoted to the analysis of physicochemical processes in the cell from the moment of the onset of initial structural damage to the manifestation of pronounced biochemical and morphological changes have come to the fore. Towards this end in view, the modifying effect of oxygen, temperature, and other agents affecting the development of radiation damage to biological objects is studied. A large number of works are devoted to the problem of energy and charge migration in an irradiated system; the role of free radicals and the relative contribution of the direct and indirect effects of ionizing radiation are analyzed.

In the following years, molecular radiobiology has achieved considerable success (primarily in determination of the mechanism of radiation inactivation of enzymes and nucleic acids) using its entire experimental and scientific data. At the same time, a new direction in radiation biology was formed, which is based on new fundamental physical and quantum-mechanical principles, the experience of quantitative radiobiology, the latest discoveries of molecular biology on the cause-effect relationship between the structure and biological functions of macromolecules. The phenomenon of cellular recovery from radiation damage, described in the 60s, thanks to the development of cell culture methods, begins to acquire an explanation at the molecular level, i.e. the mechanism of repair of radiation damage to DNA was discovered and analyzed in detail. This is the largest contribution of radiobiology to the science of life. A complex enzymatic system is turned out to be functioning in the cells, which supports the structural integrity of the genome. These enzymes are capable of recognizing and correcting defects in the structure of DNA resulting from radiation exposure. The functioning of reparative systems depends on the state of intracellular metabolism and the intensity of energy processes. The molecular mechanism of such well-known radiobiological effects as the dependence of radiation damage on the conditions of post-radiation cell cultivation, the state of metabolic systems and other physiological factors becomes clear. For modern radiation biology, it becomes generally accepted to consider the final radiobiological effect as a result of the interference of two oppositely directed processes: the realization of the primary lesion and its restoration by reparative systems. The structural and metabolic theory of radiation damage, developed by A. M. Kuzin, is becoming increasingly widespread.

2.3. Structural and Metabolic Theory

The theory, which has been actively developed by A. M. Kuzin since 1965, is based on the idea that under the influence of ionizing radiation in the cell not only purely radiation-chemical damage occurs, but, thanks to biochemical amplification mechanisms, highly reactive products are synthesized in the body, leading to the additional damage to biologically important macromolecules and the formation of low molecular toxic metabolites. In the theory under consideration, a large consideration is given to the radiation damage to nuclear macromolecules, disruptions of cytoplasmic structures and their normal functioning due to their inherent order. The damage of such a strictly coordinated system in one or several links leads to membrane disruption and the conjugation of important metabolic processes: inactivation of enzymes, disorder of control systems and other serious consequences.

In the monograph "Structural and Metabolic Theory in Radiobiology" (1986), A. M. Kuzin made an attempt to analyze the numerous facts registered by radiobiology over the past decades. The author concludes that this theory is a general theory of the effect of radiation on biological objects, starting from the cellular level and ending with highly organized multicellular organisms.

At the same time, this theory cannot be recognized as universal since it does not determine the quantitative relations between the accumulation of primary radiotoxins (PRT) in the cell and the degree of its damage, etc.

2.4. The Stochastic Theory

In the 70s, interesting studies were appearing that were devoted to the formal-statistical analysis of radiobiological processes. They were based on the achievements of quantitative radiation biology and the ideas about the dynamic nature of the formation of radiation damage. The classical notions of the presence of a static target were being replaced by the dynamic models that took into account the probability of the damage being realized and their recovery due to reparative processes. They included the "stochastic hypothesis" of O. Hoog and A. Kellererer, and the "probabilistic model" of Yu. G. Kapultsevich.

Thus, in the view of probability theory, the "stochastic theory" considers various disturbances of the biological system that arise during the life process or under the influence of radiation, trying to describe them with models that are most consistent with the concepts of dynamic biochemistry and molecular radiobiology. In this case, the targets are all components of the living system, and the recorded reaction is due to a superposition of a variety of events. In 1966, a monograph "Stochastic Radiobiology" by A. Hoog and A. Kellerere was published, which was translated into Russian in 1969. It is significant that the stochastic hypothesis takes into account both physiological and radiation-

induced processes in their dynamics. At the same time, the classical theory of the target considers the effects caused by irradiation as strictly determined by the primary acts of energy absorption. With the mathematical tools (using systems of differential equations) in the stochastic theory, the quantitative effect of any modifying factor on the corresponding dose dependencies is taken into account.

So, in accordance with the basic primary concepts, the stochastic concept offers a more "biological" interpretation of the dose – effect curves compared to explaining them from the standpoint of the target theory. But at the same time, two undeniable determining factors of the classical theory of targets remained – *the discreteness of the radiation agent and functional in homogeneity of the biological object*. The stochastic hypothesis leads to the understanding that the exponential curve indicates a system without compensatory mechanisms, and the sigmoid curve corresponds to the systems that have such mechanisms whose effectiveness decreases with increasing the dose.

The main feature of this theory is a *strictly quantitative approach;* however, the mathematical tools of this theory are quite complicated, and this makes it difficult spread its use widely.

2.5. The Probabilistic Model of Radiation Damage to a Cell

After studying the vegetative propagation of irradiated cells (yeast), Yu. G. Kapultsevich in 1978 suggested a "probabilistic model of cell radiation damage."

According to this model, different cells exposed to the same dose are affected to varying degrees in accordance with the principle of hit. In contrast to classical concepts, both potential and realized damages occur with a probability of less than a unit. Realized lesions (or the changes induced by them) are inherited during cell division with a certain probability depending on the number of these lesions, which leads to the failure of cell division. In this case, the probability of the damage manifestation may depend both on the biological (genetic) characteristics of the cells and on the conditions of their cultivation.

The main difference between the probabilistic model and the classical ones is the following; according to the latter, the radiosensitivity of the cell is determined only by the volume of the target and the critical number of hits. In the view of the probabilistic model, the problem of radiosensitivity seems to be more complicated. Yu. G. Kapultsevich formally divides the process of radiation damage to the cell into three stages:

The first stage of radiation damage is the implementation of impact events; as a result, primary potential damage is formed.

The second stage of radiation damage is the realization of the potential damage.

The third stage is various secondary violations of the normal course of intracellular processes caused by the implementation of damage. Therefore, at this stage, it is possible to recover cells from the effects of realized injuries or their compensation; therefore, the probability of manifestation of realized injuries is not equal to a unit. It depends on the biological characteristics of the cell and on the cultivation conditions.

However, neither the model itself nor the analysis of cell reactions to irradiation can reveal the nature of the damage that underlies these reactions. In addition, the conclusions were obtained in the study of yeast cells, which makes it difficult to verify their applicability to the description of radiation reactions of mammalian cells.

The mathematical constructions developed in the above-mentioned studies allow obtaining the quantitative information on the nature of the trigger events of radiation damage and the features of their implementation in cells based on experimental 'dose-effect' curves.

In recent years, many radiation-biophysical studies have addressed the issue of the degree of specificity of the response of biological systems to radiation exposure.

Back in the 60s of XXth century V.P. Paribok suggested that the well-known ability of reparative systems to eliminate radiation damage to DNA is just one of the manifestations of a non-specific reaction of a living system to a damaging effect. Thus, it is assumed that there are systems in cells that support the native state of its structures and responsive to any damaging effect. In of L. X. Aidus' studies, an analysis of *the mechanisms of the nonspecific reaction of cells to damaging effects* was made; the author, along with ionizing radiation, attributed the effect of various radioprotectors to them. According to his hypothesis, under the influence of a damaging agent, the same type of changes occur, including impaired membrane transport and the corresponding concentration gradients of low molecular weight compounds that locally accumulate in cell compartments, are sorbed on macromolecules, and change their conformational mobility. In the end, a state of paranecrosis can occur, which is reversible with moderately damaging effects. At the same time, the ratio of speeds of competing processes of realization and reparation of "hidden damage" in the unique structures responsible for cell death changes.

The understanding of the molecular mechanisms of the nonspecific response of cells to a damaging effect certainly needs further definition. However, the very question of the non-specificity of the processes and systems under consideration allows us to consider the well-known radio-biological phenomena as the manifestations of the general biological response of living systems to any damaging effects. This means that the methodological approaches, mathematical apparatus and methodological techniques accumulat-

ed by radiation biology are becoming important for modern biology as a whole.

At present, it is planned to unite the most valuable but extreme information in theoretical radiobiology. Obviously, this is the immediate future of radiation biology. It can be expected that the synthesis of physicochemical, molecular, and traditional biological (radiobiological) approaches will soon answer the key question of radiation research: what the mechanism of the initial triggering processes of radiation damage is and how to more effectively manage it.

Part 3. DNA DAMAGE AND REPAIR

3.1. Chromosomal Aberrations and Their Classification

Chromosomal aberrations (chromosomal rearrangements, chromosomal mutations) are a type of mutation that changes the structure of chromosomes. A change in the structure of chromosomes is associated with its direct damage (the break of one or two chromatids), subsequent reunification of the resulting gaps (either with the preservation of the previous structure or erroneously) as well as redistribution, loss or partial doubling of genetic material.

Chromosomal aberrations (chromosomal rearrangements, chromosomal mutations) are the type of mutations that changes the structure of chromosomes. A change in the structure of chromosomes is associated with its breakage by direct damage (the breakage of one or two chromatids), the subsequent reunion of the resulting breakages (either with the previous structure or mistakenly) as well as the redistribution, loss or partial doubling of genetic material.

Chromosomal mutations are characterized by changes in the position of sites, sizes and organization of chromosomes. Parts of the same chromosome or different chromosomes may be involved in such rearrangements.

The standard classification of radiation-damaged chromosomes is based on the identification of 2 main types – chromatid aberrations (the breakage of only one chromatid) and chromosome aberrations (both chromatids are damaged). Chromatid aberrations (single fragments) are nonspecific, but certain chromosomal aberrations (their formation is associated with the release of more energy as a result of radiation exposure and the formation of several breakages at the same time) can be attributed to the action of radiation.

The following types of chromosomal rearrangements are distinguished: deletions, inversions, duplications, translocations (balanced and unbalanced, including dicentric and ring chromosomes as well as isochromosomes).

Some of them are already widely used as markers of radiation exposure. First of all, they include unbalanced translocations: the frequency of dicentric and ring chromosomes. As a result of the development of the technique of cytogenetic analysis (using differential staining of chromosomes and FISH analysis), it became possible to use balanced translocations for these purposes, which have less effect on the viability of host cells.

If rearrangement changes the structure of one chromosome, this rearrangement is called *intrachromosomal rearrangement* (inversions, deletions, duplications, and ring chromosomes); however, if the two are different it is called *interchromosomal rearrangement* (duplications, translocations, and dicentric chromosomes).

Chromosomal rearrangements are also divided into *balanced and unbalanced.* The balanced rearrangements (inversions, reciprocal translocations) do

not lead to the loss or addition of genetic material during formation; therefore, their carriers, as a rule, are phenotypically close to normal. Possible complications are associated with a change in the position of the gene, which may affect the viability of the carrier by deviations of various levels.

The unbalanced rearrangements (unbalanced translocations, deletions, duplications, and the formation of paired fragments) change the ratio of genes, their interposition or cause their complete or partial loss, and, as a rule, their carrier rate is associated with the significant deviations from the norm.

Chromosomal rearrangements play an important role in the evolutionary process and speciation, impaired fertility, the development of human cancer, congenital and hereditary diseases.

The main condition for the occurrence of chromosomal rearrangements is the appearance of breaks of both strands of the DNA helix. Double-stranded DNA breaks can occur in a cell spontaneously or under the influence of various mutagenic factors: primarily physical (ionizing radiation) and sometimes chemical or biological (transposons, viruses) nature.

It was established that double-stranded DNA breaks occur programmed during prophase I of meiosis as well as during maturation of T and B lymphocytes during specific somatic V (D) J recombination.

Defects and errors in the process of repairing double-stranded DNA breaks lead to the appearance of chromosomal rearrangements.

Deletion is the loss of a chromosome region. There are *terminal and intercalary* deletions. Terminal deletions are associated with the loss of the terminal region of the chromosome, and intercalary deletions are associated with the loss in the inner region of the chromosome (fig. 12).

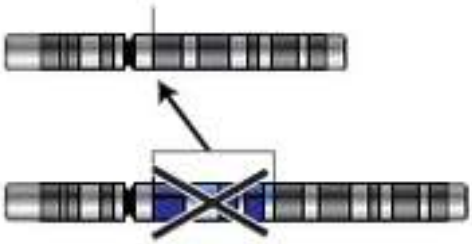


Fig. 12. Intercalary deletion

If after the deletion formation, the chromosome retains the centromere, it is transmitted similarly to other chromosomes during mitosis, while the sites without a centromere, as a rule, are lost. When conjugating of homologous chromosomes during meiosis, in the normal chromosome, a deletion loop is formed at the site corresponding to the intercalary deletion of the defective chromosome, which compensates for the absence of the deleted fragment.

Congenital deletions in humans rarely capture the extended regions of chromosomes, and usually such aberrations lead to the death of the embryo in

the early stages of development. The most studied disease caused by a fairly large deletion is feline scream syndrome, described in 1963 by Jerome Lejeune. It is based on a deletion of the short arm of chromosome 5. Patients are characterized by a number of deviations from the norm: dysfunction of the cardiovascular and digestive systems, underdevelopment of the larynx with a characteristic scream that resembles feline meowing, general developmental delay, mental retardation, moon-shaped face with eyes wide apart. The syndrome occurs in 1 out of 50,000 newborns.

Modern methods for detecting chromosomal abnormalities, primarily fluorescence hybridization *in situ*, have made it possible to establish a relationship between microdeletions of chromosomes and a number of congenital syndromes. Microdeletions, in particular, are due to the well-known Prader-Willi syndrome and Williams syndrome.

Duplication is the multiplication of chromosome fragments. Duplications are a class of rearrangements that combines both intra- and interchromosomal rearrangements. In general, any duplication is the appearance of an additional copy of the chromosome region, which can be located immediately behind the region that is duplicated, and then this is *tandem duplication* (fig. 13), either in a new site or in anothWer chromosome. A new copy can form a separate small chromosome with its own telomeres and centromeres, and then this is *free duplication.*

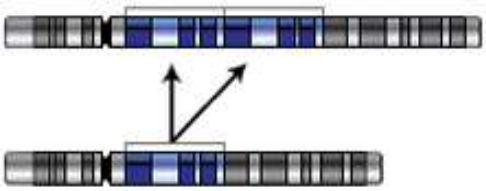


Fig. 13. Tandem duplication

Tandem duplications appear in germ cells during meiosis as a result of unequal crossing-over (in this case, the second homolog carries a deletion) or in somatic cells as a result of non-allelic homologous recombination during the repair of double-stranded DNA breaks. In the process of crossing over, in heterozygotes, when a chromosome with tandem duplication and a normal chromosome is conjugated, as in a deletion, a compensation loop is formed.

In almost all organisms, a multiplicity of genes encoding rRNA (ribosomal RNA) is normally observed. This phenomenon is called gene redundancy. So, in *E. coli*, rDNA (DNA encoding rRNA) accounts for 0.4% of the total genome, which corresponds to 5–10 copies of ribosomal genes.

Another example of duplication is the mutation of *Bar* in *Drosophila*, discovered in the 1920s by T. Morgan and A. Stertevant. The mutation is due to duplication of the locus 57.0 of the X chromosome. In normal females

(B^+/B^+), the eye has 800 facets, in heterozygous females (B^+/B) the eye has 350 facets, in homozygotes for mutation (B/B), there are only 70 facets. Females with a triplicate gene – double Bar (B^D/B^+) were also found.

In 1970, Susumo Ono in the monograph "Evolution by Duplication of Genes" developed a hypothesis about the evolutionary role of duplications supplying new genes without affecting the functions of the original genes. The proximity of a number of genes in nucleotide composition but encoding different products speaks in favor of this idea. These are trypsin and chymotrypsin, hemoglobin and myoglobin, and a number of other proteins.

Inversion is the reverse of the order of the genes of the chromosome region, i. e. 180 ° the rotation of the chromosome region. There are *paracentric* and *pericentric* inversions (fig. 14, 15). During paracentric inversions, the inverted fragment lies on one side of the centromere, and in pericentric inversions, the inverted fragment lies on opposite sides of the centromere.

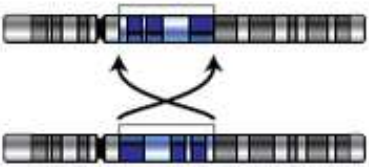


Fig. 14. Paracentric inversion

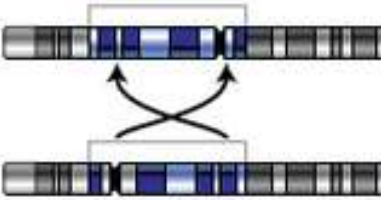


Fig. 15. Pericentric inversion

During inversions, there is no loss of genetic material; therefore, inversions, as a rule, do not relatively affect the phenotype of the carrier if the effect of the position of the gene is not taken into account. However, if heterozygotes by inversions (i. e., an organism carrying both a normal chromosome and a chromosome with an inversion) during metosis in gametogenesis, crossing over occurs within the inverted region, there is a possibility of the formation of abnormal chromosomes, which in turn can lead to partial elimination of germ cells, as well as the formation of gametes with unbalanced genetic material.

More than 1 % of the human population is carriers of pericentric inversion in chromosome 9, which is considered a normal variant (fig. 16).

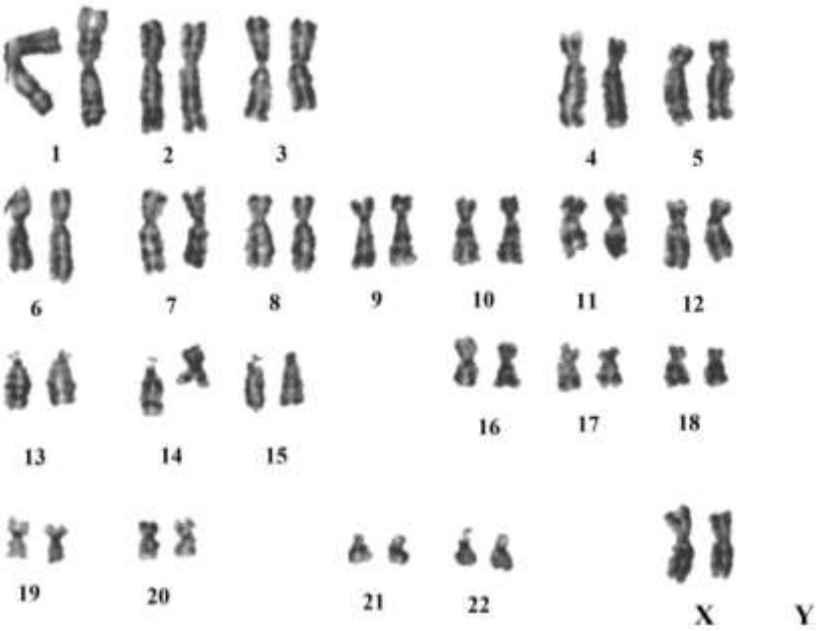


Fig. 16. Metaphase plate with pericentric inversion in chromosome 14 (differential staining of chromosomes, x2000)

Translocation is an interchromosomal rearrangement, in which there is a transfer of a section of one chromosome to another. Separately, the most common types of translocations are distinguished, for example, *reciprocal (balanced*) and *non-reciprocal (unbalanced) translocations*. A **special case** is represented by *Robertson translocations*, or centric fusions.

In reciprocal translocations, two non-homologous chromosomes exchange regions, while the genetic material is not lost but changes its position (fig. 17), which can affect the phenotypic manifestation of this aberration due to a change in the position of the translocated material. In Robertson translocations, two non-homologous acrocentric chromosomes are combined into one with the loss of material of short arms (fig. 18).

The first centric fusion was described by W. Robertson in 1916 comparing karyotypes of closely related locust species.

Reciprocal translocations are not accompanied by the loss of genetic material; they are also called *balanced translocations*. They, as a rule, do not appear phenotypically. However, in reciprocal translocation carriers half of the gametes carry unbalanced genetic material, which leads to a decrease in fertility, an increased likelihood of spontaneous miscarriages and the birth of children with congenital anomalies. The frequency of heterozygotes for reciprocal translocations is estimated as 1 in 600 married couples. The real risk of hav-

ing children with an unbalanced karyotype is determined by the nature of reciprocal translocation (the specifics of the chromosomes involved in the restructuring and the size of the translocated segments) and can reach 40 %.

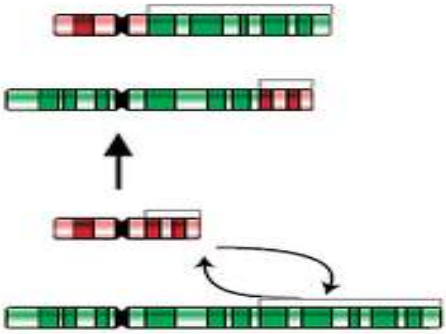


Fig. 17. Reciprocal translocation

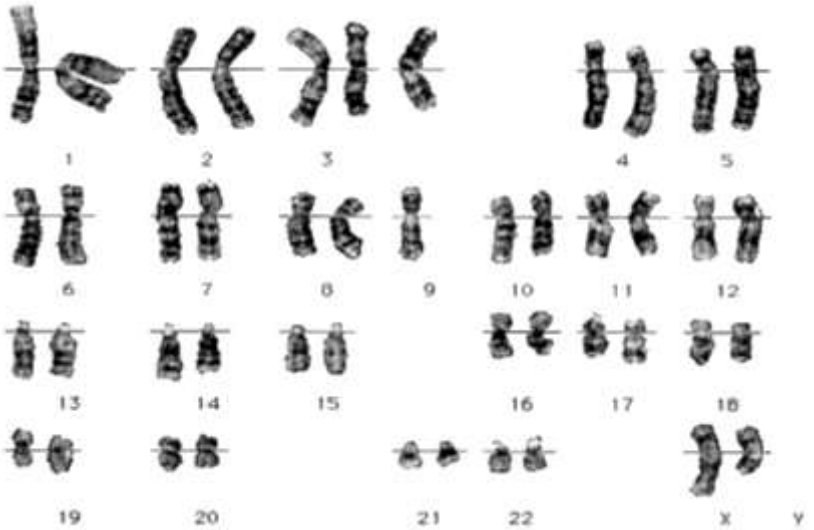


Fig. 18. Balanced Metaphase Plate with Balanced Translocation t (9p;Xq)

An example of reciprocal translocation is a Philadelphia chromosome (*Ph)* type translocation between chromosomes 9 and 22. In 95 % of cases, it is this mutation in hematopoietic progenitor cells that causes chronic myeloid leukemia. This restructuring was described by P. Novell and D. Hungerford in 1960 and named after the city in the USA, where both worked. As a result of this translocation, the ABL1 gene from chromosome 9 is combined with the *BCR* gene of

chromosome 22. The activity of the new hybrid protein leads to cell insensitivity to the effects of growth factors and causes its uncontrolled division.

Robertson translocations are one of the most common types of congenital chromosomal abnormalities in humans. According to some reports, their frequency is 1: 1000 newborns. Their carriers are phenotypically normal; however, they have a risk of spontaneous miscarriages and the birth of children with an unbalanced karyotype, which varies significantly depending on the chromosomes involved in the fusion as well as on the gender of the carrier.

Most Robertson translocations (74 %) affect chromosomes 13 and 14. In the structure of prenatal diagnosis, carriers der (13; 14) and der (14; 21) are leaders. The latter case, namely, Robertson translocation involving chromosome 21 leads to inherited Down syndrome.

Robertson translocations are probably the reason for the differences between the number of chromosomes in closely related species. It was shown that various species of Drosophila have from 3 to 6 chromosomes.

Robertson translocations led to the appearance in Europe of several twin species (chromosome races) in mice of the *Mus musculus* species group, which, as a rule, are geographically isolated from each other. The set and, as a rule, gene expression during Robertson translocations do not change, so the species are almost indistinguishable externally. However, they have different karyotypes, and fertility during interspecific crosses is sharply reduced.

A special case is represented by unbalanced translocations, which include radiation exposure markers – dicentric and ring chromosomes, which will be discussed below. Unbalanced translocations are accompanied by a nonequilibrium redistribution of genetic material and centromeres, which significantly affects the viability of carrier cells.

Isochromosomes consist of two copies of one arm of the chromosome connected by a centromere so that the arms of the formed chromosome are mirror images of each other (fig. 19). In a certain way, an isochromosome is a gigantic inverted duplication with the size of an entire arm and a deletion of the other arm.

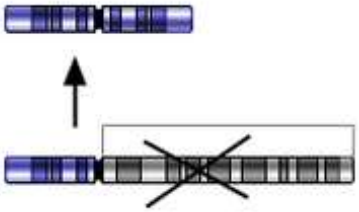


Fig. 19. An Isochromosome

Patients with 46 chromosomes, with one that is an isochromosome, are monosomics on the genes of the lost chromosome arm and trisomics on the genes that are present in the isochromosome. If the isochromosome is complementary, this patient is a tetrasomic on the genes presented on the isochromosome. In general, the smaller the isochromosome is the less the genetic imbalance is, and the more likely the survival of the fetus or child with such a rearrangement is. Therefore, it is not surprising that the most common of the described cases of autosomal isochromosomes involve chromosomes with small arms. Some of the most frequent participants in the formation of isochromosomes are the short shoulders of chromosomes 5, 8, 12, 18.

Two mechanisms can be suggested to explain the occurrence of isochromosomes. They appear:

– due to the abnormal transverse separation of the centromere during cell division;

– as a result of improper merging of the ends of the isochromatid gap formed in the pericentromeric region.

In the first case, an incorrect separation of the centromere in meiosis II occurs. In the second case, te translocation occurs that includes the whole arm of the homologous chromosome (or sister chromatid) in the area directly adjacent to the centromere. Formally, the last isochromosomes can be called isodicentric since they have two centromeres, but they are usually not cytogenetically distinguishable since they are very close to each other. The most common isochromosome, the long arm isochromosome of the X chromosome, is i (Xq) in some female patients with Turner syndrome.

Isochromosomes for a variety of autosomes are described; they include the short arm isochromosomes of chromosome 8, i (18p) and the short arm of chromosome 2, i (12p).

Isochromosomes are also often detected in karyotypes of benign and malignant neoplasms.

3.2. Classification of Radio-Induced Chromosomal Aberrations

Mutagenic effects that cause double-stranded DNA breaks lead to the appearance of chromosomal rearrangements in cells. The well-characterized mutagen inducing chromosomal aberration is ionizing radiation. Karl Sachs, whose fundamental work “Chromosome Aberrations Induced by X-Rays” was published in 1938 [8], is considered to be the progenitor of radiation cytogenetics.

To classify radio-induced chromosomal abnormalities, such a classification of aberrations has been developed, but it only partially coincides with the classification used in medical genetics. In this classification, *chromosomal and chromatid types of aberrations* are distinguished, which, in turn, can be *exchangeable and simple, stable and unstable*.

The type of chromosomal aberration is largely due to the phase of the cell cycle with the cell located at the time of irradiation.

When cells are irradiated at the G_0-G_1 stage of the cell cycle, a chromosome type of aberrations are observed in metaphases. The most characteristic among them are the so-called exchange chromosome aberrations, namely, dicentric and ring chromosomes formed as a result of improper reunification of double-stranded DNA breaks. Dicentric and ring chromosomes are usually accompanied by a fragment of a chromosome that does not contain centromeres, the so-called chromosome acentric fragment (fig. 20).

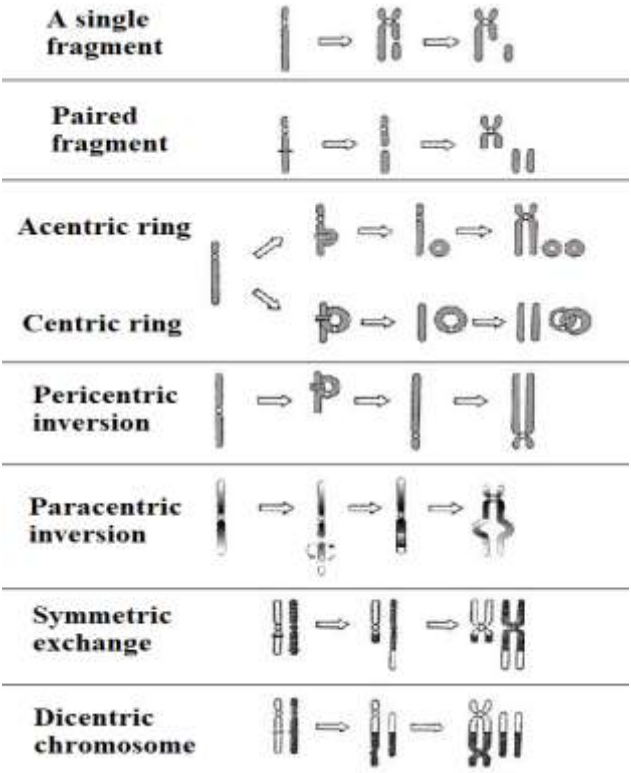


Fig. 20. The main types of chromosomal aberrations recorded in classical cytogenetic analysis

Translocation is also related to metabolic aberrations of the chromosome type. Unrepaired double-stranded DNA breaks lead to deletions of the chromosomes and the formation of acentric chromosome fragments, which can be observed in the nearest mitosis. Dicentrics, rings, and acentric fragments are poorly transmitted in a series of cell divisions and disappear in dividing cells over time, so they are referred to as unstable chromosomal rearrangements.

Translocations that do not lead to the loss of genetic material are freely transmitted to daughter cells in mitosis; therefore, they are classified as stable aberrations.

Dicentric chromosomes (dicentrics) are a fairly common type of abnormal chromosome (normally their frequency varies between 0.05–0.10 %), when two chromosome centric segments from different chromosomes, which are formed as a result of the breaks of both chromatids in non-homologous chromosomes, join end-to- end with the loss of acentric fragments (fig. 21).

Dicentric chromosomes, despite two centromeres, can be relatively stable if one of the two centromeres is inactivated, or if the centromeres in anaphase coordinate their movement to the same pole. Such chromosomes are formally called pseudo-dicentric. Most often pseudo-dicentrics consist of sex or acrocentric chromosomes (Robertson translocations).

Fig. 21. Irradiation effect: paired fragment (1) and a metaphase plate with a dicentric chromosome (2) (Romanovsky–Giemsa stain, x2000)

The ring chromosome is a closed double-stranded DNA molecule, the natural structure of chromosomes in many prokaryotes, some viruses as well as DNA molecules that make up plastids and mitochondria of eukaryotes. In some viruses, the ring chromosome consists of a single-stranded DNA or RNA molecule.

Ring chromosomes can also be the result of structural chromosomal aberration resulting from two-hit events in two chromatids with the loss of telomeric regions and the subsequent closure of free ends into ring structures.

Small ring chromosomes can be formed during fragmentation of chromosomes and also in the form of so-called “minutes” (micro rings) form in apoptotic cells. The stability of these aberrations is determined by the nature of the reunion of the free ends; when they cross-circuit, they cause the formation of anophase bridges and, as a result, are lost during the first division (fig. 22).

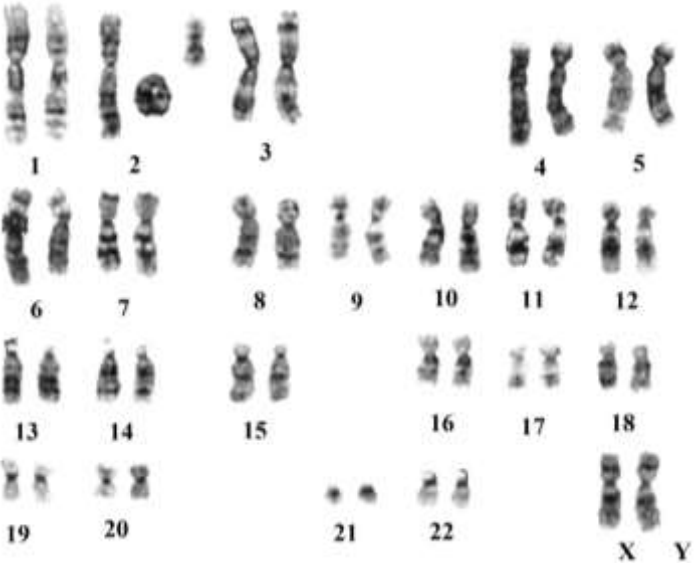


Fig. 22. Metaphase plates with a ring chromosome and a paired fragment on chromosome 2

If irradiation causes the appearance of double-stranded DNA break in a chromosome region that already has undergone doubling during replication in the S phase of the cell cycle, this can lead to the formation of chromatid type aberrations. The most typical aberrations of a chromatid type are tetraradials (metabolic aberrations arising from the improperly joining of two double-stranded DNA breaks located on chromatids of different chromosomes) and chromatid fragments (unrepaired double-stranded DNA break).

Dicentrics and rings, as well as some chromatid-type metabolic aberrations, often lead to the formation of bridges in the anaphase of mitosis, which can be detected using the anaphase-phase method for analyzing chromosomal aberrations.

3.3. Radiation-Induced Gene Mutations

It is believed that in other cases, smaller intramolecular rearrangements caused by ionization entail genuine gene mutations, i.e. such intramolecular rearrangements of groups of atoms that do not lead to the changes in the structure of the chromosome. Recently, it has also been revealed that the relation-

ship between irradiation and the mutation process may not be as direct as it seemed before. The radiation energy is absorbed, apparently, not only in the chromosomes but also in their environment, which can cause chemical changes, which in turn cause gene mutations or fragmentation of chromosomes.

Gene (point) mutations are changes in the number and / or sequence of nucleotides in the DNA structure (insertion, loss, movement, substitution of nucleotides) within individual genes, leading to a change in the quantity or quality of the corresponding protein products. Substitutions of the bases lead to the appearance of three types of mutant codons:

– with a changed meaning (missense mutations),

– with unchanged meaning (neutral mutations),

– meaningless (nonsense mutations).

As a result of the missense mutation in the polypeptide encoded by this gene, one amino acid is replaced by another; therefore, the phenotypic manifestation of the mutation depends on the functional significance of the affected domain. So, the replacement of amino acids in the active centers of proteins can be accompanied by a complete loss of their functional activity. For example, the missense mutation in the 553rd codon of the FAC gene, which leads to the replacement of leucine with proline, makes the product of this gene incapable of complementing a functional defect in the cells of Fanconi anemia cells.

Not every amino acid replacement will affect the functional activity of the protein, and, Detection methods for chromosomal rearrangements as a result, the mutation that has occurred may remain undetected. This explains the fact that there is a mismatch in the frequency of mutations in a particular gene and the occurrence of mutants in it. In addition, due to the degeneracy of the genetic code, not every substitution of the base will lead to missense mutations. Perhaps, it will turn out to be neutral.

Substitution of nucleotides in the coding regions of genes, which are not accompanied by amino acid substitutions due to the degeneracy of the genetic code, leads to neutral mutations that do not significantly affect the function of the corresponding protein or its structure.

As a result of the nonsense mutation, the codon that determines any amino acid turns into one of the stop-codons that does not translate on the ribosomes (UAA UAG, UGA). The appearance of such a codon not at the end of the structural gene but inside it leads to premature termination of translation and termination of the polypeptide chain. Nonsense mutations have the greatest damaging effect since the proteins that are formed upon premature termination of translation are not capable of modification and are often not protected from the action of proteolytic enzymes and quickly degrade.

Insertions, moving or dropping out of individual bases or their short sequences within a gene, cause a reading frame shift. The nature of such mutations was studied by analyzing the amino acid sequence of T4 phage proteins

encoded by the wild-type e + gene and three different mutant genes that shift the reading frame.

Some single mutations turned out to be the result of simultaneous changes in several neighboring nucleotides. A single mutation with a frame shift is likely to result from the insertion of two adjacent nucleotides but not one. When mutations occur with a shift of the reading frame, all triplets below the duplication or deletion site in the course of reading change, and the likelihood of stop-codons and, accordingly, translation termination is increased.

From the point of the structural and functional organization of genes, the substitutions, insertions, loss, and movement of nucleotides occurring inside them can be grouped into the following:

1) mutations in the regulatory regions of genes that cause quantitative changes in the corresponding products in 5 'and 3'-untranslated regions of the genes and manifest phenotypically (clinically) depending on the threshold level of proteins,where their function is still preserved:

• mutations in the promoter region (for example, a regulatory element with the PuCPuCCC sequence and inside the TATA box in the p-globin gene) reduce the level of protein product synthesis;

• мутации в сайте полиаденилирования снижают уровень транскрипции (характерны для афроамериканцев, страдающих талассемией);

• mutations in the polyadenylation site reduce the level of transcription (typical for Afro-Americans with thalassemia);

2) mutations in the coding regions of genes:

• mutations in exons can lead to premature termination of protein synthesis (for example, in the case of thalassemia, when, as a result of mutations inside the exon of the hemoglobin gene, the protein chain is shortened and does not have activity);

• mutations in introns, which are capable of generating new splicing sites and competing with normal (initial) ones, in the end, replace them (for example, the occurrence of substitutions in the hemoglobin gene that slows splicing is known for both B0 and B +- thalassemia);

• mutations in splicing sites (at the junctions of exons and nitrons) that disrupt the processing of the primary RNA transcript and result in the translation of senseless proteins (for example, elongated by improper excision of introns or shortened by excision of exons).

3.4. The Methods of Detection of Chromosomal Rearrangements

Chromosomal rearrangements were first discovered in Drosophila using genetic analysis. In some crosses, the ratio of the number of descendants in different classes differed very much from the expected one, and this was explained by the presence of rearrangements in the parents' chromosomes.

Deletions, duplications and translocations were discovered by K. Bridges in 1916, 1919 and 1923, respectively. The first inversion was described by Alfred Stertevant in 1921, comparing the order of genes in chromosome 3 in *D. melanogaster* and *D. simulans*.

The first cytological observations of chromosomal rearrangements were made on the polytene chromosomes of the Drosophila salivary glands. Only after some time, chromosomal rearrangements were shown on mitotic chromosomes.

Cytologically, chromosomal rearrangements can also be detected in the prophase of the first division of meiosis at the pachytene stage due to the synapse of homologous chromosome regions. A similar analysis was first performed by Barbara McClintock in 1930 when studying translocation in maize [10; 11].

In medical genetics, chromosomal rearrangements are identified and analyzed using cytogenetic methods. Most often, analysis of chromosomal rearrangements is carried out cytologically at the metaphase stage. The most common and affordable cytogenetic method is the classical cytogenetic analysis based on the frequency of dicentric chromosomes and the method of differential G-staining of chromosomes (G-banding).

Since the late 1980s, chromosome rearrangements have been detected using fluorescence hybridization *in situ* using DNA probes for individual chromosomes or chromosome loci, which significantly facilitates research and allows (as in the case of differential staining of chromosomes) to take into account the frequency of stable translocations, which makes it possible to evaluate the effect of radiation exposure in a remote time period.

One of the most accurate methods for detecting small duplications and deletions at present is the method of comparative genomic hybridization on the preparations of metaphase chromosomes or DNA microarrays.

Duplications and deletions can also be detected with genome-wide SNP genotyping. It should be noted that these two methods do not allow to detect the balanced chromosomal rearrangements and, unlike other cytogenetic methods, do not make the analysis of chromosomal aberrations at the level of a single cell possible; that is, they are insensitive to cases of mosaicism.

Studying the features of the frequency distribution of radiation-induced chromosome abnormalities has allowed us to form a new direction in assessing the dose effects of radiation effects, which is called biological dosimetry.

The fundamental difference between biological and physical dosimetry is as follows:

– physical dosimetry is based on the characteristics of radiation and, based on models, gives an average population dose;

– biological dosimetry is based on an analysis of the consequences (effects) of radiation exposure in the body, i.e. represents an integral assessment of the damaging effect of the radiation factor, which is conventionally expressed in physical units.

Thus, the biodosimetry approach is initially individualized since the effect of radiation exposure is mediated by specific characteristics of the body, including individual radiosensitivity.

A different approach suggests the possibility of the difference in the results of physical and biological dosimetry for the same individual. However, at the population level in a sufficiently large random population, the results of both methodological approaches should be fairly close.

In the biomedical aspect, the biodosimetry approach has clear advantages over physical dosimetry, i. e. the ability to evaluate the biological effect of radiation taking into account the characteristics of a particular individual at the time of exposure, which is especially important when determining adequate treatment methods and prognostic assessment of possible consequences.

At the same time, the methods of biological indication and biological dosimetry are quite complex and time-consuming, which creates great difficulties for using them at the population level. In this situation, physical dosimetry is clearly more effective.

Thus, physical and biological dosimetries are complementary methodological approaches that provide the most reliable result for complex use.

In the biological assessment of dose loads, it is usual to distinguish biological dosimetry and biological indication.

The tasks of biological dosimetry are solved by the methods, the ultimate goal of which is the result that determines the specific value of the additional dose and expressed (rather conditionally) in physical units by comparing the magnitude of the effect with the standard dose-response curve – the effect obtained, as a rule, in the experiment.

The main task of biological indication is to establish the fact of radiation exposure and to determine its severity according to the principle of "more – less".

As a rule, the methods of biological indication are simple and less labor-consuming in comparison with the methods of biological dosimetry, which makes it possible to use them for the purposes of pre-screening of large populations.

At the same time, there seems to be no strict difference between these concepts since the same methodological approaches under various conditions can be used both for bio dosimetry and for bioindication. Thus, classical cytogenetic analysis, without the use of additional methodological and mathematical techniques, is a very reliable source of biological dose for a rather short period after irradiation; later its data can be used only for biological indication.

To date, a whole range of bio-dosimetry (bioindication) methods has been developed (fig. 23).

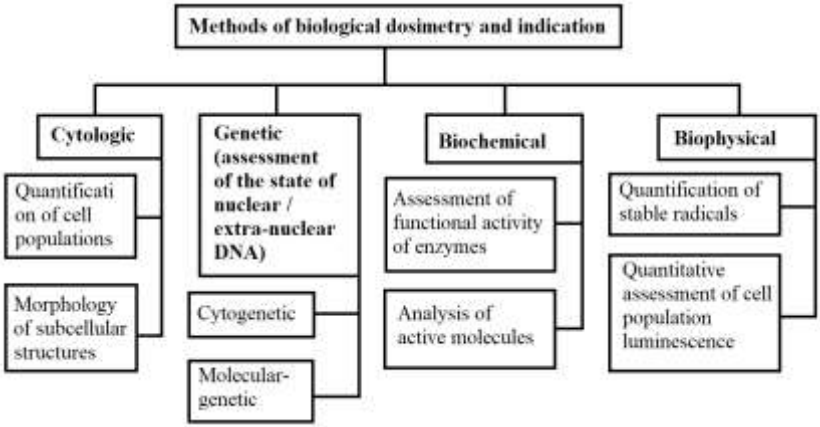


Fig. 23. Classification scheme of the main methodological approaches of bioindication and / or biodosimetry

3.5. DNA Repair

The first experiments on the mutagenic effect of ionizing radiation were performed on lower fungi and were carried out by G. N. Nadson and G.F. Filippov in the 30s of the XX century. Then, a series of works in which the biological effect of ionizing radiation at other experimental objects was checked followed. Drosophila (G. Müller, 1927), corn and barley (L. Stadler, 1928) were used for them.

