

In 2009, the research programme of the Laboratory of Radiation Biology (LRB) determined by first-priority theme 04-9-1077-2009/2011 «Research of Biological Action of Heavy Charged Particles with Different Energy» was mainly focused on the following areas: fundamental radiobiological and radiation genetic research with heavy charged particle beams; research on the effects of accelerated heavy charged particles on

eye structures (the lens and retina); molecular dynamics research; mathematical modelling of induced mutagenesis in bacterial cells; radiation research and radiation protection at JINR's basic nuclear facilities and environment. Special attention was paid to the participation of young researchers, students, and postgraduates in the LRB's current events and in conferences and seminars where the LRB took part.

RADIOBIOLOGICAL AND RADIATION GENETIC RESEARCH

Radiation Genetics of Lower Eukaryotes

In 2009, research was continued on the regularities in the mutation process in *Saccharomycetes*. Regularities were studied in the occurrence of spontaneous mutations and mutations induced by electromagnetic radiation (ultraviolet light) and ionizing radiation (γ rays) depending on the checkpoint control functionality and the mitochondrial activity. In this work, genetic systems were used to test recombination (interchromosomal and intrachromosomal recombination, including recombination accompanied by the formation of deletions), deletions, and frame shift mutations. To suppress checkpoint control, mutants of the *rad53* kinase, which participates in the activation of numerous ways of checkpoint control, were used. Experiments showed that the *rad53* mutation suppresses deletion mutagenesis, and the *RAD53* gene participates in nonhomologous end joining (NHEJ) accompanied by the formation of deletions. Besides, the influence of the mitochondrial functionality on the level of mutagenesis was studied. The respiration activity in mitochondria was suppressed in experiments by the ρ^- and ρ^0 mutations of respiratory failure. It was shown that respiratory disorder influences the survival rate, recombination, and formation of deletions, while no influence on the formation of the frame shift mutations was observed.

Research was started on the participation of mutations localized in the genes coding the subunits of protein complexes (e.g., the CDC28 kinase, SAGA histoneacetyltransferase, and regulator of the Sir2 deacetylase localization) in cell apoptosis and aging. It was shown earlier by the Radiation Genetics of Yeasts Research Group that these genes participate in repair and checkpoint control [5, 7].

The CDC28 kinase plays a key role in the cell cycle regulation. Studies of the pleiotropic manifestations of the *cdc28-srm* [Gly20Ser] mutation and the *cdc28-13* [Arg283Gln] mutation is topical because disorder in the functioning of the homologous kinase in human cells leads to the malignant transformation of cells.

The use of dynamic modeling allowed the determination of the structure changes induced by the corresponding mutations in the homologous human CDK2 kinase. The *cdc28-srm* mutation consists in a substitution for the third glycine in the GxGxxG conservative sequence in the so-called G-rich loop in the small lobe of the kinase subunit; it is located against the T loop in the large lobe of the kinase subunit. Although the importance of the G and T loops is established, their role is still poorly studied. To investigate the structure of kinases, including CDC28, the crystal structure of the human CDK2 kinase is used now. The analysis of the nanosecond dynamics of the CDK2/ATP complex progression has been continued [6].

