Physics – Uspekhi **59** (4) 411 – 418 (2016)

60th ANNIVERSARY OF THE JOINT INSTITUTE FOR NUCLEAR RESEARCH (JINR) PACS numbers: **87.53. – j**, **87.56. – v**

Radiobiological research at JINR's accelerators

E A Krasavin

DOI: 10.3367/UFNe.0186.201604e.0435

Contents

Abstract. The half-a-century development of radiobiological studies at the Joint Institute for Nuclear Research (JINR) is reviewed on a stage-by-stage basis. With the use of the institute's accelerators, some key aspects of radiation biology have been settled, including the relative biological effectiveness (RBE) of various types of ionizing radiation with different physical characteristics; radiation-induced mutagenesis mechanisms, and the formation and repair of genetic structure damage. Practical space radiobiology problems that can be solved using high-energy charged particles are discussed.

Keywords: radiobiology, space radiobiology, problem of relative biological effectiveness, radiation-induced mutagenesis, damage repair, ionizing radiation

1. Introduction

The Joint Institute for Nuclear Research (JINR) is a unique scientific center operating various kinds of equipment used in nuclear physics to generate ionizing radiation with different physical characteristics. JINR has for many years attracted specialists from many countries with the opportunity to carry out basic research not only in physics but also in biology. Accelerated charged particles with different energies provide a powerful tool to solve many actual problems facing modern radiobiology.

The application of ionizing radiation with different physical characteristics over half a century has greatly contributed to the solution to *fundamental problems* of radiation genetics, including elucidation of mechanisms behind the biological action of ionizing radiation, induced mutagenesis, and particularization of physical events triggering formation of mutations.

Solving a number of very important problems that have emerged in past decades requires detailed investigation into the mechanisms of biological action of high-energy charged particles. One of them relates to practical space radiobiology. The increased distance and duration of spaceflights brought to the forefront the problem of evaluation of dangerous biological action of high-energy heavy ions and the development of radiation safety procedures to safeguard spacecraft crews.

Accelerated heavy ions (largely carbon nuclei with an energy of 200–300 MeV per nucleon) have begun to be successfully used for the treatment of oncological diseases. The optimal distribution of the absorbed radiation dose in a tumor treated with heavy ions makes this form of radiotherapy highly efficient for clinical application.

Equally important remain such problems as standardization of radiation exposure of personnel to mixed ionizing radiation fields. They necessitate research on stochastic effects of radiation influence induced by emissions differing in linear energy transfer (LET).

The first radiobiological experiments at JINR date back to 1959 when they were conducted at the proton synchrocyclotron of the Laboratory of Nuclear Problems (LNP). At that time, the USSR encountered a number of important problems that emerged in the early days of the space age in connection with the exploration of near-Earth space. The pressing need to solve these problems gave impetus to large-scale radiobiological studies and in the long run determined the range of research using JINR equipment. Artificial satellites and spacecraft launched in that period detected a high level of ionizing radiation doses in near-Earth space. It turned out that various forms of ionizing radiation existing in outer space have complicated charge and energy spectra. When preparing the first animal and human space flights, it was not clear how the living organisms would behave in response to multicomponent radiation impact, e.g., high-energy protons produced inside the Sun and arriving from the depth of the Galaxy. It became possible to address this issue on Earth by irradiating biological objects at proton accelerators generating particles with an energy of 660 MeV. The objective of those studies was to determine the relative

E A Krasavin Joint Institute for Nuclear Research,
Laboratory of Radiation Biology,
ul. Joliot-Curie 6, 141980 Dubna, Moscow region, Russian Federation
Tel. +7 (496) 216 57 40. Fax +7 (496) 216 59 48
E-mail: krasavin@jinr.ru

Received 6 July 2015
Uspekhi Fizicheskikh Nauk **186** (4) 435 – 443 (2016)
DOI: 10.3367/UFNr.0186.201604e.0435
Translated by Yu V Morozov; edited by A Radzig

biological effectiveness (RBE) of high-energy protons, i.e., their efficiency compared with that of X-rays or gamma-radiation acting on living organisms.

Specialists from various research institutes in this country [in the first place the Institute of Medico-Biological Problems (IMBP), USSR Ministry of Health] conducted experiments designed to elucidate the action of protons with an energy from 25 to 645 MeV on animals (rats, mice, dogs, monkeys), plants, mammalian, and human cell cultures. Specifically, reactions of different cell and tissue systems to acute, fractioned, and chronic proton radiation were investigated. The modifying influence of various physical and chemical factors on radiation effects was also studied. Extensive explorations were carried out to evaluate the danger of radiation exposure during short- and long-term space flights, to determine allowable radiation exposure levels, to develop methods for physical protection from cosmic radiation, etc. It was revealed that the damaging action of high-energy protons estimated from the totality of biological effects is actually similar to that of 'standard' X-rays and gamma-quanta [1].

2. Lethal and mutagenic action of accelerated heavy ions on cells

Thenceforth, radiobiological research with the application of basic JINR equipment was successfully continued by radiobiologists affiliated with JINR-based LNP and its Sector of Biological Research set up in 1978. A main objective of these studies that remained as such for a few decades was to elucidate mechanisms responsible for the difference between RBEs of ionizing radiation with different physical characteristics and, therefore, different LETs. These mechanisms are still unclear, despite extensive research in many laboratories throughout the world,

A number of mathematical models were elaborated to explain consistent features of the lethal action of radiation differing in LET on cells of different origins. However, it proved impossible to account for the dubious LET dependence of RBE, the main difficulty being RBE is ambiguously determined by various factors, both purely physical (reflecting specific features of energy transfer to the cellular matter) and biological. Although the dual nature of RBE was known long before (moreover, studies were undertaken to differentiate between physical and biological constituents in order to derive relevant formulas for calculating RBE coefficients), mechanisms underlying the differences in RBEs of different types of ionizing radiation remained unknown because an important issue, namely, the possible LET dependence of the biological constituent, was disregarded. As a result, a misconception arose that the LET dependence of RBE is totally determined by the microscopic radiation energy distribution imparted on the genetic structure responsible for realization of the radiation-induced effect.