By the mid-1960s the universality of the mutagenic effect of ionizing radiation has been proven for a large number of species belonging to any of the biological kingdoms.

From the point of physics and chemistry, in order for a mutation to occur, a consistent implementation of the following steps is necessary:

1) direct interaction of the energy of ionizing radiation with the substance of the cell (an event associated with the release of energy of ionizing radiation within the cell). At this stage, the ionization of molecules occurs as well as the formation of free electrons and unstable ions. If an event is realized in the immediate vicinity of DNA (in the nucleus), this event directly affects its structure;

2) ion recombination occurs, which leads to the formation of electronically excited molecules. These molecules are further decomposed into free radicals due to the instability of their state (indirect effect).

3.5.1. Radiation Induced DNA Damage

Radiation-induced DNA damage can be direct or indirect. Direct damage leads to the occurrence of a positively charged radical, an electron, and an electronically excited DNA molecule. With an indirect type of DNA damage,

radiolysis of water occurs, which, depending on the type of cell and the stage of its growth and development, can make up to 90 % of the cell mass. As a result of ionization, water molecules can form:

1) free radicals of hydroxyl (HO*),

2) hydrogen (H*)

3) and hydrated electron (e_{hydr}).

These radicals can subsequently interact with an excited water molecule and tissue oxygen, additionally forming reactive oxygen species (ROS):

1) hydrogen peroxide (H_20_2),

2) hydroperoxide radical ($HO*_2$),

3) superoxide ($0*_2$) and

4) atomic oxygen (0*).

ROS can act as inducers of cellular enzymes of antioxidant defense such as superoxide dismutase, catalase and glutathione peroxidase.

The main danger to the DNA molecule is the highly reactive hydroxyl radical HO*, which can induce up to 70 % of all damage that occurs in the DNA.

In the process of normal cellular metabolism, active forms of oxygen and substances that potentially have a toxic effect are also formed, which may ultimately make it difficult to determine the genetic changes that have arisen precisely due to radiation-induced ROS and cytotoxins, additionally formed under the influence of ionizing radiation in small doses. Therefore, accurately predicting the degree of dose-response dependence is a very difficult task.

On average, the number of endogenous double-stranded DNA breaks in a cell, which occurs per day, is 103 times higher than similar disorders induced by the natural background radiation. But under the chronic effects of additional doses of ionizing radiation with a low power line, the probability of the occurrence of radiation-induced double-stranded breaks relative to other types of DNA changes is 104 times higher than those induced endogenously. Unlike ROS, which are normally formed in the cell, the radicals that occur along the radiation track are locally clustered in high concentrations. For this reason, they are also capable of causing clustered changes in macromolecules. In this case, densely ionizing radiation (for example, α-particles) causes more complex and heavier reparable disturbances than rare-ionizing radiation (hard ultraviolet, X-ray and γ-rays).

Such local clusters of DNA damage can consist of several breaks or groups of modified bases located at a close distance from each other. In this case, the repair enzymes are no longer able to reliably cope with their task. It also happens that single-stranded breaks are located on opposite DNA strands and can easily become double-stranded during repair, and the restoration of several closely spaced double-stranded breaks can be carried out in violation of the nucleotide order (“sticky ends” reunite without taking into account their affiliation according to the principle “cross-links which is closer").

However, there are mechanisms in the cells both to prevent the occurrence and to repair DNA damage. For example, free radicals can be inactivated when they are captured by so-called molecular traps. Interceptors of free radicals include ascorbate, tocopherol, and other substances.

As a result of oxidative damage to DNA the following can be formed:

1) modified bases;

2) sites (places) with dropped nucleotides of one of the complementary DNA strands;

3) destruction of deoxyribose in nucleotides;

4) the appearance of single and double strand DNA breaks;

5) DNA / protein crosslinking, which play an essential role in reading genetic information and in DNA replication.

There are several repair mechanisms in the cell, and each corrects a certain type of structural DNA damage. At the same time, in mammalian cells there is an enzymatic cascade that ensures the elimination of modified oxidized nitrogen bases.

In the process of free radical DNA damage, more than 100 types of oxidized bases are formed; the most common is 8-oxo-2'-deoxyguanosine (8-oxoG) (fig. 24).

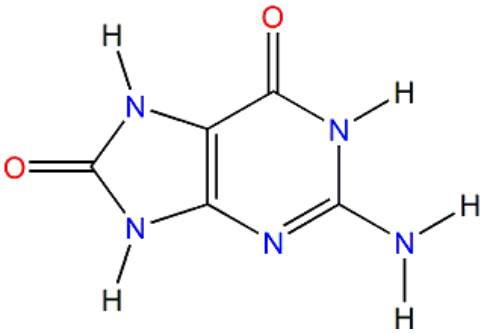


Fig. 24. Structural form of 8-oxo-2'-de-oxyguanosine

Usually, in case the oxidized bases are present in the DNA, the cell uses excision repair of the bases as the main mechanism for the restoration of the original DNA sequence, using a native double-stranded DNA strand as a matrix. If excision repair is still not effective enough, the changes in the DNA are copied during replication. This leads to the formation of a mutant genotype and may contribute to carcinogenesis.

In case it is impossible to complete the repair before replication in the cell begins if it plays a significant role in the body and is important for maintaining its viability, the mechanism of tolerance to DNA damage is activated, which avoids stopping replication. In this circamstances, replication through unreduced sites occurs using special DNA polymerases that work inaccurately

enough to skip the defective site. This process is called "DNA synthesis on a damaged matrix."

If free radicals interact with deoxyribose, the break of the sugar-phosphate of DNA backbone can occur, resulting in the formation of single and / or double-strand breaks. If single-strand breaks are localized close to each other, it can easily turn into double-strand breaks, which are the most dangerous for the cell; otherwise, repair systems easily repair the damage due to the safety of the second DNA strand.

It was shown that double-strand breaks occur when exposed to x-rays even in very small doses – about 1 mGy. An irradiation dose of 1 mGy results in averagely of only one track per nucleus, which induces double-stranded breaks in about 3 % of irradiated cells. However, due to the fact that under small impacts, non-homologous repair is not fully activated, double-stranded breaks caused by such doses are extremely dangerous. In the range of radiation doses from 0.01 to 1 Gy, up to 30 double-stranded DNA breaks per cell arise.

Double-strand breaks are the primary molecular events leading to cell death, and their number, as a rule, correlates with the severity of the cytotoxic effect of. The wrong repair of double-strand breaks can lead to chromosomal exchanges, and as a result, unstable dicentric chromosomes or acentric chromosome fragments are formed, leading mainly to a decrease in the viability of the host cell, as well as to the formation of balanced translocations, the main threat of which is associated with the subsequent possible malignancy of the cell carriers due to the launch of the oncogenic cascade of genes due to the redistribution of genetic material (the so-called “position effect").

To eliminate double-stranded DNA breaks in the cell, there are mechanisms of homologous recombination and non-homologous reunion of broken ends. The choice of a particular repair method by a cell also depends on the stage of the cell cycle. Non-homologous reunion occurs mainly at stage G_1, homologous recombination occurs at stages S and G_2.

Since homologous recombination uses DNA fragments with complete homology for several hundred nucleotides, its distinguishing feature is its error-free character, i. e., the restoration of the original DNA sequence at the site of disruption is quite effective. Non-homologous reunification, on the contrary, completely ignores the principle of homology and therefore can itself be a source of errors, which is manifested in changes in the nucleotide sequence at the repair site and, as a result, leads to the fixing of a mutational event in the genome or stimulates the expression of epigenomic variability. Usually, this mechanism leads to the formation of microdeletions or insertions in the gap site, less often, to large chromosomal rearrangements. The mechanism of the formation of chromosomal aberrations in the case of improper restoration of double-strand breaks is not yet fully understood, but its danger for maintaining genetic uniformity is already obvious.

Summing up the information presented above, it can be stated that the final product of radiation mutagenesis is due to the opposition of two processes: DNA damage and repair (fig. 25).

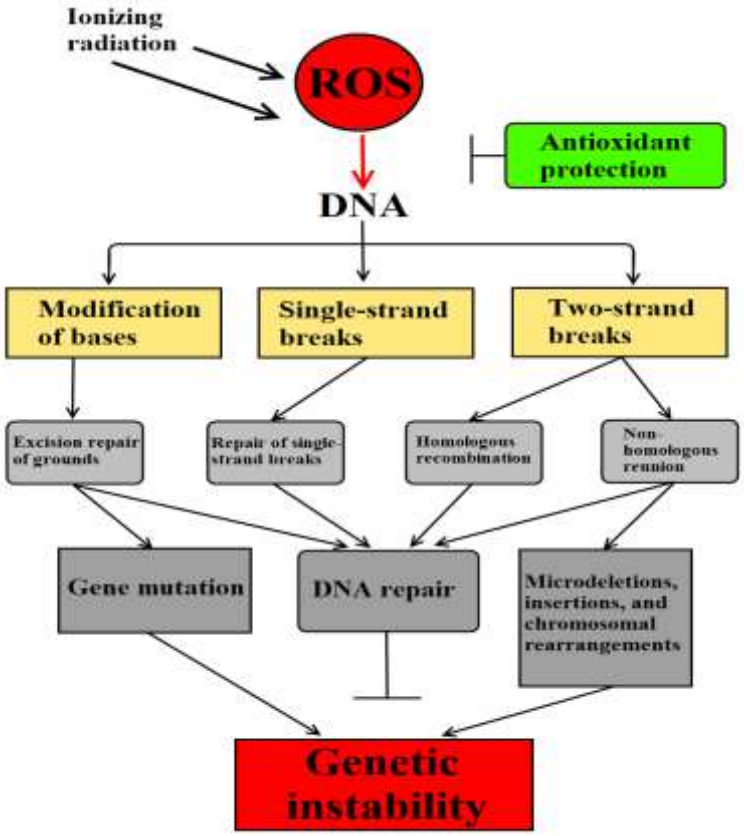


Fig. 25. Schematic Diagram of The Processes of Radiation-Induced Damage and DNA Repair (IR is Ionizing Radiation, ROS is Reactive Oxygen Species)

Although ionizing radiation does not have a specific mutagenic effect, the nature of the induced disturbances makes them difficult to restore in a cascade of repair enzymes. Apparently, these particular disorders of the genetic material lead to the development of delayed genetic instability and tumor transformation of cells. It should also be noted that a violation of any of the repair mechanisms leads to an increase in the radiosensitivity of a particular cell and the organism as a whole and leads to the formation of genetic instability.

However, irradiation does not always lead to the induction of mutations in germ cells of directly irradiated individuals. In some cases, such epigenetic

changes are induced that lead to an increase in the level of mutagenesis and can occur in an offspring of irradiated organisms.

3.5.2. Postreplicative DNA Repair

One of the special forms of repair is its post-replication form. It occurs when so much damage occurs in DNA that during excision repair the cell does not have time to completely eliminate them, and also if genes that control the synthesis of enzymes involved in excision repair are damaged. As a result, after replication of such DNA in the daughter chain, “gaps” are formed at the site of injuries in the mother thread.

The pyrimidine dimer delays the progress of DNA polymerase during replication. It stops. Continued replication occurs with the Okazaki fragment.

Reparation proceeds by recombination (exchange of fragments) between two newly formed double DNA helices. An example of such post-replicative repair is the restoration of the normal DNA structure when thymine dimers (T-T) occur when they do not spontaneously resolve under the influence of visible light (light repair) or during pre-replicative excision repair.

Covalent bonds arising between adjacent thymine residues make them incapable of binding to complementary nucleotides. As a result, the breaks (gaps) recognized by the repair enzymes appear in the newly synthesized DNA chain. The integrity of the new polynucleotide chain of one of the daughter DNA is restored through recombination of the other daughter DNA with the corresponding normal mother chain. The break formed in the mother chain is then filled by synthesis on the polynucleotide chain complementary to it. The manifestation of such post-replicative repair, carried out by recombination between the chains of two daughter DNA molecules, can be considered as the not frequently observed exchange of material between sister chromatids.

3.5.3. SOS – Repair

The existence of this system was first postulated by M. Radman in 1974. He gave the name to this mechanism, including the international distress signal "SOS" in it. Indeed, this system turns on when the damage to the DNA becomes so bad that it threatens the life of the cell. In this case, the induction of different genes that are involved in various cellular processes associated with DNA repair occurs. The inclusion of certain genes, determined by the number of lesions in the DNA, leads to cellular responses of different significance (starting with the standard repair of damaged nucleotides and ending with the suppression of cell division).

The most studied is the SOS repair in E. coli, the main participants of which are proteins encoded by the Rec A and Lex A genes. The first of these is the multifunctional Rec A protein involved in DNA recombination. The

second (Lex A protein) is a transcriptional repressor of a large group of genes for DNA repair. When it is inhibited or destroyed, SOS repair is activated. The binding of Rec A to Lex A leads to the splitiing of the latter and, accordingly, to the activation of transcription of repair genes. The SOS repair system was detected not only in bacteria but also in animals and humans.

Gens	Consequences of gene activation
uvr A, B, C, D	Repair of damage to the secondary structure of DNA
Rec A	Post-replicative repair, SOS - system inductions
lex A	Turning off the SOS-system
rec N, ruv	Repair of double-strand breaks
ssb	Providing recombination repair
umu C, D	Mutagenesis caused by changes in the properties of DNA polymerase
sul A	Suppression of cell pressure

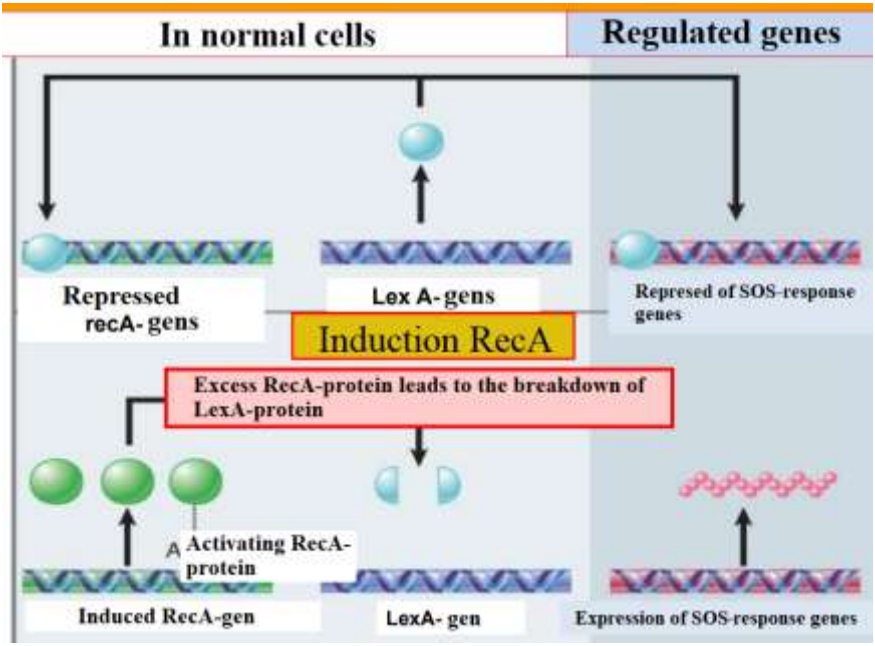


Fig. 26. SOS - DNA Repair

The onset of the SOS response is determined by the interaction of the RecA protein with the LexA repressor protein (fig. 26). The cell response to the damaging effect starts at the activation of the protease activity of the RecA protein.

The activating signal can be the presence of a single chain region at the damage site. When activated, RecA protease cuts the LexA repressor protein. The LexA protein in intact cells functions as a repressor of many operons whose genes are responsible for various repair functions.

Proteolytic cutting of a repressor (LexA protein) induces all of these operons. Currently, about 40 genes that participate in the SOS response as a result of activation of their products have been identified. All of these genes are inducible.

The LexA protein has been found to repress target genes by binding to a DNA sequence of about 20 base pairs, called the SOS block.

3.5.4. Mismatch Repair (The Repair of Mismatched Nucleotides)

During DNA replication, pairing errors occur when, instead of complementary pairs of A-T, G-C, non-complementary pairs are formed. Incorrect pairung affects only the daughter chain. The mismatch repair system must find the daughter chain and replace the non-complementary nucleotides.

Special methylase enzymes attach methyl groups to adenines in the GATC sequence on the mother chain, and it becomes methylated in contrast to the unmethylated daughter. In E. coli the products of 4 genes are responsible for mismatch repair: mut S, mut L, mut H, and mut U.

Violation of the repair mechanisms can lead to the formation of a malignant cell phenotype and, as a result, to cancer.

Part 4. RADIATION INDUCED CELL CYCLE EFFECTS

4.1. The Mechanisms of Cell Division

As the cellular theory postulates, the increase in the number of cells occurs notably due to the division of the parental cell, which previously has doubled its genetic material.

This is the main event in the life of the cell as it is, namely, the completion of reproduction of its own kind. Cell division is a non-random process but strictly genetically determined.

According to their ability to divide, all cells of an adult organism are divided into 3 types:

1. Mitotic cells, i. e. constantly dividing cells (cells of the basal layer of the epithelium, hematopoietic cells of the initial stages of maturation, spermatogonia, and etc.)

2. Conditionally postmitotic cells. These are non-dividing cells that retain the ability to divide under the action of certain stimuli. Most often, the division resumes during the regeneration of the corresponding organ or tissue (liver cells, stem cells of bone, muscle and other tissues.)

3. Postmitotic cells are non-dividing cells that have finally lost the ability to divide (the cells of all layers of the epidermis, except for the basal one; nerve cells; the cells of the heart muscle; and skeletal muscle fibers).

The life span of a cell from one division to another is called a **mitotic (cell) cycle** (fig. 27).

The life cycle of a mitotically dividing cell consists of four stages.

S-period (synthetic): Replication (doubling) of DNA and chromosomal proteins occurs in the nucleus. The DNA content in the nuclei of somatic cells (body cells) is 2 times higher than in the nuclei of mature germ cells. In the synthetic period, the amount of DNA gradually increases from 2c to 4c. The number of chromosomes does not change (2n), but each contains 2 sister chromatids.

G2-period (postsynthetic or premitotic). It is not very long and includes the synthesis of a number of substances that are necessary for the mitosis. In particular, at this time, tubulin, which is microtubule protein, is synthsized. It is necessary for the formation of the spindle. The DNA content in this period is 4c.

Mitosis. At this stage the division occurs, leading to the formation of two diploid cells (2n2c).

G1-period (presynthetic or postmitotic). During this period, the content of cytoplasmic proteins is restored; as a result, cell growth occurs (up to the size of the parental one). In addition, there is a synthesis of proteins that is necessary for replication. The DNA content in the cell is 2n. It is during this period when the decision on the entry of cells into the next cell cycle or on the termination of divisions is made. Stages G1, S, G2 together make up the **interphase** (fig. 28).

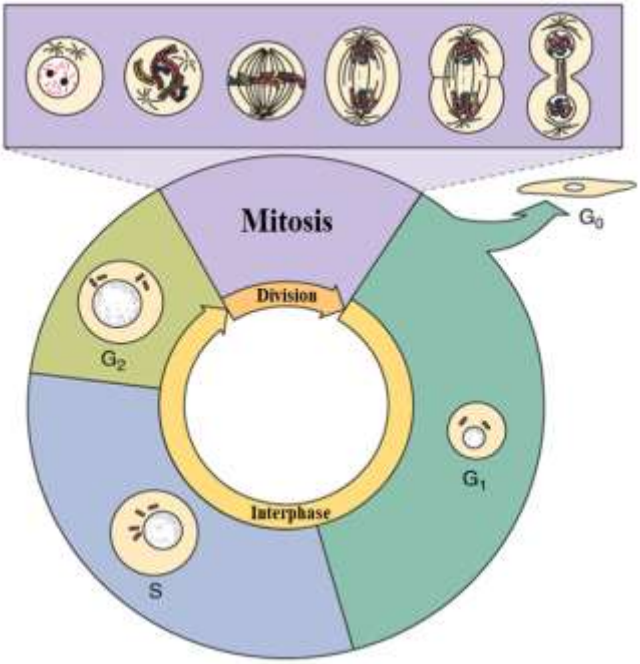


Fig. 27. The Scheme of the Cell Cycle And Its Stages

During the mitotic cycle, the change of stages occurs in a strictly ordered manner.

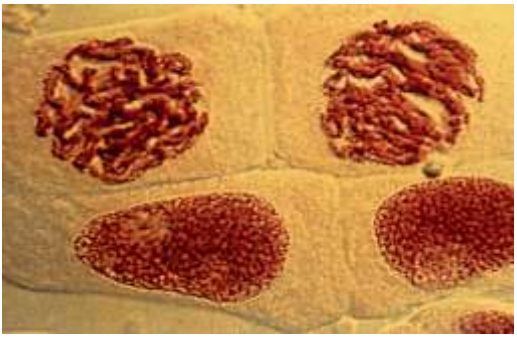


Fig. 28. Interphase (1) and Metaphase (2) of the Cell Cycle

Dividing cells can continue to divide or temporarily stop dividing and exit the cycle. These cells are said to have entered the **G_0-period**. In addition, depending on a number of circumstances, the cell may enter the process of *differentiation*, activate the self-destruction mechanism **(apoptosis)**, or undergo **blast transformation**, i.e. turn into a tumor cell

Mitosis (from the Greek word *Mitos,* meaning a *thread*) is indirect division, the main way of dividing eukaryotic cells. The biological meaning of mitosis lies in the strictly identical distribution of reduplicated chromosomes between daughter cells, which ensures the formation of genetically equivalent cells and maintain the continuity in a number of generations (fig. 29).

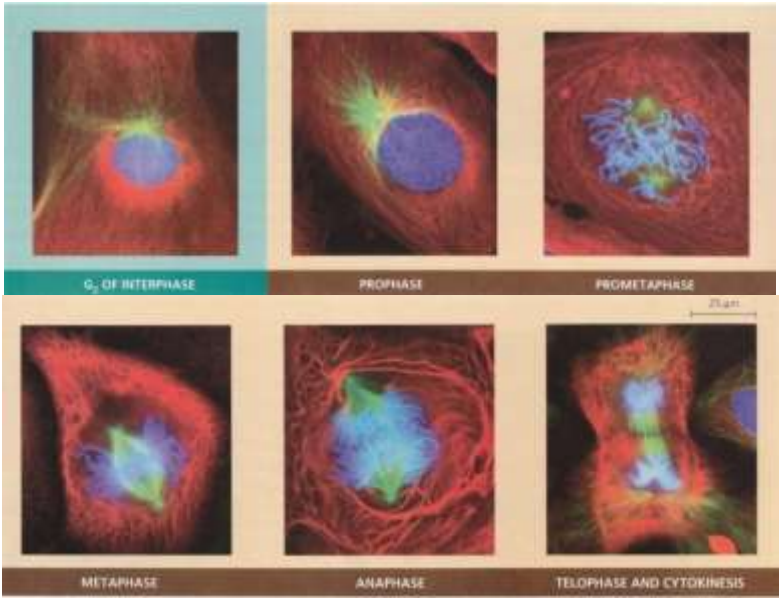


Fig. 29. Mitosis Stages

In cells that have entered the division cycle, the phase of the mitosis, indirect division, takes a relatively short time, about 0.1 time of the cell cycle.

Mitosis consists of four main phases: **prophase, metaphase, anaphase, and telophase**. Mitosis is a continuous process, so the boundaries between the phases are difficult to establish.

Prophase. It includes the cells from the G2-period of interphase; after replication in the S-period, they contain twice the amount of DNA (4c) compared to the original cell in the G1-period, which corresponds to that of a tetraploid cell (fig. 30).

At the beginning of the prophase, thin filaments, prophase chromosomes, begin to be detected in the nucleus. This is the result of the process of condensation of chromosomes, which coincides with a decrease in their transcriptional activity. As the prophase passes, the chromosomes shorten and thicken. The number of chromosomes corresponds to a diploid number, but the nucleus of prophase cells is tetraploid. Each prophase chromosome consists of two mutually helical chromatids. Sister chromatids are linked together

using **cohesin** proteins. Thus, the number of chromatids (4n) in the prophase exactly corresponds to the amount of DNA (4c).

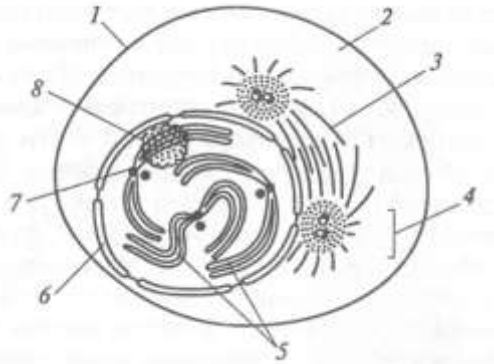


Fig. 30. Mitosis prophase: 1 – plasma membrane; 2 – cytoplasm; 3 – forming spindle; 4 – the pole of the spindle; 5 – condensing chromosomes; 6 – nuclear shell; 7 – centromere; 8 – a decaying nucleolus

As a result of condensation, RNA synthesis on the chromosomes completely stops. Due to the inactivation of chromosomal genes, **the nucleoli disappear and the nuclear membrane is gradually destroyed**. A thin plate (**nuclear lamina**) of protein nature is connected with the inner surface of the inner membrane of the nucleus. This plate is formed by intermediate filaments. The nuclear lamina is destroyed by the depolymerization of intermediate filaments, and the nuclear membrane itself breaks up into small bubbles.

A similar process takes place in the cytoplasm: the amount of granular endoplasmic reticulum decreases, it breaks up into short tanks and vacuoles, and the number of ribosomes on its membranes drops sharply. The number of polysomes is significantly reduced both on the membranes and in the hyaloplasm, which determines the overall decrease in protein synthesis in dividing cells. The Golgi apparatus also breaks up into small bubbles.

In prophase, spindle formation also occurs. The spindle of division is formed with the participation of centrioles and without them. Without centrioles, a spindle of division forms in the cells of higher plants and some protozoa. In some other simplest and lower fungi, spindle formation may occur inside the nucleus; in this case, the nuclear membrane does not collapse during mitosis (closed mitosis). In animal cells, the main role in the formation of the fission spindle is assigned to centrioles. The centrioles diverge to opposite ends of the cell and form the poles of the fission spindle. Microtubules of the fission spindle depart from centrioles to centromeric sections of chromosomes.

Metaphase often takes about a third of the time of the entire mitosis. During the metaphase, polymerization of the protein of tubulin completes the formation of the fission spindle, and the most spiralized chromosomes line up in the equatorial plane of the cell and form the so-called **metaphase plate (maternal star).**

The fission spindle includes three types of microtubules*: kinetochoric* (which bind each chromatid to one of the centrioles. Kinetochor is a special protein complex in the centromere region), *polar* (which go from one of the diplosomes to the center of the spindle where they overlap with microtubules from the other pole), and *astral* (which are directed from the centromere to the cell surface).

Gradually, on the chromosomes, cohesin complexes between sister chromatids are destroyed. Towards the end of the metaphase, the process of separation of sister chromatids from each other is completed. Their arms lie parallel to each other and are connected only in the centromere zone.

Anaphase begins suddenly. This is the shortest stage of mitosis (fig. 31).

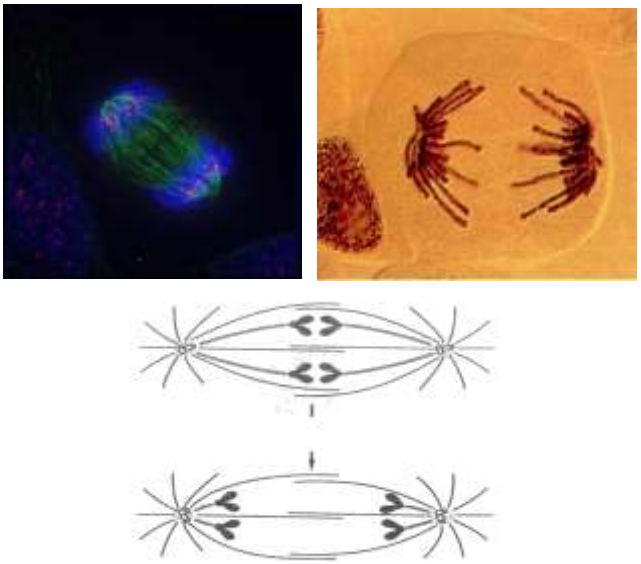


Fig. 31. The Anaphase of mitosis:
1 – spreading force arises between the microtubules from the opposite poles, pushing them aside; 2 – pulling force acts directly on the poles, pulling them apart

Chromosomes lose centromeric ligaments and synchronously begin to move away from each other towards the opposite poles of the cell. At the same time, they are oriented of the centromere sites towards the corresponding pole, and telomeric - towards the equator of the cell. The movement of chromosomes is due to the reduction of microtubules. The kinetochore micro-

tubules are shortened (disassembled) and the polar microtubules become elongated (which leads to the divergence of the poles themselves). In addition, **translocator proteins** are involved in the process. They move the chromosomes along kinetochore microtubules and move the polar microtubules apart from each other.

Telophase begins with the stopping of chromosomes and ends with the beginning of the reconstruction of a new interphase nucleus and the division of the original cell into two daughter cells (cytokinesis) (fig. 32).

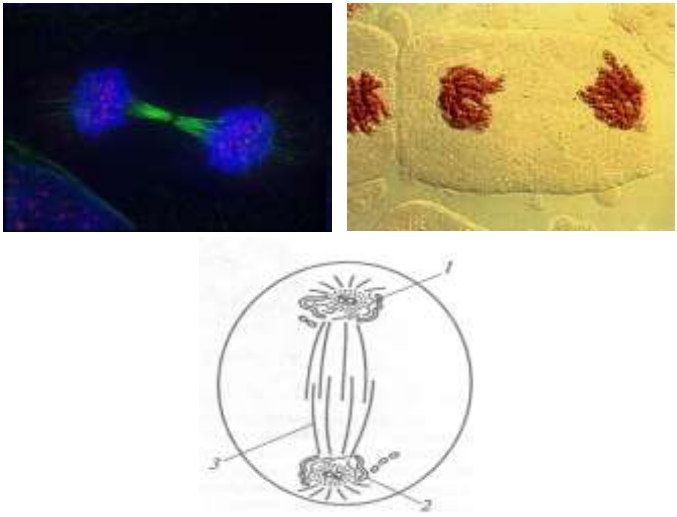
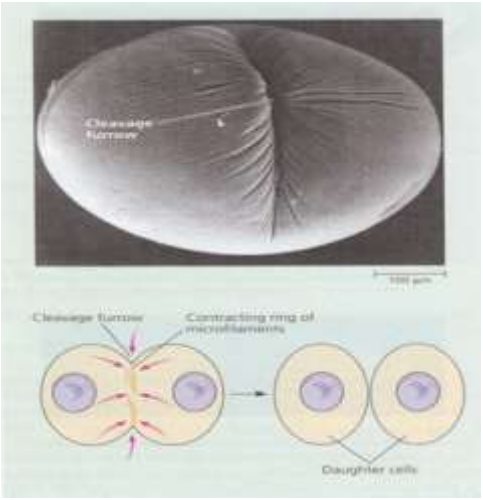


Fig. 32. Telophase of the Mitosis:
1 – decondensable chromatids; 2 – the resulting nuclear shell; 3 – pole microtubule

Vesicles formed in the prophase from the destroyed nuclear membranes are associated with chromosomes. Complexes of nuclear pores are re-embedded in their walls. Through the pores, proteins penetrate into the vesicles, forming intermediate filaments, which in turn form a nuclear lamina. Due to this, the vesicles merge. Initially, they form a double shell **around each chromosome**. It turns out to be mini-nuclei **(karyomers).** Later, the karyomers, which are associated with one diplosome, merge. Chromosomes gradually decondens, and nucleoli begin to form.

In the telophase, the mitotic division spindle is destroyed.

To separate the cell along the equator, an actomyosin ring is formed, which gradually contracts, pulling together the plasmolemma and forming a constriction of an ever-decreasing diameter (fig. 33, 34).



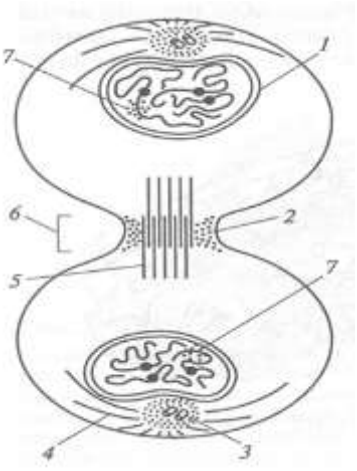
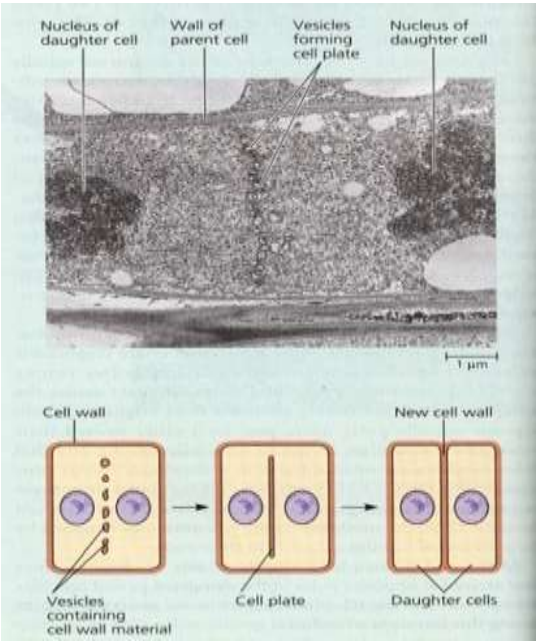


Fig. 33. The Cytokinesis of an Animal Cell: 1 – nuclear envelope around decondensable chromosomes; 2 – contractile ring forming a division groove; 3 – centrioles; 4 – interphase microtubules; 5 – the remains of the pole microtubules; 6 – residual corpuscle (the region of overlapping of microtubules); 7 – newly formed nucleolus



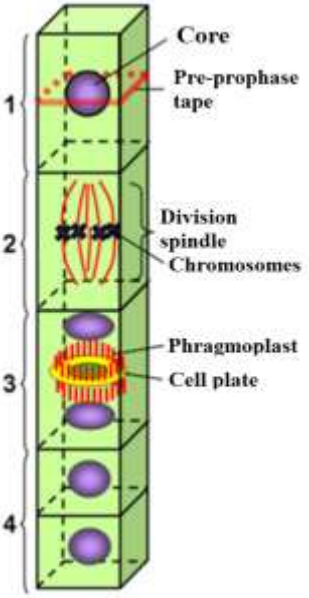


Fig. 34. Cytokinesis of a Plant Cell

4.2. Regulation of the Cell Cycle

A key role in the change of the phases of the cell cycle is played by special enzymes – *protein kinases*. This is the so-called *cyclin-dependent kinases* (Cdk). Kinase is a complex consisting of two polypeptides. One of them, CDK, binds ATP and contains an active center. However, it has kinase activity only when it is **bound to another polypeptide called cyclin** (fig. 35).

The T-loop changes its position, which provides access to the substrates. Reorientation of the side chains of certain amino acids also occurs, as a result of which the changes necessary for the transfer of phosphate groups are induced.

In most organisms, many cyclins act on each cell cycle; what is more, these different proteins appear and disappear at different stages. Moreover, depending on the name of the phase of the cell cycle during which they are present, cyclins are divided into three classes: Gl-cyclins, which are responsible for promoting the cycle from phase Gl to S-phase; S-phase cyclins, which are necessary to initiate DNA replication; and M-phase or mitotic cyclins, which initiate mitosis. In many cells there is more than one representative of each class of cyclins; even simple eukaryotes such as S. cerevisiae yeast contain nine different cyclins. All nine interact with the same catalytic subunit. Since the catalytic subunit in each CDK complex is the same, cyclin subunits must not only activate the kinase subunit but also recognize proteins that need to be phosphorylated.

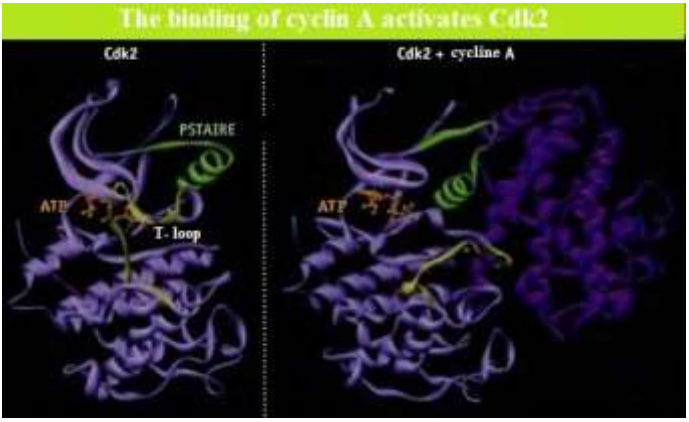


Fig. 35. The binding of cyclin to CDK causes conformational changes in it

In Metazoa, various classes of cyclins are usually denoted by letters. Cyclin D is associated with G_1, and cyclins A and E with S-phase. Cyclins A and B are associated with mitosis.

Along with many classes of cyclins, for the majority of multicellular eukaryotes, the presence of several representatives of each class (which indicate cyclin B_l, B_2, etc.) is characteristic. Thus, the total number of cyclins is quite

large. For example, the human genome encodes at least 12 cyclins that are involved in the regulation of the cell cycle. Why does a cell have so many cyclins? Along with many classes of cyclins, the presence of several representatives of each class (which indicate cyclin B_1, B_2, etc.) is characteristic of most multicellular eukaryotes. Some cyclins are found only in certain parts of the cell, for example, in the nucleus, or in the centrosome, while others are not present in all but only in some animal tissues. The level of cyclins can also be regulated in time, i. e., various cyclins that are typical to any single phase of the cycle can, depending on time, either appear or disappear. The meaning of such regulation is not yet clear, but it is possible that it is necessary for the cell cycle to correspond to the type of the cell. Thus, for large animals, which are characterized by the presence of many types of cells, a large number of cyclins is required.

Although various representatives of the cyclin family appear at different cycle times and in different parts of the cell, they have common molecular properties. In the primary structure, they have a sequence of approximately 150 amino acids called the cyclin domain. This sequence is the region through which cyclin binds to the catalytic subunit of CDK. Outside the cyclin domain, the primary sequence of cyclins varies significantly in composition although many cyclins contain a hydrophobic region involved in the recognition of the substrate. Despite the differences, there is significant functional redundancy among cyclins, and one cyclin can replace another but not always with the same result. Such redundancy was first detected in yeast and is now indicated for mouse cells. For example, in the absence of cyclin E, mice develop and live normally. It is, therefore, possible that other cyclins compensate for the loss of this cyclin, which is involved in the initiation of the S phase; however, they usually do not perform its functions.

Metazoa cells also contain several cyclin-dependent kinases. CDK1 kinase plays an important role in mitosis, while CDK2, CDK4, and CDK6 are involved in the early phases of the cell cycle.

Most kinases bind to one or two different cyclins, with each CDK having its own set of cyclins. Therefore, from time to time, there are many different CDK-cyclin complexes in the cell. Different combinations of CDK subunits and cyclins make it possible to achieve very fine regulation at certain moments of the cell life and at a certain place. This is related to both a single cell and a multicellular organism. It should also be mentioned that for each CDK-cyclin complex there is a certain set of substrates. The stages of the cell cycle in which various cyclins act are presented in fig. 36.

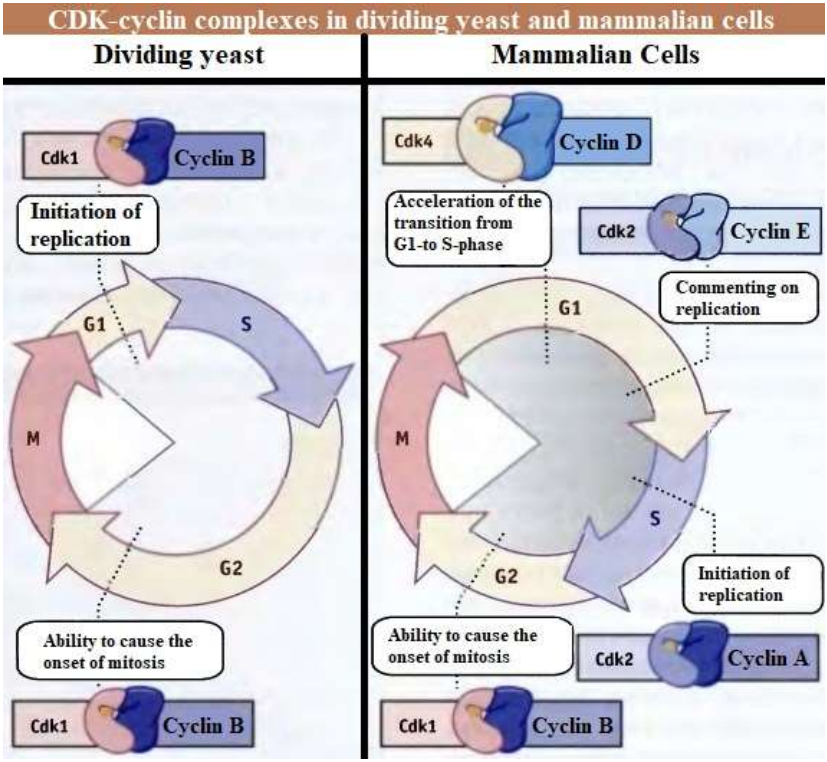


Fig. 36. CDK-cyclin complexes in yeast and mammalian cells

(In cells of dividing yeast, various transitions are controlled by a single CDK complex (Cdk1-cyclin B – left figure. In mammalian cells, different CDK-cyclin complexes perform the same tasks (the right figure).