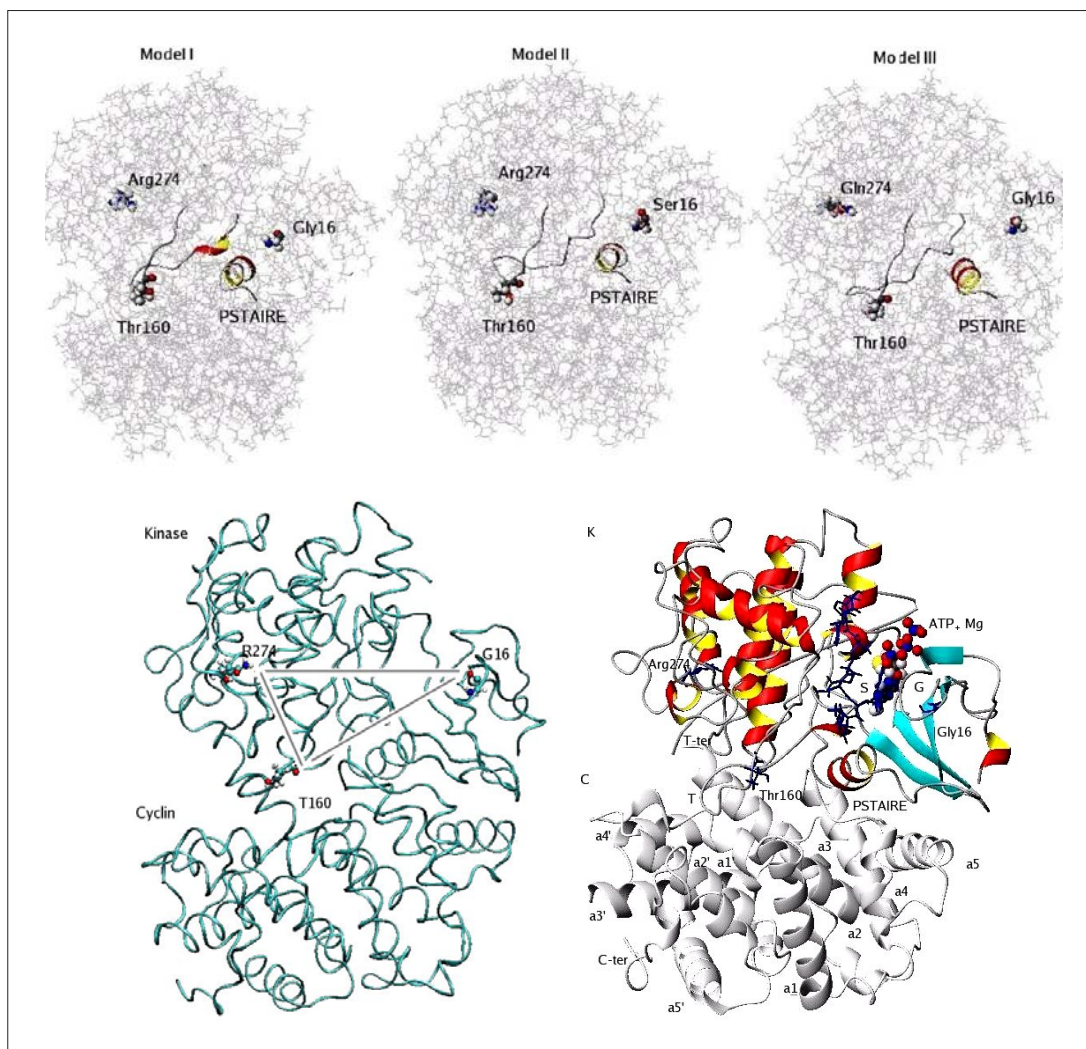


Fig. 1. A model of the CDK2 kinase and cyclin A. Shown are the positions of the studied amino acid residues G16 in the G loop of the small lobe, R274 in the large lobe, and T160 in the T loop of the large lobe of the kinase are shown

The molecular dynamics modeling of the mutant structures of the kinase with substitutions for the CDK2-G16S amino acid in the G-rich loop of the small lobe and CDK2-R284Q amino acid in the large lobe showed the importance of these amino acids and their influence on the CDK2 kinase conformation, which is observed as an increase in the distance between the G and T loops in the corresponding mutant forms. The obtained results show that mutations destabilize the local structure in the T loop area (Fig. 1). The Arg284 → Gln284 mutation in the distant C-end area has a more pronounced effect and leads to the loosening of the kinase structure and an increase in the distance between the G and T loops. Research on the tertiary structure of the native and mutant proteins is promising for the development of medicines.