Experiments conducted at heavy-ion accelerators of JINR's Laboratory of Nuclear Reactions showed [2] that the biological effectiveness of ionizing radiation differing in quality in terms of its lethal action on prokaryotic and eukaryotic cells depends on two factors of different natures, viz. the physical characteristics of radiation and the biological properties of the cells, i.e., their ability to recover after radiation-induced lesions (Fig. 1).

The main conclusion drawn from the results of experimental and theoretical studies that provided the basis for the solution of the RBE problem is that the DNA repair ability

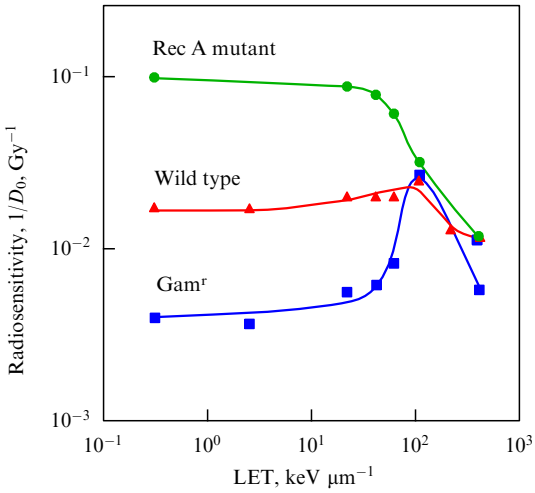

Figure 1. Dependence of the radiosensitivity of bacteria with blocked DNA repair ability (Rec A mutant), normal repair (wild type), and enhanced effectiveness of repair (Gam^r) on LET of heavy charged particles.

depends on LET, because the character of lethal lesions varies and is LET-dependent, too. It was shown that radiation with increasing LET values enhances the yield of clustered difficult-to-repair DNA lesions. Bearing in mind these findings, radiobiologists at JINR conducted a wide spectrum of research on the mutagenic action of radiation with different physical characteristics. Mathematical models were proposed describing the lethal and mutagenic action of different types of radiation on bacteria and peculiarities and mechanisms of induction of direct and reverse mutations in prokaryotic cells [3].

Information on the total yield of different types of mutations and the formation frequency of specific mutations in irradiated cells with different genotypes is of paramount importance for understanding molecular mechanisms behind the formation of both genetic and structural mutations in cells under the effect of ionizing radiation with different physical characteristics and a wide range of LET values. Such data are very difficult to obtain in mammalian cell experiments, even if they very important for solving many practical and theoretical problems. In this context, investigations into bacterial cells are indispensable for the elucidation of mechanisms of induced mutagenesis, since the structural and functional organization of the genetic apparatus in these organisms is known perfectly well; moreover, there are various repair-deficient mutants.

The available data on mutagenic action of radiation with a wide range of LETs [4] provided valuable insight into the mechanisms of the above processes. Studies on the induction of direct and reverse mutations revealed the powerlike dependence of the frequency of mutations on the irradiation dose (Fig. 2).

On the logarithmic scale, dose dependences have the form of straight lines with a tangent of the tilt angle ranging 1.7–1.8, which suggests their close-to-quadratic powerlike character. The highest efficiency of mutation induction is observed in experiments with accelerated helium ions having a LET of ≈ 20 keV μm^{-1}. Mutagenicity decreases under the influence of ions with higher LETs.

The quadratic character of the dependence of mutation frequency on the irradiation dose from heavy charged