Different protein kinases are active in different phases of the cell cycle. Some are active in the G_1 period, but others at the time of cell DNA replication, or in mitosis. During a relatively short period of activity, each kinase phosphorylates a large number of proteins, which leads either to their activation and to the launch of the main events of the cell cycle or to inhibition of their activity, in which the repetition of the previous cycle event is prevented. For example, the CDK, which initiates mitosis, phosphorylates the proteins of the lamina, with the destruction of the nuclear membrane. A large set of other proteins that regulate the assembly of the mitotic spindle is also phosphorylated. Each of them phosphorylates certain proteins involved in the corresponding phase of the cycle; they change their configuration and, thus, activate or inactivate them. The Cdk molecule itself is not active; to activate it, the binding of a special protein, cyclin (C) is required. The name "*cyclins*" is due to a cyclical change in the concentration of a given protein during the cell

cycle. During the interaction, the active *cyclin-Cdk complex* is formed (fig. 37).

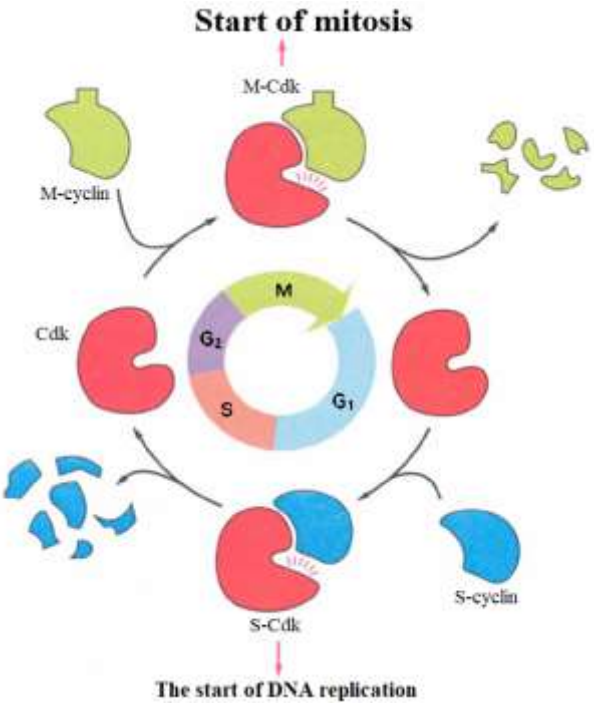


Fig. 37. General Scheme of Cell Cycle Regulation

There are several types of cyclins and several types of Cdk (different cyclins are indicated by Latin letters, and different Cdk – by Arabic numerals fig. 38).

For a cell to enter the mitotic cycle (MC), it must receive a mitogenic signal on the membrane, which must reach the nucleus. Mitogenic signal transmission begins with the activation of growth factors (proteins).

The cyclin-Cdk complex of the next stage of the cycle should provide:

1. Inactivation of the complex of the previous stage;
2. Stimulation of the events of its stage;
3. The formation or activation of the complex of the next stage.

The main checkpoints:

1. G_2 checkpoint – G_2 – M – entrance to mitosis.
2. M checkpoint (spindle checkpoint) – the control of the beginning of anaphase.
3. G_1 checpoint – the start of the mitotic cycle.
4. Postmitotic – "decision" of the fate of the cell.

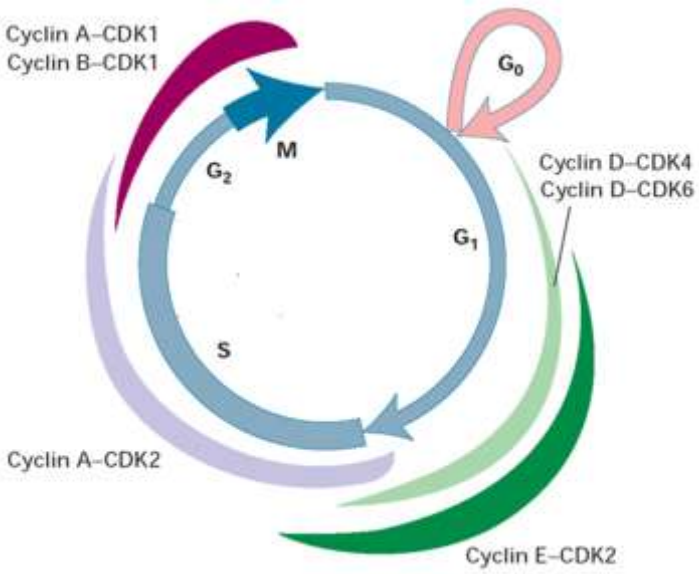


Fig. 38. Cyclins of Higher Eukaryotes

The complexes CD (1–3) -Cdk4 and 6 start the cycle. They function at postmitotic initial stage (G_1) and cause the corresponding intracellular events (cell growth and protein synthesis for replication).

For realization of mitotic division, an external stimulus is required, i.e., the action of specific growth factors (mitogens).

During the cycle, the cell carries out self-control of its own condition. For this, there are several checkpoints in the cycle (fig. 39).

1. The check point of the G_1-period. The cycle is stopped if double-stranded breaks in DNA are detected, chromosome segregation is incorrect, or the microtubule system is destroyed.

2. The check point of the S-period. The stop of the cycle is carried out in case of a lack of nucleotides in the cell.

3. The check point of the G_2-period. The cycle stops in case of an incomplete replication of any chromosome regions or large DNA damage that is left over from the previous period.

4. The check point of the metaphase of mitosis. The cycle is stopped in case of incorrect assembly of the spindle.

The main thing that is controlled is the cocndition of genetic material (fig. 40).

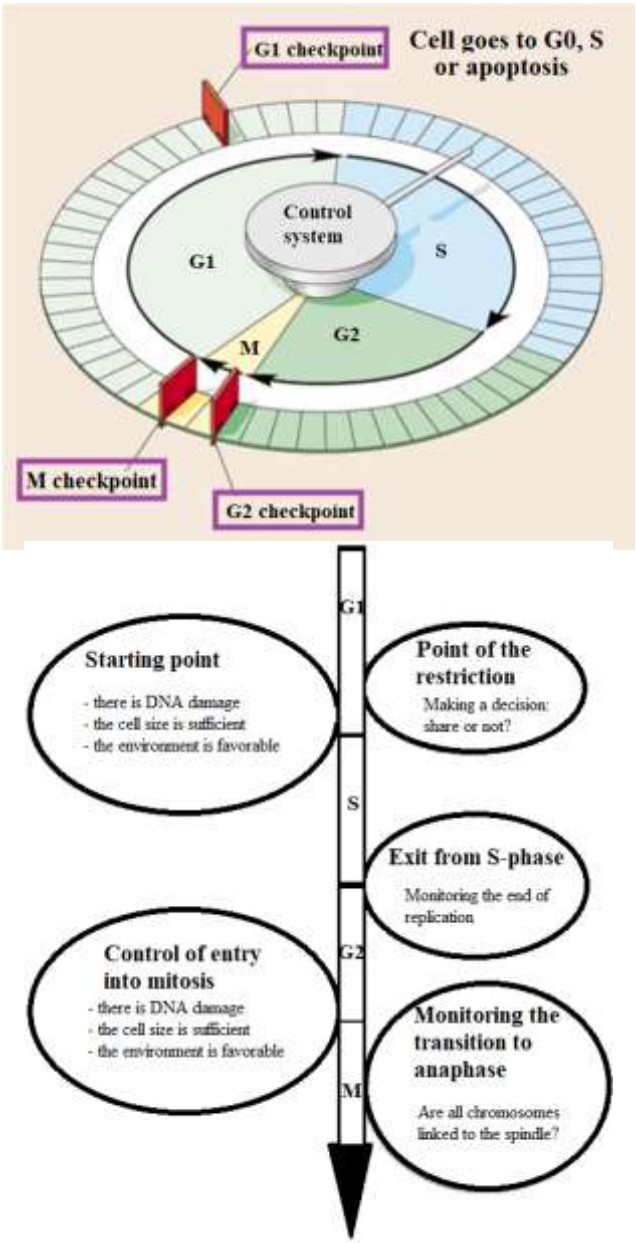


Fig. 39. Check Points of Cell Cycle

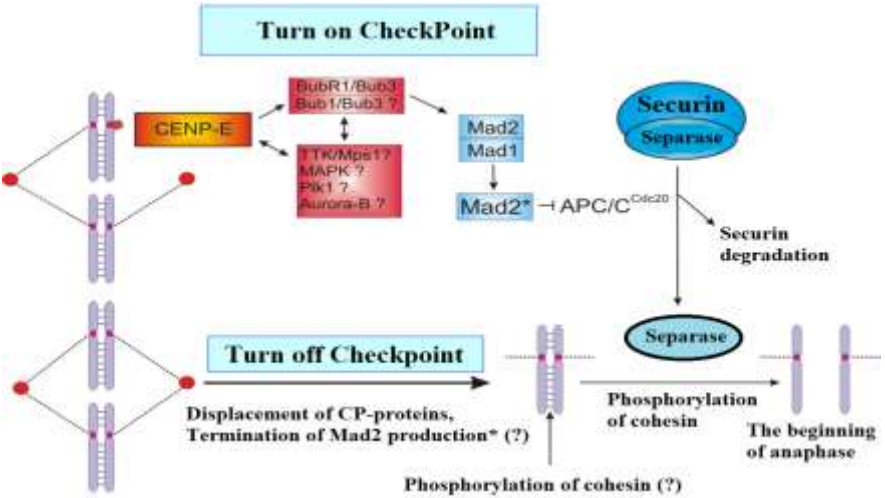


Fig. 40. Scheme of the Control of Cell Cycle

Depending on the results of the "check", one of the options for further actions is selected:

– Non-stop transition to the next phase of the cycle;

– Fairly long delay at the current stage to correct detected defects, if possible.

– Starting the mechanism of apoptosis (programmed cell death), if the violations are unimprovable.

For example, in the pre-mitotic period, the concentration of cyclin B increases in the cell. Cyclin B combines with the inactive form of cyclin B of the dependent protein kinase and activates it. Active protein kinase phosphorylates and, thereby, activates a number of enzymes and other proteins necessary for mitosis (in particular, proteins involved in the formation of the mitotic spindle and in the separation of chromosomes).

The double-stranded breaks are assumed to be recognized by a special protein kinase. In most cases of chromosomal damage, the p53 protein plays a central role in stopping the cycle. It is synthesized in the cell constantly, but will be destroyed very quickly. In the presence of chromosomal damage in the cell, DNA protein kinase phosphorylates p53, and it becomes active. Active p53 is a transcription factor for the gene of the protein p21, which is an inhibitor of all cyclin-Cdk complexes. For this reason, the cycle stops, in whatever period the cell is. If the damage to the chromosomes is large enough and their improvement is delayed, the long-lasting activity of p53 begins to stimulate, as a transcription factor, a group of genes that trigger apoptosis. As a result, a sophisticated self-destruction program begins in the cell. A defective cell breaks up into fragments and is phagocytosed by neighboring cells.

Despite the large number of protective mechanisms (repair enzymes, monitoring the processes of mitosis, apoptosis, and etc.), they are not hundred per cent. Under certain conditions, the formation of cells with a defective genotype is possible. An unbalanced set of chromosomes may appear in the cell (in case of chromosome non-disjunction due to violation of the fission spindle); polyploid cells may form (as a result of non-separation of the cytoplasm); in addition, various mutations may be present in the cell. In the case of modification of the genes that are responsible for the processes of cell division, the cell may lose control over the division and turn into a tumor. 120–150 human genes and a certain amount of viral genes can be related to oncogenesis.

Summarizing the above, we emphasize that the transition of cells to different stages of the cycle is regulated by CDK-cyclin complexes. Cyclins are the proteins; their level in the cell varies depending on the phases of the cycle. Thus, the specificity of the various phases of the cell cycle is determined by the activity of CDK, which is regulated by cyclins.

4.3. The Role of Intracellular Proteins in Cell Cycle Regulation

The check points in the cell cycle are necessary to determine the completion of each phase. The cell cycle stops in the case of:

1. DNA damage in the G_1 period.
2. Incomplete DNA replication in the S phase.
3. DNA damage in the G_2 period.
4. Violation of the connection of spindle division with chromosomes.

One of the check points in the cell cycle is mitosis, which does not develop into the anaphase if the spindle is assembled incorrectly and in the absence of the complete connections between microtubules and kinetochores.

DNA damage prevents cells from entering the S period or into mitosis. If this damage is not catastrophic and can be repaired by reparative DNA synthesis, the block of the cell cycle is removed, and the cycle reaches its completion.

If DNA damage is significant, in some way or another, the p53 protein is stabilized and accumulated; its concentration is normally very low due to its instability. The p53 protein is one of the transcription factors that stimulates the synthesis of the p21 protein, which is an inhibitor of the Cdk-cyclin complex. This leads to the fact that the cell cycle stops at the stage of G_1 or G_2. With a block in the G_1 period, a cell with DNA damage does not enter the S phase since this could lead to the appearance of mutant cells, among which there may be tumor cells. Blockade in the G_2 period also prevents the mitosis of the cells with DNA damage. Such cells, with a blocked cell cycle, subsequently die by apoptosis, which is the programmed cell death.

When mutations leading to loss of normal activity of the protein p53 genes (allelic exclusion), or when changes (mutations) also causing reduction of the functional activity of cell cycle blockade does not occur, the cells enter mitosis, which leads to the appearance of mutant cells, most of them is unviable, the other – gives rise to malignant cells.

The Bcl-2 gene family can also modulate cell cycle progression. Under suboptimal growth conditions, the Bcl-2 gene ensures the transition of cells to a resting state and delays entry into the cell cycle. This effect is genetically separated from the survival function because inhibition of the cell cycle but not the survival function is suppressed by the loop removal or tyrosine-28 Bcl-2 mutation. Inhibition can occur with a protein that can bind this region of Bcl-2, such as calcium neurin phosphatase.

T cells expressing Bcl-2 produce less IL-2, the cytokine required for progression to S phase, apparently due to a decrease in translocation to the core of the transcription factor NFAT. Translocation of NFAT requires the presence of concomitant migration of calcium neurin, which can sequestered on cytoplasmic membranes by Bcl-2. Whatever the mechanism, the cell cycle inhibition effect may have developed to reduce the oncogenic effects of Bcl-2.

p53 (p53 protein) is a transcription factor that regulates the cell cycle. p53 acts as a suppressor of malignant tumors. The protein got its name according to its molecular weight, 53 kDa. The real molecular weight of the protein is 43.7 kDa. The error in the initial determination of molecular weight is caused by the presence of many proline residues in p53, which slow down the movement of the protein in gel.

The human gene encoding the p53 protein is called *TP53* (italics indicate that it is the name of the gene but not the protein). This gene is located on chromosome 17 (17p13.1).

Gene localization in the genome of other organisms is the following

Mouse − chromosome 11.

Rat − chromosome 10.

Dog − chromosome 5.

Pig − chromosome 12.

The human p53 protein consists of 393 amino acid residues and has 5 domains. They are

– a transcription-activation domain (TAD), amino acid residues 1-42.

− a proline-rich domain that is important for the apoptotic activity of p53, amino acid residues 80–94.

− DNA-binding domain („zinc finger"), amino acid residues 100-300.

− the domain that is responsible for oligomerization, amino acid residues 307–355. Tetramerisation is very important for p53 activity *in vivo*.

− C-terminal domain involved in the removal of the DNA binding domain from DNA, amino acid residues 356–393.

The p53 protein is a product of the *TP53* tumor suppressor gene and is expressed in all cells of the body. In the absence of damage to the genetic apparatus, the p53 protein is in an inactive state, and when DNA damage occurs, it is activated. Activation consists in acquiring the ability to bind to DNA and activate the transcription of genes that contain a nucleotide sequence in the regulatory region called the p53-response element (the region of the DNA that p53 binds to, fig. 41).

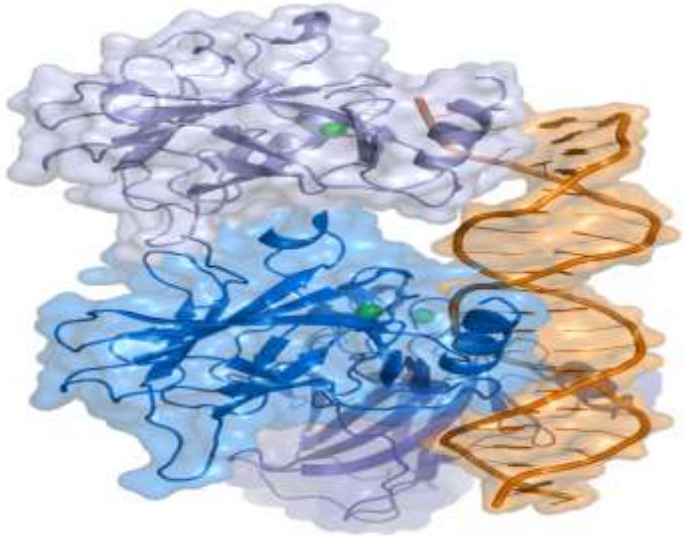


Fig. 41. p53 Complex with DNA

Thus, p53 is a factor that triggers the transcription of a group of genes and which is activated by the accumulation of DNA damage. The result of p53 activation is cell cycle block and DNA replication; with a strong stress signal, it is the start of apoptosis.

The p53 protein is activated upon the damage of the genetic apparatus, as well as upon the stimuli that can lead to such damage, or if they are a signal of an unfavorable state of the cell (stress state). The function of the p53 protein is to remove from the pool of replicating cells those cells that are potentially oncogenic (hence, the figurative name of the p53 protein − *the guardian of the genome* – the genome keeper). This idea is confirmed by the fact that the loss of function of p53 protein can be established in ~ 50 % of cases of human malignant tumors. The leading role in the regulation of p53 protein activity is played by post-translational modifications of the protein and its interactions with other proteins.

P53 protein activation occurs in response to numerous stressful stimuli:

1) the immediate DNA damage (classic stimulus);

2) the damage to the segregation apparatus of genetic material (for example, mitotic spindle);

3) the decrease in the concentration of free ribonucleotides;

4) hypoxia;

5) heat shock;

6) high concentration of NO (nitric monoxide);

7) ionizing radiation.

In rapidly dividing (proliferating) cells, an increase in the concentration of p53 protein was detected. The significance of the increase in p53 concentration in this case is that cells that rapidly replicate DNA are more prone to damage than, for example, non-dividing cells in the G_0 phase. Therefore, an increase in p53 concentration is the preparation of a cell for a quick response to the possible occurrence of DNA damage. Obviously, to stop the cell cycle under the conditions of stimulation of proliferation by extracellular growth factors, a higher concentration of p53 is required than under the conditions of the G_0 phase. Due to the strict post-translational control of p53 protein activation, a high concentration of p53 protein does not lead to its activation.

The concentration of p53 protein increases as a result of the inhibition of translation of its mRNA translation. Inhibition of translation occurs as a result of binding of regulatory proteins to nucleotide sequences in the 3'-untranslated region of mRNA. Modification of the p53 protein leads to its activation. Latent (inactive) p53 protein is localized in the cytoplasm (at least at some stages of the cell cycle); active p53 protein is localized in the cell nucleus. In the absence of a stress stimulus, p53 protein has a short half-life (5–20 min depending on the type of cell). Protein activation is associated with an increase in its stability. In the regulation of stability (and activity) of the p53 protein, the main role belongs to the Mdm2 protein.

Activated p53 protein is a specific transcription factor. The genes with transcription stimulated by the p53 protein encode the protein components of the apoptotic program (proapoptotic components) and the proteins that regulate the cell cycle.

The activated p53 protein suppresses the transcription of a number of genes. This suppressive effect is not associated with the suppressor function of the Mdm2: p53 complex since this complex suppresses the transcription of those genes that are activated by the p53 protein (unrelated to the Mdm2 protein). At the same time, the suppressor effect of p53 protein concerns another set of genes. Repression of transcription is at least partially due to the fact that the p53 protein forms complexes with non-specific transcription factors; among them there is the TBP protein (*TATA-box binding protein*; the protein that binds to the TATA sequence), CBF protein (*CCAAT binding factor;* a protein that binds to the CCAAT sequence) and SP-1 protein.

The p53 gene encodes at least two proteins with slightly different regulation (two forms result from alternative splicing of pre-mRNA). There are also

data that indicate the possibility of the existence of a whole group of proteins related to the p53 protein; the p73 protein is described most of all.

The increased p53 protein synthesis leads to the induction of p21 protein synthesis – a cell cycle inhibitor.

Violation of the normal regulation of the cell cycle is the cause of the appearance of most solid tumors. In the cell cycle, as it has been already mentioned, the passage of check points is possible only in the case of the normal completion of the previous stages and the absence of breakdowns.

Tumor cells are characterized by changes in the components of check points of the cell cycle. During inactivation of cell cycle checkpoints, dysfunction of some tumor suppressors and proto-oncogenes, in particular p53, pRb, Myc, and Ras, is observed. The p53 protein is one of the transcription factors that initiates the synthesis of the p21 protein, which is an inhibitor of the CDK-cyclin complex, which leads to cell cycle arrest in the G_1 and G_2 period.

Thus, a cell in which DNA is damaged does not enter the S phase. With mutations leading to the loss of p53 protein genes or with their changes, the cell cycle blockade does not occur; the cells enter into mitosis, which leads to the appearance of mutant cells, most of which are non-viable, and others gives rise to malignant cells.

The role of the p53 gene in the genesis of cancer is in no doubt.

Today it is well known, that p53 is a critical tumor suppressor that maintains the genetic stability in mammals by playing multiple roles in cell cycle arrest, apoptosis, senescence and differentiation. All these functions of p53 could prevent the passage of DNA damage to the daughter cells. As a transcription factor, p53 functions by directly regulating the expression of hundreds of genes, products of which are responsible for mediating the p53-dependent functions. It has been mentioned already, that p53 is consisted of two N terminal transactivation domains, a core DNA-Binding domain and a C terminal oligomerization domain. Loss of p53 function is required for the progression of most cancers. In this context, p53 is somatically mutated in over 50 % of all human cancers, and most of these mutations were missense mutations within the DNA-binding core domain. Based on their impact on p53 structure and function, p53 mutation can fall into two general classes: DNA contact mutations, which change the residues directly involved in contact with DNA but have modest impact on p53 conformation; structural mutations that dramatically alter the p53 conformation. Among all p53 mutations, there are four hotspot mutations at residues 175, 248, 249 and 273. While both R248W and R273H mutations are contact mutation, R175H mutation is a structural mutation. All hotspot mutations abolish the wild type tumor suppression function of p53.

Expression of mutant p53 is correlated with the poor prognosis of the patients. Accumulating evidence has shown that p53 mutants not only lose

their wild type p53 activity, but also acquire new oncogenic activities to promote cancer and drug resistance. For example, p53 mutants can help to transform cells, increase resistance of cells to chemotherapy, apoptosis. Down-regulation of p53 mutants in cancer cells can reduce cell proliferation, tumorigeneic potential and chemotherapeutic resistance. Therefore, in order to improve the efficacy of therapy for human cancers expressing p53 cancer mutants, it is critical to elucidate the oncogenic gain of function of p53 cancer mutants. Previous studies have suggested that one mechanism to achieve the gain of function of p53 cancer mutants is through protein-protein interaction between p53 mutants and cellular proteins, leading to the disruption of the function of the cellular proteins. For example, certain p53 cancer mutants can interact with p73 and suppress p73 function.

To investigate the impact of the p53 cancer mutations on the structure and function of p53, several groups have introduced the common p53 cancer mutations into the corresponding residues of the endogenous mouse p53 genes. These p53 knock-in mutant mice develop tumors with a similar kinetics as the p53-/- mice but with a more complex tumor spectrum, suggesting that these p53 mutants gain new oncogenic function. In order to better recapitulate the impact of the common p53 cancer mutations on the structure and function of p53, we employed the humanized p53 knock-in (HUPKI) allele to develop mouse models that express the most common p53 cancer mutants in the physiological context. By studying the common p53 contact mutation (R248W and R273H) knock- in mice, we recently discovered a gain of function of these p53 contact mutants in inducing genetic instability by inactivating ATM function. ATM is the master regulator of cellular responses to DNA double-stranded break (DSB) damage. Since genetic instability, a hallmark of cancer, plays critical roles in tumor progression and drug resistance, it is important to determine whether this gain of function is common to other p53 cancer mutants. By establishing and analyzing the most common p53 structural mutant (R175H) knock-in mice, we provide compelling evidence that it is a common gain of function of p53 cancer mutants to induce genetic stability by disrupting the Mre11/ATM pathways.

Recent studies have described that radiation induces genomic instability, which manifests in the progeny of surviving cells as a persistent decrease in plating efficiency (delayed reproductive death), increased chromosome instability, and increased mutation rate. Despite extensive studies into the process of delayed phenotypes, very little is known about the mechanism of genomic instability. Delayed chromosome instability is characterized by dicentric chromosomes, chromatid gaps and breaks, indicating that delayed DNA breakage is induced in the surviving cells several generations after irradiation.

Induced genomic instability manifests as the induction of various delayed phenotypes such as delayed lethal mutation or delayed reproductive death, delayed chromosomal instability, and delayed mutation induction. Alt-

hough genomic instability has been reported commonly in mammalian cells exposed to ionizing radiation, the mechanisms underlying the initiation and manifestation of radiation-induced genomic instability are not fully understood. Furthermore, other studies have suggested that an extranuclear target or a bystander effect may also be involved in the induction of instability. Therefore, more than one mechanism may be involved in the initiation of radiation-induced genomic instability in survival cells. If the cell does not die, it may acquire genomic instability and lead to a population of cells with abnormally high susceptibility to gene and chromosomal instability, mutation and other delayed effects. Studies using inbred strains of rodents have clearly shown genotype-dependent differences in response to radiation exposure, including susceptibility to radiation-induced cellular transformation and tumor formation, as well as differences in susceptibility to radiation- induced chromosomal instability.

More specifically, ionizing radiation causes DNA double strand breaks, which are the initiators for reproductive death, chromosomal aberration, apoptosis, and mutation. Because all of these are manifestations of radiation- induced genomic instability, it is highly possible that delayed DNA damage is associated with delayed phenotypes. DNA double strand breaks are well known to accumulate and activate *p53*. Recent studies have described that DNA double strand breaks are recognized by ATM, and ATM-mediated phosphorylation of P53 protein accumulates and activates *p53* [23]. It regulates transcription of the downstream genes such as *p21WafI/Cip1, gadd45, Reprimo, BAX, PIG-3,* and *p53AIPI* [7; 24; 25]. As a result, activated *p53* causes cell cycle arrest, apoptosis, senescence-like growth arrest, and involved in base excision repair [26]. It was estimated that one single DNA double strand break is enough to activate *p53* [27], therefore, it is highly possible that DNA breaks arising in the progeny of surviving cells activate P53 protein. Moreover, radiation-induced genomic instability could be the driving force underlying the development of radiation-induced carcinogenesis by accumulating genetic alterations. If *p53* function is a guardian of the genome, there should be delayed *p53* activation in the progeny of surviving cells.

It is well known from many studies that cells lacking normal *p53* function exhibit no detectable Gi-S arrest in response to radiation. Following genotoxic damage, *p53* is induced and acts to restrain proliferation by inducing the expression of genes which lead to growth arrest (such as p21) or apoptosis (bax). And by inhibiting proliferation following DNA damage, *p53* action prevents the accumulation of potentially oncogenic mutations. Loss of *p53* function has quite a varied effect on the radiosensitivity or chemosensitivity of tumor cells lines. In primary mouse fibroblast cells which do not exhibit significant apoptosis in response to ionizing radiation, there is little to no preferential radioresistance of the *p53* null cells over the normal cells.

p53 also functions to suppress cellular immortalisation. Normal primary somatic cells are capable of undergoing a finite number of divisions in culture until they undergo cellular senescence, characterised by growth arrest, a large flat morphology and insensitivity to further mitogenic stimulation. In contrast, many tumour cells exhibit unlimited division potential, indicating that they have bypassed the barriers to immortalisation, such as cellular senescence. *p53* appears to play a direct role in controlling the onset of cellular senescence.*p53* transcriptional activity increases with ageing of the cells, wild-type *p53* activity is necessary for growth arrest in senescence and a high percentage of cells that escape from senescence have lost*p53* activity.

Persistent genomic instability or a mutator phenotype can result in the higher frequency of genetic alterations in cancer cells, which cannot be explained by random mutation. Therefore, it has been hypothesized that radiation-induced genomic instability could be the driving force underlying radiation carcinogenesis. However, some studies have shown that delayed DNA damage occurs in the progeny of surviving cells. Unscheduled DNA breaks may result in accumulation of genomic alterations. Because activated P53 protein in response to ionizing radiation has been thought to protect the integrity of the genome, delayed activation of P53 may also play a role as a guardian of the genome. The results of research by Clutton et al support that activated *p53* causes delayed cell death including delayed apoptosis. Therefore, radiation-induced genomic instability causes accumulation of genetic alterations, and delayed activation of *p53* plays an indispensable role in eliminating the damaged cells and maintaining genomic integrity. Although several studies have described the expression of delayed phenotypes, the molecular mechanisms involved in delayed induction of DNA damage have not been determined. It was proposed that potentially unstable chromosome regions resulting from DNA repair of double strand breaks are involved in delayed DNA breakage. Previous studies demonstrated that dicentrics were the hallmark of chromosomal instability, and they provided a chance to cause DNA breakage during anaphase. Although several mechanisms may be involved in the induction of delayed DNA breakage, it may be explained in part by the mechanism of manifestation of radiation-induced genomic instability. Additional studies are required to clarify the mechanism that causes delayed DNA breakage for prevention of accumulated genetic alterations in the progeny of surviving cells exposed to ionizing radiation.

Experimentally, numerous studies have shown that ionizing radiation can increase the frequency and decrease the age at which tumors are observed in mice. This observation is apparent in animals carrying mutations in known tumor suppressor genes and even more so in animals deficient in genes shown to have a role in DNA repair and maintenance of chromosome stability. For example, exposure of neonatal *p53-/-*mice to ionizing radiation markedly decreases the latency to tumor formation. A similar decrease in tumor latency

has been observed in irradiated *p53*+/-animals. This treatment also increased the frequency of malignant lymphomas and decreased the incidence of sarcomas in these animals. In contrast, exposure of*p53*+/-mice to ionizing radiation did not alter the incidence of mammary tumors, and in fact no mammary tumors were identified on analysis of 33 treated *p53*+/-animals. These findings indicate that exposure to ionizing radiation can decrease tumor latency and alter the frequency with which specific types of tumors arise in p53-deficient animals. These results also suggest that differential exposure of humans and mice to such DNA-damaging agents alone cannot explain the extremely low incidence of mammary tumors in p53-deficient mice as compared to patients with the Li-Fraumeni syndrome. An additional explanation for the failure of *p53*-deficient mice to develop mammary tumors may be the increasingly frequent finding that differences in the genetic background on which a tumor suppressor is studied can dramatically alter the tumor spectrum. It has been shown that the incidence of teratocarcinomas is elevated in *p53*-/-mice on the 129/Sv genetic background as compared with *p53*-/-mice on a mixed C57BL/6-129/Sv background. The role of *p53* in tumor suppression is further highlighted by the creation of *p53*-/-mice, which are highly cancer prone and develop a large spectrum of tumors. This suggests that different strains of mice contain sets of alleles that modify the impact of the loss of*p53* in a cell type-specific manner.

Type of *p53* mutation depends on mutagen nature. The assessments on the influence of mutagen on the *p53* mutation prevalence and spectrum such as environmental tobacco smoke and residential radon for lung tumors, as well as ultra violet for the induction of skin cancer had been conducted by some researchers independently. Mutations in *p53* are the most common defects in lung cancer and may be a pathway through which environmental carcinogens initiate cancer. From investigation by Taylor et al on the detection of *p53* mutations in lung cancers from uranium miners with high radon exposure was shown that 16 (31 %) of 52 large-cell and squamous-cell cancers from miners contained the same AGG to ATG transversion at codon 249, including cancers from 3 or 5 miners who had never smoked. This specific mutation has been reported in only 1 of 241 published *p53* mutations from lung cancers. They proposed that codon 249 mutation may be a marker for radon-induced lung cancer. Other examination conducted by Hollstein et al revealed that none of the lung tumors examined harbored the hotspot mutation. Five of the 50 tumors, however, did indeed harbor exon 7 mutations, as determined by subsequent mutation analysis of exon 7. These mutations were dispersed among various codons and may be attributable to heavy tobacco smoking in this cohort. In support of this interpretation, they found no mutations in exons 5–8 of the *p53* gene in 13 iatrogenic liver cancers induced by injection of Thorotrast, an alpha-emitting radiocontrast agent. They propose that if the *p53* tumor suppressor gene is a target for the carcinogenic action of alpha-particle radiation, loss of suppressor function may occur preferentially by mechanisms

such as intrachromosomal deletions, rather than by base substitution mutations. Specific UV-induced mutation spectrum in the *p53* gene of skin tumors from DNA-repair-deficient xeroderma pigmentosum patients was found by Dumaz et al.[48]. All the mutations were located at dipyrimidine sites, essentially at CC sequences, which are hot spots for UV-induced DNA lesions. Sixty-one percent of these mutations were tandem CC–>TT mutations considered to be unique to UV-induced lesions; these mutations are not observed in internal human tumors. All the mutations, except two, must be due to translesion synthesis of unrepaired dipyrimidine lesions left on the non-transcribed strand. These results show the existence of preferential repair of UV lesions [either pyrimidine dimers or pyrimidine-pyrimidone (6−4) photoproducts] on the transcribed strand in human tissue. Literature suggests that the incidence of various tumors is determined largely by the genetic background on which mutations are studied. In addition, population studies and studies in animals suggest that environmental factors, together with genetic factors, determine overall risk for development of specific types of tumors. Environmental agents together with genetic factors can increase the frequency and decrease the latency of mammary tumors, leading to an incidence similar to that observed in Li-Fraumeni syndrome. Furthermore, it suggests that the risk of development of a particular type of tumor by individuals deficient in *p53* after exposure to damaging agents can be influenced by modifier alleles [49].

Shortly summarizing the data above, it is possible to conclude that the p53 protein has been implicated in multiple cellular responses related to DNA damage. When working properly, P53 binds DNA to activate genes that direct cells with damaged DNA to cease dividing until the damage can be repaired. Cells with such damage include cancer cells, since all cancers track to genetic flaws of one kind or another, whether inherited or acquired. If repairs cannot be made, P53 commands the cells with damaged DNA to self-destruct so they are no longer a danger to the body. Alterations in any of these cellular responses could be related to increased genomic instability. The *p53* gene regulates the G1 cell cycle checkpoint in response to DNA damage due to ionizing radiation. However, the significance of *p53* mutation in radiation sensitivity and its underlying mechanisms still remains unclear. To elucidate this case, some researches have measured the effects of *p53* mutation not only on cell cycle delay, but also on apoptosis and radiation sensitivity using mouse cells transfected with different forms of *p53* mutations or human tissues. Other results revealed that cell survivals determined by clonogenic assay show that *p53* mutant cells are generally more sensitive to ionizing radiation than cells with wild-type *p53*. These results suggest that mutant forms of *p53* represent a phenotype that affects the radiation sensitivity and is not dependent on the apoptotic pathway. These findings also provide the possibility that the observed instability results from these DNA breaks lead to delayed chromosome rearrangements, delayed cell death, and so forth, many generations after irra-

diation and that activation of *p53* function may eliminate cells that have potentially accumulated genomic alterations. In recent years, there has been an exponential increase in the number of *p53* mutations identified in human cancers and its database consists of a list of point mutations in the *p53* gene of human tumors and cell lines.

4.4. Activation of Check Points of the Cell Cycle During Irradiation

It is well known that irradiation of cells leads to a delay in their passage through the G_1, S, and G_2 phases of the cell cycle (table 10). This is due to the activation of checkpoints that detect DNA damage and retard cells for repair processes.

Table 10

Activation of cell cycle checkpoints

Cycle Phase	Signal Proteins	activated when cell irradiation in the phase	Properties
G_1	ATM, p53,p21	G_1	It blocks the entry of cells into the S phase
S	ATM, Chk1/ Chk12, CDC25A/ CDC25C, BRCA1, BRCA 2	S	It slows the passage of cells along S- Phase
Early G_2	ATM, Chk1/ Chk12, CDC25A/ CDC25C, BRCA1, BRCA 2	G_2	It blocks the entry of cells into mitosis
Late G_2	ATR, Chk1, CDC25A/ CDC25C	all phases	It leads to the accumulation of cells in the G_2 phase

In fact, check points are certain moments in the cell cycle, and their activation can block or slow down the entry of cells into the next phase of cell division. In response to irradiation at various points in the cell cycle, repair processes activate four checkpoints. Initially, it was believed that these are delay points that allow cells to have more time for repairing damage in DNA. To some extent, this idea is fair, especially considering the importance of check points in preventing the conservation of mutations that might otherwise arise due to the presence of unpaired or incorrectly paired DNA bases. However, only a limited amount of data is known that supports this role of control points in the cellular response to radiation exposure during a single exposure (according to the survival test).

Cycle progression of a cell in the G_1-, S-, G_2- or M-phase occurs due to the action of cyclin-dependent kinases (CDKs). These kinases phosphorylate various proteins and trigger the process which is necessary for the passage of a cell through a cycle. CDK is active only when it is associated with its partner – cyclin (hence its name), and various cyclin-CDK complexes are active at different points in the cell cycle. For example, the cyclin D-CDK4 complex is active in the G_1 phase, and the cyclin B-CDK1 is active in mitosis. To activate checkpoints, cyclin-CDK complexes must be inhibited. In the case of irradiation, this is achieved in two ways. The first way involves the activation of certain proteins that directly inhibit the cyclin-CDK complex; these are cyclin-dependent kinase inhibitors (CDKIs). The second way is to change the degree of phosphorylation and activity of the CDK enzyme itself. The activity of certain CDKs is often under the control of phosphorylation, and one or another kinase may be active in a phosphorylated or dephosphorylated state.

4.4.1. G_1-Block of Cell Cycle

The check point located between the G_1- and S-phases of the cycle plays an essential role in the cell making a decision to switch to division. Therefore, it depends on the growth factors, food sources and other components that provide proliferation. The transition from G_1- to S-phase is under the control of transcription factor E2F. This factor regulates the activity of many genes; their products are involved in the initiation of DNA replication in the S phase, and during the G1 period it is kept inactive due to binding to the retinoblastoma protein (Rb). As the cell moves from G_1- to the S-period, the Rb protein is phosphorylated by Gl-nmoraH-CDKs, which include cyclin D-CDK4 and cyclin E-CDK2. In this case, Rb is cleaved from E2F, and the latter begins to function as a transcription factor initiating the transition of the cell into the S phase.

Irradiation leads to ATM-dependent stabilization and activation of *p53*. One of the genes under positive *p53* control is CDKI *p21* (CDKNlA). Protein *p21* inhibits the G_1-cyclin-CDK complex; this prevents the phosphorylation of Rb and the transition of the cell to the S phase. As a result, when cells are irradiated in the G_l phase, their transition to the S phase of the cycle is delayed, and this delay is provided by the proteins *p53* and *p21*.

4.4.2. The Checkpoint in S-Phase

The remaining check points of the cell cycle, which are activated upon irradiation, are controlled by two kinase proteins with similar properties – Chkl and Chk2 (fig. 42). Proteins Chkl and Chk2 are activated by phosphorylation and are substrates of ATR and ATM kinases, respectively. In cells that are in the S phase at the time of irradiation, a dose-dependent decrease in the rate of DNA synthesis is observed, as a result of which the overall duration of the replicative

period significantly increases. The protein that prevents the passage of cells through the S phase is CDK2 kinase, which is activated upon dephosphorylation. The removal of phosphate groups in CDK2 occurs under the action of phosphatases CDC25A and CDC25C. After activation of the Chkl and Chk2 kinases, they phosphorylate CDC25A and CDC25C, causing their inactivation or degradation. As a result, the activation of Chkl and Chk2 under the action of ATR and ATM leads to an increase in the amount of the phosphorylated form of CDK2, and thus, the passage of the cell through the S phase slows down.

Although the activation of ATM-Chk2 and ATR-Chkl and the inhibition of CDC25A / C is the main mechanism for triggering the functioning of the checkpoint in the S-phase, some other DNA repair proteins also affect this process.

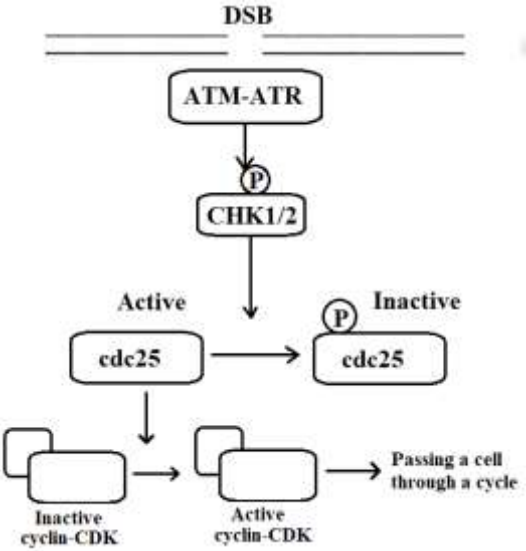


Fig.42. Activation of checkpoints in the S- as well as in the early and late G_2-phase occurs by a similar mechanism

When double-strand breaks (DRs) appear in DNA, a protein is activated that is mutated during ataxia of telangiectasia (ATM) and / or AT kinase (ATR). This leads to phosphorylation of Chkl / 2 kinases. Kinases phosphorylate and inactivate CDC25A / CDC25C proteins. The latter are necessary for the cell to pass through the S phase and enter mitosis since they activate complexes of the cyclin-cyclin-dependent kinase in both phases of the cycle. Thus, when Chk1 / 2 is phosphorylated by ATM, checkpoints are activated in the S and G_2 phases of the cycle.

These include the BRCA1 and BRCA2 proteins, the main function of which is to participate in homologous recombination. This indicates

a complex relationship between the processes of activation of the check point and DNA repair.