Radiation Cytogenetics

In 2009, research was continued to estimate the variability of individual sensitivity of chromosome apparatus to high-LET radiation. For this purpose, aberrations

induced in the G0 and G2 cell cycle phases in human blood lymphocytes irradiated with γ rays and charged particles of different LET were analyzed. Whole blood samples were irradiated with γ rays (4 donors), protons (7 donors), ^{12}C (9 donors), ^7Li (2 donors), ^{11}B (3 donors), and ^{20}Ne ions (4 donors). The ion beam energies and LET values were, respectively, as follows: protons — 170 MeV and ≈ 0.5 keV/ μm ; ^{12}C — 480 MeV/amu and 10.6 keV/ μm ; ^7Li — 30 MeV/amu and 20 keV/ μm ; ^{11}B — 32 MeV/amu and 55 keV/ μm ; ^{20}Ne — 53 MeV/amu and 170 keV/ μm . Chromosome aberrations induced in the G0 and chromatid aberrations induced in the G2 cell cycle phases were measured both with conventional metaphase technique (in normal mitotic cells) and with the premature chromosome condensation (PCC) technique (calyculin A-induced premature chromosome condensation). For chromosome aberration analysis, lymphocytes exposed to protons, ^7Li , ^{11}B , and ^{20}Ne ions were harvested at several sampling times (48–70 h). A fluorescent in situ hybridization (FISH) analysis of stable and unstable chromosome aberrations

was performed in prematurely condensed chromosomes of peripheral blood lymphocytes (PBL) exposed to protons, ^{12}C , ^7Li , and ^{11}B and incubated for 48 h.

A clear interindividual variability was observed for both chromosome and chromatid aberration yields. Generally, the results show a greater interdonor variability of aberration frequencies for charged particles than for γ rays. Some disagreement concerning the level of radiosensitivity was seen between conventional metaphase analysis and the PCC method. To compare the variability in aberration frequencies induced by different radiation types, the coefficient of variability was calculated for both chromosome and chromatid aberrations. On the average, the frequencies of aberrations induced in G2 show a greater interdonor variability than those induced in G0 [8].

Also, aberrations scored by the conventional Giemsa method and PCC + FISH were used for the assessment of the interindividual variability of the F (the ratio of dicentric to ring frequencies) and C (the ratio of complex to simple aberrations frequencies) ratios in chromosomes of several donors' peripheral blood lymphocytes irradiated with protons and heavy ions of different LET. The F ratio was found to be dose-dependent for γ rays, whereas no dependence on the dose and sampling time was seen for charged particles. Results of the PCC + FISH analysis show a considerably higher F

ratio for protons than for heavier ions. On the average the C ratio was found to be corresponding to LET. The interdonor variability of the C ratio was greater than that of the F ratio. These results explain very well the difference between data obtained at other laboratories over the world and confirm the importance of PCC + FISH analysis for determining the quality of radiation in biological dosimetry [9].

In 2009, the study of the chromosome aberrations in human blood lymphocytes irradiated with protons at the therapeutic beam of the JINR Phasotron in different cell cycle phases won the second prize for research and applied work of the 13th Conference of JINR's Association of Young Scientists and Specialists.

Using a morphological method involving fluorescent staining of samples by a mixture of dyes (ethidium bromide and acridine orange), regularities were studied in the induction of apoptosis by ^{60}Co γ quanta in human lymphocytes at different times after irradiation: 0, 24, 48, and 72 h. The variability of the results was observed among donors of different ages (21–62 years) [1, 2].

Regularities were studied in the induction of apoptosis in human lymphocytes in different phases of the cell cycle after irradiation with γ quanta and Bragg peak protons (Fig. 2). It was shown that cells in the G2 and S phases are the most sensitive against this criterion [3].