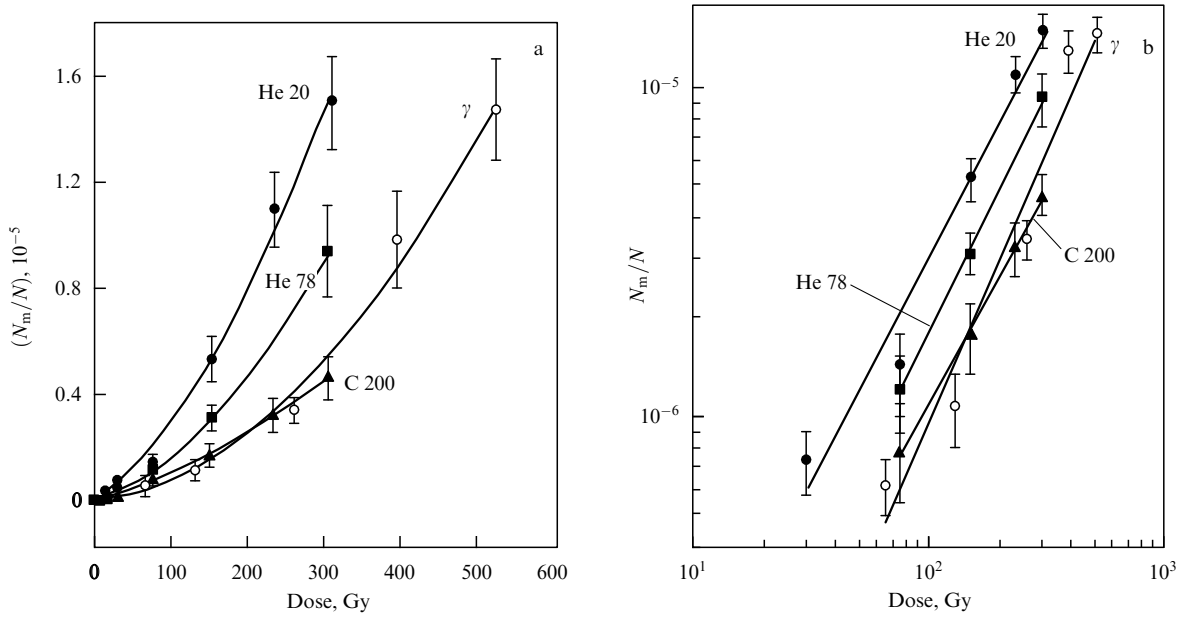

Figure 2. Formation frequency of tonB mutations under the influence of radiation with different LETs (He 20, He 78, C 200 denote LET values (in keV μm^{-1}) of respective heavy charged particles: (a) linear scale, and (b) logarithmic scale.

particles is preserved due to a number of factors. A microdosimetric analysis gives evidence that treatment with different doses of ionization radiation makes it possible to distinguish three subpopulations in an irradiated cell population, viz. intact surviving cells, fatally damaged nonsurviving cells, and moderately damaged cells that fall into the group of surviving cells after repair is completed. The fraction of intact cells increases and that of fatally damaged cells through which track core particles passed decreases with increasing LET. Due to this, most mutations occur in the subpopulation damaged by passing δ-electrons and in the small cell fracture through which one or more track core particles passed, provided these cells managed to repair damages and survive. These observations accounted for conservation of the character of dose-dependent mutagenesis, despite irradiation with different LETs.

Because the character of δ-electron energy transfer to matter under the effect of electromagnetic and corpuscular radiations does not change, the form of D-dependence of N_m/N, where N_m/N is the ratio of the number of mutant cells to the total cell number in the irradiated population, and D is the dose, remains unaltered under the effect of radiation that differs in quality. This means that heavy charged particles with high LET magnitudes ($\geqslant 100$ eeV μm^{-1}) kill the most cells when the particle tracks pass directly through sensitive cell structures, whereas surviving cells undergo so-called δ-electron mutagenesis. When cells are affected by accelerated light or heavy charged ions of high-energy particles with $LET_\infty \leqslant 100$ keV μm^{-1} and the tracks have a direct impact on sensitive structures, 'track core mutations' take place. It becomes clear from the above why the character of dose-dependent curves of mutagenesis in *E. coli* and *Bacillus subtilis* cells is preserved as the particles' LET increases.

The data obtained at heavy ion accelerators have led to the conclusion that the quadratic shape of mutagenesis curves ensues from the necessity of realization and 'interaction' of two mutually independent 'encounter' events. One of them is related to a permutation lesion in the locus of interest, while the other to the emergence of a lesion inducing the SOS-repair system that promotes fixation of changes in bacterial DNA in the form of mutations.

Because SOS-repair is a key factor in the realization of induced mutagenesis, the analysis of modern molecular mechanisms underlying the SOS-system [1] organization in *E. coli* bacteria is of primary importance.

A molecular model of induced mutagenesis was elaborated to describe the main transformation pathways of primary lesions in the mutant DNA structure (permutation lesions) [5]. According to this model, the conversion of a permutation lesion into a point mutation under the effect of ionizing radiation is a result of action of various enzymatic mechanisms, including (among the main ones) the multienzyme complex composed of inducible DNA polymerase V ($UmuD'_2C$), RccA protease, SSB proteins, and DNA polymerase III subunits.

The molecular model provided a basis for the development of a mathematical model describing the process of mutagenesis in *E. coli* cells under the exposure to UV radiation [6]. In essence, a new approach to the theoretical description of induced mutagenesis in bacterial cells was proposed. It was the first model describing the process of induced mutagenesis by a detailed mathematical depiction of key protein–protein interactions involved in the SOS response of *E. coli* bacteria. It proved possible to follow up, in the framework of a single model approach, the entire process from the appearance of a primary lesion in the DNA structure to its conversion into a mutation. The constructed models allowed predicting for the first time the dynamics of the concentration of dimeric umuD gene products and two regulatory complexes of the SOS-response system: $UmuD_2C$ and UmuDD'C. This approach made possible detailed simulation of the mechanism of translesion-synthesis respon-

[1] The protective system starting up in a cell in response to DNA lesions occurring during the cell cycle and not eliminated by DNA repair, and the onset of mutagenesis. (*Editor note*).

sible for the fixation of permutation lesions as mutations. Computations with the use of the proposed model have led to a relationship between the effectiveness of translesion-synthesis and the yield of gene mutations. Calculations done by the example of the regulatory lacI gene of *E. coli* demonstrated consistency of theoretical and experimental data on the formation frequency of lacI$^-$ mutations depending on the fluence of UV radiation energy.

These approaches open up possibilities for the further mathematical analysis of the major stages of the mutagenesis process in *E. coli* cells under the effect of ionizing radiation with different physical characteristics. Certainly, it is a more complicated but solvable problem, provided experimental data are available on the kinetics of formation and degradation of the main gene products contributing to the creation of the DNA polymerase V multienzymatic complex.