4.4.3. The Checkpoint in G_2 Phase

In the G_2 phase, there are two additional check points with the functions that are similar to those for the points of the S phase of the cell cycle. The early check point in the G_2 phase is also monitored by ATM-Chk2-Cdc25 A / C and functions when cells are irradiated in the G_2 phase. This point is activated by exposure to relatively small doses of radiation (a dose of 1 Gy is sufficient), blocking the passage of cells through the cycle at the end of the G_2 period. In this case, ATM-Chk2-Cdc25A / C acts on the mitotic complex cyclin B-CDK1, specific sites in which are dephosphorylated and activated, the same way it occurs with CDK2 in the S phase. This point is called early G_2 because it refers to cells that are irradiated in the G_2 phase, and their entry into mitosis is quickly blocked. As a result, through a short period of time after irradiation, a decrease in the number of mitotic cells is noted.

The check point in the late G_2 phase provides a continuous G_2 block and functions upon preliminary irradiation of cells in the G_1 or S phase. The passage of such cells in a cycle may be temporarily blocked during the activation of check points in the G_1 and S phases, but when they enter the G_2 phase a few hours after that, they have one more delay before they enter mitosis. Unlike the check point in the early G_2 phase, this block is highly dependent on the dose of radiation. So, after irradiation in high doses, the block can last for many hours. Also, unlike the others, this late G_2 phase point is ATM independent. Instead, the signal is transmitted in the main direction from the ATR to Chkl and to the CDC25A / CDC25C. Thus, the check point in the late G_2 phase resembles the points in the S and early G_2 phases; however, it is activated by a completely different type of the damage in the DNA. This point is not activated by DR, and its functioning is triggered by the damage that persists after the completion of DNA repair processes, and their level is sufficient to activate ATR.

4.4.4. The Features of Check Point Functioning

In the cells of most tumors, some or all check points of the cycle happens to be turned off due to genetic changes that result from malignant transformation of cells. Recently, activation of check points has been associated with the suppressors of tumor growth; their functioning is impaired by the induction of proliferation under the influence of oncogenes. It is believed that this occurs after the activation of oncogenes, inducing “untimely replication” and the formation of damage in the DNA. During normal functioning, check points block the further proliferation of such cells; thus, tumor development is actively suppressed. This point of view is supported by the evidence that, in

many cases, the blocking of tumor growth that occurs in the early stages is associated with the activation of check points.

Mutations in the genes encoding *p53*, BRCA1, or other components of DNA reparation, which affect the activation of check points, do not allow the irradiated cell to stop in the cycle. This may have important consequences for the occurrence of genetic instability after irradiation and for the development of tumors; however, there is little reason to assume that in the absence of the functioning of check points the radiosensitivity of cells changes. Although it is believed that the role of check points in the G_1 / S-, S-, and early G_2 phases is to provide the cell with additional time for damage reparation, this factor is apparently more important for the quality of reparation, and not for its volume.

The only exception is the check point in the late G_2 phase, the activation of which, unlike other points, depends on ATR and not on ATM. There is the evidence that it is a determinant of the radiosensitivity of cells. For example, ATR inhibitors that inactivate this point have radiosensitizing properties. Thus, for the reasons that are yet unknown, premature entry into mitosis of cells located at a check point of the late G_2 phase leads to an increase of their death.

Although the check points in the G_1- / S-, S- and early G_2 phases may not affect the radiosensitivity of cells after a single exposure, they can change it with repeated (fractionated) irradiation. The check points in intact cells and their absence in the cells of many tumors affect the cellular composition of the population at certain points in time after irradiation and, therefore, indirectly determine its sensitivity to repeated irradiation. For example, two populations of identical cells can be considered; however, in one population, the block at the check point of the G_1 phase is absent. 24 hours after irradiation, cells in the population of which this point is absent may appear in the G_1 phase in a smaller amount, and in the S phase in a larger amount, compared with the population in which this control point is intact. Since the radiosensitivity of the cells in the G_1 and S phases of the cycle is different, the two cell populations may respond differently to subsequent irradiation at this time. Therefore, all check points affect the response of cells to radiation, which is given in the dose fractionation mode.

Along with radiation injuries that cause cell death and the secondary morphological changes associated with cell mortality, such morphological lesions occur that do not lead to such an outcome. For example, peroxidation of membrane lipids as a result of IR is a well-known process. Membrane defects can be stably maintained by the cell since the membrane repair system uses the existing structures as a primer. Structural heredity was first demonstrated in the works of T. Sonneborn on *Paramecium aurelia*, and then on other ciliates. As suggested by A. M. Olovnikov, membrane memory can be one of the causes of accelerated radiation aging.

The cell response to radiation morphological injuries is currently poorly understood. It is known that in *Saccharomyces cerevisiae* yeast, cell cycle stop can be caused by cell cycle disruptions, which are not associated with DNA damage.

In other words, there are the check points of the integrity of cell structures, i. e. the "checkpoint" has been found meaning the control of not only replication and mitosis, but also cytokinesis. Moreover, ionizing radiation, along with the induction of checkpoint-genes and DNA repair genes and oxidative stress, also induces the genes involved in the construction of the cell wall. Radiation induces the reversible depolarization of the mitochondrial potential (AW), causing the release of ROS from mitochondria to the cytoplasm.

It is shown that the inhibitors blocking this process also prevent the formation of a signal from the checkpoint kinase cascade. The ROS sensor is an actin cytoskeleton. In response to oxidative stress in actin, a disulfide bond between the two cysteines Cys285 and Cys374 is formed. Oxidation and hyperstabilization of actin subsequently serves as an apoptotic signal associated with the accumulation of ROS.

The reaction of cells to the effects of ionizing radiation can be assumed to involve morphological check points that "scan" the integrity of cell structures, in violation of which the cell division stops, and subsequent recovery processes occur. Their launch or activation of programmed cell death is regulated by a morphological "checkpoint", which has common steps with the regulation mechanisms of the "checkpoint" – the control of DNA damage. The kinase of CDK1 / CDC28, which is the main regulator of cell cycle progression, serves as a target not only as a checkpoint of monitoring of DNA damage, but also as a checkpoint of monitoring of morphology and cytokinesis.

In yeast, a cytoplasmic checkpoint was found to control the position of the nucleus. Presumably two components can be distinguished in the stopping of division before the anaphase: one is the suppression of chromosome discrepancies (Pdsl); the second one is the prevention of the dynein-dependent movement of the spindle into the kidney, which occurs normally in the anaphase. The interaction of the spindle pool with the cortex of the kidney activates the system of the exit of MEN mitosis, including Cdcl4 phosphatase, which triggers the telophase and enters the next cell division cycle.

MEN's participation in checkpoint of the control of mitochondrial inheritance has been demonstrated. In mdm 10D cells, there is a defect in the release from the nucleolus of the central player MEN of phosphatase Cdcl4, which affects its ability to activate substrates in the isthmus of the kidney and SPB.

Part 5. CELL DEATH AND SURVIVAL MECHANISM

The concept of **radiation cell death** to date combines the phenomena of **interphase and reproductive** cell death with two types of cellular thanatogenesis - **apoptosis** and **necrosis**. The ability of hematopoietic stem cells to eliminate radiation damage, to survive and repopulate through proliferation and differentiation, is an important condition for the survival (**radioresistance**) of mammalian organisms after irradiation in moderate lethal doses. On the contrary, the genetically determined inability of cells in a certain state to repair a significant part of radiation damage, leading to their death, is the basis of the body's **radiosensitivity.**

As will be seen from what follows, all the above radiobiological effects and phenomena impaired cell movement in the cycle, their reproductive death, apoptosis and necrosis, the occurrence of mutant clones, tumor transformation, metabolic phenomena involved in the formation of the state of radioresistance and radiosensitivity of the body have a molecular biochemical basis.

In recent years, the correlation and key role of cell cycle regulation processes, DNA repair and apoptosis in the formation of the radiobiological effect at the level of critical organs and the body as a whole has become apparent.

5.1. Early Response to the Damage

This phenomenon is typical only to proliferating cells and is manifested by an immediate stop of the cells moving along the cycle in any of its phases immediately after irradiation. The check point is a mechanism in cell transition s from one phase of the cycle to another, which detects the damage in the DNA and reacts to this with an appropriate signal. The mechanism of the check points induces the transcription of genes that control the formation of protein factors, which are necessary to eliminate a detected defect in the DNA structure by its repair. It is controlled by the Tp53 gene using the protein of the same name encoded by it.

Tumor suppressor protein Tp53 is "the guardian of the genome." Tumor suppressor protein Tp53 (tumor suppressor protein 53, i. e., with the molecular weight of 53 kDa) is an essential component of each proliferating cell and is one of the important ones in coordinating the adaptive responses of cells to various damaging effects, initiating the detection of genetically defective cells, activation of their repair or destruction by apoptosis. It is called a tumor suppressor precisely because of its ability to prevent tumor tissue degeneration.

Normally, after synthesis in the cell cytoplasm, the nuclear protein Tp53 forms a tetramer, which moves into the nucleus and enters the complex with

another protein – MDM2. After that, the Tp53 protein undergoes chemical modification immediately by ubiquitination and immediately becomes the subject of degradation in the S26 proteasome located near the nuclear membrane. As a result, the half-life of the Tp53 protein is normally about 20 minutes, and its content in the cells is determined as "traces". It is believed that in an intact cell it is not manifested itself. With radiation damage to DNA in doses that cause the formation of at least one double-strand break, another protein, ATM (the product of the ATM gene – ataxia telangiectasia-mutated-which mutation is the basis of the human ataxia-telangiectasia disease) acts as a molecular sensor of such damage. Having an affinity to double-stranded DNA breaks and the properties of a protein kinase for MDM2 and Tp53 proteins, ATM, upon binding to DNA at the break site, goes into an active state and phosphorylates these proteins. Phosphoryl groups in MDM2 and Tp53 create steric barriers to the formation of their complex with each other, and Tp53 after further phosphorylation and acetylation goes into a stable state. Its half-life in the cell increases many times (up to 2 hours), and its content is 20 times. Having the ability to interact with other proteins and, most importantly, with different parts of the chromosomal DNA, the Tp53 protein translates the regulatory elements of many genes into an active state i.e., causes transactivation (activation of transcription) of DNA. The main of the activated genes are *MDM2* (its product is a negative regulator of the function of the Tp53 protein); *p21,* which is an inhibitor of the cyclin-dependent kinase responsible for stopping the cell cycle at the G_1 / S transition; as well as *CADD45* (growth arrest and DNA damage, which stops the cell growth with DNA damage); and *14-3-3σ,* which controls DNA synthesis, its repair, cell advancement in a cycle, and cyclin B inhibition.

Under the action of the protein products of these genes, G_1 / S and G_2 / M blocks appear, DNA synthesis and the transition of cells to mitotic division are stopped, and the activation of DNA repair eliminates radiation-induced damage, acquitting the risk of doubling defects in the daughter cell genome. At the same time, the block at the G_2 / M border of the cell cycle is provided by that part of the intracellular regulatory signaling network, which is called the MKK6-p38γ cascade, known as ERK6 or SAPK3, associated with the activation of the *ATM* gene. The total time from the onset of cell delay in the G_1, S, or G_2 period to the inclusion of the apoptosis signal (a condition for DNA repair from damage) is a determinant of the radiosensitivity of the lines of human blood cells. Mutations in the Tp53 gene prevent the induction of apoptosis and make these cells more radioresistant.

The importance of the biological role of the Tp53 protein in the cell was one of the first to be very accurately described in 1992 by Lane, who called this protein the "guardian of the genome" (fig. 43).

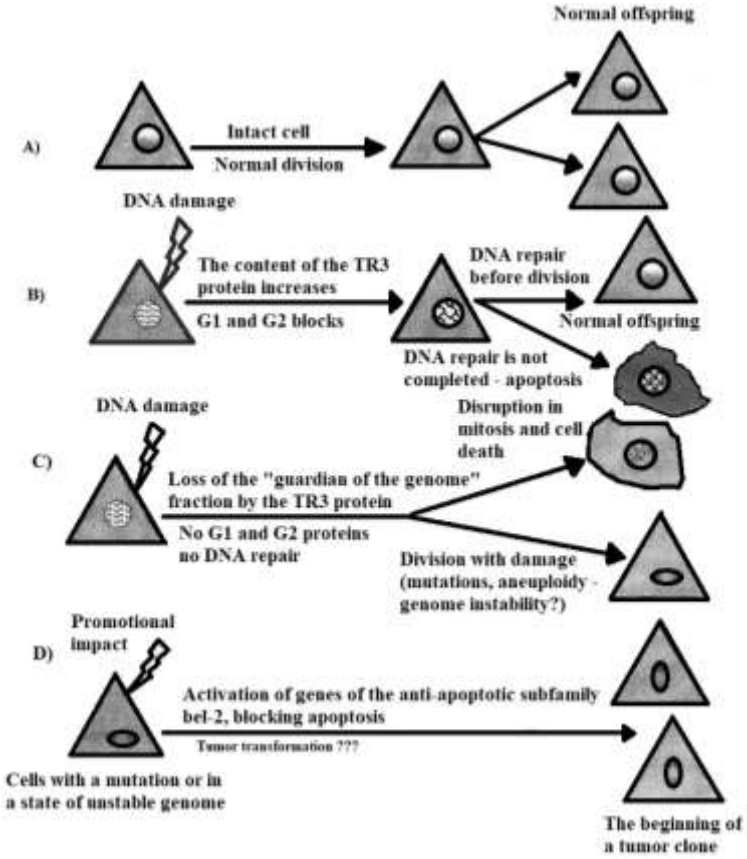


Fig. 43. The function of the protein and p53 gene as a "guardian of the genome" and the possibility of tumor cell transformation in the event of the loss of this function as a result of somatic mutation

In the absence of damaging effects (option A), the intact cell during normal division produces normal offspring. With radiation damage to DNA (option B), the Tp53 protein is stabilized and its content increases, which leads to G_1 and C_2 blocks of the cell cycle stopping the cell division.

During the repair, the structure of DNA is restored, the blockade of the cell cycle stops, and the cell divides giving normal offspring. If DNA repair does not end, the same Tp53 protein forms a signal to destroy the cell by apoptosis (shown in black). In this way, the function of the p53 protein is realized in maintaining the constancy of the genome as its "guardian".

With the loss of this function (the damage to the Tp53 gene and the formation of the mutant Tp53 protein), the third option arises: the cell cycle with the damaged DNA proceeds smoothly, but the defect is manifested in mitosis,

and then the cell either dies or gives birth with a loss of the part of genetic material, mutational changes and the instability of the genome (lower part of fig. 42). The promoting effect of radiation on such cells with a defective function of the Tp53 protein as a "guardian" of the genome (option D) causes the increased production of reactive oxygen formations, activation of the genes of the apiapoptotic subfamily of bcl-2 proteins, and a number of other effects, which, according to many researchers, result in tumor transformation. The molecular mechanisms of apoptosis are called upon to counter such a development of events.

5.2. Reproductive Death

The cell cycle, which is a universal link in the chain of events of cell reproduction, is conditionally divided into two phases – **interphase**, when the cell grows and prepares for reproduction, and **mitosis**, when the nucleus and then the rest part of the cell divides. Hence, the name of the two types of radiation-induced cell death are **interphase** and mitotic (**reproductive)**.

Reproductive death is distinguished by:

• the connection with the process of cell division, i.e. its predominance in dividing cell populations;

• a different dependence of the manifestation on time: the maximum is not in the first hours after irradiation, but in a later period (in the bone marrow of rats no earlier than 10 hours, on average, 16–20 hours after irradiation;

• the sigmoidal nature of the dose dependence, in contrast to the two-component at interphase, and each of the components can be approximated by a straight line;

• the ability to change the time of appearance of dead cells by changing the time of entry of the cell population into mitosis;

• a close relationship with the appearance of chromosomal aberrations, which are eliminated from the tissue along with the cells that die when they enter mitosis.

The most likely cause of reproductive cell death is considered to be the damage to their DNA during its replication during the second half of the S-period. A quantitative assessment of the ratio of the severity of interphase and reproductive cell death in individual tissues after irradiation was only partially done: interphase cell death predominates in lymphoid tissue, reproductive – in the bone marrow; however, the proportion of interphase dying cells is very significant.

It is known that violation of the DNA structure in the form of single-stranded and double-stranded breaks is the cause of sublethal, potentially lethal and lethal damage to the cell. Double-stranded rupture, disrupting the nuclear DNA, leads to lethal aberration of chromosomes – asymmetric chromosomal exchange and deletion, which causes reproductive cell death.

Consequently, the failure or inefficiency of DNA repair from double-strand breaks, being a key link in the molecular mechanism of reproductive cell death, creates the molecular basis for the cellular depletion of radiosensitive organs. Providing the conditions for increasing the efficiency of DNA repair from double-strand breaks can be one of the ways to reduce the radiation effect at the tissue and, accordingly, the body levels.

At the same time, it is obvious that the reproductive death of cells with molecular defects in the hereditary material after exposure to ionizing radiation is not a pathological event in its physiological essence, but it is the part of the normal biological mechanism for maintaining genome constancy. The essence of this mechanism is the repair of the damaged DNA, and if it is impossible to completely restore it - in the elimination of defective cells through the phenomenon of death. And only the extent of its manifestation, depending on the radiation dose, indicates that this normal mechanism is used to overcome potential pathology in populations of proliferating cells of the body.

5.3. Apoptosis and necrosis

As far back as the 50s of the 20th century, it was found that a significant part of the cells of the hematopoietic and lymphoid tissues die immediately after irradiation with the formation of polymer DNA fragments – polydeoxynucleotide (PDN). The electrophoretic characteristic of PDN revealed the molecular weight dispersion of its constituent DNA fragments forming a typical "ladder" on the electrophoregram. This "ladder", becoming a marker of apoptosis, served as a powerful tool for the development of research in the study of the mechanisms of radiation and physiological types of apoptosis. Their results regarding the biochemical mechanisms of radiation cell death with the formation of PDN have long been summarized in the work.

Analyzing the data characterizing the manifestations of radiation cell death, K. P. Hanson in 1979 first had the idea that radiation death in the interphase of the most radiosensitive type of cells – lymphocytes and the biochemical mechanisms of its manifestation (from the cellular to the organism level) are nothing more than the events of the implementation of the program contained in the genome of each cell of its natural death. A little earlier, in 1972, a group of authors described the pathomorphological nature of interphase cell death and pointed out the need to distinguish two heterogeneous phenomena – apoptosis and necrosis.

Apoptosis is considered as a programmed and genetically-mediated form of cell death, when external or internal signals give the cell an impulse to form (or activate a certain number of pre-existing) enzymes that lead to self-destruction. In contrast, **necrosis** is a result of external cell damage leading to disruption of the ordered cellular metabolism with the loss of ability to maintain ionic homeostasis. During apoptosis, the cells

lose contact with their immediate environment, decrease in size, and their chromatin is condensed. DNA degrades along inter-nucleosome linker sites to form fragments which sizes are the multiples of 180–200 polynucleotides. Cross-linking occurs in the membranes of apoptotic cells, leading to local hardening of these structures. Apoptotic cells "pack" their contents into small membrane-bound vesicles called apoptotic bodies, which are quickly phagocytosed by macrophages and closely spaced epithelial cells without an immune response. In contrast, necrotic cells eject their intracellular contents provoking an inflammatory reaction. Apoptosis develops in the separate cells; necrosis develops in the groups of cells with mutual contacts.

Apoptosis is a form of natural cell death and a condition for the natural renewal and change of cell populations. A pathological form of death, it becomes only in certain conditions. **Necrosis** is always a manifestation of unnatural death, it never occurs in normal cell kinetics, with normal embryonic development and metamorphosis. Necrosis is determined not by factors inherent to the cell itself, but by environmental influences that reach a damaging force. But apoptosis and necrosis develop when exposed to a variety of damaging agents such as ultraviolet, X-ray or γ-radiation, oxygen radicals, heat, heavy metals or cytotoxic substances. Which type of reaction will occur – survival or death – depends on the intensity of the exposure. At low levels, stress reactions are activated to ensure cell survival. Medium and sometimes high levels of damaging effects activate the program of their suicide, which is realized in apoptosis. High levels of damage lead to a sudden loss of the cell ability to maintain homeostasis with the outcome in necrosis. Radiation-induced necrosis of mammalian cells causes irradiation only in doses close to the minimum, absolutely lethal or exceeding them.

Table 13 presents a detailed description of apoptosis as a biological phenomenon and its differential diagnostic differences from necrosis.

Molecular Mechanisms of Apoptosis. The apoptosis mechanism, surprisingly similar in different animal species, proceeds in three stages:

– induction (signal stage);

–activation of implementation paths (control and enforcement);

– degradation (stage of destruction - structural changes).

The last two stages involve a family of cysteine-containing enzymes called caspases (the first letters of the term: cysteine with aspartate specificity proteases, formerly ICE: interleukin-lp-converting enzyme). In mammals, this family consists of 12 enzymes, divided into two classes: regulatory caspases (such as caspase-8, FADD-like interleukin-lp-converting enzyme, also denoted as MACH, Mch5, and caspase-9 - MCH6, and effector caspases such as caspase 3 (apopapain) and caspase 6 (MCH2).

Signal stage. The activation of cell cycle and DNA repair points has two outcomes: the resumption of cell proliferation or apoptosis. The choice is de-

termined by the duration of activation of the jNK- regulatory path by a damage signal (fig. 44).

Table 11

Comparative Characteristics of Apoptosis and Cell Necrosis

Indicator	Apoptosis	Necrosis
Trigger Factor	The signal perceived with membrane receptors, or lack of physiological xx signal	Toxic and membrano-tropic agents, inade-quate environmental conditions
The Speed of De-velopment	1-12 h	Within 1 h
Localization of Primary Damage	In the nucleus	In the membrane
Causes of Cell Death	DNA degradation, impaired cell en-ergy	Violation of the mem-brane integrity
Cell Resizing	Reduction (corrugation)	Increase (swelling)
Changes in Nucleus	Chromatin condensation, pycnosis, fragmentation	Swelling
Changes in the Cytoplasm	Cytoplasm condensation, contrac-tion of granules	Lysis of granules
Changes of the Cell Membrane	Loss of microvilli, formation of knobs	Integrity violation
DNA Condition	Breaks with the formation of large at first, then small fragments	Disordered degradation
Volatility	dependent	independent
Dependence on the Synthesis of Mac-romolecules	dependent	independent
Examples of Manifestation	Metamorphosis, negative selection of lymphocytes, hormone-dependent atrophy, interphase radiation-induced death of lymphocytes	Cell death from hy-poxia, the effects of toxins, viral cytolysis, complement depend-ent cytolysis
Identification Methods		
Morphological	Cell wrinkling	Cell swelling
Tinctorial	Decreasing stain with DNA trope dyes	Perception of supravital dyes
Cytofluorimetric	Hypodiploidy, reduction in cell size	–
Electrophoretic	The formation of discrete fractions	Smudged stain during

	("ladders") during electrophoresis	DNA electrophoresis

Disturbing signals or the signals of easily repaired damage are short-term, in response to them the cell enters the proliferation pathway. Signals of hard-to-repair damage maintain the JNK signaling pathway in an active state for a long time, and this leads to the activation of apoptosis mechanisms. Depending on whether the signal comes from DNA or from membrane structures, Tp53-mediated and Tp53-independent apoptosis are distinguished.

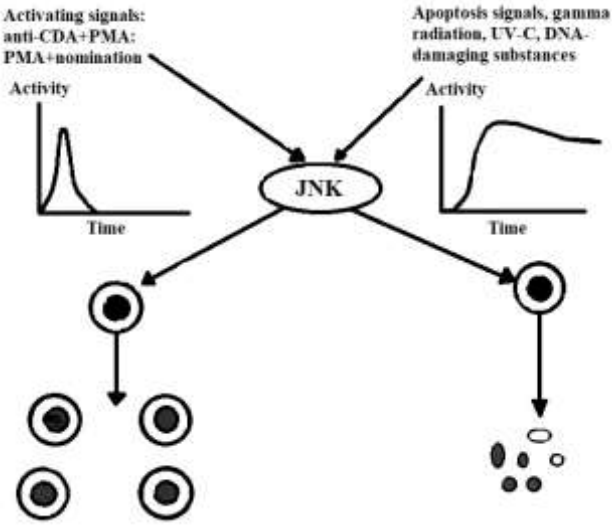


Fig. 44. Duration of induction of the JNK signaling pathway is a determining factor for cell proliferation or apoptosis

Tp53-mediated apoptosis is based on DNA damage; its elimination is caused by the activation of the tumor suppressor gene Tp53 as a result of accumulation of p53 protein (due to its stabilization under the influence of protein kinase activity of ATM and ATR proteins – ataxia teiangiectasia-related). The key event in the launch of Tp53-mediated apoptosis is the transcriptional activation of the *Noxa* gene when the cell cycle stops at the checkpoints and DNA repair. The reason for the activation of transcription of the *Noxa* gene is not yet known.

The Stage of Control and Execution. The product of the *Noxa* gene enters the mitochondria, interacts with the Bax protein, which is part of the proapoptotic subfamily of the Bc1-2 family proteins causing the damage to the mitochondria. Violation of the membrane potential of mitochondria leads to the release of cytochrome c from the intermembrane space into the cytoplasm. Cytochrome c together with Apaf-1 (apoptotic protease-activating fac-

tor-1) in the presence of DATP or dADP activates caspase-9, which in turn – caspase-3, which is involved in the final destruction of the cell.

The second mechanism of p53-dependent control of apoptosis implementation is well characterized. As a result of transactivation with p53 protein of the corresponding genes, ligands are synthesized and bound to their receptors called death receptors (DR). These receptors are CD95 (the previous name for FasL or Apo-IL); tumor necrosis factor b; DR3, DR4 (TRAIL – TNF-related apoptosis-inducing ligand (formerly Apo-2L) and DR5 (KILLER). The latter two are characteristic only of human tumor cells.

The CD95 signaling pathway, typical of all receptors, is shown in fig. 45.

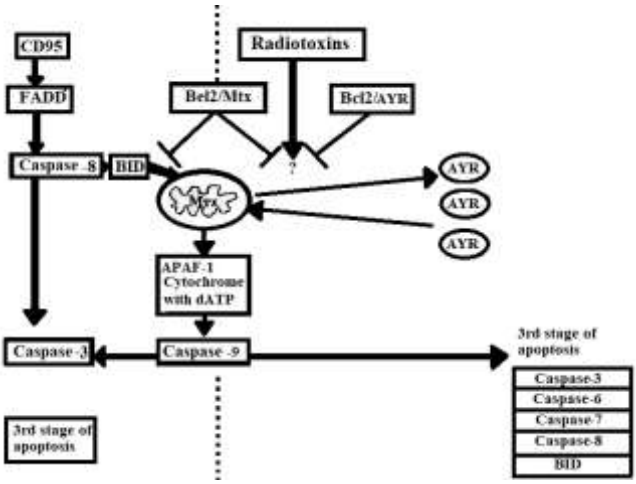


Fig. 45. Schematic model of the effect of subcellular localization of Bc1-2 on two forms of apoptosis - using the Tp53-regulated pathway through CD95 and through the Tp53-independent mitochondrial mechanism

The CD95L ligand binds to its own receptor, the activated receptor via the FADD (Fas-associated death domain protein) adapter molecule leads to autocatalytic activation of procaspase-8 into caspase-8. The latter activates effector caspase-3. If caspase-8 activation is weak, it can be enhanced through the mitochondrial mechanism. In this case, the Bid factor from the same family is included in the process, which usually proceeds with the participation of the apoptosis-promoting factor Bax from the Bc1-2 family, but its anti-apoptotic activity is suppressed by the Bel-2 protein. The initial stages of radiation-induced cell death proceed under the control of the Bc1-2 protein, which affects the interaction between mitochondria and the endoplasmic reticulum, which is higher than caspase activation. The endoplasmic reticulum can cause disturbances in calcium homeostasis and affect other proapoptotic molecules associated with it.

Tp53 ~ independent apoptosis. There is another variant of the mitochondrial pathway, different from the one that shown in Figure 42, when a protein called apoptosis-inducing factor (AIF) directly activate caspase-3.

Another Tp53 protein-independent apoptosis mechanism is the sphingomyelin / ceramide signaling pathway typical to the stress-induced apoptosis in various normal and tumor cells. It is regulated by protein kinase C, which performs an antiapoptotic function, and is triggered by an increase in the content of ceramide (as a result of an increase in its synthesis) or the breakdown of sphingomyelin biomembranes (under the action of ceramide synthase or sphingomyelinase activated by lipid peroxides, respectively).

Before the third, terminal stage of apoptosis, apoptotic signals are counteracted by the control mechanism with the participation of a number of protein families with anti-apoptotic functions, in particular, for example, the bcl-2 family. Recently the protein BAR (biiunctional apoptosis regulator) has been identified, which is capable of suppressing the transmission of apoptotic signals both on the external (via receptors) and internal (from biomembranes) pathways. Thus, the regulation of apoptosis is an example of the balanced mechanism with multiple duplication of balances, which provides the reliable control over the maintenance of cellular homeostasis in the process of overcoming the consequences of the damage by the cell. On this basis, a wide search and study of potential apoptosis modifiers is currently underway, some of which, undoubtedly, can turn out to be effective anti-radiation agents. *The Stage of Destructive Changes*. When the apoptosis signal enters the effector caspases and leads to their activation, the process becomes irreversible (fig. 45). Effector caspases, usually located in an inactive state in the cell cytoplasm, after a cascade of activations from the regulatory caspases pass into the nucleus and attack a total of about two dozen structural and functional proteins, including signal transduction and cytoskeletal proteins. Most are split by caspases-3 and -7, and the lamin is selectively split caspase-6. Caspase splitting of the DFF450 cytoplasmic inhibitor of the apoptosis-specific endopuclease DFF40 (DNA fragmentation factor) leads to the activation of this DNAase, its transition into the nucleus, and the implementation of internucleosome fragmentation of genomic DNA.

Fig. 46 shows a diagram of the molecular and biochemical events underlying radiation apoptosis and necrosis.

An important statement of this scheme is that when exposed to ionizing radiation in a wide range of doses, molecular and biochemical mechanisms of cell response to external and internal factors retain their activity and remain able to perform their functions. Moreover, the mechanism of physiological apoptosis is preserved, and, in a certain dose range, it acts as the main molecular basis of cellular devastation, which is one of the most significant clinical manifestations of acute radiation pathology. Radiation necrosis occurs only with general or local effects in doses of an absolutely lethal range leading to significant violations of mitochondrial oxidative phosphorylation, inactiva-

tion by the action of radiation radicals and reactive oxygen species of genetic mechanisms for regulating cellular vital functions mediated by DNA and corresponding regulatory networks.

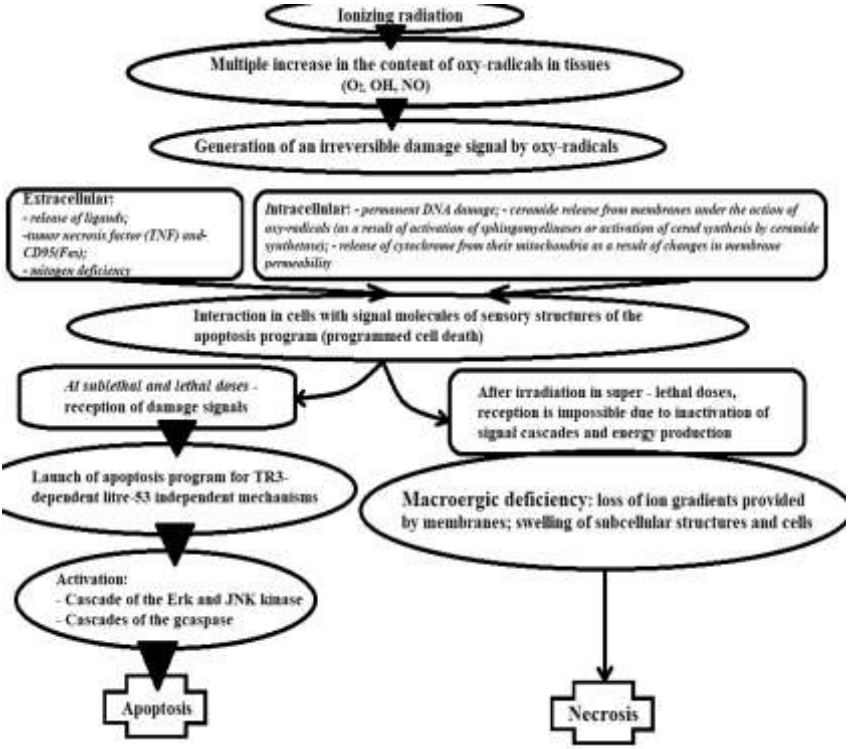


Fig. 46. General scheme of molecular events leading to the occurrence of radiation apoptosis and necrosis

In the case of necrosis, due to the loss of K +, Na + -ionic gradients, the intracellular spaces are overflowing with water, which leads to immense swelling of the cell. The strength of the plasma membrane turns out to be unable to withstand the increase in pressure of osmotic forces, and the membrane ruptures "dumping" the cellular contents from the microstructures swollen to the limit into the intercellular space. An immediate reaction from the tissue to the appearance of this material is inflammation.

Herewith, the main difference between apoptosis and necrosis is that apoptosis is a highly ordered process, implemented according to the nature-established genetic program for eliminating excess or defective cells using physiologically capable molecular structures and mechanisms. Necrosis is a consequence of the complete inactivation by the damaging agent of these structures and mechanisms in the group of cells that form the tissue site, as

a result of which the coordination of the processes of metabolism and the interaction of subcellular structures is completely lost, and the enzymatic reaction takes on the character of an indiscriminate "all against everything" attack.

5.4. The Definition of Apoptosis. The Signs of Apoptosis

Apoptosis (from Greek – apo – separation and ptosis – drop) is a programmed process of cell destruction caused by internal (intracellular) or external (extracellular) physiological and pathological factors that activate the genetic program of cell death and its removal from tissue.

Analyzing this definition, we can draw the following conclusions:

• Firstly, the process of cell death is programmed in its genetic apparatus. In other words, the cell at birth already carries the mechanisms of its death; that is, the genome of the cell contains the genes, the activation of which triggers the mechanism of its death;

• Secondly, apoptosis can be initiated both during normal physiological processes and during the development of certain pathology;

• Thirdly, the mechanism of cell death can be triggered both by factors that form in the cell itself (that is, intracellular factors) and by the signals transmitted to the cell from other cells.

Scientists who assigned the term "apoptosis" to the phenomenon of programmed cell death had in mind a certain artistic image: autumn fall of foliage. In this way, doomed and calm trees lose their foliage. Indeed, the separation of leaf cuttings from a tree branch occurs due to apoptosis of the plant cell layer. Thus, the artistic image involuntarily coincided with the essence of the physiological process.

The phenomenon of apoptosis – programmed cell death – was described by researchers much later than it was done in relation to necrosis.

So, Rudolf Virchow as early as 1859 described the histological changes that occur in dying cells.

This was a process that Virkhov called "degeneration", "necrosis", "cell death" and emphasized that these changes are typical to irreversible changes in tissues.

However, shortly after this, in 1864, the well-known zoologist and theorist of evolutionary doctrine August Weisman first described the local death of cells during metamorphosis in insects (the transformation of a larva into an adult). From a modern point of view, this description corresponds to embryonic apoptosis.

Later, a detailed description of cell death as a physiological phenomenon was given in 1885 by the German cytologist V. Fleming, who described the disintegration of ovarian epithelial cells into particles (later called apoptotic bodies) determining the process of the rapid disappearance of fragments of the cytoplasm and nucleus formed during cell decay, like chromatolysis.

The physiological death of cells in embryos in 1950 was described in detail by L. Glusman, who called it "programmed cell death." This researcher clearly understood that he was dealing with a special type of cell death but supposed that this phenomenon was inherent only in embryogenesis and was fundamentally different from cell death typical to an adult organism.

Most of the works related to the description of apoptosis of tumor cells, cells of the immune system and some other tissues date back to the end of the last century. The term “apoptosis” itself was first used in the article by three researchers; they were J. F. R. Kerr, A. H. Wyllie, A. R. Currie, who published the materials on programmed tumor cell death in the British J. of Cancer.

The significance of apoptosis and its role in physiological and pathological processes was confirmed by the awarding of the Nobel Prize in 2002 to three researchers: Sidney Brenner (S. Brenner), John Salston (J. Sulston) and Robert Horvits (R. Horvitz) for the investigations on the problem of programmed cell death. In particular, S. Brenner in the 60s of the last century discovered genes that control "life and death" of the cells in the process of their development. D. Salston first discovered and described mutations in the genes of apoptosis, and R. Horwitz found the mechanisms of the relationship between the genes involved in the process of apoptosis

Currently, biologists and pathologists distinguish two types of cell death: necrosis and apoptosis. In order to sequentially and carefully deal with the mechanisms of apoptosis, it is necessary to at least briefly describe the morphological and biochemical differences of these two processes.

The earliest sign of apoptosis detected at the electron microscope level is the sharply defined consolidations of nuclear chromatin in the form of a homogeneous mass. In addition, there is some condensation (consnolidation) of the cytoplasm. Then, the nucleus and cytoplasm break up into fragments, and cytoplasmic fragments are separated by a cytoplasmic membrane; that is, the integrity of the membrane in this case is one of the signs of apoptosis.

As a result of apoptosis, the cell turns into a set of apoptotic bodies surrounded by a membrane, where the tightly packed organelles may look intact. In some bodies there is no nuclear component, and in others there is (sometimes even several); moreover, the chromatin is always very dense, sharply defined and condensed at the nuclear membrane.

Apoptotic bodies are rapidly absorbed by neighboring cells, where they are utilized by lysosomes. The surrounding cells come closer together, so that changes in the cytoarchitectonics of tissues do not occur. There are also completely no signs of inflammation. Some apoptotic bodies (for example, in the surface epithelium) are desquamated.

In tissue culture, it was found that the process of condensation of the cytoplasm and its decay into apoptotic bodies occurs within a few minutes. In

the body, the process of apoptosis also occurs quite quickly: phagocytosis and utilization of apoptotic bodies occur within 15–120 minutes; therefore, researchers often cannot catch the apoptosis process.

Ultrastructural manifestations of necrosis significantly differ from the pattern typical to apoptosis. Mainly, they come down to wrinkling of organelles and disintegration of the cytoplasm. Although chromatin in necrotic cells, as well as in apoptosis, condenses at the nuclear membrane, its compact masses are less homogeneous and much less clearly defined at the edges of the nucleus. After the formation of these masses (*or even in parallel with this process*), cell and intracellular membranes, including lysosome membranes, are destroyed, which leads to the release of lysosomal enzymes, proteolysis and cell breakdown. At a later stage of necrosis, chromatin from the nucleus disappears; that is, karyolysis develops. Necrosis is usually accompanied by exudative inflammation and, if a large number of cells are involved in the process, it ends with the formation of a scar. In other words, unlike apoptosis, in case of necrosis, tissue cytoarchitectonics do not recover.

5.4.1. Causes of Apoptosis

Cell death in the body can occur in 2 ways: necrosis and apoptosis.

Apoptosis is a type of cell death when the cell itself is actively involved in the process of its death, i. e. cell self-destruction occurs. Apoptosis, unlike necrosis, is an active process; after exposure to etiological factors, a genetically programmed cascade of reactions is launched, which is accompanied by the activation of certain genes, the synthesis of proteins and enzymes leading to effective and rapid removal of cells from the tissue.

This phenomenon is the result of various factors leading to cell death. These factors can be non-specific, such as temperature, toxic agents, oxidizing agents, free radicals, gamma and UV radiation, bacterial toxins, etc. In all these cases, apoptosis is induced, but cell necrosis develops with an increase in the dose of the corresponding agent. Since apoptosis is a physiological phenomenon, there must be factors in the body that lead to programmed cell death. At present, it is known that these can be both intracellular signals and external ones that mediate their action through receptor systems, which are not toxic or destructive themselves.

Among physiological fatctors, hormones are of greatest interest. It is known that they can act both as inducers and inhibitors of cell death depending on the stage of its differentiation (for example, sex hormones). A central place in the study of the apoptogenic effect of hormones belongs to the study of the effect of glucocorticoids (GC) on lymphoid cells. The sensitivity of T cells to GC depends on the stage of development of the lymphocytes. Bone marrow pre-T cells and immature thymus T cells are sensitive to physiological doses of GC. Certain subpopulations of mature T-lymphocytes (*natural killer cells or cytotoxic T-*

lymphocytes) undergo apoptosis under the influence of GC. Pre-B cells and immature B cells also die. Mature B lymphocytes are not sensitive to GC.

Another physiological regulator is cytokines (an extensive group of proteins that regulate cell proliferation and differentiation upon binding to specific receptors on target cells). Cytokines are divided into three large groups (depending on the structure and function): growth factors, the tumor necrosis factor family, and helical cytokines (*interleukins and interferons*). The effect of cytokines on cells is also ambiguous: for some cells they act as an inducer, for others as an inhibitor of apoptosis. It depends on the type of a cell, the stage of its differentiation and functional state. The presence of physiological factors in the body – inducers and inhibitors of apoptosis — allows us to conclude that the programmed cell death depends on the ratio of these regulators.

All in all, the following conclusions can be drawn:

• During embryogenesis, apoptosis plays an important role in the destruction of various tissue primordia and the formation of organs.

• Aging cell; which have completed their developmental cycle, for example, lymphocytes, which have exhausted their supply of cytokines; undergo apoptosis.

• In growing tissues, a certain portion of daughter cells undergo apoptosis. The percentage of dying cells can be regulated by systemic and local hormones.

• The cause of apoptosis may be a weak effect of damaging factors, which, at a higher intensity, can lead to necrosis (hypoxia, ionizing radiation, toxins, etc.).

5.4.2. The Participation of Apoptosis in Physiological and Pathological Processes

Apoptosis is one of the fundamental processes in the life of cells of organisms located at the most different levels of evolutionary development. It is enough to indicate that the main works related to the genetics of apoptosis were carried out on roundworms – nematodes.

It was found that the genes that control apoptosis (*stimulating apoptosis and inhibiting this process*) in nematodes and humans are few, which differ from each other. That is why, the physiological processes in which apoptosis takes part are similar for most living organisms.

In describing what physiological processes do we encounter the phenomenon of apoptosis?

First of all, it is an autonomous apoptosis proceeding in the process of embryogenesis. There are three categories of autonomous embryonic apoptosis: morphogenetic, histogenetic and phylogenetic apoptosis. Due to morphogenetic apoptosis, various tissue rudiments that are not needed for the forming organism are destroyed (for *example, the destruction of cells in the interdigi-*

tal spaces). Histogenetic apoptosis promotes the differentiation of organs and tissues. This type of apoptosis, in particular, accompanies the differentiation of the genital organs from tissue primordia (for example, regression of the rudiments of the Mueller ducts in men, from which the fallopian tubes, uterus and upper part of the vagina form in women). Phylogenetic apoptosis causes the elimination of rudimentary organs and structures in the embryo (*for example, the involution of pronephros, the "primordial kidney", the paired excretory organ in lower vertebrates, which does not develop in higher vertebrates*).