Experiments with accelerated heavy charged ions revealed that the frequency of formation of deletion mutations, unlike that of gene mutations, increases linearly with radiation dose for all kinds of ionizing radiation [4] (Fig. 3). Ions with LET $\approx$ 50 keV μm^{-1} show the highest effectiveness. Accelerated ions with a higher LET produce a weaker biological effect. In other words, dose dependences for the induction of deletion mutations in *E. coli* cells are quite different from those for gene mutations, discussed in the foregoing. In the latter case, a close-to-quadratic powerlike dependence is observed. Dose dependences for the induction of deletion mutations described by linear functions are due to different mechanisms than those underlying gene mutations. The linear character of the dependence for deletion formation under the effect of γ-irradiation of bacterial cells is attributable to the fact that the molecular basis for primary lesions leading to deletions under conditions of γ-irradiation of bacterial cells, unlike that for gene mutations, is constituted by double strand DNA breaks rather than by DNA base lesions. Induction of the SOS-repair system playing the key role in the formation of gene mutations is not required to convert permutation lesions of a given type into structural mutations.

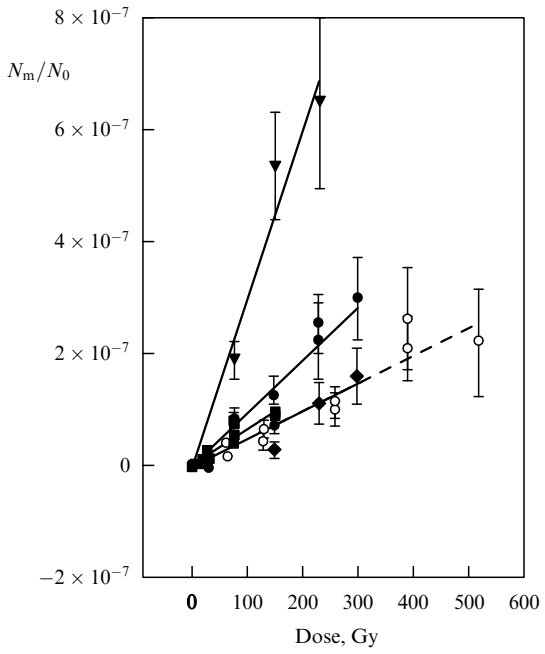

Figure 3. Dependence of occurrence of tonBtrp-mutations on the dose of radiation with different LETs. Light circles — γ-rays, dark circles — He ions (20 keV μm^{-1}), triangles — He ions (50 keV μm^{-1}), squares — He ions (78 keV μm^{-1}), and diamonds — ^{12}C ions (200 keV μm^{-1}).

Results of our studies indicate that the biological effectiveness of heavy charged particles evaluated from the induction of deletion mutations increases with increasing LET, just as well as for lethal irradiation effects and the induction of point mutations. However, the position of maxima in the LET dependence of RBE for the irradiation effects of interest is not invariant (Fig. 4).

The highest RBE values are associated with irradiation by particles having LET $\approx$ 100 keV μm^{-1}. The maximum estimated based on induction of deletion mutations occurs at LET values on the order of $\approx$ 20 keV μm^{-1}. For deletion mutations, this value approaches $\approx$ 50 keV μm^{-1}. Based on these estimates, it was concluded that the difference between positions of the maxima of the LET dependences of RBE for lethal and mutagenic effects of irradiation are due to the different character of DNA damage leading to the realization of both effects. In the former case, DNA base lesions prevail, while in the latter case DNA double-strand breaks (DSBs) occur.

The microdosimetric analysis of the yield of cluster DNA single-strand breaks (SSBs) and DSBs depending on LET values reveals that both types of dependences are described by curves with a local maximum. For cluster SSBs, however, the position of the maximum is shifted by almost an order of magnitude toward lower LET values. This observation can explain the difference between positions of the maxima in the LET dependences of RBE for lethal irradiation effects and induction of gene mutations.

Irradiation of mammalian cells by heavy ions revealed their strong mutagenic action [7]. RBE of heavy ions studied with respect to the action of γ-quanta is described by a curve with the local maxima at LET $\sim$ 80–100 keV μm^{-1}. We assumed that the mutagenic process in mammalian cells can be associated with violation of structural integrity of the chromosomal apparatus manifested as a cellular chromosomal instability. With this in mind, we undertook investigations designed to distinguish single mutant colonies to be used for growing subclones; their cytogenetic analysis was then performed [8]. The cytogenetic analysis demonstrated a

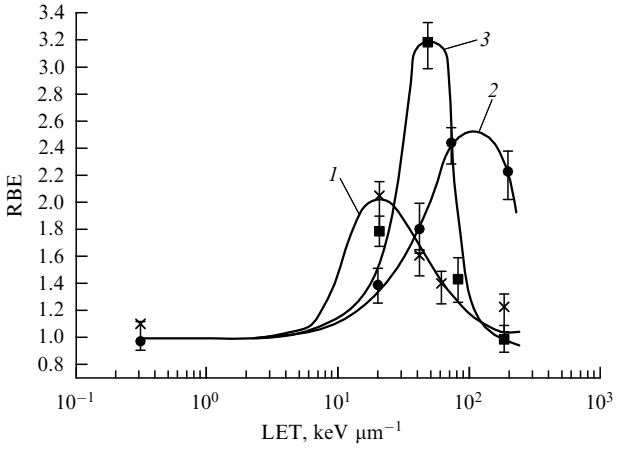

Figure 4. LET dependence of RBE evaluated based on different irradiation criteria: *1* — induction of tonB-mutations, *2* — lethal effect, and *3* — induction of tonBtrp-deletions.

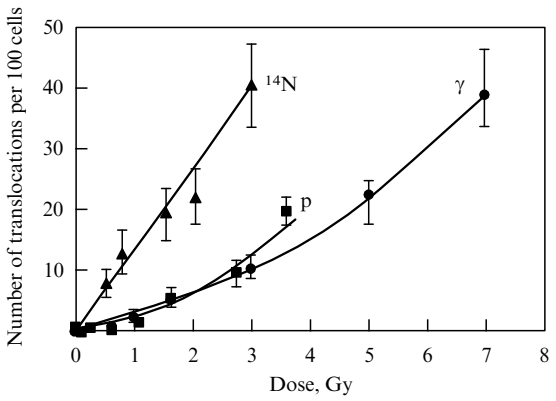

Figure 5. Dose dependences of the occurrence of chromosome-1 translocations after irradiation of human blood lymphocytes by γ-rays (circles), 1-GeV protons (squares), and ^{14}N ions (triangles).

heterogeneity of spontaneous and radiation-induced HPRT-mutagenic subclones in terms of the studied cytogenetic characteristics (mitotic activity, aneuploidy, frequency of chromosomal aberrations). It was evidenced that the consequences of mutagenic events manifest themselves as the development of genomic (in the number of chromosomes in the cell) and chromosomal (in the frequency of chromosomal aberrations) instabilities in populations of the progenies of mutant cells.

Irradiation by different types of accelerated heavy ions allowed consistent patterns of induction of stable and unstable chromosomal aberrations in human cells to be elucidated [9]. For the total number of chromosomal aberrations, the powerlike dependence of the effect on the dose of rarely ionizing radiation (protons and γ-quanta) was documented (Fig. 5). The dependence was modified into a linear one under the influence of heavy ions. However, the effect weared off at high doses of such irradiation as a result of prolonged mitotic arrest, especially in heavily damaged cells with multiple chromosomal aberrations. We used DNA samples specific to chromosomes 1 and 2 of the human lymphocyte genome. These are the largest chromosomes in the human genome and are the most susceptible to damaging when impacted by such an unfavorable factor as ionizing radiation. The fluorescence *in situ* hybridization (FISH) analysis revealed the high frequency of such stable aberrations of these chromosomes to be translocations. RBE coefficients for radiation with LETs of 80 keV μm^{-1} amounted to 3 or more.

3. DNA damage and repair under the effect of accelerated heavy ions

As is shown in Section 2, heavy charged ions induce many effects totally different from those caused by electromagnetic radiation. The difference is largely due to specific features of heavy charged particle energy transfer to cell genetic structures. In the case of irradiation by γ-quanta, the absorbed dose is transferred to bulk matter in small portions during numerous randomly distributed acts. The same dose can be transferred to the same bulk matter by a single passing heavy charged particle. The specific character of heavy ion energy transfer to genetic structures is responsible for the induction of DNA lesions qualitatively different from those caused by electromagnetic forms of ionizing radiation. This concerns, first and foremost, the formation of the most severe damage, such as DNA DSBs.

The passage of a heavy charged particle through a DNA segment not only disturbs the integrity of the two complementary strands of DNA but also damages other molecular structures adjacent to a given site. Such cluster lesions are most difficult to repair by cell reconstitution systems. They make up a molecular basis of cell death, their malignant transformation, and a variety of chromosomal mutations.

To elucidate consistent patterns and mechanisms of formation and repair of radiation-induced DNA DSBs, the JINR Laboratory of Radiation Biology (LRB) undertook investigations into the action of radiation with different physical characteristics on human cells with the use of efficient modern methods allowing the study of such processes in cell nuclei, e.g., the immunochemical staining technique using antibodies conjugated with various fluorescent dyes and specific to individual proteins (DNA foci method) and DNA comet assay.

The DNA-foci method is based on the ability of certain proteins to 'recognize' DNA DSBs in cell nuclei and participate in repair processes. One of the early stages in the formation of cell response to DNA DSB and activation of reconstitution systems is γH2AX histone phosphorylation. Phosphorylated H2AX (γH2AX) histone can be detected near DNA DSBs and serve as a signal for recruiting other proteins into DNA DSB sites. H2AX phosphorylation events can be visualized as isolated nuclear foci by the immunochemical staining technique based on specific antigen–antibody binding. A specific antibody (primary antibody) can be synthesized for each protein or antigen. The primary antibody binds to the protein being studied; then, the specific secondary antibody attaches to the primary one. The secondary antibody carries a fluorescent label that allows the protein of interest to be visualized. This method can be employed to visualize some proteins, besides γH2AX histone, involved in DNA DSB repair (e.g., 53BPI).

An international team of radiobiologists used the immunochemical staining technique and confocal microscopy [10] to obtain 3D images of human fibroblast nuclei irradiated by γ-quanta of ^{60}C (LET = 0.3 keV μm^{-1}) and accelerated ^{11}B ions (LET = 135 keV μm^{-1}) (Fig. 6). To study damaged DNA repair kinetics under the effect of ^{11}B ions, the samples were irradiated frontally with respect to the cell monolayer.

Sample irradiation by a beam at a small angle (10°) made it possible to analyze both the formation and the cluster structure of DNA lesions along the particle's track. To quantitatively estimate the induction and repair of DNA damage, we counted co-located γH2AX and 53BP1 foci (DNA DSB markers).

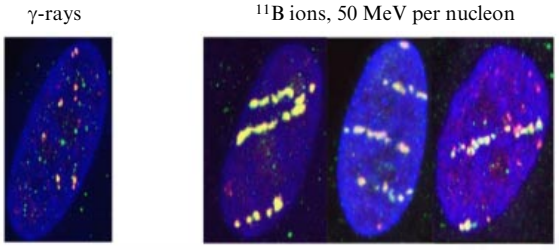

Figure 6. Formation of DNA-foci in human cells treated with γ-rays or accelerated boron ions.