Apoptosis is also physiological proceeding in slowly and rapidly proliferating cell populations.

In the first case, it means maintaining the tissue homeostasis, removing cells from the tissue that are not capable of mitosis due to their aging and "freeing up the space" in the tissue for young and actively dividing cells. For the second case, it means ensuring the differentiation and development of cellular elements of rapidly proliferating cell populations (*for example, hematopoietic tissue*).

The participation of apoptosis in physiological processes includes the so-called "hormone-dependent involution of organs and tissues." An example of this process is endometrial rejection during the menstrual cycle and the regression of the mammary gland in women after cessation of breastfeeding.

The role of apoptosis in a number of pathological processes is extremely important. Cursorily, it should be pointed out that apoptosis is typical to the following pathological processes when it can have both a sano- and pathogenetic significance:

• apoptosis of cells with DNA damage. Most often, we encounter DNA damage caused by hard radiation or prolonged exposure to ultraviolet radiation. In the event that the reparase systems of the cell are not able to "heal" the damaged DNA; the genes responsible for the initiation of apoptosis are turned on and the cell dies.

Thus, apoptosis prevents the possibility of the appearance of a clone of mutant cells, which always threatens with serious consequences for the body;

• apoptosis of tumor cells. To a certain extent, this is a special case of apoptosis of the previous type. Acquisition by cells of the property of uncontrolled reproduction without the effect of maturation as a result of exposure to viral oncoproteins, carcinogens or radiation on the genome of a cell can lead to the appearance of a clone of malignant cells, which is fraught with the development of malignant tumors;

• apoptosis of cells of ischemic organs and tissues. Ischemia of organs and tissues can lead to both necrosis and apoptosis. In the first case, a scar will form in the tissue, in the second - there will be no scar, but the number of normally functioning cells will decrease. The phenomena of apoptosis are dis-

tinctly identified in the peri-infarction zone with myocardial infarction; apoptosis is “guilty” of cardiomyocyte death in the final stages of heart failure;

• atrophy of hormone-dependent organs as a result of apoptosis with a lack *(absence*) of the corresponding regulatory hormone. In the pathology of the endocrine system, the so-called "withdrawal syndrome" is well known, which is a severe pathology associated with cell death and, as a result, the cessation of the production of corticosteroids by the adrenal glands during the cancellation of long-term therapy with corticoid drugs of some pathological processes. Another example of this process is atrophy of the prostate after castration;

• apoptosis of cells in the state of "cellular stress". Overheating of cells, exposure to cells of reactive oxygen species (oxygen radicals) can lead to the initiation of apoptosis;

• apoptosis of cells infected with viruses. This is a very important protective function of the body. The death of a virus-infected cell, on the one hand, impedes the cycle of its propagation, on the other hand, prevents tissue malignancy due to the appearance of a rapidly proliferating clone of cells mutated by the action of viral oncoproteins. It should be noted that some viruses (for example, Epstein-Barr virus), penetrating the cell, are able to synthesize proteins that inhibit apoptosis. However, some viruses (for example, the AIDS virus) can cause apoptosis of T-helpers and, thereby, lead to the development of immunodeficiency;

• apoptosis of the host cells induced by cytotoxic T-lymphocytes during transplantation of immunocompetent tissue. In immunology “the graft versus host” reaction is well known. When transplanting immunocompetent tissue (*for example, bone marrow*), immune transplant cells can destroy recipient cells. In this case, cell destruction occurs both due to damage to cells by proteolytic enzymes of T-killers and due to the induction of apoptosis in the host cells.

5.4.3. The Role of Enhancing or Attenuating of Apoptosis in the Development of Pathological Processes

Both strengthening and weakening of apoptosis can play an almost decisive role in the development of many pathological processes. An abnormal increase in apoptosis during fetal development can lead to a “minus tissue” effect, which is often lethal and ends in fetal death.

The excessive apoptosis of cardiomyocytes in Down disease can lead to the development of cardiomyopathy.

Many types of pathology of the blood system are also explained by an increase in the level of apoptosis of hematopoietic progenitor cells. As a result, diseases such as severe combined immunodeficiencies, aplastic anemia, pancytopenia develop. Most often, this pathology is the result of insuffi-

cient production of the so-called "survival factors", for example, interleukin 7 (IL-7), which is a cytokine that inhibits apoptosis of stem and other progenitor cells.

The excessive apoptosis plays a leading role in the development of neurodegenerative processes (*Alzheimer's disease, Parkinson's disease and others).*

The excessive apoptosis of T-helpers in AIDS is the main pathogenetic mechanism of this immunodeficiency. On the other hand, the excessive apoptosis of cells infected with viruses or damaged by microbial toxins plays a positive role, interrupting the progression of viral and microbial infections.

Cytotoxic therapy (*the use of cytostatics and radiation therapy*), causing damage to the DNA of malignant cells, on the one hand, blocks their mitotic cycle, and, on the other hand, induces apoptosis.

The weakening of apoptosis can also contribute to the development of pathological processes. First of all, this situation well demonstrates the phenomenon of weakening apoptosis in cancer. Malignant tumors are the most active and rapidly developing ones; in their development, due to their characteristics, the apoptosis of tumor cells is inhibited. When a tumor develops, there is a kind of competition between two processes: the development of apoptosis and the multiplication of tumor cells. If the degree of apoptosis of malignant cells is high, their clone does not form and the tumor does not develop. If the growth rate of tumor cells overtakes apoptosis, a malignant neoplasm occurs in the body.

The increased production of factors inhibiting apoptosis in the cells of the immune system, as well as the formation of extracellular factors that block apoptosis (for example, the appearance of soluble receptors of certain cytokines that can induce apoptosis) can lead to the development of a number of autoimmune processes up to the manifestation of systemic autoimmune pathology (for example, systemic lupus erythematosus).

Some of these phenomena, which demonstrate the development of pathological processes associated with both an increase and a decrease in apoptosis, will be discussed in more detail in the following sections.

5.4.4. Pro- And Anti-Apoptotic Cell Factors

We have already seen that during a number of pathological processes in the body, both the acceleration and attenuation of apoptosis can have a cardinal effect. Substances involved in the regulation of apoptosis, as a rule, are proteins, and their synthesis is controlled by the corresponding genes.

The same genes that regulate the level of apoptosis can be found in living beings of various stages of evolution. Apoptosis inhibiting genes include the Bcl-2, Ced-9, BHRF1, and MCL-1 genes. However, the genes that synthesize proteins and stimulate apoptosis have been described (p53, Bax, bcl-xS). It should be noted that pro-antiapoptotic proteins are able to combine with

each other forming homo- and heterodimers. For example, when a Bcl-2 protein apoptosis inhibitor is combined with a Bax apoptosis activator protein, the outcome (*inhibition or activation of apoptosis*) will be determined by which protein will prevail in this combination.

Further, for greater clarity and some simplification of the considered mechanisms and schemes of apoptosis, only p53 protein will be indicated as a factor stimulating apoptosis and Bcl-2 protein as the main factor inhibiting apoptosis

It has already been mentioned above that apoptosis is induced in cells having unrepaired DNA damage. In this case, the destruction of the cell prevents the appearance of clones of mutant cells; their existence can lead to very serious consequences (*for example, the development of a malignant tumor*).

Unrepaired DNA damage (Fig. 42) leads to the activation of two genes: p21 and p53. The production of p21 protein of the same gene provides the blockade of the mitotic cycle (a mutant cell should not produce similar mutant cells).

It is known that the cell (mitotic) cycle begins with the G_1 phase - preparation for DNA synthesis. It is followed by the phases S – the phase of DNA synthesis and phase G_2 – postsynthetic. The cycle of cell mitosis ends.

Two other points in the life of the cell that entered the mitotic cycle are also very important. These are the so-called “checkpoints”: at the boundary of the G_1 / S phase and at the boundary of the G_2 / mitosis phase. At the level of checkpoints, the integrity of DNA, the absence of its mutations and deletions are checked. In the cells with the damaged DNA, the cell cycle is blocked, and the cell enters the apoptosis stage (fig. 47).

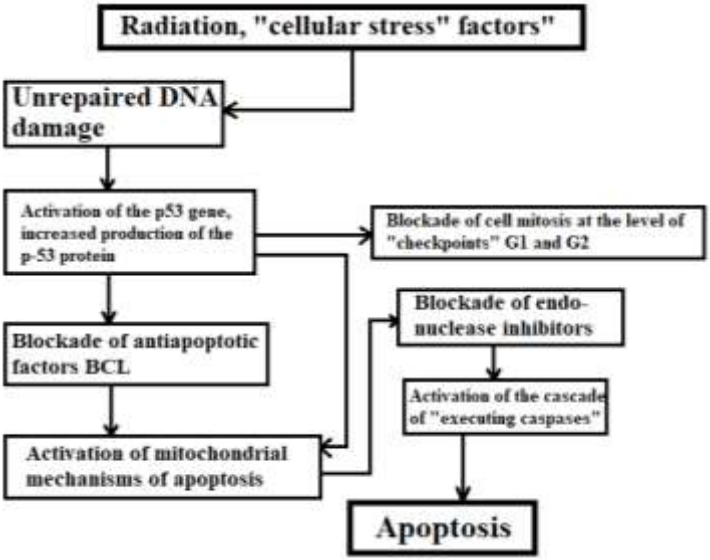


Fig. 47. The Mechanism of Apoptosis Induced by Intracellular Factors

Activation of the p53 gene and the synthesis of the same protein trigger the apoptosis mechanism. At the same time, p53 protein, on the one hand, blocks the antiapoptotic mechanisms of the Bcl-2 protein embedded in mitochondrial membranes, and, on the other hand, it opens the pores of the mitochondria and allows the cells that activate intracellular proteases, the so-called "executing caspases", to enter the cell protoplasm (the mitochondrial mechanism of apoptosis and the role of caspases in this process will be discussed later).

Active caspases cause proteolysis of nuclear proteins, activate endonucleases, and provide proteolysis of cytoplasmic proteins. Ultimately, this leads to fragmentation of the cell nucleus, fragmentation of the cytoplasm and the formation of apoptotic bodies. Apoptosis is complete.

5.4.5. "Instructive" Apoptosis

The apoptosis process can also be initiated by external signals, which one cell transmits to another. In this case, the role of signaling molecules belongs to certain cytokines (tumor necrosis factor alpha – TNFa, tumor necrosis factor beta, also known as lymphotoxin – TNFb, nerve growth factor, and some others).

The importance of this type of apoptosis, which was called "instructive", is significant for normal activity, especially the immune system (*antitumor immunity, protection of natural antigens, for example, testes or the lens of the eye from the body's immunocytes, etc.).*

The developmental pattern of "instructive" apoptosis is presented in fig. 48.

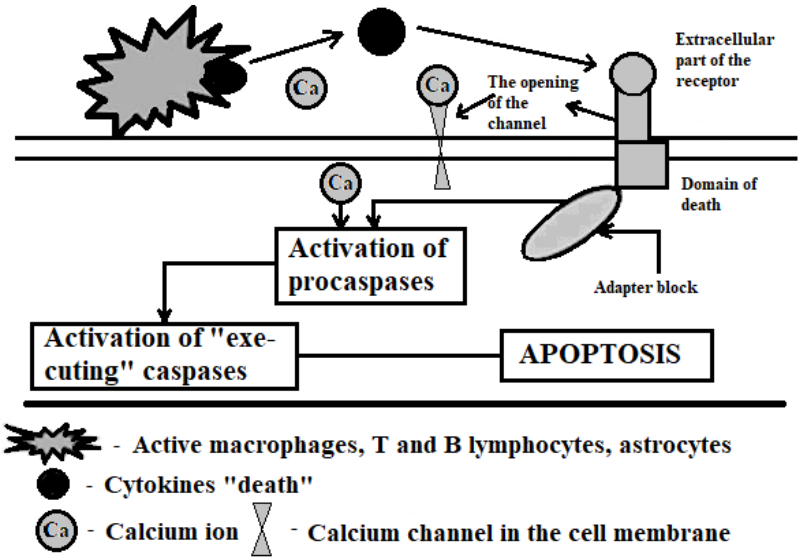


Fig. 48. The Pattern of Development of "Instructive" Apoptosis

An interesting story is the discovery of the TNFa cytokine. At the end of the 18th century, doctors noticed that in some patients, malignant tumors disappeared after they had passed an infectious disease. At the beginning of the twentieth century, the American physician W. Coley tried to treat cancer patients by administering drugs obtained by filtering cultures of gram-positive and gram-negative bacteria. In some cases, that therapy was successful. Then, in the middle of the twentieth century after the discovery of lipopolysaccharide – a substance that is part of the membranes of microbial cells – it was shown that this substance is able to induce tumor necrosis. However, in 1975, thanks to the works of L. Old and his colleagues, it became clear that tumor necrosis was not caused by lipopolysacchride itself, but by a certain protein factor that was produced by macrophages when they came in contact with bacteria. This protein factor is called the "tumor necrosis factor." By the end of the twentieth century, it became clear that TNF is produced not only by activated macrophages, but also by T-lymphocytes, neutrophils, mast cells, astrocytes and natural killer cells (NK cells). It has now been determined that TNF is able to induce apoptosis in a wide variety of cell structures including tumor cells. In addition, as a pro-inflammatory cytokine, TNF is also able to cause cell necrosis as a result of their death in the hotbed.

An important element of the mechanism of "instructive" apoptosis is cell receptors that can bind to these inflammatory cytokines (fig. 49).

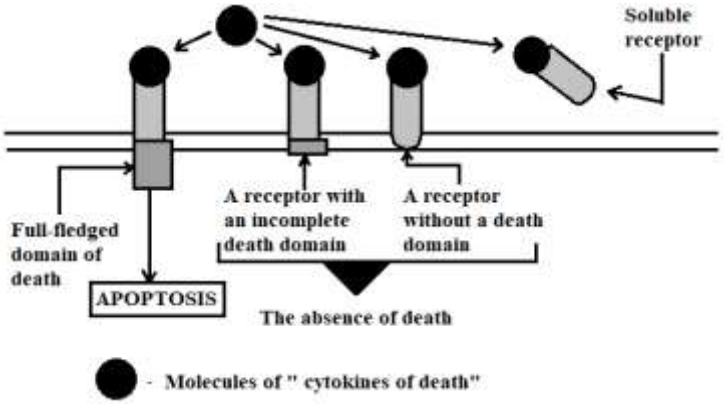


Fig. 49. Cell Defense Mechanisms Against Apoptosis ("Decoy Receptors")

An important element of the mechanism of "instructive" apoptosis is a cell receptor that can bind to these cytokines (Fig. 49). These receptors (protein macromolecules) belong to the superfamily of tumor necrosis factor receptors alpha and, due to their special function, are called "death receptors" (Death Receptors). The intracytoplasmic part of these receptors is called the "death domains" (fig. 49). After connecting of their ligands (TNFa, TNFb, and others) with these receptors, the activated death domain, through a complex enzyme system (adapter protein), carries out autocatalytic processing of prokases, which, in turn, activate the kinases that make up the "execution kinases" cascade. Their enzymatic effect carries out the apoptosis according to the already known pattern.

It should be known that a certain role in the activation of the kinase cascade is played by Ca^{2+} ions, which penetrate the cell through calcium channels, the opening of which is also facilitated by the activation of death receptors.

Cells are able not only to comply with the cell death ligands, but also to defend themselves from their effects. Such protection can be carried out in two ways (fig. 49). First, cells are capable of synthesizing inferior death receptors that are either devoid of a death domain or have an inferior death domain. In both cases, the connection of TNF with the death receptor does not lead to the realization of apoptosis since the effect of the ligand on the receptor is not transmitted to the apoptosis executive unit. Secondly, the cell is able to "desquam" the extracellular part of the receptors, which at the same time become the so-called "soluble receptors". TNF molecules appearing in the intercellular space are firmly connected with them and can no longer impact the real cellular death receptors.

5.4.6. Embryonic Apoptosis

In the process of embryo development, apoptosis can serve both a positive and a negative purpose. The triggering factors for apoptosis of embryonic cells in

most cases are the deficiency of apoptosis inhibiting factors in the intercellular medium, lack of growth factors, or inability of embryonic cells to perceive the effects of these factors, as well as deprivation of embryonic cells of adhesion substrate (fig. 50). Apoptosis of nerve cells can also be induced if they do not form or lose synaptic connections with their neighbors. By the way, the latter mechanism works not only in the embryonic nervous system but also in an adult body.

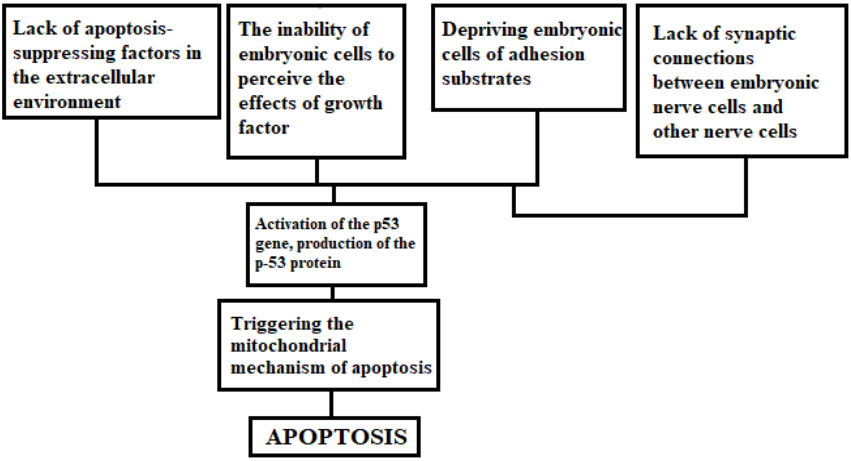


Fig. 50. The Scheme of Embryonic Apoptosis

5.4.7. Apoptosis of Aging Cells

Currently, it is known that somatic cells are fully differentiated cells that are capable of a limited number of divisions, that is, they comply with the so-called "Hayflick limit" (*named after the scientist L. Hayflick, who first described this phenomenon).*

The limitation in the number of divisions of completely differentiated cells is explained by the fact that the chromosomes of such cells have specialized structures at their ends – telomeres (*single-stranded DNA*), which, with each division, is shortened by 300 to 400 nucleotides. Since telomeres play a crucial role in the stabilization of chromosomes during replication, their absence stops mitosis at points G_1 and G_2. An exception to this rule is the so-called "immortal" cells, which include germ cells, stem totipotent cells, as well as cancer cells that can divide an unlimited number of times. This phenomenon was explained by the experiments of two scientists – Grieder and Blackburn, who in 1985 isolated the telomerase enzyme from such cells, which can compensate for the shortening of chromosomes by completing nucleotides (*telomeres*) in them. Telomerase is a ribonucleoprotein complex containing a matrix for the synthesis of telomeric DNA repeats. In other words, telomerase is a kind of reverse transcriptase.

However, cell division arrest is an alarming signal for genetic programs responsible for cell safety. It has been previously said that in a cell that has received a certain damage, genes are activated (p21, p53), which block mitosis in the "checkpoints" G_1 and G_2. The stop of mitosis in cells that have reached the Hayflick limit, according ro the feedback principle, causes the activation of the p53 gene and the production of the p53 protein that induces apoptosis. An aging cell ceases to exist (fig. 51).

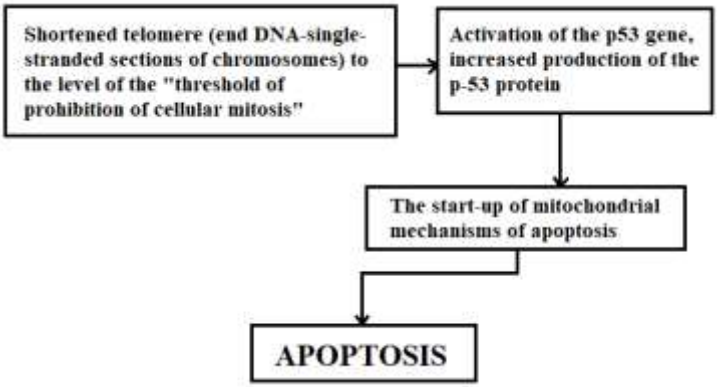


Fig. 51. Apoptosis of aging cells

5.4.8. Malignant Tumors and Apoptosis

When studying the problems of carcinogenesis, it was noted that one of the most effective methods of controlling the body against malignancy of cells is their apoptosis. If the immune mechanisms of the fight against the cells of malignant tumors are turned on only when abnormal mutant cells have already appeared in the body, the apoptotic mechanism responds to the possibility of malignancy of the cell even at the moment when the primary DNA damage is detected.

In this case, the prerequisite for the activation of apoptosis mechanisms is the absence of the effect of activity of different systems that tried to "heal" DNA damage. Unrepaired DNA damage due to the mechanisms that are yet poorly understood ensures the inclusion and activation of the tumor suppressor p53 gene. The increased production of p53 protein causes a number of successive events:

- activation of the p21 gene and production of the p21 protein, which blocks the mitotic cycle at the levels of G_1 and G_2;
- blocking of anti-apoptotic factors (in particular, Bcl-2 protein and some others);
- triggering the mitochondrial apoptosis mechanism;
- the increased synthesis of "death receptors" of the cell;

• completion of apoptosis due to the activation of the cascade of "executing caspases" (fig. 52).

• This is how the events develop if the development of apoptosis is ahead of the proliferation rate of malignant cells. However, if anti-apoptotic mechanisms preserve the life of a mutant cell, if it manages to give rise to a clone of its descendants, the tumor grows rapidly with all consequences of this process.

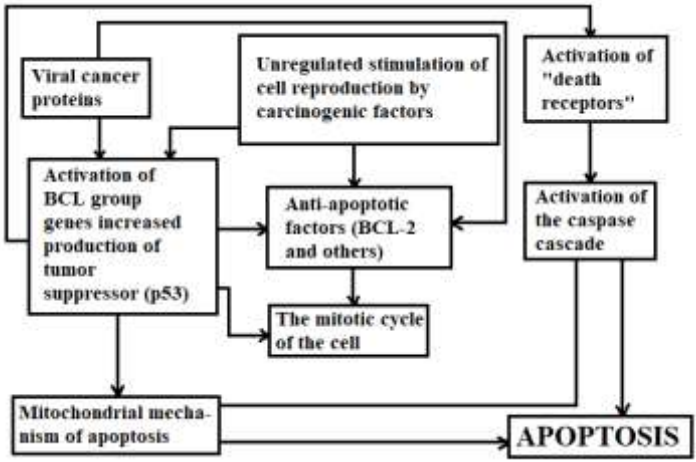


Fig. 52. The role of the p53 tumor suppressor in the fight against cell malignancy

5.4.9. Radiotherapy and Apoptosis

Apoptosis is found in a tumor both before and after radiation treatment. Apoptosis is characterized by the damage to individual cells, the absence of an inflammatory reaction in the tissues, rapid phagocytosis and digestion of dead cells. Changes in cells during apoptosis consist in the marginalization of chromatin and its breakdown into fragments, condensation of the cytoplasm, cell division into parts with separation of the latter in the form of apoptotic bodies. It is believed that during apoptosis, activation of self-destruction mechanisms occurs instead of degeneration processes, as it occurs in necrosis.

The factors causing cell apoptosis and its mechanisms are being investigated, but it has been established that radiation, hyperthermia and chemotherapy of tumors accelerate the process. Therapeutic effects change the histophysiology of the tumor, proliferation (*division*), differentiation and growth of cells, their coombination and movement, the intensity of specific functioning (*if it is preserved*), the ability to cellular and tissue regeneration, sensitivity to environmental influences, etc. The proliferative ability of tumor cells is not only a target for finding new medicinal chemotherapeutic agents and improving the known methods of treating malignant neoplasms, but it is also reliable

direct morphological criterion of treatment efficacy, as well as one of the leading factors of pathomorphism.

As it is known considering proliferative ability, tumor and normal tissue consist of two subpopulations of cells: proliferating (*growth fraction, proliferative pool*) and resting. The latter is formed by the cells that temporarily leave the cell cycle and retain the ability to divide (*phase G_0*), as well as non-proliferating cells. Ionizing radiation, especially chemotherapeutic drugs, acts mainly on the growth fraction, i. e., on all rapidly growing tumors in which most of the cells are in a state of proliferation (*for example, leukemia, lymphosarcoma, uterine chorionepithelioma, Ewing sarcoma*) and are highly sensitive to chemotherapy and radiation exposure.

Stage of initiation of apoptosis. Numerous studies of the cell cycle in tumors are of interest from two points of view. First, cell cycle information is necessary to understand the nature of tumor growth. Secondly, the development of optimal radiation and chemotherapy models for tumors is associated with a detailed knowledge of the laws of cell reproduction in tumors. The main method for determining the parameters of the cell cycle is the analysis of changes in the percentage of labeled mitoses after administration of H-thymidine.

Radioautography, like no other method, "revived" morphology and contributed to the transformation of the latter from formally dynamic as it has been so far into truly dynamic. But, at the same time, a serious drawback of radioautography recorded at the level of light microscopy is that it relates the centers of intensive synthesis of a substance entirely to the nucleus or to the cytoplasm and not to those organelles in which they are actually carried out. In addition, modern technical capabilities limit the use of the method in the clinic due to its duration, the need for repeated biopsies and the possibility of the harmful effect of the isotope on the patient. Despite the limitations of the method, it was possible to obtain the data on the duration of different phases of the cell cycle in some human tumors.

The method of incubation of biopsy and surgical material in an environment with H-thymidine in vitro is of great practical importance. This method determines the label index equal to the ratio of labeled cells to the total number of cells multiplied by 100. Data from different authors for various tumors and even for different sites the same neoplasms often differ significantly, which is associated largely with the errors of the method. Therefore, to obtain reliable results, it is necessary to examine several pieces of tissue. The method of autoradiography in vitro has been successfully used to control tumor treatment.

There are 2 examples. The mitotic regime of rectal adenocarcinomas with various methods of preoperative irradiation varies as follows. In unirradiated, highly differentiated adenocarcinoma, the mitoti c index was 25.6 %, in moderately differentiated – 35.2 %. The prevalence of metaphases (78.9 %

in highly differentiated and 70.2 % in moderately differentiated adenocarcinoma) over other phases of mitosis was determined, which is typical to human tumors. Pathological mitoses accounted for 29.3% in highly differentiated and 23.2 % in moderately differentiated adenocarcinomas (*lag of chromosomes in the metaphase, scattering of chromosomes, a three-group metaphase, etc.*). After irradiation, there is a significant decrease in the level of mitotic activity and an increase in the number of pathological mitoses. In a highly differentiated adenocarcinoma after irradiation with a dose of 20 Gy, the mitotic index is reduced to 15 %, and the proportion of pathological mitoses is increased to 86.7 %; in moderately differentiated adenocarcinoma, up to 29.4 % and 61.2%, respectively. At a dose of 40 Gy, mitotic activity in a highly differentiated adenocarcinoma is 46 %, in a moderately differentiated adenocarcinoma – 7.2 %, and the proportion of pathological mitoses increased to 91.3 % and 100 %, respectively. Especially sharp changes in the proliferative ability of cells were observed with a combination of radiation and chemotherapy.

The mitotic regimen of laryngeal cancer was studied during radiation treatment using the radiosensitizer of metronidazole. Radiation changes were characterized by a decrease in the cell population due to necrosis and apoptosis, a decrease in the proliferative pool of cells, pathological mitoses, and inhibition of DNA synthesis. So, before treatment, the label index was 6.05 (*non-keratinizing cancer 7.33; keratinizing cancer 5.0*), after treatment – 2.50 (P> 0.001) (*non-keratinizing cancer 3.24, P> 0.01; keratinizing cancer – 1.60, P> 0.001*).

When comparing different total doses, there were no significant differences in the magnitude of the label index (*20 Gy – 2.70; 32 Gy – 2.35).*

It should be noted that the reliability of the measurement results in one patient *(different parts of the center and periphery of the tumor before and after radiation therapy*) and the groups of patients did not always coincide. For example, a low index could be seen in keratinizing cancer before treatment and in both histological types of cancer after treatment. Therefore, the problem of the significance of proliferation parameters for assessing the radiosensitivity of a tumor cannot be related to a finally solved one.

The effect of synchronization of the mitotic activity of tumor cells, which was used in the practice of radiation and chemotherapy should be mentioned. The essence of the effect is that with the help of a drug (*for example, 5-fluorouracil*), the cells are blocked at a certain phase of the cell cycle, and after drug withdrawal, all of them synchronously move to the next phase of the cycle. Knowing the time taken by the cells to pass this interval, it is possible to expose the tumor to radiation or polychemotherapy in one of the most sensitive phases of the cycle. Thus, in the complex treatment of patients with colorectal cancer using 5-fluorouracil, deep and widespread damage to cancer cells and tumor structures, up to their complete destruction, were found in the tumor. The wide practical use of the effect of synchronization of the mitotic activity of tumor cells is

difficult due to the need for a long (15–20 h) administration of 5-fluorouracil to the patient, drug toxicity, lack of reliable control of changes in the temporal parameters of the cell cycle and the presence of resting cells in the tumor.

Part 6. CELL SURVIVAL CURVES

6.1. Reproductive and Interphase Cell Death Curves

The frequency of radio-induced chromosomal aberrations is specific to a strict dependence on the dose, power and nature of ionizing radiation, which allowed to create cytogenetic methods of biological dosimetry.

The mechanism of occurrence of chromosomal rearrangements remains far from clear. The frequency of chromosomal rearrangements depends on external agents (ionizing radiation, chemicals) and the physiological state of the body.

In the animal body, the cells of certain tissues (hematopoietic, genitals, or intestinal mucosa) actively divide, reproducing their own kind; the cells of other tissues (kidneys, liver, heart, muscles, neurons, etc.) rarely divide or do not divide at all. Accordingly, two types of cell death are distinguished: *reproductive and interphase*.

Reproductive death is a violation of the ability of dividing cells to unlimited reproduction: after 1–2 divisions the defective cell progenies die. During interphase death, soon after irradiation, the irradiated cells die. For all dividing and most non-dividing cells, the interphase death occurs only at doses of hundreds of Gy. The exception is lymphocytes and germ cells at some stages of their development; they die during the interphase already at doses of several tens of Gy.

The causes and patterns of reproductive and interphase death are different. The reproductive death is the most studied . It occurs as a result of the damage to the DNA molecule resulting in the breakdown of one or both of its strands, which prevents the further reproduction of normal cells.

The dependence of the proportion of cells that retained their reproductive ability after irradiation at a dose of D has the form:

$$N(D)/N(0) = \exp(-SD) = \exp(-D/D_0).$$

Here, $N(0)$ and $N(D)$ is a number of cells before and after irradiation; the value $S = 1/D_0$ defines cell radiosensitivity, D_0 is the dose reducing the number of surviving cells by e times. For most dividing cells $D_0 = (1{,}2 \div 2{,}0)$ Гр. Often the exponential part of the dose curve is preceded by the part of the curve with a smaller slope (fig. 53)

The radiosensitivity of dividing cells depends on many factors and can be artificially increased (sensibilization) or reduced (protection). Accordingly, D_0 decreases or increases.

The most effective natural sensibilizer is oxygen. In its absence, the damage to various biological objects (macromolecules, cells, or organisms in general), as a rule, is weakened. In this case, D_0 for cells increases by 3 times. With increasing linear ionization density, the radiosensitivity of cells and tissues increases.

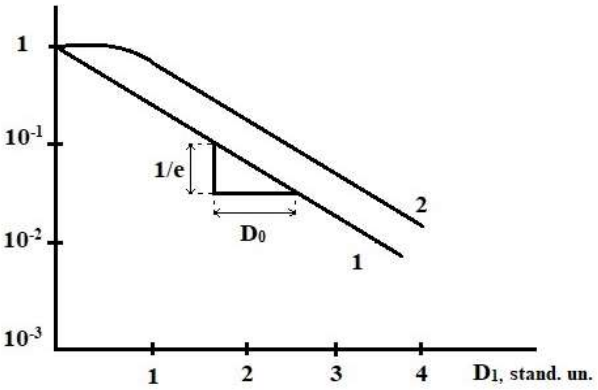


Fig. 53. The dependence of reproductive cell death on dose D; along the ordinate axis, there is the proportion of cells that have retained reproductive ability; 1, 2 are different forms of dose curves

DNA damage causing reproductive death of a cell is not fatal for it due to the existence of powerful recovery (repair) systems. Part of the primary damage resulting from ionization is repaired by chemical reducing agents that are found in the cell.

The main reducing agent is the amino acid glutathione. It competes with intracellular oxygen, fixing the primary damage, and prevents their recovery. Damages that persist after this physico-chemical stage of repair are effectively eliminated by enzyme systems that specifically repair various types of genetic damage.

The final damaging effect of radiation is due to the unrepaired part of the primary DNA damage. Their fraction under ordinary conditions is small, which determines the relative stability of living cells to the action of ionizing radiation. The possibility of increasing radiosensitivity, artificially suppressing the ability of dividing cells to repair or reducing their radiosensitivity, creating the conditions for better repair of potentially damaged DNA, is also associated with this.

The mechanism of interphase cell death has been studied less, and the reason for the sharp difference in the radiosensitivity of lymphocytes from other types of cells is also unclear. In contrast to reproductive death, changes leading to interphase death are observed in all cells, and not the proportion of dead cells, but the average time of death of the entire population changes with the radiation dose (fig. 54). The reason for the differences, apparently, is that the interphase death is caused by the damage not to the unique structure of DNA but to membranes and other multiple cell structures.

The radiation death of the whole organism of mammals is associated with the devastation of populations of dividing cells and tissues of the so-

called critical organs that are necessary for life. Such organs are hematopoietic and digestive. In the blood-forming organs (bone marrow, spleen) and the small intestine, there are actively dividing cells, which are the stem cells for all functioning blood cells and small intestine cells responsible for the absorption of nutrients. Reproductive death of stem cells, reducing their number below a critical level compatible with life, leads to the death of the body.

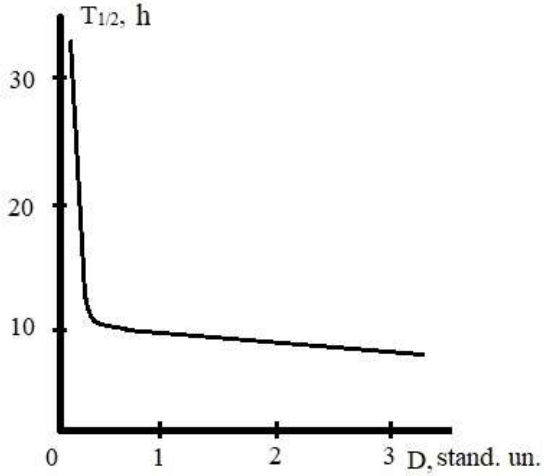


Fig. 54. Dose-dependent interphase death of lymphocytes; the time of death of half the irradiated cells ($T_{1/2}$) is along the ordinate axis

Fig. 55 shows the dose-response curve of mammalian survival rate under irradiation of the whole organism. The mortality dose of 50 % of individuals in the population (LD_{50}) is different for mammals of different species, but the shape of the dose curve and the causes of death are the same.

At doses of the order of LD_{50}, the blood formation system is critical for the body; at high doses – the mucous membrane of the small intestine. In the first case, some animals die within 10–14 days, in the second one – 4–7 days after irradiation.

At D> 1 Gy up to the absolute lethal dose, surviving individuals exhibit radiation sickness of various severity.

There are a number of preventive measures to protect the body from radiation. Two classes of chemical protective substances (radioprotectors) are the most effective when they are administered 10–15 minutes before irradiation. These are the compounds containing thiols and indolyl-alkylamines. The former, like intracellular glutathione, contribute to the physico-chemical repair of primary lesions, competing with oxygen and, apparently, contributing to enzymatic repair. The second ones constrict the blood vessels, and, thereby,

also weaken the damaging effect of oxygen in the irradiated cells of critical organs.

In some cases, it is necessary to increase the radiosensitivity of cells, for example, with radiotherapy of tumors. Sensibilizers can be the so-called electron-acceptor compounds, the role of which is similar to the action of oxygen, but they penetrate deeper into the tumor.

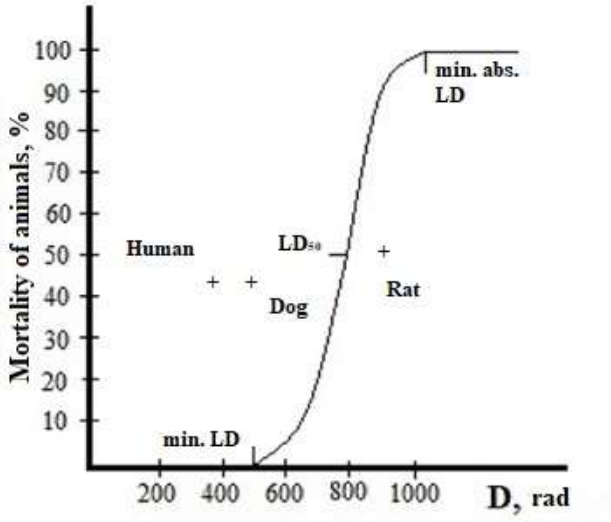


Fig. 55. Dose curve of mammalian death

6.2. Energy Metabolism and Radiation Damage to Cells

Energy support of cell activity is realized in **mitochondria** that are specialized organoids for extracting energy from food substrates and transforming it into macroergic ATP bonds. Being self-renewing structures with a relatively short half-life (in rat hepatocytes, it is about 10 days), they have their own DNA and ribosomes to ensure self-reproduction. Mitochondrial DNA contains the genetic code for 25–125 mitochondrial oxidative proteins and, as a result, these organoids in the cell are genetically relatively autonomous.

The number and shape of mitochondria depends on the activity and type of cells. Somatic mammalian cells contain from 500 to 1000 of these organelles, localized near the sites of energy expenditure or storage: along contractile structures, in invaginations of basement membranes to ensure ion transport against concentration gradients, in the region of neuron synapses, etc. Constructed according to a unified plan, in different cells they have a rod-shaped, dumbbell-shaped, filiform or round shape and sizes from 1 to 10 microns. The outer surface of mitochondria is formed of two membrane layers

that form the intermembrane space. The inner membrane forms outgrowths (cristae), the intramitochondrial matrix contains DNA, ribosomes and inclusions. Carbohydrate, fatty and lipid substrates are converted by the Krebs citric acid cycle enzymes and fatty acid oxidation (located only in the mitochondrial matrix) to NADH2, succinate, acetyl coenzyme A, β-glycerophosphate, which are oxidizable substrates. Electrons are transferred from oxidized substrates to oxygen sequentially in a chain: flavoproteins I and II, ubiquinone, cytochromes b, c_1, c, a, b, a_3 in accordance with the oxidation potential (fig. 56).

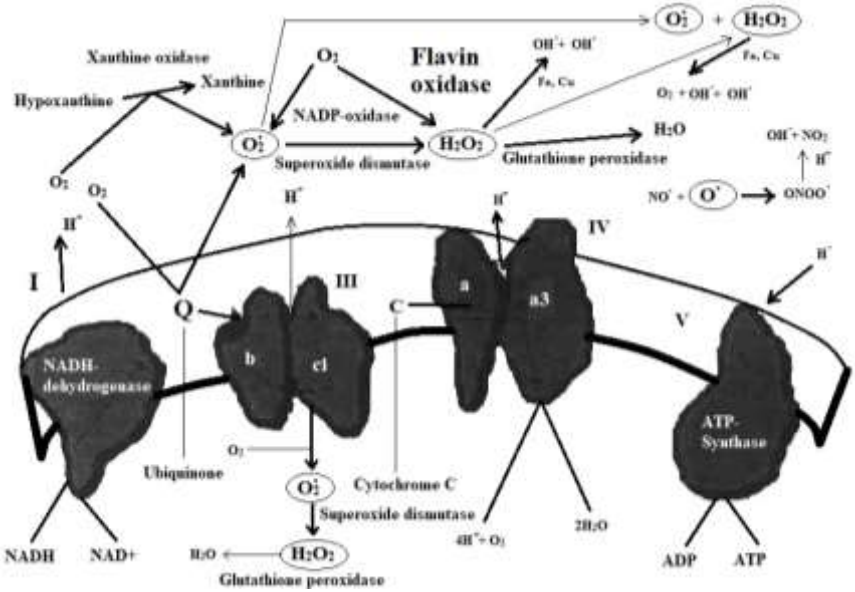


Fig. 56. The scheme of metabolic generation of reactive oxygen species in a cell

In the process of transition of electrons in the respiratory chain from a high energy level to a lower one, oxidative phosphorylation is carried out ; the oxidation energy is cumulated in 3 ATP molecules. The electron transfer and phosphorylation chain enzymes are located predominantly in the inner membrane. The combination of oxidation and phosphorylation is due to the activitu of the ATP-synthetase complex (proton-translocating ATPhase) (also located in the inner membrane of the mitochondria) by converting the chemical oxidation energy into electrical energy (proton gradient), and then back into chemical energy of macroergic bonds.

Along with the generation of ATP, mitochondria are involved in the metabolic production of reactive oxygen species.

Based on the concepts of the key role of energy exchange in the life of living systems from the cellular to the organism level and observing radiation-

induced changes in phosphorylation in the subcellular structures of the organs of animals and humans after irradiation in a wide range of doses up to the minimum absolutely lethal, some researchers have found a direct indication on the leading role of bioenergetic disturbance in the development of radiobiological effects and with sufficient certainty have argued that one of the primary mechanisms of the biological effect of ionizing radiation is the suppression of bioenergy processes.

However, the observed changes, which were taken for radiation disturbances in ATP generation systems in tissues (they turned out to be an artifact), contradicted the resistance to ionizing radiation at a dose of 8 Gy of energy-dependent lipid synthesis in hepatocytes (activation of cholesterol biosynthesis after 5 minutes and phospholipids – after 40–60 min up to 48 hours), the formation of milk proteins in the lactating mammary gland (50 Gy), maintaining the mobility of mature sperm (dose of 200 Gy). This series of evidence of the preservation or even activation after irradiation of the functional activity of plasma structures in cells that are resistant to radiation was continued by establishing the ability of endocrine system cells to provide the production of pituitary hormones and adrenal glands in the phenomenon of post-radiation hypercorticism, liver cells in the synthesis of seromucoids and other acute phase proteins radiation damage, in the formation of glycogen by gluconeogenesis from protein degradation products of dying cells of other organs, on end, mechanisms for the implementation of radiation effects in radiosensitive tissues, such as cell cycle arrest, DNA repair, apoptosis, which occur with high ATP consumption with mandatory replenishment due to phosphorylation processes. All these data indicate a different mechanism of radiation injury formation other than a violation of bioenergy.