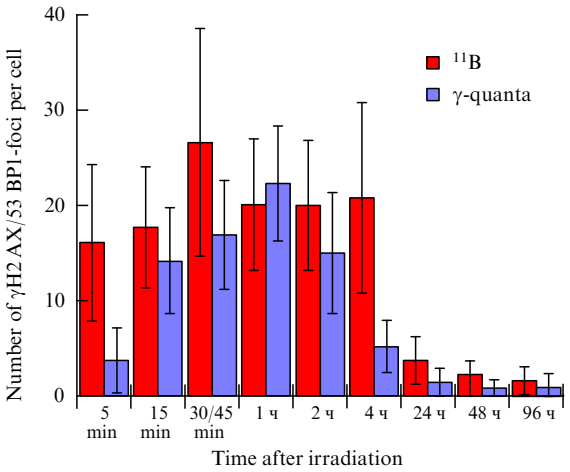

Figure 7. γH2AX/53BP1-foci formation and elimination kinetics under the effect of ^{60}C γ-quanta and accelerated ^{11}B ions.

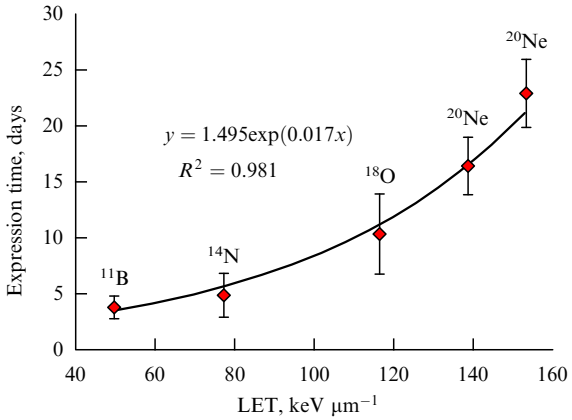

Figure 8. Maximum level of radiation-induced mutagenesis in the cells of Chinese hamsters depending on the expression time and LET of accelerated ions.

These experiments were designed to study the kinetics of the formation and elimination of radiation-induced γH1AX/53BP1 foci in fibroblast nuclei under the action of ^{60}Co γ-quanta and accelerated ^{11}B ions. It was shown that accelerated ^{11}B ions induced more γH2AX/53BP1 foci in human fibroblasts than ^{60}Co γ-quanta did (Fig. 7).

The maximum yield of gamma-radiation-induced foci (~ 25 foci per cell) is reached 1 hour after the onset of the treatment; most foci (~ 80%) are eliminated within 4 hours. The highest yield of γH2AX/53BP1 foci under the effect of accelerated ^{11}B ions (~ 72 foci per cell) occurs within 45 min of post-irradiation incubation. The number of radiation-induced foci 24 hours after the cell irradiation by accelerated ^{11}B ions is much greater than in cells treated with γ-quanta of ^{60}Co, which suggests that accelerated ions induce more severe lesions. The different character of DSB formation in DNA under the effect of γ-radiation and heavy particles was demonstrated by comparing materials after their irradiation by γ-quanta of ^{60}Co and accelerated ^{11}B ions at a dose of 1 Gy in the plane lying normally to the direction of beam propagation and at an angle of 10° to it. In the latter case, a particle propagating through the nucleus made a track consisting of a few closely located radiation-induced foci. It was shown that cluster DNA lesions are formed along the particle's track as early as the first minutes after irradiation.

Because DNA DSBs serve as a signal initiating apoptosis, i.e., programed cell death, the qualitative and quantitative differences in DNA DSB formation and, in particular, reduction of repairability in the latter case impacted by electromagnetic radiation and heavy ions must manifest themselves in the realization of the cells' apoptotic response. This conjecture was confirmed in experiments on human blood lymphocyte irradiation by γ-quanta and accelerated oxygen and neon ions (LET = 170 and 180 keV μm^{-1}, respectively) [11].

Cytogenetic scientists at JINR LRB performed large-scale heavy-ion accelerator-based research on mutagenic action of ionizing radiation with different levels of quality on mammalian cells. Cells of Chinese hamsters were irradiated by γ-quanta and accelerated ions of ^{11}B, ^{14}N, ^{18}O, ^{20}Ne (in a LET range from 50 to 153 keV μm^{-1}) to study the peculiarities of formation of HPRT-mutations. It was shown that the manifestation of mutations depends on the time of seeding irradiated cells into a 6-thioguanine supplemented selective nutrient medium (mutation expression time) and radiation LET. The rate of spontaneous and radiation-induced mutations during a four-day expression amounted to 1.2×10^{-5}. A longer expression resulted in a three-fold increase in the mutagenesis rate, up to the maximum level.

The position of this maximum depended on LET of accelerated ions. Its increase shifted the maximum toward a longer expression (Fig. 8). For example, the highest mutagenic activity was observed within 11 and 23 days after irradiation by ^{18}O ions (LET ~ 116 keV μm^{-1}) and neon ^{20}Ne ions (LET ~ 153 keV μm^{-1}), respectively. These time periods correspond to approximately 40–50 cell generations (one cell division cycle in Chinese hamsters lasts roughly 11–12 hours). Thereafter, the rate of radiation-induced mutagenesis fell to that of spontaneous mutagenesis 30–45 days after seeding. It was concluded based on the results of earlier studies that a rise in the level of radiation-induced mutagenesis can be attributed to enhanced chromosomal instability in the population of irradiated cells, and its manifestation at different expression times depends on the severity of primary damages.