6.3. Regulatory Networks of Cell Response to Damaging Effects

In the last one and a half decade, with the discovery and study of molecular and biochemical mechanisms of regulation of the cell response to various damaging effects, the response manifested by changes in growth, development (differentiation), proliferation or apoptosis, there is a significant improvement and extending the ideas about the real mechanisms of the formation of the radiation effect.

It has been established that there is a system for conducting signals from receptors (sensory link) to the genetic apparatus of the cell, which is formed by the cascades of mitogen-activated protein kinases (MAPKs). Each of the cascades is a module of three types of protein (peptide) protein kinase molecules: MAPKK (MAPK kinase kinase) -> MAPKK (MAPK kinase) -> MAPK. Sequential kinase phosphorylation of the upper level of the underlying enzyme ensures its activation and signal transmission to include cellular programs for differentiation, movement, division or cell death by apoptosis; 3

different kinase cascades composed of enzymes differing in molecular structure were found in mammals. A cellular response consisting in a change in proliferation, differentiation and development (aging) is provided by a MAPK called ERKl / 2 kinase (extracellular signal-regulated kinase 1 and 2); the response, manifested by inflammation, apoptosis, developmental change, is provided by two types of MAPK: JNK / SAPK (c-Jun N-terminal kinase / stress activated protein kinase - terminal cell kinases / stress-activated protein kinases) and p38 MAPK. Thus, different cell responses to certain stimuli are provided by the corresponding kinase modules, i. e. implemented through various channels. Moreover, the "set" of modules in different types of cells can be different.

At least three effector transcription systems are included in the implementation of the cell response to disturbing effects with the participation of the above modules: a system integrated by the p53 protein, a family of transcription factors of the nuclear factor kappa B (NF-kB) involved in the regulation of acute phase and surface protein genes cell receptors, and cytokines and protein activator of transcription AP-1.

Recent data show that, interacting with cells, ionizing radiation initiates compensatory activation of the mentioned MAPK signaling pathways similarly to other damaging agents. Emerging biosignals play a critical role in controlling cell survival and repopulation after irradiation and remain dependent on the type of cell. Some of the signaling pathways activated by irradiation are those that are normally mitogen-dependent (for example, the ERK kinase pathway). Other MAPK-signaling pathways excited by radiation (JNK and p38 MAPK), as well as signals coming from DNA damage, include mechanisms below the death receptors and procaspases. The expression and release of ligands of autocrine factors such as transforming growth factor a and tumor necrosis factor can enhance the responses of MAPK signaling pathways in irradiated cells and involve neighboring unirradiated cells in the radiation reaction.

6.4. Biological Membranes and the Radiobiological Role of the Prooxidant and Antioxidant System of the Cell

Biological membranes, being the main component of most organoids, create a dense network of compartments with a huge total surface area throughout the entire cell volume. In combination with the fluidity of the lipid component, this makes inevitable the interaction of membrane structures with the products of radiolysis of water, participation in the formation of radiation effects. Simultaneously, in many sections of the membranes, radiation initiates a chain autocatalytic reaction of lipid peroxidation. As a result, upon irradiation in sufficiently high doses, the membranes and associated enzyme ensembles degrade, and enzymes are released from their specific locations with a shift in metabolism towards ca-

tabolism. But the main consequence is the generation of signals for MAPK kinase cascades. These signals may include growth factor (s), inflammatory cytokines, stress factors, in particular, ROS – O_2:, $H_2.O_2$, OH·, NO·.

The intensification of free radical oxidation of lipids accelerates their circulation in membranes with the elimination of easily oxidized fractions; the lipid component of the membranes is enriched with oxidation-resistant molecules. As a result, the intensity of oxidative processes in lipids decreases and returns to normal, meaning the cell transition to a new metabolic state. Thus, oxidative transformations in lipids are an important component of the system of regulation of the functional activity of membranes, ensuring the initiation of adaptive reactions in the cell.

From the scheme given above (Fig. 51) it is obvious that a normal cell constantly produces ROS, nitric oxide NO- and its derivative, peroxynitrite. They appear in the process of oxidative metabolism with the participation of xanthine oxidase, NADH oxidase, aldehyde oxidase, flavin-containing hydrolases, cyclo- and lipoxygenases, as well as NO synthases, to ensure normal functioning.

In blood cells of the phagocytic type, O_2: and OH radicals perform the function of protecting the body from foreign genetic aggression. However, their production in any other cells indicates that the formation of such radicals is not a metabolic "noise" but has a positive biological function of genome protection similar to that realized by phagocytes. The generation of radical forms of oxygen activates tyrosine protein kinases and neutralizes the aggression against cellular organelles of foreign genetic material by inactivating it and prevents the accumulation of intrinsic macromolecules in the cell that have become foreign due to spontaneous structural changes.

The constant metabolic production of oxyradicals in the cell, reaching 10^{10} free radicals per cell per day, could pose a potential danger to its life by being able to damage the membrane and genetic structures of the nucleus and mitochondria, especially during stress exposure when this production increases significantly. However, the occurrence of oxidative catastrophe in the cells is prevented by the detailed system of antioxidant protection. This system is formed from enzymes (superoxide dismutase, which ensures the O_2 dismutation in the presence of water:,: into hydrogen peroxide; catalase and glucate peroxidase – an Se-containing enzyme that decomposes hydrogen peroxide to water, other enzymes), ceruloplasmin protein, which binds excess transition metals, and low molecular weight natural antioxidants – ubiquinones, glutathione, ascorbic acid, a-tocopherol. It protects the biomembranes of organoids from damage by radicals. The antioxidant barrier (in combination with activation of DNA repair, apoptosis, and immune mechanisms of elimination of mutated cells) under normal life conditions is practically insurmountable for free radicals. In those cases when the ROS content exceeds physiological values, their signal function is realized in the activation of regulatory networks of cell response to damaging effects.

There is about 500 direct experimental evidence for this fact. However, under the action of ionizing radiation, the superoxide anion radical O_2: hydrogen peroxide H_2O_2, nitric oxide NO perform both a signal function and the role of factors damaging DNA and individual links of regulatory cascades.

The signal function of ROS when changing their content in the cell consists in the activation of kinase cascades: ERK, which leads to the release of the nuclear factor Kappa B (NF-kV), and JNK, which causes the expression of the tumor suppressor protein p53 gene (which controls the cell cycle reconciliation points, repair DNA and the launch of the apoptosis program), as well as the *PIC 1–14* genes (p53-induced gene 1–14) involved in the maintenance of redox homeostasis in the cell, in particular, mitochondial protein encoding ferredoxin, PIG3 is the cytoplasmic homologous oxidoreduction pelvis, NADH-quinone oxidoreductase 1, PIG8 protein, modifying apoptosis in tumor cells.

Non-radiation stressful effects also cause an increase in ROS production in the cells, often ten-fold. In turn, radiation from a natural radiation background at a dose of 0.1 Gy / year leads to an increase in mutations by 10^{-7} per cell per day. But the realization of the damaging effect of ROS, produced metabolically under non-radiation influences and arising in radiation-chemical reactions, has certain differences.

In the first case, ROS arise in the structures of the cytoplasm and, migrating, have a damaging effect both on nearby membrane structures and on organoids in general, including the nucleus, when the antioxidant defense opposing them is ineffective due to the exhaustion of its total capacity in this part of the cell. This leads to local disorganization of cellular metabolism and can cause damage to the genetic material, membrane degradation, chromosome aberrations and gene mutations. However, no matter how wide the "breakthrough" of such radicals is, there is never a burst in cell death by apoptosis and the subsequent clinical evidence of radiation sickness.

The reason for the fundamental differences between the action of metabolic radicals and ionizing radiation is that the anti-oxidative defense is always a barrier to the exchange of radicals (formed in the cytoplasm) to the cell organoids. Under the action of ionizing radiation, free radicals, including ROS, arise in the entire irradiated volume of the cell both in organoids and in the cytoplasm. At the time of irradiation, more than half of the radiation-induced ROS is beyond the antioxidant barrier and, being shorter than the metabolic ROS from critical targets, interacts with the most radiosensitive biomolecules, managing to realize a damaging effect in organoids even before it reacts with the antioxidant system protection. Therefore, even against the background of activation of signaling MAPK cascades, which organize the response of cells to damage, the observed radiobiological effect turns out to be dependent on the radiation dose.

6.5. Molecular Mechanisms of Hypersensitivity to Radiation

Hypersensitivity to low doses of radiation means a reduced level of cell death in culture compared with the predicted level that can be supposed based upon the linear-quadratic model. The subsequent return to linear-quadratic values or their excess corresponds to the range of the increased radio resistance. Thus, the phenomenon of hypersensitivity means that small values of the absorbed dose of ionizing radiation can lead to a greater effect than higher values of the absorbed dose.

Hypersensitivity is observed in tumor cells irradiated *in vitro* and in some normal tissues when irradiated *in vivo* (fig. 57). Indeed, cell lines of almost all human tumors show hypersensitivity to radiation that, as a rule, occurs at doses less than 50 cGy. At the same time, they retain the ability to p53-induced apoptosis, with which hypersensitivity is apparently associated. In the range of 50–100 cGy, on the contrary, there is an increase in radio stability in comparison with the calculated values corresponding to the linear-quadratic model. Over 100 cGy, cell survival is adequately described by the linear-quadratic equation. Thus, at present, hypersensitivity is postulated as a "default reaction" for most types of cells at a dose of less than 20–30 cGy and is detected for qualitatively different types of irradiation.

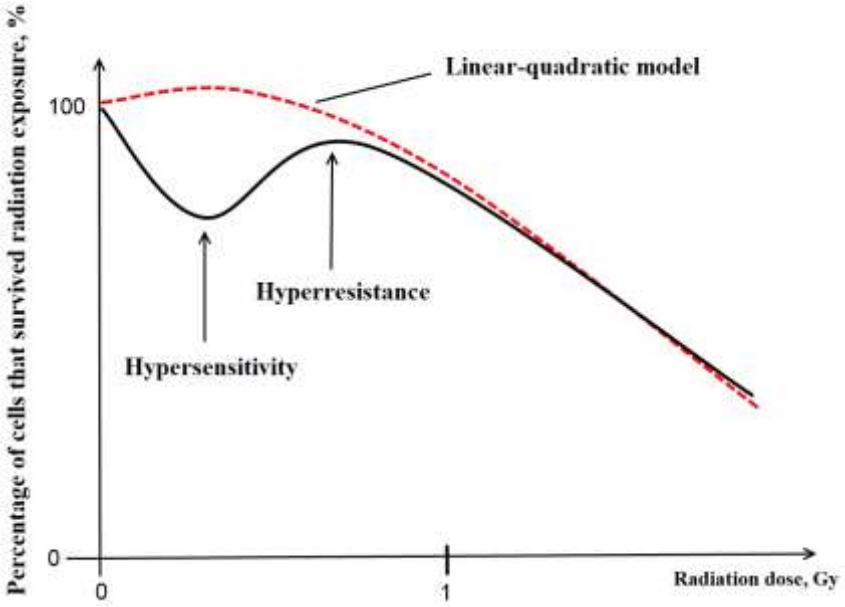


Fig. 57. The dependence of the survival rate (%) of cells in culture on the dose of ionizing radiation (Gy)

Hypersensitivity can be explained by the presence of a subpopulation of cells having the increased sensitivity due to a certain stage of the cell cycle or genetic predisposition. In turn, the resistance increases with the dose increase, as the defense mechanisms such as inducing DNA repair or suppressing apoptosis begin to be more effectively induced.

It was shown that hypersensitivity with the subsequent radioresistance is manifested only in cell subpopulations located at the stage of the G_2 cell cycle during irradiation, while the clonal survival of the subpopulations at S and G_1 stages corresponds to a linear-quadratic model.

It is assumed that as a result of activation of the check point G_2 of the cell cycle, DNA repair is activated, but only if a certain threshold dose is exceeded. Below this dose, the cells in stage G_2 will go into mitosis with unrepaired potentially fatal DNA damage. Thus, the radiosensitivity of cells in this case is not associated with the initial number of double-stranded breaks and not even with repair kinetics, but with the number of residual, unrepaired double-stranded breaks.

So, in the case of artificial suppression of proliferation of cell culture for some time after irradiation, clonal survival is increased. This may be due to the fact that it takes time to induce the effective repair of damage.

Pretreatment of the protein biosynthesis inhibitor with cycloheximide prior to irradiation leads to hypersensitivity without subsequent increased radioresistance. On the contrary, sensitivity decreases or disappears during pretreatment of the H_2O_2 cell culture. In accordance with the concept of the threshold for repair, pre-irradiation in doses of 20 or 100 cGy (several hours before the main exposure) relieves hypersensitivity, while a dose of 5 cGy is ineffective. Thus, the relationship between hypersensitivity and adaptive response is obvious: an adaptive response can be considered as a suppression of hypersensitivity. At the same time, if cycloheximide is present both at the stage of H_2O_2 pretreatment and during irradiation, the adaptive response disappears, but hypersensitivity and the subsequent increased radioresistance take place. It is possible that the adaptive response is a consequence of hypersensitivity – the death at the sensitive stage G_2 of those damaged cells in which cell cycle delay was not induced to repair their DNA. When restoring the ability of protein synthesis and resuming the cell cycle, cultures again show hypersensitivity and induced radioresistance.

Obviously, with an increase in the dose of radiation, a change in hypersensitivity to radioresistance is a consequence of the activation of the "inducible" response mechanism (C_2/M cell cycle checkpoint). Two different G_2/ M cell cycle checkpoints were detected. There is an ATM-independent postpone delay in the G_2/M stage, which is observed only a few hours after irradiation and is dose dependent. Another check point G_2/M is activated immediately after irradiation and is not induced at doses less than 30 cGy. In addition, it is ATM dependent. Hypersensitivity is apparently associated with the inability

to induce precisely the early G_2/M response. The relationship of hypersensitivity and DNA repair is supported by numerous facts.

Hypersensitivity also occurs with instability of the terminal sections of chromosomes - telomeres. Congenital dyskeratosis in humans, which a disease associated with a decrease in the level of telomerase activity in germ and stem cells, leads to accelerated shortening of telomeres and hypersensitivity to AI. Mice whose telomeres are 40 % shorter than normal are hypersensitive to γ radiation due to the induced disturbances in the gastrointestinal tract, lymphoid organs and kidneys. They have an increased level of chromosomal damage and apoptosis compared with irradiated wild-type individuals. Thus, telomere function is one of the determinants of the radiosensitivity of the body.

So why did the DNA repair system that have a threshold of damage appear? Possibly, it can be explained that this system appeared in order to ensure the effective elimination of the few cells damaged by small doses of AI, thus reducing the risk of mutations during the repair itself (by the type of nonhomologous reunification of the ends of the chromosomes) and the probability of survival of the unrepaired cells.

In general, low-dose hypersensitivity reflects the differences in DNA signaling and repairing at very low doses compared to medium or large. In this regard, it is interesting to compare this phenomenon with other effects of small doses. There is a close relationship between hypersensitivity and the adaptive response. It should also be noted that intercellular contacts have an important part in hypersensitivity to small doses of radiation, which brings this phenomenon closer to the bystander effect. At the same time, like other effects of small doses of radiation, hypersensitivity is not always manifested (table 12).

Table 12

The mechanisms of hypersensitivity and the increased radioresistance of cells

Dose, cGy	The Effect	The Mechanism
0.01–30	Hypersensitivity	single unrepaired double-stranded DNA breaks causing mitotic cell death and / or p53-dependent apoptosis
30–100	Increased Radio Resistance	the induction of an early ATM dependent G_2 / M cell cycle checkpoint
>100	Linear-Quadratic Dose-Response Relationship	cell death is directly proportional to the dose of radiation (damage)

Thus, according to modern concepts, when irradiation in doses does not exceed 30 cGy, there is an increased frequency of cell death (hypersensitivity) associated with the formation of unrepaired double-stranded DNA breaks. With an increase in doses in the range of 30–100 cGy, the increased radioresistance of cells is observed. A further increase in dose leads to effects that are fully consistent with the linear-quadratic survival model.

However, the following fact remains unclear: how the hypersensitivity phenomenon found in cultures of most types of cells is associated with integral indicators of the viability of organisms, for which hypersensitivity to small doses of radiation is also detected.

Part 7. Radiobiology of Normal Tissue Damage

7.1. The Basics of Specific and Individual Radiosensitivity

Soon after the discovery of the biological effect of ionizing radiation, it was found that the radiation doses leading to death for different objects of living nature vary within very wide limits. Lymphocytes, for example, die at a dose of several tens of rad, and unicellular organisms withstand radiation in doses of the order of hundreds of thousands of rad. It can be stated that each biological object has its own measure of sensitivity to the action of ionizing radiation, which in radiobiology is called *radiosensitivity.*

An example of extremely low radiosensitivity is bacteria found in the channel of a nuclear reactor, where the dose rate reaches 10^7 rad per day. They are able to live and reproduce under these conditions, so they are called micrococcus radioresistant (Micrococcus radiodurens). However for most other microorganisms, their semi-lethal doses are in the range of 10–45 thousand rad. So, Micrococcus sodensis is about 25 times more radiosensitive. For E. coli the semi-lethal dose is 50 Gy, while for ciliates and amoebas this indicator is 3000–5000 Gy. These examples show that there are significant differences in radiosensitivity among a variety of protozoa.

In addition, even in one organism, various *cells* and *tissues* differ greatly in radiosensitivity, and, along with sensitive (hematopoietic system and epithelium of the small intestine mucosa) there are stable (muscle, nervous and bone) tissues, which are commonly called *radioresistant.*

Today, the problem of radiosensitivity is crucial in radiobiology since knowledge of the nature of the differences in natural radiosensitivity and radioresistance and the mechanisms of its regulation is not only of theoretical general biological significance, but also promises important practical results. First of all, it is important for artificial control of radiation reactions of tissues, i.e. the possibility of their weakening, when it comes to protecting the body, or, conversely, selective (local) enhancement when irradiating malignant tumors.

When comparing radiosensitivity, it is imperative to use adequate methods and criteria.

At first glance, it seems that radiosensitivity can be characterized by any detected reaction to radiation, regardless of its value for the viability of a particular object. Then a comparison of various objects should be made according to the degree of manifestation of this reaction. Meanwhile, many radiation reactions are strictly specific for certain objects (in particular, for certain tissues and systems), and are absent in others.

For example, such a universal response of cells to radiation as delayed division is easily detected in actively proliferating tissues, but for obvious reasons it cannot be detected in tissues where cell division is weak or absent.

A variety of functional reactions, which are a manifestation of the highly differentiated properties of individual tissues, organs and systems, cannot serve as comparative indicators of radiosensitivity. These include the activation and inhibition of specific metabolism, the production of certain enzymes, hormones and other substances.

A mandatory requirement for the criterion is its strict quantitative dependence on the radiation dose. Either *the degree of survival* of the studied objects as a result of irradiation in certain doses or quantitative indicators of damage that are uniquely associated with a certain relationship with survival in this dose range are usually used as the most integral criterion for radiosensitivity.

Most often, quantitative quantities such as LD_{50} or LD_{100} are used for this purpose, which characterize the radiation dose causing death of 50 % or 100 % of objects, respectively, at different times after irradiation depending on the type of irradiated organisms. The LD_{50} values, as it will be considered below, in nature differ quite significantly not only between individual taxonomic series, but also within them and even within the same species.

There is a lot of data indicating a wide range of differences of *species* in radiosensitivity in the natural environment (table 13).

Table 13

Range of differences in dose sensitivity to γ-radiation (LD_{50}) in the natural environment

№	Object	$ЛД_{50}$, Гр
1	a sheep	1,5–2,5
2	a dog	2,5–3,0
3	a human	2,5–3,5
4	mice	6,5–15,0
5	rats	7,0–9,0
6	birds	8,0–20,0
7	fish	8,0–20,0
8	insects	10.0–100.0
9	plants	10.0–1500.0

Thus, the term **"radiosensitivity"** means the reciprocal of the ratio of the doses of ionizing radiation causing quantitatively equal effects of the same type in the compared biological systems.

As it can be seen from fig. 58, there is a certain pattern of changes in radiosensitivity when comparing different taxonomic groups of organisms: warm-blooded> cold-blooded> invertebrates> protozoa. As it can be seen

from fig. 58, the radiosensitivity increases with the complexity of the organization of biological objects.

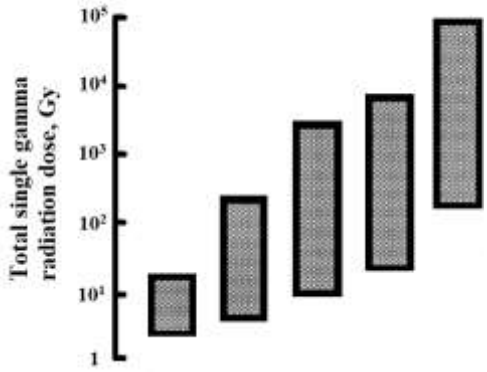


Fig. 58. Estimated values of the range of radiosensitivity (LD_{100}) in organisms belonging to different phylogenetic groups: 1 − mammals; 2 − other vertebrates; 3 − invertebrates; 4 − plants; 5 – microorganisms

The observed significant differences in radiosensitivity among representatives of various taxonomic groups are due not to the physical features of the absorption of energy of ionizing radiation, but primarily to the biological characteristics of objects, such as:

1) the nature of their structural and functional organization;
2) the differences in adaptive and regenerative capabilities;
3) the specificity of the initial reactions of primary radiation damage;
4) the intensity of metabolic and proliferative processes, etc.

In addition, the degree of radiosensitivity can vary significantly within the same species; that is, there is such a thing as *individual r*adiosensitivity of organisms. Moreover, for a given individual, it depends on the *age, gender, physiological state of the biological object*, and a number of other factors. Young and old organisms are more radiosensitive than mature ones (fig. 59). Within the same organism, cells and tissues vary greatly in their radiosensitivity. Therefore, in order to correctly assess the effects of irradiation of the body, it is necessary to analyze the consequences of its irradiation at various levels: cellular, tissue, organ, systemic, and organismic.

As early as 1906, Bergonier and Tribondo showed that the radiosensitivity of cells is directly proportional to their proliferative activity and inversely proportional to the degree of differentiation of its constituent cells. This can explain the increased radiosensitivity of young individuals whose cells actively divide in the process of growth and development of the body. It's interestingly that this general biological pattern is observed not only in mammals, but

also in other organisms (fig. 60), which is used for practical purposes, for example, in the fight against destructive insects.

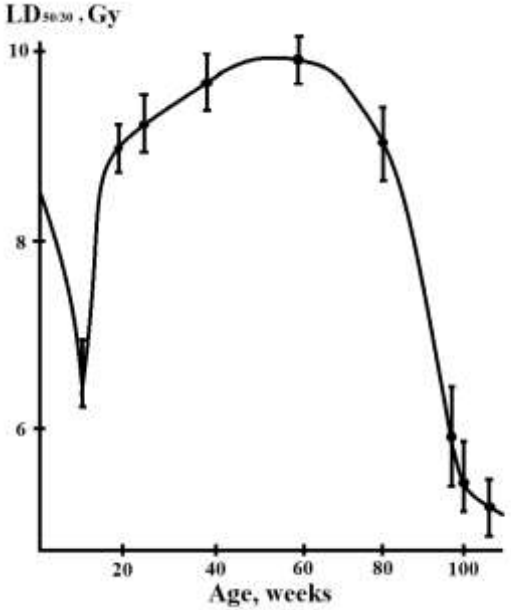


Fig. 59. The values of the lethal dose $LD_{50/30}$ for mice of different ages

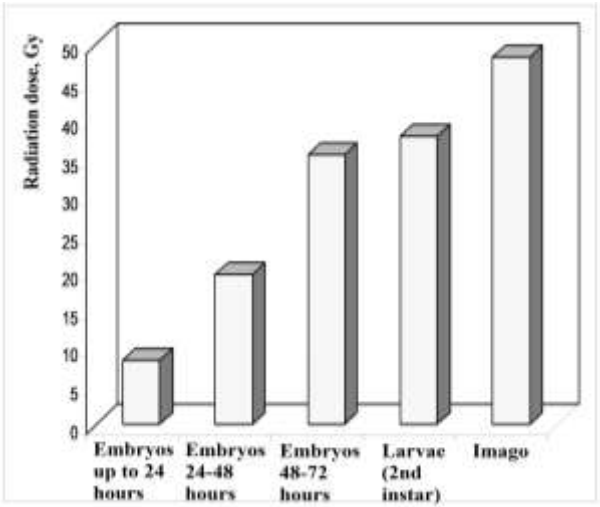


Fig. 60. LD_{50} values of acute γ-exposure for the Colorado potato beetle at different periods of development

The physiological (e. g., sleep, vigor, fatigue, or pregnancy) or pathophysiological state of the body (chronic diseases or burns) will also affect the individual radiosensitivity of the body.

In addition, men have greater radiosensitivity compared to the female body.

At the tissue level, the Bergonier-Tribondo rule is also applied. In the body, the most radiosensitive ones will be tissues that have a reserve of actively multiplying poorly differentiated cells, for example, hematopoietic tissue, gonads, and small intestine epithelium. The least radiosensitive and the most radioresistant ones are highly specialized low-renewing tissues, for example, muscle, bone, and nervous.

At the organ level, radiosensitivity depends not only on the radiosensitivity of the tissues that constitute the organ, but also on its functions.

7.2. Radiation Reactions of Individual Organs and Tissues Testis

They constantly reproduce spermatogonia, which have a high radiosensitivity, and spermatozoa (mature cells) are more radioresistant. At radiation doses higher than 0.15 Gy (0.4 Gy / year), cellular devastation occurs. When irradiated in doses of 3.5–6.0 Gy (2 Gy / year), permanent sterility occurs.

Ovaries

The ovaries of an adult woman contain a population of irreplaceable oocytes (their formation ends early after birth). The impact of a single exposure at a dose of 1–2 Gy on both ovaries causes *temporary infertility* and the cessation of menstruation for 1–3 years.

With acute exposure in the range of 2.5–6 Gy, *persistent infertility* develops. This is due to the fact that the formation of female germ cells ends in the early stages after birth, and in the adult state the ovaries are not capable of active regeneration. Therefore, if irradiation causes the death of all potential eggs, the fertility is lost irreversibly.

Visual organ

Two types of eye lesions are possible: inflammatory processes in the conjunctiva and sclera (at doses of 3–8 Gy) and cataract (at doses of 3–10 Gy). In humans, cataracts appear when irradiated at a dose of 5–6 Gy. Neutron radiation is the most dangerous one.

Digestive organs

The small intestine has the greatest radiosensitivity. Further, according to the degree of radiosensitivity decrease; then the oral cavity, tongue, salivary glands, esophagus, stomach, rectum and colon, pancreas, and liver follow.

The cardiovascular system

In vessels, the outer layer of the vascular wall has greater radiosensitivity due to the high content of collagen.

The heart is considered to be a radioresistant organ; however, with local exposure in doses of 5–10 Gy, myocardial changes can be detected. At a dose of 20 Gy the endocardial damage is noted.

Excretory organs (kidneys)

The kidneys are quite radioresistant. However, irradiation of the kidneys in doses of more than 30 Gy during 5 weeks can lead to the development of chronic nephritis (this can be a limiting factor when conducting radiation therapy of tumors of the abdominal organs).

7.3. Cellular Radiosensitivity

Cells are the basic blocks of life where the initial effects of radiation exposure are formed, leading to lesions, which are later manifested at higher levels of biological organization: tissue, organ, systemic, and organismic. Therefore, in radiobiology, special attention is paid to the processes that develop after irradiation in the cells.

In a living cell, metabolism with the external environment between individual intracellular structures is constantly carried out. Molecular damage that has occurred in cells at the initial stages of the action of ionizing radiation changes the course of metabolic processes involving damaged structures. Since the localization and nature of the primary lesions in one or another molecular structure of the cell are largely probabilistic, the metabolic changes associated with them are very diverse.

Violation of metabolic processes, in turn, leads to an increase in the severity of molecular damage in the cell. This phenomenon is called the biological amplification of primary radiation damage. However, along with this, repair processes develop in the cell resulting in the complete or partial restoration of structures and functions.

The cellular radiobiological phenomenon has been analyzed since the discovery of x-rays and radioactivity. During this period, extensive experimental material on the nature of morphological and biochemical changes in the irradiated cell has been accumulated, the kinetic laws of the development of radiation damage have been studied, and a quantitative analysis of cell death in the irradiated population has been carried out. Currently, intensive research is being conducted on the analysis of the primary physicochemical processes occurring in the irradiated cell.

It has been shown that at the cellular level, radiosensitivity depends on the following factors:

1. The activity of reparative systems.
2. The level of sulfyhydryl groups.
3. The level of radioprotectors.
4. The level of antioxidant systems, etc.

The task of modern radiation biology is to study the central link, i. e., the sequence of processes that occur from the moment of the occurrence of a few initial injuries to the occurrence of the tested biological effects including cell death. For this, a wide arsenal of physicochemical, biophysical and biochemical methods of analysis is used, and the original approaches based on fundamental physical concepts about the interaction of photons with matter and various model systems: isolated molecules, various multicomponent systems, subcellular organoids, cultured cells of various tissues are considered.

7.4. Molecular and Biochemical Basis of the Most Significant Radiation and Medical Effects

Radiation and medical effects are radiation effects that cause human health disorders. According to pathogenetic criteria, they are divided into somatic and genetic, and according to the time of manifestation after irradiation they are immediate, delayed and long-term. According to modern concepts, the immediate consequences are acute and chronic radiation sickness and radiation death of the body, as well as embryotoxic and teratogenic effects as a result of intrauterine irradiation. Radiation sickness refers to the somatic effects of a non-stochastic (deterministic) nature. Such effects occur under the action of radiation in a certain dose for each irradiated individual. They are characterized by a dependence of the severity of the manifestation on the radiation dose. Unlike stochastic effects, for which any increment of the dose over the natural background radiation can increase the probability of their occurrence, deterministic effects become recorded only above a certain dose level, i. e. have a threshold due to the presence of mechanisms for the elimination of radiation damage at the molecular and cellular levels, regeneration and compensation – at the organ and organismic levels.

The long-term effects are manifested in the recovery from the early clinical manifestations of acute lesion – atrophy and sclerosis of organs exposed to acute radiation in significant doses. The long-term effects of radiation include clinical manifestations of radiation carcinogenesis and radiation aging. In this case, radiation carcinogenesis is considered to be a genetic effect having a stochastic (probabilistic) nature since the dependence of its manifestation on the radiation dose does not exist for the severity of the process but for the probability of occurrence in each individual. In other words, at the level of an individual bioobject (cell, organism), it is typical for manifestation according to the principle of “yes-no” (“all or nothing”) and the absence of

a threshold on the graph of the dependence of the manifestation frequency on the radiation dose.

Among the genetic consequences of the action of radiation there are the mutations, which include the already mentioned gene (point) mutations and chromosomal aberrations in somatic and germ cells. These changes in the genetic material of somatic cells are reliably associated with the radiation dose only when it exceeds 0.1 Gy; they do not show any determined clinical damage, and so far they are considered to be the basis for biodosimetry in radiation accidents. Their occurrence in germ cells, according to the data on survivors of the atomic bombing in Japan, after a single exposure even in moderate doses, causes negligible harmful effects on the health of subsequent generations. These consequences are hardly detected from the "noise" of naturally occurring mutational effects using the most sophisticated epidemiological techniques of the last five decades. Experimental data on animals are more distinct and indicate the appearance of disturbances in the development of offspring of the 1st and 2nd generations related to the geender of the irradiated parents.

The phenomenon of radiation sickness is considered as radiation-induced thrombocyte, lymphocyte, and leukocytopathies with the pronounced immunodeficiency, homeostasis disorders and a possible outcome in a lethal effect, the probability of which increases with increasing radiation dose from sublethal to completely lethal. Its cellular basis is a violation of the kinetics of cell populations. This disorder is manifested by cellular depletion of critical organs (bone marrow, small intestinal mucosa) due to radiation-induced death of some precursor cells including stem cells, natural death of mature cells and impaired proliferation of the remaining ones, and is replaced by repopulation of organs as a result of proliferation of surviving stem cells, committing of early progenitors mature cells in the directions of differentiation and reproduction of cell clones.

Part 8. Non-Target Effects

8.1. Radiation-Induced Genomic Instability

It was initially postulated that the cell inherits only those changes that were formed in the DNA before entering the first mitosis after irradiation. The researchers proceeded from the fact that DNA damage is fixed in the mutation during the first round of DNA replication after irradiation or due to the erroneous repair of the initial radiation-induced damage. However, subsequently, in experiments on cell cultures *in vitro*, evidence began to accumulate about the occurrence of an unstable state of the genome in the descendants of irradiated cells that were not subjected to direct irradiation. Moreover, it was proved that, in a series of mitoses, the spectrum of chromosomal aberrations, for example, can change not only quantitatively (due to the elimination of coarse genetic anomalies due to death of carriers), but also qualitatively – a number of mutations occurred *de novo* without additional mutational effects. The confirmation of the fact that the total number of mutations remains significantly increased compared with the control level was common to all studies.

Today, this phenomenon is known as radiation-induced genetic instability. It is characterized not only by an overall increased level of mutations, but also by destabilization of chromosomes, cell death and a high risk of tumor transformation. Both rare-ionizing and densely-ionizing radiation, i. e. from a biological point of view, this phenomenon is quite universal.

Genetic instability can be considered as one of the delayed effects of radiation exposure, which has been recorded in the descendants of irradiated cells for many generations. Considering the previously mentioned Hayflick phenomenon, it can be stated that for multicellular organisms such an "imprint" can remain throughout life, i. e. in individuals affected by the effects of IR; biological effects of this kind can persist (and be manifested) throughout the remaining period of life. Usually, the induction of genomic instability takes place as a result of acute irradiation in doses of 2–12 Gy. However, there is the evidence of the induction of genetic instability and radiation in small (up to 0.1 Gy) doses.

Genetic instability can be fixed not only in *in vitro* experiments, but also *in vivo*. Thus, irradiation of mouse embryos with x-rays or neutrons at the zygote stage or two blastomeres leads to an increase in the frequency of aberrations in the first, second and third mitoses following the irradiation. Irradiation of adult animals induces the prolonged genetic instability in the epithelial cells of the mammary gland. But it is after all well known that the consequence of genetic instability *in vivo* is an increased risk of tumor transformation of somatic cells.

Despite the fact that genetic instability is one of the most intensively studied delayed effects of exposure, to date there is no single explanation of the causes of its occurrence. It is assumed that it is a universal reaction of the genome to any adverse effect that violates the integrity of the genome. The

following are the best-studied mechanisms of induced genetic instability to date:

1) the dysfunction of metabolic biochemical processes in the mitochondrion leading to a permanent increase in the level of reactive oxygen species;

2) the major changes in the structural organization of chromatin, both mutational and epigenomic in nature;

3) the violation of the function of telomeres or their complete loss and the formation of so-called "sticky" ends;

4) the genetic changes due to the insertion of genes of retroviruses;

5) the activity of mobile genetic elements.

As a group of American researchers led by V. F. Morgan managed to find out, there is strong evidence of the participation of free radicals in the formation of radiation-induced genetic instability.

First, the increased levels of reactive oxygen species are often observed in genetically unstable clones. Secondly, the treatment of cells with free radical interceptors reduces the frequency of induction of genomic instability after irradiation. Apparently, an increase in the level of ROS in carrier cells of genomic instability occurs due to their enhanced production in mitochondria as well as due to a decrease in the activity of superoxide dismutase that are responsible for their inactivation.

It is believed that physiological mitochondrial dysfunction in genetically unstable cells may be the main cause of chronic oxidative stress, which is a factor in the destabilization of the genome. However, this mechanism, which quite reasonably explains the fact of genomic instability in a single somatic cell, does not explain the existence of the fact of hereditary transmission of an unstable phenotype to the offspring of unirradiated cells.

It is well known that not only IR can induce genetic instability, but also many other DNA-damaging factors. The fact that radiation-induced genetic instability is manifested over a long period after irradiation suggests a mechanism by which the original DNA damage is "remembered" by the surviving cells.

It is suggested that improper repair of radiation-induced double-stranded DNA breaks can lead to the formation of non-lethal but potentially unstable chromosome regions inherited for many generations after irradiation. These unstable sites can be activated and manifest in the form of time-delayed DNA damage. This hypothesis has received experimental confirmation. In particular, it was proved that the presence of large deletions representing potentially unstable regions at the locus of the HPRT gene encoding the enzyme hypoxanthine-guanine phosphoribosyltransferase, X chromosomes contributes to a high level of induction of delayed chromosomal instability.

It is possible that potentially unstable regions appearing in the same chromosome can have a destabilizing effect on the genome as a whole. This assumption seems quite logical because a violation of the integrity of the ge-

nome, which has evolved over the course of millennia and allowed the genome to work as a single mechanism, is violated when such sites appear, and the formation of a new stable state is accompanied by multiple transitional forms, which manifest themselves in a number of "unstable" generations. So, for example, in experiments on Drosophila, an increase in the level of induction of recessive lethal mutations in unirradiated chromosomes of ovules fertilized by irradiated sperm was found. To test the role of a single chromosome in the formation of genetic instability, the irradiated human chromosome was transplanted into an unirradiated mouse cell. It was shown that the irradiated chromosome *per se* is unstable even in an unirradiated environment. To determine the trigger for the delayed chromosomal instability, the authors analyzed chromosomal aberrations and found that the irradiated chromosome has an increased frequency of fusion of telomeric ends. Hence, there is the assumption that irradiation does not directly affect telomeres but mediates their dysfunction, which acts as a trigger for delayed instability.

The mechanisms of an epigenetic nature are likely to be involved in the formation of genomic instability. This, in particular, is confirmed by the fact that for more than 20 passages after irradiation, methylation of CpG islands in human keratinocytes of the HPV-G line remains unregulated.

The phenomenon of radiation-induced genetic instability can also be due to the activity of mobile genetic elements. Unlike the direct DNA damage that is repaired or fixed in the form of stable mutations, the activation of mobile genetic elements can lead to several transposition cycles, cause the formation of unstable mutations and contribute to a multiple increase in DNA damage after the action of IR. In support of the role of mobile elements in the formation of genomic instability is proved by the fact of their insignificant content in the genome of some radio-resistant organisms. This confirms the important role of the induced transposition of mobile genetic elements in the process of destabilization of the genome, and the possibility of multiple transpositions can serve as an explanation for its conservation over many cell generations.

Thus, the analysis of the above information allows us to identify two main mechanisms of the formation of post-radiation genetic instability: mitochondrial and chromosomal (fig. 61).

Mitochondrial dysfunction after irradiation leads to a constant increase in the level of reactive oxygen species and oxidative stress, and in the chromosomes; IR causes changes that do not appear immediately but lead to an increased level of mutations and chromosomal rearrangements in subsequent generations of irradiated cells.

Radiation-induced genetic instability can be induced not only by irradiation, but also by culturing cells in a medium collected from irradiated cell cultures. This fact emphasizes the important role in the formation of the radiation

response of not only intracellular but also extracellular factors, and directly relates radiation-genetic instability to the bystander effect.

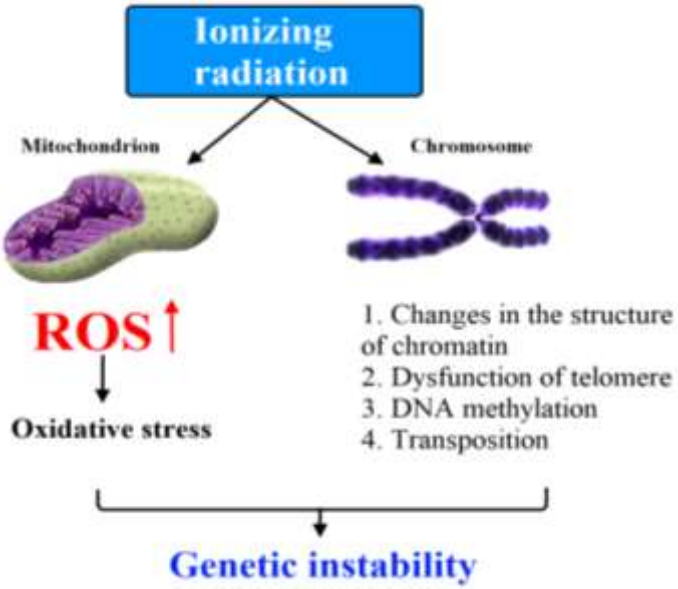


Fig. 61. Mitochondrial and chromosomal mechanisms of the formation of radiation-induced genetic instability

8.2. Bystander Effect

The biological effects of IR were traditionally believed to occur in irradiated cells as a result of direct DNA damage. This meant that

1) biological effects occur only in irradiated cells;

2) the radiation transport through the cell nucleus is a prerequisite for the occurrence of a biological reaction and

3) DNA is the main target molecule in the cell.

However, eventually the evidence of the existence and non-target effects of radiation, i. e. the effects in cells that caused the appearance of genetic abnormalities, such as mutations, chromosomal aberrations, and changes in gene expression that themselves were not directly exposed to radiation appeared. In this case, the damage signals are transmitted from cell to cell mainly through slit-like contacts, and the genetic effects observed in witness cells arise as a result of activation of oxidative stress. The possible effect of such non-target effects of radiation on the biological response of tissue and organ cells when exposed to low-dose studies is also discussed.

For many years in radiobiology there has been dominated the opinion that the effects of radiation should be studied directly in irradiated cells. In addition, according to leading researchers of that period, all the effects came down to the consequences of unrepaired or incorrectly repaired nuclear DNA damage during direct ionization or in the interaction of DNA with water radiolysis products. Effects in nuclei that are not directly affected by IR, as a rule, were not considered and, moreover, were considered just impossible. However, in recent decades there has been a change in the basic paradigm of radiobiology, and the "non-target" effects including the radiation-induced witness effect have taken their rightful place in both theory and experimental research.

In 1974 A. Brooks injected a small amount of plutonium, which is concentrated in certain parts of the liver, to Chinese hamster individuals. Moreover, the analysis of radiation-induced damage to chromosomes showed an increase in cytogenetic damage in the whole liver regardless of the local dose distribution, which is greater the closer the cell is to the radioactive particle. The data were interpreted in this way: all liver cells were at risk of inducing chromosomal damage despite the fact that only a small fraction of the organ cells was directly exposed to direct radiation exposure.

A modern surge of interest to this problem is associated with Nagasawa and Little's study, who showed that when a monolayer of cultured cells is irradiated with α-particles, in which the tracks directly receive less than 1 % of the nuclei, and more than 30 % of the cells have an increased frequency of sister chromatid exchanges. Soon, bystander effect was confirmed by a number of researchers.

The discovery of bystander effect showed that the effect of small doses can be more pronounced per dose unit and more dangerous than previously thought because the number of cells manifesting the negative effects significantly exceeds the number of cells directly affected by IR.

Despite the fact that the value of bystander effect is less than with a direct lesion, it makes a significant contribution to the risks associated with the effect of small doses. Unfortunately, it must be noted that not everyone shares this point of view – Publication 103 of the International Commission on Radiation Protection (ICRP), which defines mechanisms for assessing the effects of radiation on the human body, explicitly states that it is not accepted for consideration due to insufficient knowledge. However, it is obvious that its effect is of great importance in assessing the risk of tumor formation (realized through the mechanism of genetic instability) when small doses of α-particles emitted by radon and its decay products are irradiated.

On the other hand, bystander effect can be induced in tumor cells located adjacent to those irradiated with cancer radiotherapy, and thus, the effect of radiotherapy can also be affected by genetic effects in the non-irradiated part. This is also supported by the data that in the case of the main tumor and small secondary tumors formed by metastases, when the main tumor is irradiated,

the mass of it and secondary (non-irradiated) tumors decreases at the same time. In addition, an inflammatory reaction may develop in the irradiated tissue by the mechanism of bystander effect.

Bystander effect can be induced by various types of radiation: x-ray and γ-radiation (with low LEP, α-particles and heavy ions (with high LEP). A similar effect is determined for ultraviolet radiation and thermal exposure. With radiation exposure, bystander effect is detected with the dose of 5 mGy. At this dose, the level of DNA damage to the target cell corresponds to only five single-stranded breaks, 10–15 damaged bases, and only one double-stranded break in every fifth cell.

The effect is stably manifested in the dose range of 1–50 cGy. It is caused even if only one cell is irradiated with just one α-particle. Obviously, bystander effect is significant only at low dose loads when the number of target cells is small. Its induction at high doses reaches a plateau; all cells become directly irradiated. Apparently, the nature of the effect lies in the communication between the irradiated ("target") cell and the surrounding unirradiated ("non-target") cells through direct physical contacts (for example, gap junctions between cells), culture medium or tissue fluid.

As a rule, such a "non-target" effect is unfavorable for the cell. It leads to different consequences; it reduces the survival of clones of unirradiated cells, induces cell cycle delay, the delayed cell death, apoptosis and neoplastic transformation, leads to chromosomal instability, promotes mutagenesis, causes sister chromatid exchanges, and modifies gene expression.

In general, this is an almost complete description of the previously mentioned instability of the genome. One of the mechanisms of its occurrence is a chronic increase in the level of formation of free radicals.

However, in some cases, bystander effect promotes cell proliferation or their terminal differentiation, i.e. leads to "useful" effects. In this regard, it is worth noting that hereditary radioresistance can also be transmitted from irradiated to non-irradiated cells, as shown, for example, in I. E. Vorobtsova and I. S. Kolesnikova's works. Thus, adaptive response mechanisms and bystander effects may overlap.

Moreover, it has already been established that the effects that occur in bystander cells can be transmitted to their offspring, i.e. they are hereditary, which is also typical to genomic instability. Thus, it becomes very clear that there is a close relationship between genome instability and bystander effect, and the state of genomic instability can be a direct result of bystander effect. In other words, bystander effect can be both a cause and a consequence of induced instability since it can be caused by inflammation resulting from exposure. In cells with bystander effect, hypersensitivity to small doses is not detected. This is due to the fact that hypersensitive cells die during irradiation, and more stable cells become genetically unstable. Thus, at low doses, bystander effect may dominate other forms of cell response to radiation.

Apparently, bystander effect is evolutionarily conservative. It is noted in protozoa and primitive multicellular organisms. For example, it was found in fusion yeast *Schizosaccharomyces pymbe*. Bystander effect is found in sponges after exposure to UV radiation and hydrogen peroxide. Bystander effect is also typical to crustaceans, and among vertebrates, in addition to mammals, it is noted in fish. For example, irradiated living trout releases into the water the substances that cause bystander effect in fish placed in this water.

The blood plasma of people irradiated with radiotherapy or as a result of accidents causes the so-called "clastogenic" effect in unirradiated cells. The so-called "clastogenic factors" were found even in blood taken more than 30 years after irradiation. Thus, the plasma of irradiated animals and humans contains substances that can induce clustered damage in unirradiated cells. Clastogenic plasma factors were first described by W. Parsons and colleagues in 1954, who observed the damage to the red bone marrow in the sternum of children with chronic granulocytic leukemia in when their spleen was irradiated. Normal human peripheral blood lymphocytes cultured in a medium containing the plasma of irradiated people also had significantly more chromosomal aberrations than lymphocytes cultured in a medium with non-irradiated plasma. Therefore, extracellular radiation-induced DNA damaging factors can exist for a long period of time after irradiation. Apparently, these factors are not the result of radiation-induced depletion of protective mechanisms or changes in the ratio of normal plasma components. Most likely, these are molecules or cell components released into the bloodstream as a result of exposure to IR. It should be noted that the clastogenic effect, like other effects of small doses, is not determined in all experiments.

The exact molecular nature of clastogenic factors is still unknown. Inflammatory cytokines and free radicals are suggested as potential ones. Interceptors of free radicals reduce or bting to nothing the activity of clastogenic factors. There is also the possibility that clastogenic factors do not appear as a result of irradiation, but they might be endogenous viruses. The clastogenic factors and signals causing bystander effect are likely to be the same. At least both are induced by IR and cause genetic damage in unirradiated cells. It should be noted that clastogenic factors can arise not only during irradiation. They are found in blood plasma when inhaled asbestos, heart attack, hepatitis C, Crohn's disease, scleroderma, in patients with chromosomal instability syndromes, and etc.

The bystander effect does not feed into the existing radiobiological paradigm according to which the cell or its DNA is recognized as the main target of the action of IR since this effect is a multicellular type of radiation response in the three-dimensional areal of the tissue. It is known that the function of a cell in the surrounding tissue is regulated by water-soluble small molecules and signal peptides, as well as by the extracellular matrix. Apparently, during irradiation a steady violation of signaling mechanisms occurs, which leads to

the damage to neighboring unirradiated cells. In the same way, an adaptive response and radiation-induced genomic instability can occur.

There are several models that explain the nature of intercellular signals responsible for bystander effect through:

1) gap junctions,

2) plasma membranes directly,

3) either interactions between receptors and their auto- or paracrine ligands.

When exposed and non-irradiated cells come into contact, gap junctions present in almost all body cells are of great importance in signal transmission. Possible candidates are long-lived organic radicals. Such molecules are known to be able to exist for about a day and cause mutations and cell transformation.

The bystander effect is more pronounced in cell populations with defects in the repair of double-stranded breaks. In later studies, it was found that the isolation of a signal by an irradiated cell with bystander effect does not depend on the presence of unrepaired DNA damage and the reparative ability of the cell. The signal is equally effective regardless of whether it is produced by a wild-type cell or a cell with mutations in the repair of single- or double-stranded breaks. The spectrum of mutations in bystander effect is similar to spontaneous but differs from that observed with direct irradiation of nuclei. Mutations in bystander cells are usually point mutations, while deletions predominate in irradiated cells.

According to the mechanism of bystander effect, the so-called “death effect” occurs when genetically unstable cell clones can be cytotoxic to the parent clone. Transfer of the filtered medium from unstable progenitors of irradiated cells to unirradiated ones in a number of experiments leads to total induction of the death of the latter.

The mitogenic bystander effect is also known: irradiation with small doses of a-particles stimulates the proliferation of unirradiated cells treated with a supernatant obtained from irradiated ones. Molecular biochemical changes occurring in the bystander cell itself are being intensively studied. ATM and ATR-dependent pathways can play an important role in the response of the bystander cell.

So, bystander effect belongs to the group of so-called “non-target” effects of small doses of radiation, when unirradiated cells receiving a cytokine or free radical signal from irradiated cells change their physiology in such a way that, in some cases, damage to the DNA of the bystander cell occurs through ROS generation; in other cases, stimulation of its differentiation, proliferation, adaptive response, or death are observed (fig. 62). As a result, the bystander effect is directly related to the effects of small doses such as apoptosis, genetic instability, and an adaptive response.

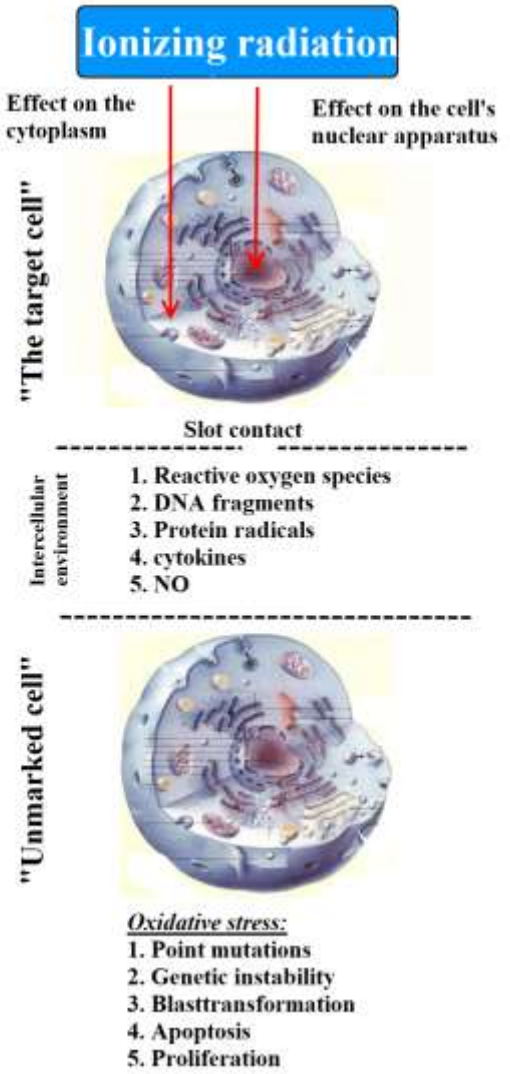


Fig. 62. Hierarchy of the events determining bystander effect

8.3. Adaptive Response

Almost all organisms (from bacteria to mammals) have the ability to adapt quickly, which ensures their survival in continuously changing environmental conditions. The increased resistance of the cell or organism to the damaging effect of the factor after the preliminary exposure to such a factor in a small dose is called the “adaptive response”. This is a widespread phenome-

non that is observed in all studied organisms. Thus, the main biological significance of the adaptive response is to protect the cell and the body from high doses of dangerous agents.

In 1960 J. Maysin et al. obtained the data on the increase in the resistance of rats to the action of a lethal dose of x-rays after preliminary irradiation at a dose of 5 cGy. Although the increase in survival was not statistically significant, this study showed for the first time the presence of a modifying effect of small doses on radiosensitivity. Experimental evidence for the existence of a radio-adaptive response was first obtained by G. Olivieri et al. in the study of the mutagenic effect of x-rays on human lymphocytes. It was found that when human lymphocytes were grown on medium supplemented with low concentrations of labeled thymidine, which is a source of chronic radiation at a dose of 30–40 cGy, a significant decrease in the level of induction of chromosomal rearrangements by acute irradiation at a dose of 150 cGy occurs. Later, it was shown that the radio-adaptive response leads to a decrease in the level of various cytogenetic disorders, mutations, prevents cell death and neoplastic transformation, and increases the activity of antioxidant enzymes and DNA repair systems. Thus, the radiation-induced adaptive response is manifested in a decrease in sensitivity to a damaging dose of radiation (the acquisition of radiation resistance) after preliminary irradiation in a small (adaptive) dose.

Recently it was also found that small doses of radiation (100 mGy) lead to a qualitative change in the patterns of genetic expression of cells, which is different from the expression patterns induced by irradiation in large doses. The transcription factor p53 is of key importance in determining the cellular response to radiation. The main targets of p53 are the genes for cell cycle control and apoptosis. In the high-dose range, p53 provides cell cycle block and cell repair or death in the case of unrepairable DNA damage.

In the study of adaptive response mechanisms, genome-wide studies of radiation-induced genes using expression microarray technology play an invaluable role. It was shown that in response to irradiation at a dose of 3 Gy, the activity of 87 genes is induced, while 227 is repressed. At a dose of 10 Gy, 156 genes are repressed and 660 genes are induced. Most of them are involved in cell cycle control, cell death, DNA repair and metabolism.

Thus, *in vitro* studies have shown that the mechanisms of DNA repair at the checkpoints of the cell cycle and the response to DNA damage are involved in the radio-adaptive response.

General irradiation of the animal organism can lead to an increase in the radiostability of both somatic and germ cells. An adaptive response can be observed on the example of the development of radiostability of a whole multicellular organism. The role of heat shock proteins in the adaptive response is of particular interest since it implies the commonality of molecular-cellular stress response systems for different types of stress and the existence of cross-

adaptation to various environmental factors (temperature, IR, oxidative stress). At the same time, the role of heat shock proteins in the formation of resistance to IR in small doses and at the level of the whole organism remains unclear (fig. 63).

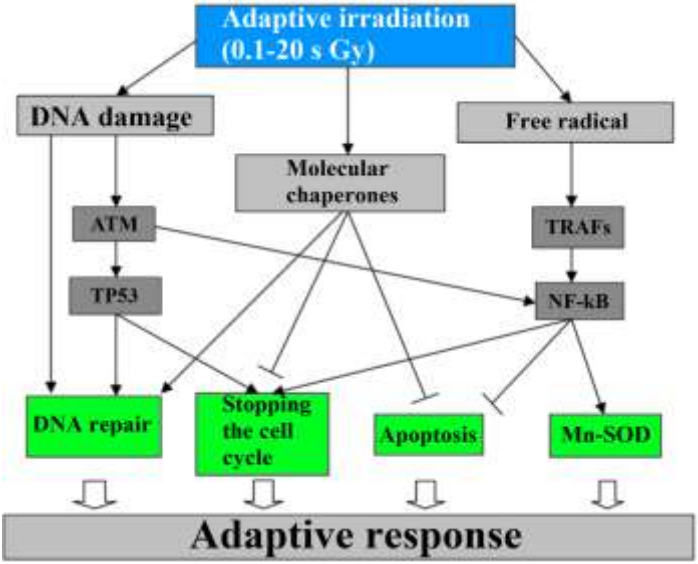


Fig. 63. The mechanisms involved in the induction of an adaptive response

As a result, the adaptive response is one of the most specific effects of radiation in small doses.

The optimal induction of radioadaptation occurs in the dose range of 0.1-20 cGy. The triggering of intracellular signaling pathways as a result of oxidative stress and DNA damage leads to the activation of major transcription factors. Their mediated effect of molecular chaperone activation, antioxidant defense, repair processes and inhibition of apoptosis prepares the cell for the effects of adverse factors and makes it more resistant to radiation in large doses.

Summarizing the somatic effects of IR in small doses, it should be noted that the cells respond to radiation-induced damage by inducing a large number of biological responses. These early events determine the fate of the irradiated cells: whether the cell will undergo mitotic death, necrosis, apoptosis, aging or, ultimately, survival and enter a new mitotic cycle. If the cell survives, the primary biological response to radiation-induced damage can affect whether the cell will normally divide or differentiate, have a limited lifespan, or acquire characteristics of genomic instability and become blast-transformed.

8.4. Radiation Hormesis

Small doses of IR are the doses when one ionizing particle passes through the nucleus of the cell and causes one act of ionization throughout the track. Depending on the size of the cell nucleus, these are the doses of the order of 100 mGy. The results of biological studies in the field of small doses of IR are quite contradictory and are not always statistically conclusive. Therefore, one of the main problems in radiobiology is the dose-response relationship for radiation-induced lesions.

Currently, there are two opposing models for assessing the risk of stochastic effects of ionizing radiation depending on the dose.

The first one is based on the extrapolation of the results obtained in the study of the effects of large doses into the field of small doses. This implies that the risk of cancer when irradiated with small doses of IR is the most accurately assessed by linear relationships without a threshold, and any arbitrarily small dose increases the likelihood of cancer and other diseases.

The second model postulates that there is a threshold dose below which radiation cannot cause diseases of a carcinogenic and or non-carcinogenic nature. This model is based primarily on the concept of radiation hormesis.

The concept of "radiation hormesis" suggests that IR, which at high doses is detrimental to living organisms, in small doses can induce positive biological processes and have a stimulating beneficial effect on the body, which is recorded as an increase in fertility, growth, cell division and an increase in the life expectancy of various biological objects

The stimulating effect of IR is also observed at high doses when radioresistant organisms are exposed to radiation, but in this case, the mechanisms of radio stimulation are apparently different than under the action of small doses.

The problem of radiation hormesis is also relevant as a problem affecting the protection of people health. If the effect of small doses is favorable for the body, it may be advisable to revise the radiation safety standards for increasing the limits of maximum permissible radiation loads for both personnel associated with working with ionizing radiation sources and the population. Providing radiation protection measures requires significant financial resources, and mitigating radiation safety standards can bring enormous economic benefits.

To date, a large number of epidemiological studies of human populations irradiated as a result of nuclear bombings or accidents involving the release of radionuclides as well as the populations living in areas with the increased natural radiation background and the contingents that are professionally in contact with ionizing radiation have been carried out all over the world. In all these studies, the effect of radiation hormesis was registered.

According to the UNSCEAR report, among the survivors of the atomic bombing of Hiroshima and who received the doses of about 10 rem, there was

a significant decrease in the overall mortality rate and, in particular, the mortality rate from leukemia compared with the unirradiated part of the population of the corresponding age. The mortality rate of men who received the doses of less than 150 cGy during the atomic bombing of Nagasaki was significantly lower than the mortality rate of men of the unirradiated cohort.

An epidemiological survey of nearly 108,000 workers in the US shipbuilding industry showed a statistically significant decrease in overall mortality and the mortality due to all malignant neoplasms in irradiated workers compared with non-irradiated workers.

A reduction in mortality from cancer has been recorded among military observers over nuclear explosions in the atmosphere in the United States and England. The death rate of Canadian military observers was 88 % of the control, while the mortality from leukemia was 40 % of the control. The leukemia mortality rate was significantly reduced in a cohort of workers in the nuclear industry in England and the United States compared with non-irradiated personnel. Mortality from cancers and leukemia among workers in Canadian nuclear industry was also 58 % lower than the nationwide mortality rate.

In 1957, as a result of the accidental release of radioactive substances in the Southern Urals, three groups of residents of 22 villages with a total of 7852 people received an average of 50, 12 and 4 cGy doses. Observations over the next 30 years showed a significant decrease in mortality from different types of tumors in all three groups. The mortality was 28 %, 39 % and 27 %, respectively, compared with the non-irradiated population. Comparison of the populations subjected to the chronic inhalation of 239Pu as a result of this accident and receiving 0.343, 1.18 and 4.2 kBq showed that the risk of lung cancer was significantly reduced compared with the non-irradiated control by 46 %, 41 % and 47 %, respectively .

The study, which covered 90 % of the US population, showed a strong downward trend in the incidence of lung cancer with the increasing levels of natural radon gas in residents' homes.

The conclusion arising from the results of epidemiological studies on the anticancerogenic effect of small doses of radiation is confirmed by numerous laboratory experiments. Thus, irradiation at a dose of 15 cGy inhibited tumor growth after the introduction of cancer cells into mice; irradiation at the same dose reduced the number of metastases in the lungs of mice and rats; irradiation at a dose of 1 cGy reduced the frequency of neoplastic cell transformation; chronic irradiation of mice for 5 days with a daily dose of 1 cGy suppressed the appearance of thyroid lymphoma in them.

Stimulation of cell division as the effect of the action of small doses of IR is observed in a number of other biological objects: mammalian cell culture, blue-green algae, and ciliates.

Screening from the natural radiation background leads to a decrease in cell proliferation. For the first time this phenomenon, expressed in a decrease

in proliferative activity in protozoa and a delay in hatching of Drosophila larvae, was discovered by G. Planel. A 20-fold decrease in the rare-ionizing component of the Earth natural radiation background led to an increase in the rate of aging and death of yeast cell strains.

There are several models trying to explain the effect of radiation hormesis. Published in 2003, M. Polyakov and L. E. Finindegan's model is the latest one. According to it, the effect of small doses of IR on a cell, unlike large doses, is dualistic. On the one hand, DNA damage occurs with the immediate launch of reparative systems; on the other hand, a signal is sent to stimulate physiological processes that neutralize DNA damage. These adaptive physiological processes do not start immediately; they are nonspecific and are aimed mainly at neutralizing non-radiation damage to DNA. The following adaptive cellular processes are distinguished:

1) the stimulation of the radical detoxification system;

2) the protection against chromosomal aberrations, which occurs by activating several DNA repair systems;

3) the removal of damage by inducing immunocompetence associated with an increase in the number of lymphocytes;

4) the apoptosis of latently damaged cells.

DNA damage of a non-radiation nature prevails over the radiation damage and, for this reason, is primarily responsible for the recorded background of carcinogenesis and body aging. At doses above 20 cGy, the level of occurring cellular radiation damage will already exceed the ability to reduce their protective mechanisms of the cell and the dose-response curves will correspond to the usual linear or quadratic-linear model.

The evidence of the anticarcinogenic effect of small doses of IR is quite convincing, but there are some doubts that remain regarding their effect on diseases of a non-carcinogenic nature and especially children's health.

The results of numerous studies indicate radiation damage in children born by women who were irradiated in diagnostic doses before or after fertilization. An increase in carcinogenic risk is directly proportional to the number of diagnostic x-rays or the received fetal dose. For example, irradiating the fetus shortly before birth at a dose of 1cGy is estimated to lead to an additional 300-800 cancer deaths per million under the age of 10 years.

For the irradiated at low doses, an increase in the level of catabolites of the lipoperoxide cascade with simultaneous and conjugate depletion of the system of essential antioxidants combined in the concept of "Chernobyl syndrome" are the early radiogenic biochemical symptoms. The number of cases of thyroid enlargement and visual disturbances (mainly dry eye syndrome) depended on the level of radioactive contamination. Children living in the Chernobyl zone had an increased level of peroxidation products.

Based on the results of their research and summarizing other experimental data, E.B. Burlakova et al. came to the conclusion that at low and ul-

tra-low intensities, IR has a unique ability to increase the biological effect by ten folds. Under these circumstances:

1) the dependence of the effect on the radiation dose is nonmonotonic, polymodal in nature;

2) the doses at which the extremes are observed depend on the irradiation power (intensity);

3) radiation in small doses leads to a change (in most cases to an increase) in sensitivity to the action of damaging factors;

4) in certain dose ranges, low-intensity irradiation is more effective than acute.

For example, depending on the intensity of irradiation in a number of objects in the low-dose area, either an antimutagenic effect or, conversely, an increase in the number of mutations or cytogenetic disorders per unit dose can be observed; stimulation of cell population growth or, conversely, the increased radiosensitivity of cells compared to the expected linear dependence can be evidenced. This is explained by the fact that at low doses comparable to the level of natural radiation, the degree of DNA damage is too small to activate an adequate level of repair of genetic damage.

Defining radiation hormesis as the "beneficial effect of radiation", scientists thereby a priori consider the increase in fertility or biomass of animals and plants (the observed effects of radiation hormesis) favorable and useful. In fact, an increase in fertility or biomass does not mean benefits to the body. For the body, this is in any case a deviation from the physiological norm. Differences from the norm can be hypo- or hyperfunctional and affect the survival in both cases both positively and negatively.

An increase in the survival, fecundity, and the life-span of individuals can lead to an increase in the burden of mutations (even if it is assumed that radiation exposure does not add to additional mutations) and the changes in age and gender proportions in the population. An increase in the radioactive background to a level that causes an increase in reproduction and survival due to different species radiosensitivity to the stimulating effect of IR can also bring changes to the community of organisms because of the increased competitive abilities of the "stimulated" species. IR can activate the reproduction of pathogens with all the ensuing consequences.

From a physiological point of view, any effect on a biological object can cause both a hypofunctional and hyperfunctional response of the corresponding body systems. Then, by radiation hormesis it is necessary to understand such events when, under the action of the IR, any vital functions, processes or physiological parameters are exceeded over the biological or physiological norm, i.e. as a hyperfunctional effect of IR at low doses and not as a favorable effect of radiation.

Anticancerogenic effect of background doses of ionizing radiation and the therapeutic effect of small doses, in particular in radon therapy are certain

to be considered. But can the effect of radiation hormesis serve as an argument in a dispute about a possible mitigation of radiation safety standards? Definitely not. The dose dependences in the field of small doses are complex and far from predictable. And radiation hormesis is just one of the effects in this area.

Part 9. Radiation Effects in Utero and Radiation Induced Heritable Damage

9.1. Transgenerational Changes During Irradiation

Irradiation of the germ cells of the parents leads to the appearance of various mutations in the offspring, which can be manifested mainly in

1) increased incidence of congenital malformations,
2) stillbirths,
3) postnatal death,
4) functional inferiority accompanied by reduced vitality,
5) increased risk of carcinogenesis,
6) genome instability.

Thus, the data accumulated to date convincingly indicate that ionizing radiation can have a pronounced effect on the stability of the genome transmitted through the germ cells of irradiated parents to their descendants. Moreover, the data published at the moment indicate that the effects in the offspring are manifested even when only one of the parents (for example, father) is exposed to radiation. This phenomenon is called "transgenerational instability" and leads to an increase in the rate of mutagenesis in the germ line of cells, which ultimately leads to an increased risk of cancer.

According to various sources, the spectrum of transgenerational changes in somatic tissues of offspring is very wide: from a high risk of developing malignant tumors to a change in behavioral reactions.

A special boost for research in the offspring of irradiated parents was received after the appearance of the data on an increase in the incidence of leukemia in children born in settlements near the nuclear fuel reprocessing plant in the families of employees (USA). It was also possible to establish that in restudies, the frequency of cancerous tumors did not exceed the control level; however, the qualitative composition of tumors in children as a result of exposure to parents changed. It was also found that there is an increase in the frequency of malignant tumors in the offspring of the irradiated parents, if they were subsequently subjected to additional exposure to carcinogens of a different nature compared with the offspring of non-irradiated ones. Moreover, the spectrum of tumors was significantly different from spontaneous. As a result of this research, it was concluded that irradiation of fathers can contribute to the onset of cancer in the offspring.

If the genomic instability leads to an increased risk of oncopathology in the offspring of irradiated parents, it should be assumed that they will have an increase in the level of somatic mutations in general. So, in 2003, Russian scientists in experiments on the offspring of irradiated male mice showed an increase in the frequency of chromosomal aberrations, micronuclei, and other

changes in genetic material. A statistically significant increase in the frequency of micronuclei in bone marrow cells in offspring of the first generation of female and male mice irradiated in doses of 0.1–0.5 Gy compared with the offspring of unirradiated animals was also found.

In the 80s of the XXth century, Lewing suggested that irradiation of males leads to an increase in the level of not only somatic mutations (i .e., in all cells of the organism except the sex cells that are responsible for reproduction), and similar effects (aberrations, chromosomal mutations) should be observed in germinative cells of offspring. The scientist proved his hypothesis in experiments that confirmed an increase in the frequency of embryonic death, a decrease in cell proliferation in the early stages of embryonic development, and an increase in the number of hereditary deformities in second-generation offspring.

The effect of ionizing radiation also leads to an increase in the mutational load in humans. The first studies of the offspring of irradiated people date back to the 30s of the XXth century. This work was carried out in the USA and Germany, they examined the offspring of women who were exposed to ionizing radiation in the pelvic area for radiotherapeutic purposes. A slight increase in the number of developmental defects was also found. Then, the data regarding the changes in health status in the offspring of male radiologists appeared. Among the offspring of these men, a significant increase in cases of congenital malformations, miscarriages and stillbirths was revealed. A high risk of leukemia and birth defects was proven for children whose fathers were exposed to the chronic effects of radionuclides in nuclear fuel processing plants and during diagnostic exposure rather than the acute effects of IR as it was in Hiroshima and Nagasaki.

However, the atomic bomb explosion in Hiroshima and Nagasaki, as it turned out, did not lead to significant genetic defects in the offspring. However, these data are not absolute, since, for example, as a result of the Chernobyl disaster, the population was exposed to chronic low-dose AI. It is well known that the maximum doses of acute, prolonged and chronic exposure were received by the liquidators of the accident in 1986–1987. So, children born in the families of liquidators had a significant increase in DNA changes if they were conceived after participating in the liquidation work compared with the comparison group, which were their brothers and sisters born before the Chernobyl accident. Studies of this kind were conducted in Russia and Ukraine; in general, the data obtained unequivocally confirmed that the cytogenetic status of children of liquidators is changing and individual radiosensitivity may increase. Unfortunately, the available scientific data is not enough to draw any final conclusions. This is often associated with the lack of reliable dosimetric information, differences in methodological approaches, sample sizes, and etc.

At the same time, these data suggest that double and single-stranded breaks that occur after irradiation of germ cells in the case of unrepaired (or incorrect repair) can be extremely dangerous ultimately leading to the for-

mation of a genomic instability syndrome and, as a result, an increased susceptibility to cancer among the offspring of irradiated individuals.

Another reason may be the radiation-induced gene mutations in the genes responsible for maintaining the stability of the genome. Nevertheless, there is the evidence of a possible epigenetic nature of the transgenerational instability of the genome:

1) it is maintained for a long period of time after the initial exposure and does not cause a reaction from the immune system;

2) its level is often too high to be explained by direct mutations of DNA repair genes.

The mechanisms of epigenomic variation in this case are likely to be DNA methylation and atypical changes in chromatin condensation in germ cells of irradiated parents. As it is known, DNA methylation occurs during spermatogenesis, and it can occur in the early stages of embryogenesis and be transmitted through many generations. Methylation significantly affects the pattern of gene expression including those that are responsible for the integrity of the genome.

On the other hand, the contribution to this process of changes occurring in response to radiation-induced oxidative stress and the inflammatory process, i. e. secondary effects arising from the free radical mechanism.

For example, a constantly high level of free radicals was found in the offspring of irradiated somatic cells in culture. It may well become a launching event for a cascade of molecular events that ultimately lead to instability of the genome. However, it must be borne in mind that the amount of cytoplasm in mature sperm is too small to transfer a sufficient number of long-lived radicals to the zygote. It is known that free radicals that occur during inflammation can affect DNA causing a variety of damage. Thus, what it involves is some kind of DNA-dependent signal inherited from the irradiated father. In addition, an unusually high level of mutational mosaicism is often observed both in germ cells and in the somatic tissues of mice in the first generation, which suggests a significant increase in the mutation rate associated with an early stage of development. In this regard, the study of issues related to mutation of not only nuclear, but also extra-nuclear DNA concentrated in mitochondrion is of particular interest. This is also supported by the fact that the previously recognized fact of transferring extra-nuclear hereditary information through the maternal line has now been rejected – sperm mitochondria can also enter the fertilized egg.

Indeed, a number of studies have already shown a steady change in the pattern of gene expression in the offspring of irradiated male mice. Transgenerational transmission of an increased risk of oncogenesis is likely to be associated with cumulative changes sequentially formed by a cascade mechanism in protooncogenes that affect the immune system, control of the cell cycle and repair cell

functions, which helps to accelerate the formation of tumors. This hypothesis is supported by studies performed using GeneChip analysis (molecular chips).

In another work, exposure of γ-radiation at a dose of 1 Gy to parents led to a significant change in gene expression even in the third generation of mice in comparison with the descendants of unirradiated parents.

Another way for transgenic gene instability to occur is replicative stress. In this case, a long delay or stop of replication of individual chromosomes can lead to the formation of chromosomal instability syndrome. There is the evidence of the preservation of transgenerational changes in the descendants of males irradiated in the late postmeiotic stage of spermatogenesis when most of the ways to maintain the integrity of the genome are not active. In addition, pre-mutation damage to sperm DNA is efficiently repaired within a few hours after fertilization. Recognition and repair of these lesions in a fertilized egg is accompanied by a suppression of DNA synthesis in both pronuclei (irradiated male and non-irradiated female) and changes the expression of DNA repair genes in the preimplantation embryo. Thus, radiation-induced damage to sperm DNA can later trigger a cascade of events in a fertilized egg leading to epigenetic modifications that persist in the cells of the embryo.

Despite the fact that the rate of mutagenesis in the cells of the offspring of irradiated parents is often significantly increased, the role of the genetic background of parents in this phenomenon has not yet been studied. The severity of transgenerational instability of the genome, as it turned out, largely depends on the genotype of the studied line of animals. The radiation-induced transgenerative genomic instability in the offspring is not always manifested, which suggests a contribution to its formation of the maternal genotype and the status of the state of the DNA repair system. Radiation-induced double-stranded breaks in the sperm head are repaired in the wild-type zygote at the beginning of the S cell cycle by the mechanism of non-homologous end reunification. Interlinear differences in the efficiency of DNA repair in the oocyte significantly affect the yield of dominant lethal mutations induced in male germ cells. In addition, given the evolutionarily established features of human ontogenesis, it is impossible, as already noted above, to mechanically transfer the data obtained on experimental animals directly to humans.

Summarizing the above, the following conclusion can be made: irradiation of at least one of the parents can lead to the appearance of signs of genetic instability in somatic and reproductive cells of offspring including the increased levels of mutagenesis and teratogenesis as well as an increased risk of oncopathology (fig. 64).

A possible signal transmitter may be unrepaired DNA breaks, free radicals, epigenetic or mutational changes in the activity of genes responsible for maintaining the stability of the genome (fig. 65).

However, when parents are irradiated, subsequent generations of offspring can transmit not only a predisposition to genetic instability, but also, on

the contrary, relative radioresistance to further adverse effects. In this case, it means the formation of the phenomenon of radioadaptation.

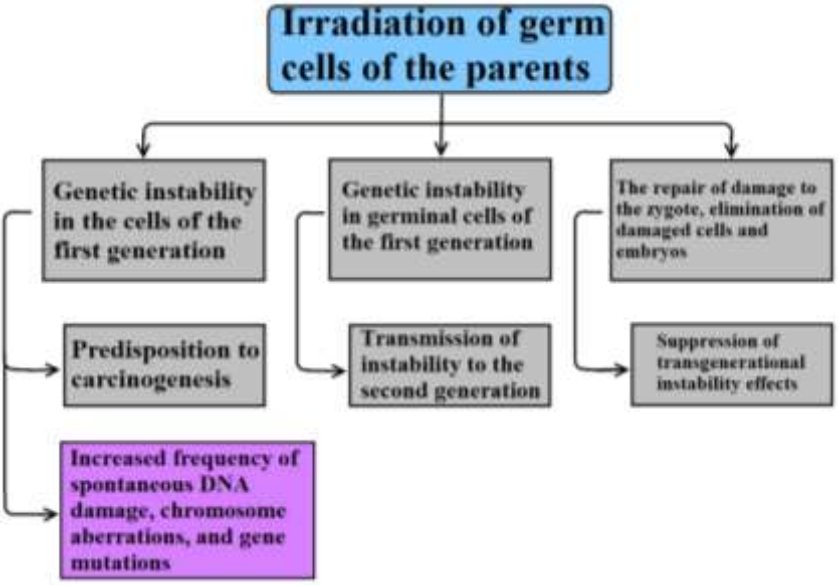


Fig. 64. The scheme of the probabilistic effects of transgenic instability of the genome

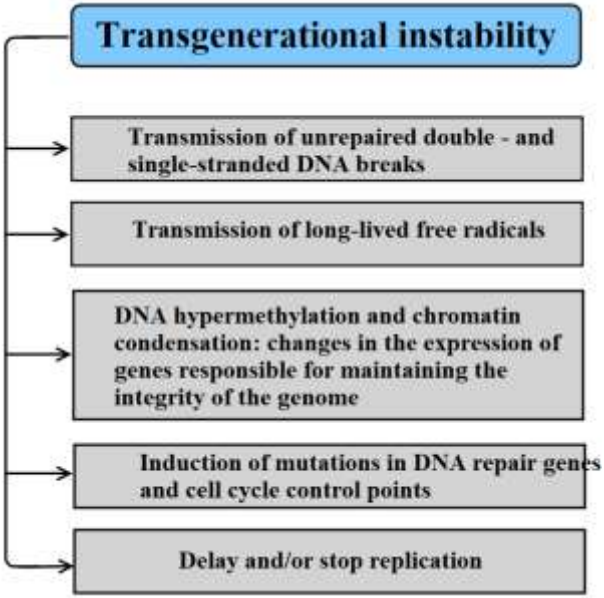


Fig. 65. Possible mechanisms of transgenic instability of the genome

9.2. The Phenomenon of Radioadaptation and its Role in the Body Response to IR

The natural radiation background on the Earth is on average about 1 mGy / year and has been at a constant level for about 4 billion years, so most organisms have never encountered high doses of radiation and in the process of evolution have not developed the system of adaptation to them. However, with an increase in the background radiation, both as a consequence of natural causes and as a result of the influence of anthropogenic factors, many organisms are able to survive a stronger radiation effect. Adaptation to IR is understood as the adaptation to radiation load that makes it possible to save viability and maintain fertility and normal functional stability of all structures of a biological object under conditions of further exposure to IR. In practice, it is not possible to conduct a full study of all three of these components; therefore, to assess the radiation adaptation, the radioresistance of the organism and the cells of its critical organs are used. It should also be noted that under natural conditions, populations are under the simultaneous influence of a large number of factors that are capable of modifying the radioresistance of an organism. The natural background of the planet fluctuates. However, its smooth fluctuations may well be compensated for in a series of generations thanks to the mechanisms of natural selection and

adaptive potential. Man, like any mammals, is able to adapt to new conditions. However, due to the technological revolution, human influence on the environment has increased dramatically. So, as a result of testing nuclear weapons in some regions of the planet, the natural background has grown by about 20 times. In this case, adaptive variability “does not keep pace with” the rapidly changing environment. A real situation is created when a person, as a biological species, is threatened with a breakdown of adaptations and its transition to the pathogenesis link. Moreover, the specificity of the effect is determined by the "weak" link in the body. In this regard, the emergence of any form of environmental pathology is possible.

At the same time, it is known that some individuals from populations living in the areas with the increased radiation background (up to several tens of mGy / year) become more resistant to high radiation doses. Thus, some individuals of Drosophila nebulosa and D. willistoni, caught from populations living in forests with a high natural radiation background (Minas Gerias, Brazil), are characterized by radiation resistance in the doses of up to 900 Gy although they sustain a greater genetic burden than individuals of control populations living in areas with low radiation background.

The studies conducted on wild populations of microorganisms, plants and animals living in the area of the 30-km zone of the Chernobyl nuclear power plant detected some of the mechanisms that underlie adaptation to radiation. When analyzing the structure of biota in territories with a high level of radiation background (0.5–1 Gy / year), it was found that species of some microscopic fungi dominated by its high melanin content dominate in its structure. Apparently, the adaptive value of this trait is due to the high radioprotective activity of melanin, which can serve as an interceptor of free radicals. The adaptability analysis of natural populations of Arabidopsis from regions with different levels of pollution around the Chernobyl nuclear power plant in the period 1986–1992 revealed the resistance of descendants of irradiated plants to high concentrations of mutagens. Similar facts were identified for other representatives of flora and fauna. The mechanisms underlying adaptation are extremely diverse: increased accumulation of antioxidants, activation of the repair system, and other mechanisms.

Thus, for example, in Arabidopsis growing in radioactively contaminated territories, a more than 10-fold decrease in the frequency of homologous recombination as well as an increase in the level of DNA methylation were found. On the one hand, a low level of recombination prevents an increase in the frequency of genetic rearrangements but, on the other hand, inhibits the repair of double-stranded DNA breaks. It is possible that in plants growing in contaminated areas, the repair is rearranged to a faster but erroneous mechanism of non-homologous recombination. Methylation essentially changes the active DNA profile through epigenetic changes. This implies the assumption that it is the epigenetic regulation that

leads to the stabilization of the genome that plays the main role in adaptation. In this case, hypermethylation can be considered as a stress response and the main mechanism of plant protection that prevents genomic rearrangements.

Some bird species caught in the 30-kilometer zone of the Chernobyl nuclear power plant have an increased mutation rate of partial albinism compared to the level of mutations that occurred before the radioactive contamination of the territories and with the level of mutations in the control plot. This type of mutation is associated with a decrease in the content of carotenoids in feathers, which indicates the extreme susceptibility of the metabolism of these pigments to radiation. In addition, exposure to radiation can lead to a decrease in the intracellular level of carotenoids, which are used due to their antioxidant activity to deactivate free radicals.

The dynamics of population mutagenesis over 22 successive generations and genetic radioadaptation were also studied in wild populations of the red-backed mouse. Two oppositely directed processes were found in irradiated populations: accumulation of mutations (the genetic burden of the population) and the formation of genetic radioadaptation. It is assumed that the frequency of genetic abnormalities in the population could be higher if there were no radioadaptation processes. Considering evolutionary processes, optimal adaptation to environmental conditions is achieved through the redistribution of the energy and plastic resources of the body between vital needs, such as growth, reproduction and maintenance of physiological life. In adverse conditions, the maintenance of vital activity becomes energetically unprofitable, and all expenditures are directed to reproduction. The results of some radioecological studies support this. So, in root voles (Microtus oeconomus Pall.), which have long lived in areas with a high content of uranium, radium, and thorium compounds, and in their descendants obtained under vivarium conditions, fertility indicators increase. Increased fertility is also observed in people living in areas with increased radiation background.

Populations of Drosophila melanogasler from various regions of the Republic of Belarus with an increased level of background radiation compared with populations of the control zones have an increased frequency of lethal mutations and increased heterozygosity. In natural populations of Drosophila, there is an increase in adaptation to adverse environmental factors, such as IR and chemical effects. After they are placed in standard laboratory conditions without the indicated effects, the adaptation persists for up to ten generations without radiation. Under laboratory conditions, which, according to the authors, has a stressful effect, even populations in control plots become more radioresistant.