The detection and selection of mutant subclones revealed mutants exhibiting retarded growth in comparison with intact controls. Growth retardation of many mutant subclones in a selective solution supplemented with 6-thioguanine could be due to mutations resulting in the reduced activity or synthesis of the HPRT enzyme. In such cases, the viability of a mutant population can be maintained only by cells that fail to utilize the purine derivative during the cycle. Unusual types of growth were reported in mutant subclones isolated from Chinese hamster cells irradiated by accelerated ^{18}O ions (LET ~ 130 keV μm^{-1}) at 0.5, 1, and 2 Gy doses. Under identical growth conditions, certain mutants exhibit nontypical morphological features differing from those of control cell population, e.g., openwork, chain, and stellate growth patterns. Colonies may appear before a cellular monolayer of mutant subclones is formed. These features are supposed to reflect initiation of malignant cell transformation.

4. Radiophysiological research

In recent years, the spectrum of studies carried out at LRB has been significantly extended to include radiophysiological research to meet the challenges of practical space radio-

biology. The crews of manned space missions are at high risk of exposure to heavy charged particles of galactic cosmic rays (GCRs). The energy range of the particles coming from the depth of Galaxy is extremely wide, up to superhigh values of $\sim 10^{20}$ eV. It appears impossible in the near future to protect the human body from the damaging action of this irradiation by physical methods.

As mentioned in Section 3, the distribution of particle energy transferred to biological structures during propagation of heavy ions (e.g., iron ions) through matter is entirely different from that in ionizing electromagnetic radiation (X-rays, gamma-quanta), which accounts for the specific action of GCR irradiation on the human body in remote space, where radiation syndromes are likely to develop, in striking contrast to those induced by rarely ionizing cosmic radiation, e.g., high-energy protons. This circumstance needs to be taken into consideration when evaluating radiation-related risks for astronauts traveling outside Earth's magnetosphere.

The currently adopted risk concept is based on the introduction of the generalized dosimetric functional as a criterion and quantitative measure of radiation hazard. The generalized dose is the sum of irradiation doses that cause *immediate* and *long-term* effects. The former develop during the flight, while the latter manifest themselves in the remaining life. When calculating doses responsible for immediate and long-term effects, coefficients are introduced to take account of the influence of radiation quality (e.g., heavy charged particles with different energies) on radiobiological effects and dose distribution in time and over the human body. Also used are coefficients of modification of the human body response to other spaceflight radiation factors. Immediate radiation effects include disorders in bone-marrow hemopoiesis, skin, and other organs and tissues of a human organism. Long-term irradiation effects are usually described in normative technical documentation from different countries with reference to the risk of *neoplastic* progression.

Moreover, the assessment of the risk of irradiation by heavy GCR nuclei during manned interplanetary missions must take into account the possibility of central nervous system (CNS) disorders [12]. Animal experiments with irradiation by high-energy iron ions at doses equivalent to the intensity of galactic iron nuclei fluxes that can supposedly be encountered during human missions to Mars demonstrated various CNS disorders, such as marked disturbance of spatial orientation and cognitive impairment. Data on the development of radiation syndromes due to the impact of heavy charged particles on brain structures resulting in derangement of its integrity, give reason to consider CNS a key system in the evaluation of the risk of irradiation impact on astronauts during interplanetary missions.

LRB investigates morphofunctional retinal lesions and variations of neurotransmitter levels in different brain regions of experimental animals irradiated by heavy charged particles [13]. The data obtained suggest a relationship between DNA damage and repair and degenerative retinal changes induced in mice by ionizing radiation (γ-radiation and protons) and the genotoxic agent methylnitrosourea (MNU). Gamma-radiation largely causes DNA SSBs uniformly distributed over the entire genome, whereas protons are more efficient in terms of induction of DSBs localized along the particle's track. DSBs are lethal lesions due to their high effectiveness in induction of apoptosis of dividing cells. The methylating agent MNU induces continuous defects in DNA, such as methylated bases, and apurinic and apyrimidinic (AP) sites. It was shown in the mid-1990s that MNU is capable of inducing apoptosis of photoreceptors in subcutaneous fat following its single administration for a dose > 60 mg kg^{-1}. In this study, MNU was used as a positive control for retinal apoptosis. Thus, the three above agents account for the main types of DNA damage and repair mechanisms. These data confirm the high radiation resistance of the mature murine retina. DNA is fully repaired following irradiation by γ-quanta and protons at a dose of 14 Gy. Enhanced expression of retinal proteins associated with cell death (apoptosis) becomes normal within 12 hours after irradiation. By this time, the repair of radiation-induced breaks in DNA is completed, meaning that these retinal proteins do not induce apoptosis but instead promote DNA repair and regeneration of damaged cells.

An increase in the radiation dose to 25 Gy caused marked morphological changes in the retinal photoreceptor layer, apparent as degradation of external photoreceptor segments and decreased density and thickness of their nuclear layer. Degradation grows with time as more photoreceptors die by apoptosis, as evidenced from the enhanced expression of proapoptotic proteins. Thus, the relatively high radiation resistance of the retina and the active mechanism of post-irradiation repair eliminating radiation-induced breaks in a DNA molecule suggest the existence of a genotoxic threshold accounting for the nonlinear character of the irradiation dose–effect relationship.

Recent studies in this field have been carried out with the use of electroretinography (ERG), providing a cumulative physiological indicator of retinal functional integrity. Registration of electroretinograms generated by flashes of white light of different intensities gives a comprehensive intravital picture of retinal functional activity in mice. The ERG profile proved to be more sensitive to genotoxic effects than morphological and cellular characteristics. This approach revealed the retina's adaptive responsiveness and ability to restore functional activity. At present, evaluation of the possible contribution of Müller glial cells to retina regeneration is underway in the Sector of Radiation Physiology, LRB, JINR. These cells make up a small population of retinal cells retaining the ability to increase their proliferation, migrate into external retinal layers, differentiate into photoreceptors, and produce endogenous neuroprotectors to safeguard retinal photoreceptors.

A series of studies with the use of 170-MeV proton beams in the Bragg peak were designed to elucidate the influence of irradiation at a dose of 1 or 2 Gy on learning ability, reproduction of skills, and concentrations of monoamines and their metabolites in the rat pre-frontal cortex, adjacent nucleus accumbens, hypothalamus, corpus striatum, and hippocampus [13]. Discrimination training with food reinforcement was conducted in a T-maze for 5 days. The animals showed a much higher learning ability on days 2 to 5 after irradiation than on day 1. The ability to perform the test decreased on day 6, the difference being significant between days 1 and 7. Irradiation by protons at 1 and 2 Gy in the Bragg peak affected neither the formation nor the reproduction of the passive avoidance reflex. The same impact decreased catecholamine levels in the pre-frontal cortex and dopamine metabolite 3-methoxytyramine concentration in the corpus striatum. The alteration of neurochemical characteristics in the above brain structures provides a logical explanation for the observed behavioral disorders.

To address the problem of irradiation by heavy GSR nuclei during outerspace flights, comprehensive investigations into the damaging action of charged particles at the molecular, cellular, and organismal levels of biological organization are needed. Of special importance in this context is the study of disorders of higher integrative brain functions; hence, the necessity to plan experiments at charged particle accelerators with the use of not only small laboratory animals but also primates. The first steps toward this goal were taken at LRB in 2013 when the 170-MeV medical beam of the LNP phasotron and the 500-MeV carbon nuclei beam of the LHEP Nuclotron were used to irradiate rhesus macaques (three animals per beam) borrowed from IMBP. The monkeys' heads (brains) received a dose up to1 Gy. The animals had been previously trained to practice computer-based problem solving tests. The aim of the experiments was to detect the deterioration of acquired skills under the effect of brain irradiation by heavy charged particles with relatively small LETs. The experiments completed, the animals were returned to IMBP to be used in routine studies.

Further extensive accelerator-based experiments are needed to solve many remaining problems.

5. Conclusion

JINR accelerators provide ample capacity for multifaceted radiobiological research concerning basic problems of radiation genetics, molecular radiobiology, and radiation physiology, as well as a wide spectrum of practical issues, the most challenging ones being space radiobiology with special reference to manned interplanetary missions and the use of charged particles for the treatment of malignant tumors. Of primary importance is the evaluation of the biological effectiveness of protons and multicharged ions (mostly carbon ions), bearing in mind the ever-growing application of medical accelerators in clinical practice. Evidently, detailed radiobiological studies using beams from such accelerators are indispensable, and the JINR's experience in this area may be of great value for the purpose.

References

1. Grigor'ev Yu G (Ed.) *Biologicheskoe Deistvie Protonov Vysokikh Energii* (*k Otsenke Radiotsionnoi Opasnosti Kosmicheskikh Poletov*) (Biological Action of High-Energy Protons (on the Evaluation of Radiation Hazard for Space Missions)) (Moscow: Atomizdat, 1967)
2. Krasavin E A *Problema OBE i Reparatsii DNK* (The Problem of RBE and DNA Repair) (Moscow: Energoatomizdat, 1989)
3. Krasavin E A, Kozubek S *Mutagennoe Deistvie Izluchenii s Raznoi LPE* (Mutagenic Action of Radiation with Different LET) (Moscow: Energoatomizdat, 1991)
4. Boreyko A V, Krasavin E A *Phys. Part. Nucl.* **42** 998 (2011); *Fiz. Elem. Chast. At. Yad.* **42** 1865 (2011)
5. Belov O V, Krasavin E A, Parkhomenko A Y *J. Theor. Biol.* **261** 388 (2009)
6. Bugay A N et al. *J. Theor. Biol.* **364** 7 (2015)
7. Govorun R D et al. *Adv. Space Res.* **30** 885 (2002)
8. Krasavin E A et al. *Phys. Part. Nucl.* **35** 797 (2004); *Fiz. Elem. Chast. At. Yad.* **35** 1483 (2004)
9. Falk M et al. *J. Radiat. Res.* **55** (Suppl. 1) i81 (2014)
10. Baranova E V et al., in *Rapid Diagnosis in Populations at Risk from Radiation and Chemicals, Proc. of the NATO Advanced Training Course on Rapid Diagnosis in Population at Emergency and Risk, Krakow-Zakopane, Poland, 19 – 24 October 2009* (NATO Science for Peace and Security Series. E, Vol. 73, Eds A Cebulska-Wasilewska, A N Osipov, F Darroudi) (Washington, D.C.: IOS Press 2010) p. 261
11. Grigor'ev A I, Krasavin E A, Ostrovskii M A *Ross. Fiziol. Zh. Sechenova* **99** 273 (2013)
12. Tronov V A et al. *Cell Tissue Biol.* **6** 219 (2012); *Tsitologiya* **54** 261 (2012)
13. Shtemberg A S et al. *Neurochem. J.* **9** 66 (2015); *Neirokhimiya* **32** 78 (2015)