The evolutionary aspects of high mutational pressure were studied in laboratory populations of Drosophila melanogaster. In populations irradiated for over 600 generations at the doses of 20, 40, and 80 Gy per generation, a step decrease in radiosensitivity was observed with an increase in the

radiation dose. Drosophila irradiated over many generations of laboratory populations show an increase in resistance not only to the action of IR but also in parallel to chemical mutagens.

Thus, the data obtained from natural and laboratory populations indicate an increase in genetically determined radioresistance and activation of the selection of stable phenotypes in response to stress factors. In this case, the reactions of organisms are universal in nature and increase the resistance to a wide range of adverse effects.

In that way, it turned out that organisms adapted to life in adverse environmental conditions, coupled with a high level of DNA damage, possess the highest radioresistance to the effects of IR. This primarily refers to organisms that can survive after complete dehydration. The bacteria of the genera Deinococcus, Geodermatophilus and Hymenobacter are characterized by extremely high resistance to the damaging effects of ionizing radiation. The bacterium Deinococcus radiodurans, which can exist under conditions of chronic exposure with a dose rate of 60 Gy / h and also tolerate acute gamma radiation at a dose of up to 15,000 Gy, is particularly famous. As studies of recent years have shown, the resistance of D. radiodurans to radiation and other adverse factors is due to at least three reasons:

1) the efficiency of the DNA repair process,

2) a system of protection against reactive oxygen species,

3) and structural features of the cell wall.

Irradiation in doses that D. radiodurans is able to survive causes hundreds of single and double-stranded DNA breaks and numerous base damage. While the cells of most organisms cannot recover after two or three double-stranded DNA breaks, in D. radiodurans the induction of up to 100 double-strand DNA breaks for each of six copies of plasmids and four copies of chromosomes does not ultimately lead to an increase in mortality and mutagenesis. A critical element in the radio stability mechanism of D. radiodurans is DNA polymerase of type A (PolA). For efficient operation, PolA needs Mg^{2+} ions, which modulate its activity and help resume DNA synthesis if it stops at the damaged site. The concentration in the Mg^{2+} cell correlates with the radiostability of D. radiodurans. Possibly, bacterial resistance is due to the presence of a certain evolutionarily developed universal mechanism for the repair of multiple breaks since in case of natural dehydration for D. radiodurans, multiple DNA fragmentation also occurs. The presence of multiple copies of plasmids and chromosomes in the bacterial genome, between which intense homologous recombination processes occur, also contributes to a more effective repair. During repair, each plasmid can participate in six, and the chromosome in more than 700 recombination events. The leading role in homologous repair of D. radiodurans belongs to the RecD protein. In addition, RecD is involved in antioxidant defense processes stimulating the activity of catalase and free radical interceptors.

Comparison of DNA sequences of the D. radiodurans genome with other organisms revealed several interesting features that partially explain the mechanisms of its radiostability. The genes involved in the control of DNA repair and recombination, as well as the stress response, were studied in this organism in sufficient detail. Apparently, some of these genes entered the D. radiodurans genome from eukaryotes due to vertical transfer because they are not found in the genomes of other bacteria. For example, three proteins have been identified that are homologous to the drought tolerance proteins of plants; their occurence correlates with radiation resistance. These facts clearly indicate that the effectiveness of DNA repair in this organism is due to several different biological mechanisms. Because of the insufficient study of the issue at the present time, it is not possible to assess the effectiveness of these mechanisms in full and build a holistic picture of their action.

It is possible that the mechanism of detoxification of D. radiodurans free radicals is associated with an unusually high concentration of Mn^{2+} ions relative to the concentration of Fe^{2+} ions in the intracellular environment of the microorganism. Unlike the radiosensitive bacterium Shewanella oneidensis, the content of Mn^{2+} in the cytoplasm of D. radiodurans is increased by 300 times while the content of Fe^{2+} ions is reduced by three-fold. The protective effect of Mn^{2+} is apparently due to its ability to intercept the superoxide radical. In contrast to Mn^{2+} , Fe^{2+} ions under the irradiation conditions enter the Fenton reaction and contribute to the formation of hydroxyl and superoxide radicals. In addition, the described mechanism can protect not only DNA but also the proteins involved in the repair system, and it is well known that the stability of genetic material is directly dependent on the radioresistance of the protein complex of the repair system.

An additional kind of adaptation that contributes to the increased radiostability of D. Radiodurans is a complex multilayer cell wall including an outer membrane and a thick peptidoglycan layer containing the ornithine aminoacid.

The ability to survive under the conditions of dehydration, when DNA is subjected to numerous breaks, apparently determines the resistance of D. radiodurans to radiation since the similarity of the recovery processes suggests the presence of common mechanisms. Apparently, this phenomenon is of general biological significance. So, drought-resistant organisms of the rotifers type, class Bdelloidea, have been recently found. They also have extreme resistance to radiation: rotifers Adineta vaga and Philodina roseola are able to survive irradiation at a dose of 1120 Gy while the drought-resistant species Euchlanis dilatata has five times less radiostability.

Thereby, when adapting to the damaging effects of radiation, universal mechanisms of resistance to the chronic effects of any genotoxic factors (for example, UV radiation or oxidizing agents) or non-static environmental

conditions (for example, drought and wet cycles or high and low temperature cycles) can be of leading importance.

All in all, we can conclude that in the genotype of any sufficiently highly organized biological organism there is a dynamic balance between the level of mutation induction and the effectiveness of the mechanisms of protection of genetic material (fig. 66).

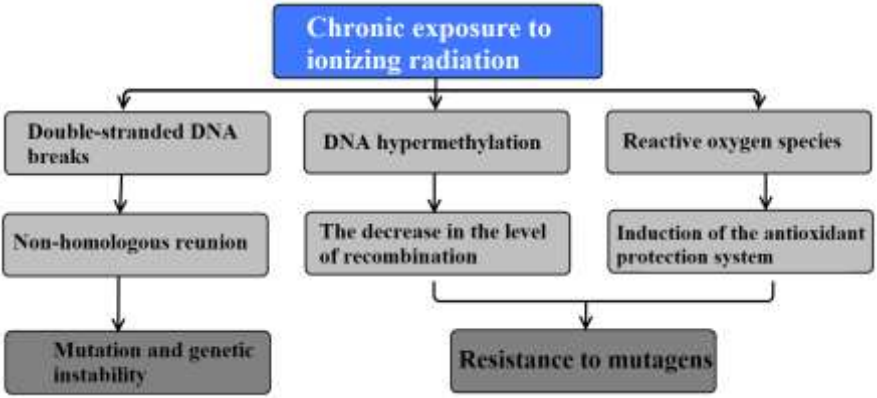


Fig. 66. A possible scheme of the mechanism of genetic adaptation to the mutagenic effect of ionizing radiation taking into account the level of mutational pressure and the effectiveness of the repair system

An increase in the mutagenic load as a result of exposure to radiation or another unfavorable factor leads to the suppression of genetic recombination and the DNA reparation system switches to a faster, but error-prone path leading to the growth of the genetic burden but allowing the individual to survive in unconventional conditions.

An equally important factor in radioadaptation is an increase in the activity of the free radical deactivation system, achieved by increasing the cell content of carotenoids and Mn^{2+} ions (bacteria), melanin (fungi), or other biologically active substances with similar activity.

Summarizing the considered phenomena, it should be emphasized that IR can cause genetic and epigenetic changes in the germ cells of parents that determine or predispose to various pathogenic conditions in offspring such as

1) teratogenesis,

2) increased incidence of disease,

3) reduced life expectancy.

During a long-term existence of a population under irradiation conditions, on the contrary, a state of radioadaptation can form, which is determined, on the one hand, by the activation of various forms of reparation and, on the other hand, by the accelerated reproduction with subsequent positive selection of the adapted forms due to natural selection. However,

when assessing the risk of exposure, not only the possible consequences of exposure in the offspring are important, but also the immediate and long-term effects of exposure to small doses of IR in somatic cells, especially in respect to humans.

9.3. Intrauterine Radiation Effects

While the effects of radiation leading to the development of cancer appear in the organs of people directly exposed to IR, hereditary effects occur as a result of the damage to the DNA of germ cells (sperm and egg cells) in the reproductive organs (testicles in men and ovaries in women) of those persons who are also exposed to IR. If DNA damage results in mutations in germ cells, they can be inherited by the offspring of the irradiated person and further to future generations. An increase in the level of these mutations will directly lead to an increase in dominant hereditary diseases. Other mutations are manifested indirectly as a result of interaction with other genes, and here the occurrence of chronic multifactorial diseases will largely depend on lifestyle or environmental factors (the so-called multifactorial diseases with a hereditary predisposition). In any case, both classes of such diseases arise naturally contributing to the appearance of hereditary defects in children. In some cases, irradiation of parents can result in congenital malformations. At the same time, studies of the incidence of congenital malformations in a large number of newborn children in areas with a high level of natural background radiation in India and China do not indicate an increase in this incedence.

The most obvious examples of the inherited consequences of the effects of IR are the results of extensive experimental studies in animals using large doses , which were carried out, in particular, on laboratory mice. As a rule, these experiments are very conclusive.

The effects of IR on the developing embryo/fetus during pregnancy can also contribute to the development of non-cancer diseases in children. In addition to the appearance of congenital malformations, the central nervous system of the unborn person is exposed to strong influence. The risks are due to two main factors:

- firstly, the dose of radiation
- and a specific stage in the development of the embryo/fetus during IR exposure

Based mainly on the results of animal studies and the few cases of pregnant women who were exposed to a sufficiently large dose of IR, ICRP concluded that the threshold for the occurrence of such effects is about 100 mGy.

There is the evidence that the risk of widespread diseases with a hereditary predisposition, in addition to cancer, may increase after IR exposure in a wide range of doses, at least from moderate to high doses of IR. The main source of the evidence is also the data from epidemiological studies on

survivors of the atomic bombings in Japan, and, in particular, the studies focused on circulatory diseases. In 2006 report, the ICRP provided an analysis of the data obtained both from a survey of survivors of the atomic bombings and from the studies of other exposed populations. When conducting this analysis, a number of difficulties arose, among them the following, as the leading ones, can be noted:

• high overall incidence of these diseases in populations that are not exposed to IR;

• the appropriate amendments to the effect of factors other than radiation exposure (for example, smoking, cholesterol, hereditary predisposition);

• the lack of evidence-based cellular mechanisms that determine their development.

The only clear evidence of an increased risk of cardiovascular disease due to exposure to IR at the doses below 1–2 Gy is also the data on survivors of the atomic bombing. Other studies that have been analyzed by the ICRP also indicate an increase in cardiovascular disease when a person is exposed to higher doses. For other non-oncological diseases in total, the same general conclusion is justified; it was concluded with respect to cardiovascular diseases; in addition, the ICRP analysis did not allow any conclusions to be drawn about the direct causal relationship between radiation at the doses below 1–2 Gy and the increased incidence of cardiovascular and other non-cancer diseases. In the field of low doses for these diseases, the relationship between the dose and the effect is not yet clear at all and is currently under intensive study.

It should also be noted that in recent epidemiological studies, in particular related to the study of the consequences of the Chernobyl accident, there is the evidence showing, so far with insufficient evidence of a possible increased risk of non-cancer diseases when irradiated with doses below 1–2 Gy and in some cases at much lower doses. However, the mechanisms of this phenomenon are still completely unclear, and the risk assessment at low doses is still problematic.

In 2006, the ICRP also evaluated the effects of IR on the immune system. In fact, it is known that different doses of IR contribute either to increase or decrease the body's potential in relation to the formation of an immunological response to an infection, cancer or other disease. In addition, in the early R.V. Petrov's works it was shown that IR is capable of inducing autoimmunity in experimental animals against their own antigens. ICRP has been the subject of many studies on the effects of IR on immunity, but it is still impossible to draw a clear and definitive conclusion as to whether AI in small doses is able to activate or suppress immunological reactions.

Finally, the ICRP notes that, as recent studies have shown, an increase in the incidence of cataracts can also be associated with the effect of IR in small doses on the human eye. For several years now, the occurrence of such disturbances in the lens of the eye has been considered a consequence of

exposure to only high doses of radiation. Mechanisms that may be relevant to explaining diseases caused by IR, for example, genomic instability and the effect of a witness, as well as new concepts and technologies that could contribute to a better understanding of the effects of IR in small doses, continue to be intensively studied on human health, as well as the biological mechanisms that explain such effects.

Part 10. Radiation Carcinogenesis

Cancer is a general term used to describe serious systemic disorders in the division, differentiation and elimination of cells in organs and tissues. Typically, cells divide and differentiate normally, and then, having worked out their lifecycle, they are coordinately eliminated in order to form organs and tissues of the body, but abnormal growth and delays in differentiation can lead to an abnormal, uncontrolled mass of cells in this organ, which is known as solid neoplasm. Such abnormal growth or development of bone marrow cells and lymphatic vessels can cause leukemia and lymphoma, respectively. Depending on a particular organ, uncontrolled growth of the neoplasm and further cellular changes can lead to the development of malignant neoplasms. There is a lot of evidence based on epidemiological studies that exposure of people to moderate and high doses of ionizing radiation can lead to an increase in the incidence of solid tumors in many organs of the human body and leukemia. More and more information is also emerging on the cellular and molecular mechanisms of the onset and possible development of oncopathology.

Cancer occurs for many reasons, it is a serious and widespread problem, which accounts for about a quarter of deaths in developed countries and more deaths in developing countries. However, if the increase in cancer incidence caused by high-dose exposures is an indisputable fact, in the case of exposure to ionizing radiation in small doses, it is quite controversial.

10.1. Epidemiological Aspects of Cancer Incidence Assessment

For several years, the International Commission on Radiological Protection (ICRP) has used a rolling review system for all studies related to the study of cancer incidence due to exposure to ionizing radiation among exposed populations. Particular attention was paid to the formation of the correct research design including the analysis of the effect on the final result of various side factors and the statistical validity of any such research in order to draw up a real overview describing the growth of cancer caused by exposure to ionizing radiation in the irradiated population. The ICRP analyzed the statistical validity of the studies, the likelihood of systemic errors, and other causes of uncertainty including those associated with the received radiation doses.

The source of epidemiological information on the incidence of cancer induced by IR is the results of a survey of people who survived the atomic bombings in Japan, the groups exposed to ionizing radiation due to their professional employment, patients who were exposed to radiation during medical procedures, and people exposed to ionizing radiation from natural sources. In recent years, it has also been possible to detect an increase in the incidence of

lung cancer in people exposed to radiation at home as a result of exposure to the naturally occurring radioactive radon and its derivatives.

Analyzing all these studies, the ICRP came to the conclusion that the single most informative data set on the effects of all types of ionizing radiation are surveys of survivors of the atomic bombings in Japan in 1945. During the atomic bombardment, people were mainly exposed to high doses of gamma radiation with a small impact of neutrons. The ICRP used this data to assess the risks of solid cancers as a result of exposure to ionizing radiation, as well as their impact on the formation of the risk of developing leukemia and lymphomas. Although statistical and other uncertainties limit the ability to analyze all available data, it was possible to study the main trends typical to radiation risks associated with gender and age at the time of exposure, and also the time passed since the exposure. The issue of how such risks for residents of different regions of the world can also be analyzed was also cosidered. To date, for some types of cancer, there is no evidence of an increased risk of its occurrence due to exposure to ionizing radiation, while for others, an increased risk appears only after the exposure to ionizing radiation at high doses.

Fig. 67 shows the different susceptibilities to the development of solid cancer in 13 different organs of the human body based on mortality data among people who survived the atomic bombing in Japan. As can be seen from this figure, there are large differences in the risk of cancer of various organs.

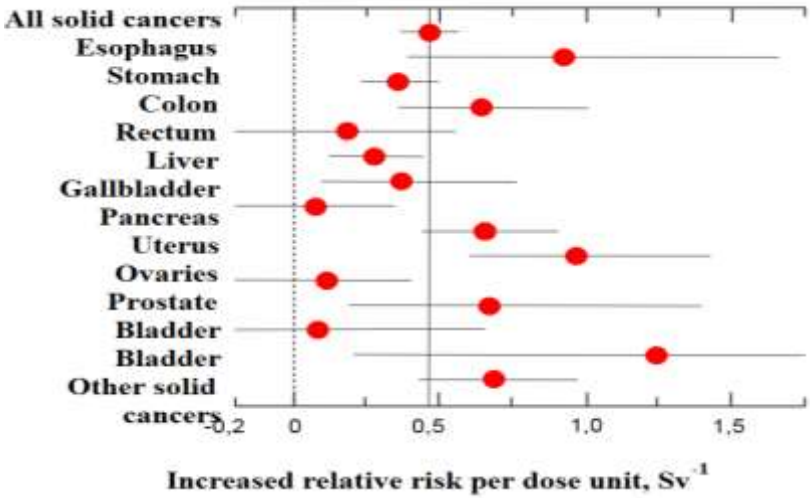


Fig. 67. Estimates of the risk of mortality from solid cancers in different organs, which were obtained by studying the data on people who survived the atomic bombing in Japan

To study the relationship between the dose received and the risk of cancer, i. e. the relationship between dose and effect, ICRP used epidemiological data. Increased comparative risk is an indicator of the increased risk of cancer in the studied populations as a result of exposure to ionizing radiation atcertain doses (a larger number indicates a higher risk). The clearest understanding of this relationship for all types of solid cancer diseases is provided by the data on survivors of the atomic bombing in Japan; this information is summarized in fig. 68. Statistically significant risk assessments are observed at doses of 100–200 mGy and higher. However, only epidemiological studies are unlikely to produce serious risk assessment results at doses much below these levels. Obtaining, based on all informative studies, a general assessment of the risk of cancer resulting from exposure to ionizing radiation throughout life is an extremely complex process.

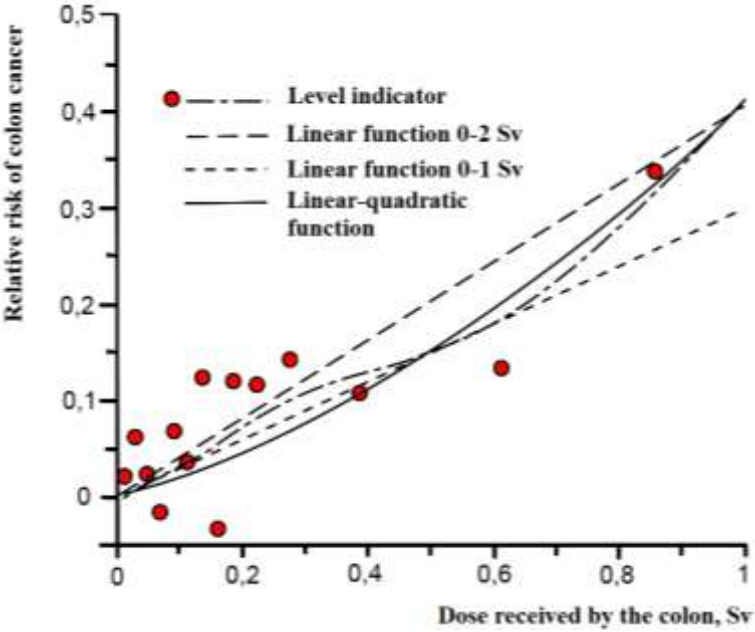


Fig. 68. Radiation dose response for solid fatal cancers based on the surveys conducted in 2002 of survivors of the atomic bombings in Japan

The current ICRP estimates of the risk of fatal cancer resulting from exposure to ionizing radiation are summarized in table 14. Risk assessments vary depending on the age of the exposed; young people have a higher level of risk. Studies of the effects of ionizing radiation during fetal development show that the embryo is especially sensitive, with an increased risk at doses of 10 mGy or higher.

Table 14

Increased risk of mortality throughout life (averagely for both sexes) * according to the UN ICRP

Dose of acute exposure (Gy)	Solid cancers in general (% at a specific dose)	Leukemia (% at a specific dose)
0,1	0,36–0,77	0,03–0,05
1,0	4,3–7,2	0,6–1,0

* Increased lifetime risk of 1.0 percent mortality means one additional case per 100 people

The effect of ionizing radiation on the survivors of the atomic bombings in Japan is very different from what we have analyzing most studies of groups of people exposed to ionizing radiation either at the workplace or from natural sources. People who survived the atomic bombardment were exposed to external radiation mainly due to gamma rays and neutrons, usually in large doses for a short period of time. Many representatives of other groups, on the contrary, were exposed to small doses for a long time, and sometimes exposure was caused by radionuclides entering the body. Epidemiological studies of the health status of employees of the Mayak nuclear complex in the South Urals in Russia and of the population living in the Techa River region, which was exposed to ionizing radiation as a result of discharge, provided valuable information on the effects of long-term effects of radionuclides entering the body in small doses. Observations of individuals exposed to ionizing radiation as a result of the Chernobyl accident also provided very useful information on the effects of external exposure in small doses on the human body and on the effects of exposure to the thyroid gland of radioactive iodine nuclides. In general, estimates of the risk of developing cancer obtained in the course of these studies do not differ much from the results obtained from a survey of survivors of the atomic bombing in Japan. Conversely, surveys of residents of areas of China and India where there is an increased natural background radiation in general do not indicate that ionizing radiation at these levels increases the risk of cancer . These and other studies provide more and more new data.

10.2. The Analysis of the Mechanisms of Radiation-Induced Oncopathology

Understanding the mechanisms of cancer development due to exposure to ionizing radiation can help to decode epidemiological data, in particular, to monitor the trends in assessing risks for small and low doses.

Over the years, the research on cancer induction has provided new evidence that this process usually begins with a change (mutation) of one or more genes. The subsequent development of cancer and the occurrence of

a malignant tumor presumably take place in several stages, and this is also associated with a mutation (or mutations) or other changes in cell genes.

The ICRP studied the findings of this kind of research, as well as many other works aimed at studying the effects of ionizing radiation at the cellular and subcellular levels. The current understanding of the problem is that the energy received by the cell after irradiation can damage all intracellular components. The main intracellular target of changes caused by IR is DNA molecules in which about 25-35 thousand genes are encoded that coordinate all functions in each cell, and if the damage caused by ionizing radiation to the gene (or group of genes) is not eliminated, the cell can die. The cell may survive, but DNA mutations will occur that affect the behavior of the cell. Even a small amount of such mutations can lead to the development of cancer. Cells have a number of special DNA recovery systems that help eliminate many forms of its damage that have arisen by chance during the life of the cell or under the influence of external factors.

The most recent studies of how ionizing radiation damages the DNA of cells, as well as cellular systems that recognize and repair damage, including the biological effects of ionizing DNA mutations, give a new insight into the possible mechanisms of cancer.

Such DNA damage is difficult to repair properly, and even with small doses of radiation there is a very small but not zero chance of DNA mutations that increase the risk of cancer. Therefore, the currently available data support a non-threshold response to a mutational component that causes cancer to develop under the influence of ionizing radiation in small doses and / or under the influence of low dose rate. Information on the nature of mutations induced by ionizing radiation shows that the process of loss of genetic information is likely to dominate this constituent component of mutations. There is also evidence that the reduction in the risk of cancer with a certain exposure to ionizing radiation in low doses and at low dose rates compared to high doses and high dose rates is associated, at least in part, with the ability of a cell to repair DNA damage after radiation exposure. In order to take into account the relative reduction in exposure to small doses and the effects of low dose rate, a correction factor is often used, known as the dose rate and dose rate; however, in the 2006 ICRP report, the linear-quadratic model was used directly to extrapolate the estimated risks at low doses, and, accordingly, the dose and dose rate exposure coefficient was not used.

The emergence and development of cancer after irradiation is not just a matter of the gradual accumulation of mutations in the DNA of the corresponding cells but a key factor in the cascade of subsequent interrelated events that ultimately result in a tumor cell that is the progenitor of the cancerous tumor. Studies have been conducted to confirm the following hypotheses:

• adaptation of cells and tissues to small doses of ionizing radiation can make them be more resistant to cancer (adaptive reaction);

• the effect of ionizing radiation on immune systems that recognize and destroy abnormal cells can affect the possibility of developing cancer;

• an additional dose of ionizing radiation can cause changes that will lead to long-term and generation-to-generation consequences for the stability of cell DNA (genomic instability) and/or cause the transmission of signals from damaged cells to their intact neighbors (bystander effect); it has been suggested that both genomic instability and the bystander effect may be factors that alter the probability of cancer resulting from exposure. These and other modulating factors, such as the occurrence of inflammatory reactions, can lead to both an increase and a decrease in the risk of developing cancer due to exposure to radiation.

The immune system that is responsible for maintaining the body's genetic homeostasis is one of the main barriers to the development of oncopathology.

At the same time, it is one of the most environmentally sensitive systems of the body, which allows it to be used as a marker of the ecological situation.

It is well known that the sensitivity of the organism in whole and its individual organs is determined by the complexity of their organization. It is quite obvious that more complex and multicomponent systems, the stability of which is maintained due to the interaction of many multidirectional processes, are more easily out of balance and, as a result, are more sensitive to any destabilizing factors. Thus, it can be stated that the sensitivity of any system is directly proportional to the degree of complexity of its organization. This statement is primarily applicable to those organs and systems of the body that can be classified as super systems.

The term "super system" means a highly integrated living system. If systems of a mechanistic type are a set of various elements that are combined and correlated in such a way that they form an organic unity, which is designed to perform certain functions, the super system is able to reproduce its elements from a common preginator. The variety of elements of the super system is formed as a result of adaptation and co-adaptation and, as a result, it is a self-regulating and self-organizing system.

On the one hand, it is a closed and self-renewing system; on the other hand, a system is open to signals from outside, capable of transforming them into internal signals and using it for self-regulation and expansion.

An example of this type of the structure is the immune system. It is formed and developed as a typical super system; its prototype can be the process of embryogenesis or the evolutionary process as a whole. As a rule, any super system is characterized by several basic criteria, which include the following:

1. A supersystem consists of many different components, or elements;

2. The components are interconnected and coordinated to perform a single function.

3. The supersystem is designed to perform specific specialized tasks.

As a rule, a super system is initially represented only by a certain form of pluripotent or totipotent precursors. The pool of the latter gradually increases due to cell division (moreover, this process often has a stochastic character), and later generations form specific receptors on the surface.

Progenitors do not have any pre-programmed relationship. On the contrary, they begin to form various relationships in the process of interaction using their receptors and adhesive molecules. Those of them that are able to interact with other surrounding cells survive, differentiate and subsequently form more mature types; cells that are unable to adapt and interact with their environment die (selection through self-adaptation). At the same time, the surrounding cells also adapt to the newly formed ones by the exchange of various signals with them (co-adaptation). Selection through self-adaptation and co-adaptation is the main principle of self-organization of super systems and underlies the normal formation of the immune system.

However, if a system develops only through adaptation and selection mechanisms, it will inevitably turn into a self-sustaining closed system. At the same time, the real super system is constantly being modernized under the influence of external signals, which include hormones, cytokines, adhesion molecules, antigens, and other factors that are perceived by cellular receptors. Receptors are used to convert an external signal into an internal one, which subsequently activates the corresponding genes. Often, external signal receptors are identical to receptors that mediate self-adaptation. Thus, the supe rsystem combines “openness” with “closeness” by using the same receptors for different purposes. For example, the immune system uses the same TCR complex for both internal selection based on the recognition of its own antigens and for the contact with external foreign antigens. As a result of these self-controlled responses to an external signal, the super system forms its own behavioral profile, which in turn forms the "self-specificity" of the individual super system.

Thanks to such a complex regulatory system, the immune system is closely interconnected with other regulatory systems of the body and, first of all, with the nervous and endocrine systems forming a single complex neuro-immuno-endocrine super system. The functioning of this supersystem is regulated by totipotent biologically active molecules: neurotransmitters, neuropeptides, hormones, as well as mediators produced by them, which cause mutual trophic effects (nerve growth factor, insulin-like substances, cytokines, IL-6, etc.)

All the components of this complex super system function on the basis of mutual regulation and the failure of functioning in one of its links in one way or another inevitably affects the others.

The above reasoning forms the conclusion that violations of the immune status in people affected by the Chernobyl accident are primarily the result of a violation of the correlated interaction of the components of this complex super system. In this regard, the cause of the violation of immune homeostasis can be both the radiation exposure itself, and, for example, post-radiation and psycho-emotional stress. All while, the changes in the function of the pituitary-adrenal and thyroid units of the neuroendocrine system were found in affected individuals, which once again emphasizes the close relationship between the various structures of this complex super system.

10.3. The Risk of Cancer Depending On the Characteristics of IR Impact

Different types of IR (e. g., X-rays, β-radiation, α-particles) differ in their effectiveness in relation to the induction of cancer. In addition, the effect of IR can be internal due to ingestion or inhalation of radioactive materials, or the external one coming from such radiation sources as, for example, diagnostic x-ray examination. The distribution of radioactive materials in the human body that has entered the body from the outside is a complex process, and although estimates of the doses received by tissues and organs as a result of this route of entry, and their effects on health are a rather difficult task, relevant studies have been developed to date models allowing such estimates to be made with a fairly high degree of accuracy.

After the accident at the Chernobyl nuclear power plant, one of the main components of the total radiation exposure was precisely the internal impact. Risk assessments for internal exposure were also obtained through epidemiological surveys of employees of the *Mayak* nuclear complex in Russia and several other groups of people exposed to IR in different countries.

Based on the accumulated data, the ICRP formulated and analyzed a database of the reasons that differentiate the hereditary and environmental predisposition of some people to certain types of cancer compared with the average indicators. There is some evidence of epidemiological studies based on a study of patients undergoing radiotherapy, which suggests that people with an increased hereditary predisposition are at greater risk of cancer after exposure. The results of experimental studies with *in vitro* cell lines and experimental animals have only reinforced this conclusion and make it possible to substantiate the position that such an increased susceptibility to IR in people predisposed to cancer can be more common than it was previously thought. Other individual characteristics (for example, age, state of the hormonal and general immune system), as well as some environmental factors (for example, exposure to toxins, diet) can contribute to an increase in individual susceptibility to radiation. However, at present, this preliminary conclusion is limited only to those cases when the family clearly shows an in-

creased predisposition to cancer. Based on epidemiological studies, in general, this is a rather rare occurrence for the population; however, a lower degree of hereditary predisposition to cancer caused by IR can be more general.

In recent years, great progress has been made in this area, thanks to the achievements of molecular genetics: the active development of the influence of IR on the level of mutagenesis in the genes of repair systems, proto-oncogenes, etc., the formation of the phenomenon of genomic instability, allows us to take a fresh look at the problem of radiation-induced cancer.

Summing up, it can be stated that epidemiological studies have not provided clear evidence that the inherited genetic predisposition to cancer, arising as a result of radiation exposure, has been transmitted to people in a number of generations. The largest and most extensive study of this problem was conducted using the data on the children and grandchildren of survivors of the atomic bombings in Japan. As a result of the analysis, the authors noted the absence of an increase in the frequency of oncological diseases in subsequent generations. Thus, these studies do not allow a direct assessment of any inherited risks due to the impact of IR. At the same time, they do not confirm the fact that there is no risk of inheriting the effects of radiation exposure, since it is difficult to detect a small excess of the incidence due to IR compared with the already sufficiently high incidence in the non-exposed populations (table 15).

However, the results of these studies are useful because give the possibility to set the top limit for assessing any IR-related risk.

Table 15

The consequences of IR for heredity (according to the UN ICRP).

The Class of the Disease	Standard Incidence (per million people)	First-Generation Risk per Radiation Dose Unit with Low LET (per million people exposed to 1 Gy)[a]
dominant (including X-ray related diseases)	16500	≈750–1500
chromosomal	4000	-[b]
chronic multifactorial diseases	650000	≈250–1200
developmental abnormalities	60000	≈2000

[a] types of radiation with low linear energy transfer (low LET) include x-rays, gamma radiation and beta particles.

[b] presumably, this partially gets into the category of risks associated with autosomal dominant diseases and diseases associated with x-ray irradiation, and partially - into the category of developmental anomalies.

However, despite the lack of evidence of the studied genetic effects of IR in humans, this kind of attempt cannot be stopped. A number of facts proves that. So, for example, to the descendants of the survivors of the atomic bombing in the first generation there is a statistically significant decrease in the size of the skull (anthropological (non-medical) microcephaly). Considering the fact that this morphological trait is polygenic in nature, it is not possible to exclude the mutagenic basis of this phenomenon.

10.4. Oncopathology Due to the Impact of IR

Cancer is the most serious of all the effects of radiation on humans even in small doses. Extensive surveys, covering about 100,000 people who survived the atomic bombings of Hiroshima and Nagasaki in 1945, showed that oncopathology seems to be the main cause of the increase in mortality in this population.

The graph (fig. 69), based on the survey of people who survived the atomic bombing, shows the approximate time period for the appearance of malignant tumors from the moment of exposure. From the graph it follows that, first of all, after a two-year latent period, leukemia develops reaching a maximum frequency 6–7 years after irradiation; then their frequency gradually decreases and after 25 years it becomes practically equal to zero. Solid tumors begin to appear approximately 10 years after irradiation, but, considering the time period, the researchers do not yet have sufficient information to construct a complete dose-response curve.

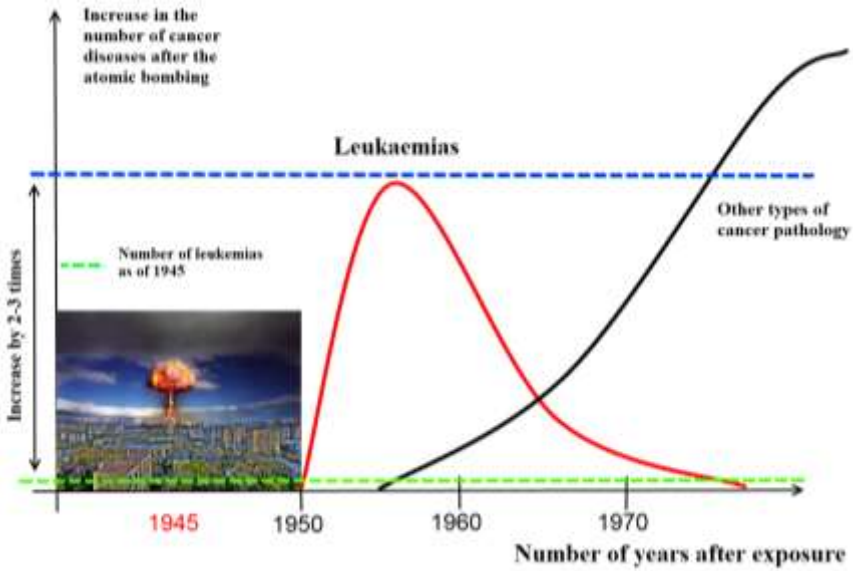


Fig. 69. The probability of cancer due to radiation exposure

UNSCEAR estimates of the risk of cancer for affected individuals also considerably rely on screening for survivors of the atomic bombing. The UNSCEAR also uses other materials including information on the incidence of cancer among Pacific Island residents when radioactive fallout occurred after nuclear tests in 1954 and among workers in uranium mines and for people who have undergone radiation therapy. But materials on Hiroshima and Nagasaki are the only source of information reflecting the results of a thorough examination for over 30 years of a large group of people of all ages and both sexes who underwent relatively more or less uniform irradiation of the whole body.

Despite all these studies, the above estimates of the probability of people getting cancer due to radiation are not entirely reliable. It should also be noted that so far there has been accumulated a lot of useful information obtained during experiments on animals; however, despite their obvious significance, they cannot fully replace information about the effect of IR on humans. To make the risk assessment of cancer for humans be sufficiently reliable, the information obtained from an examination of human populations must comply with a number of conditions:

1) the amount of the absorbed dose should be known fairly accurately;

2) the radiation should uniformly reach the whole body or at least to that part of it, where the localization of tumors is currently being studied;

3) the irradiated population should be examined regularly for several decades in order to realize all the basic nosologies of cancer diseases;

4) the diagnosis should be of sufficient quality allowing to identify all cases of cancer;

5) it is also very important to have a good control group comparable in all basic parameters (with the exception of the fact of exposure) to the group of people being monitored; this will not only determine the frequency of cancer in the absence of radiation but also reliably record the increase in the incidence (if the latter occurs);

6) both of these populations should be large enough so that the data obtained are statistically reliable (it must be remembered that, depending on the spontaneous frequency of occurrence of one or another form of oncological pathology, the number of people in control groups will vary within a fairly wide range).

None of the research materials available to the ICRP fully satisfies all of these requirements.

An even more fundamental uncertainty of this kind of data is that almost all data on the frequency of oncological diseases resulting from irradiation with IR are obtained from examining people who received relatively large doses of radiation - 1 Gy or more. There is very little information about the effects of exposure at doses associated with certain occupations, and there is absolutely no direct data on the effects of exposure received by the Earth's population in everyday life (taking into account variations in the Earth's natural background). Therefore, there is currently no alternative to the method of assessing population risk for small doses as an extrapolation of risk assessments at large and medium doses (no longer quite reliable) to the area of low radiation doses. On the other hand, it is well known that such a straightforward extrapolation can significantly cause the erroneous results, but any other alternative is currently unavailable.

UNSCEAR as well as other institutions involved in the research in this field, in its assessments relies on two main assumptions, which so far are in full agreement with all available data.

According to the first assumption, there is no threshold dose below which there is no additional risk of cancer. Any arbitrarily small dose increases the likelihood of developing cancer for the person who received this dose, and any additional dose of radiation further increases this probability. This concept is also supported by the World Health Organization (WHO).

The second assumption is that the probability or risk of a disease increases in direct proportion to the radiation dose: when the dose is doubled, the risk doubles, when a triple dose is received, it increases threefold, and so on. The UNSCEAR believes that with this assumption, risk assessment in the low-dose area is possible,, but its underestimation is hardly possible. On such a deliberately imperfect but convenient basis, all rough estimates of the risk of illness of various forms of cancer during irradiation are considered.

According to available data, the first in the group of cancers that affect the population as a result of radiation are leukemia. They cause death on average 10 years after exposure - much earlier than other types of cancer; solid tumors occur much later – 20–25 years or later.

So, according to currently available data, the mortality from leukemia among those who survived the atomic bombings of Hiroshima and Nagasaki began to decline sharply after 1970. Thus, the assessment of leukemia mortality rate due to irradiation is by far the most reliable in comparison with similar estimates for other forms of cancer. According to UNSCEAR estimates, from each dose of 1 Gy, an average of two out of a thousand people will die from leukemia. In other words, if someone receives a dose of 1 Gy by irradiating the whole body, in which the cells of the red bone marrow consistently suffer, there is one chance out of 500 that this person will die in the future from leukemia.

According current data, after leukemia the most common forms of cancer induced by IR are breast cancer and thyroid cancer. According to NKDAR estimates, approximately ten out of a thousand exposed patients have thyroid cancer, and ten out of a thousand women have breast cancer (calculated for each Gy of the individual absorbed dose).

However, both of these forms of cancer are curable in principle, and mortality from thyroid cancer, considering the post-Chernobyl experience, is especially low. Therefore, only five out of a thousand women are likely to die from breast cancer for each Gy of exposure, and only one out of a thousand exposed women appears to have a real risk of death from thyroid cancer.

Lung cancer also belongs to a group of very common forms of cancer, typical to the exposed groups of population. In addition to the above survey data for survivors of the atomic bombings of Hiroshima and Nagasaki, additional information was obtained on the incidence of lung cancer among miners in uranium mines in Canada, Czechoslovakia, the United States and Russia. It is interesting that the estimates obtained in both cases differ significantly: even if we take into account the different nature of the exposure, the probability of developing lung cancer per unit dose of radiation for miners of uranium mines was 4-7 times higher than for people who survived the atomic bombing. The UNSCEAR considered several possible reasons for this discrepancy, among which, apparently, the fact that miners are on average older than the population of Japanese cities at the time of exposure is of great importance. According to current UNSCEAR estimates, from a group of one thousand people whose age at the time of exposure exceeds 35 years, it is likely that five people will die from lung cancer per each Gy of the average individual dose.

Cancer of other organs and tissues, as it turned out, is much less common among irradiated populations. According to the NCCAR estimates, the probability of dying from cancer of the stomach, liver or colon is

approximately 1/1000 for each Gy of the average individual dose of radiation, and the risk of cancer of the bone tissue, esophagus, small intestine, bladder, pancreas, rectum and lymphatic tissue is even smaller and amounts to approximately 0.2 to 0.5 cases for each thousand irradiated and for each Gy of the average individual dose.

Children are more sensitive to the action of IR than adults, and when the fetus is irradiated, the risk of developing any form of cancer is apparently even greater. Some studies have indeed reported that infant mortality from cancer is significantly higher among those children whose mothers were exposed to x-rays during pregnancy.

However, the scientific community has a number of data indicating that there are discrepancies between the data for Japan and other sources: for example, there are significant differences in the prevalence of cancer in the affected population, both for breast cancer and for thyroid cancer. In both cases, the data for Japan provide a significantly lower incidence of cancer than other sources. In both cases, the UNSCEAR adopted large values as estimates. These contradictions once again emphasize the difficulty of obtaining real estimates of oncogenic effects in the low-dose region based on extrapolation of information related to high-dose doses and obtained from a very limited number of sources. The difficulty in obtaining more or less reliable risk assessments is further increased due to the uncertainty in estimating the doses received by survivors of the atomic bombing. New information from other sources actually cast doubt on the correctness of previous calculations of absorbed doses in Japan, and all of them are currently being re-considered.

Since obtaining real estimates is associated with great difficulties, it is not surprising that at present there is no consensus on the problem of how high the risk of cancer with small doses of IR is. Further research is needed in this area. It would be especially useful to conduct a survey of people receiving additional dose due to their professional activities and/or specific environmental conditions. Unfortunately, the lower the dose is, the more difficult it is to obtain a statistically significant result. It is estimated, for example, that if the UNSCEAR estimates are more or less correct when determining the incidence rates for all types of cancer among the personnel of nuclear fuel cycle enterprises receiving an average individual dose of about 10 mGy per year, it will take several million person-years to obtain a significant result. And it will be much more difficult to get a significant result when examining people who are affected only by the radiation background from the environment.

There are a number of issues that are even more complex but urgently require to be studied. IR, for example, can in principle have an effect on various chemical and biological agents modifying their characteristics, which, in principle, can lead in some cases to an additional increase in the frequency of cancer.

It has long been suggested that IR may accelerate the aging process and, thus, reduce life expectancy. The UNSCEAR recently reviewed all the evidence in favor of such a hypothesis but did not find enough convincing evidence to support it, both for humans and animals, at least at moderate and low doses obtained from chronic exposure. Irradiated groups of people do have a shorter life span, but in all known cases this is entirely due to the higher incidence of cancer.

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