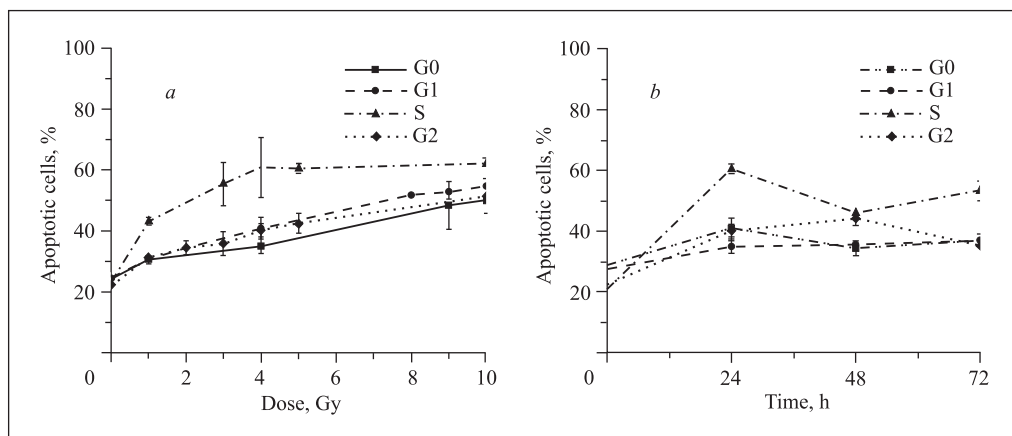


Fig. 2. a) Apoptosis induction in human lymphocytes in different cell cycle phases; b) apoptosis kinetics in different cell cycle phases

The influence of inhibitors of the replicative and reparative DNA synthesis — cytosine arabinoside (Ara-C) and hydroxyurea (HU) — on the kinetics of γ -induced apoptosis in human lymphocytes was studied. It was shown that in the presence of inhibitors, the induced apoptosis frequency increases, which correlates with the active accumulation of enzymatic DNA DSBs in these conditions. As is known, DSBs are the molecular basis of the events triggering apoptosis (Fig. 3).

The influence of the combined effect of γ radiation and lipid A obtained from *Escherichia coli* on the phagocyte, lysozyme, and peroxidase activities of hu-

man leukocytes was studied. It was found that a low concentration of lipid A significantly influences human cells under γ irradiation by increasing their phagocyte and lysozyme activity. Besides, under low doses of irradiation, lipid A decreases peroxidase activity. On the grounds of the obtained results, a conclusion was made that under ionizing radiation lipid A has a modifying effect on the phagocyte, lysozyme, and peroxidase properties of the cells forming immune response [4]. Figure 4 shows the modifying effect of lipid A on the relative immune activity of human leukocytes irradiated with ^{60}Co γ quanta.

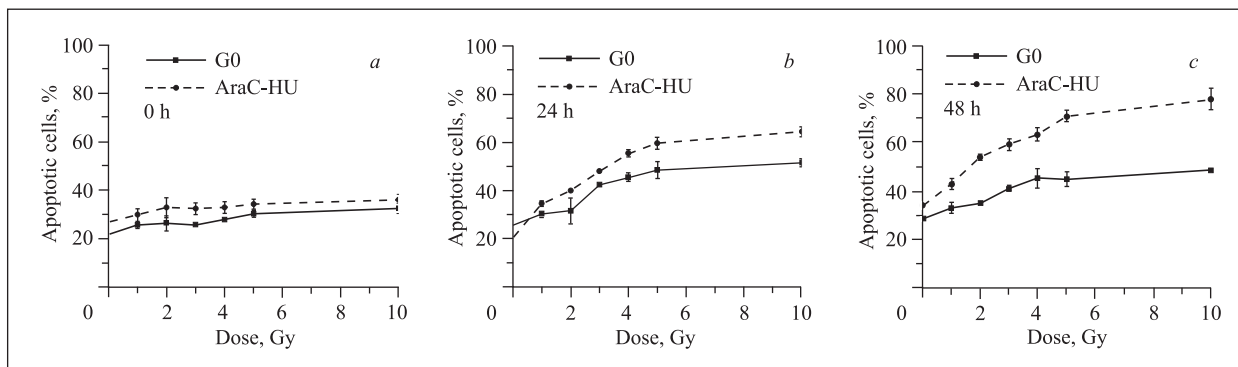


Fig. 3. Induction of apoptosis in human lymphocytes by γ irradiation in the presence of inhibitors of the replicative and reparative synthesis of DNA (after 0 h (a), 24 h (b), and 48 h (c))

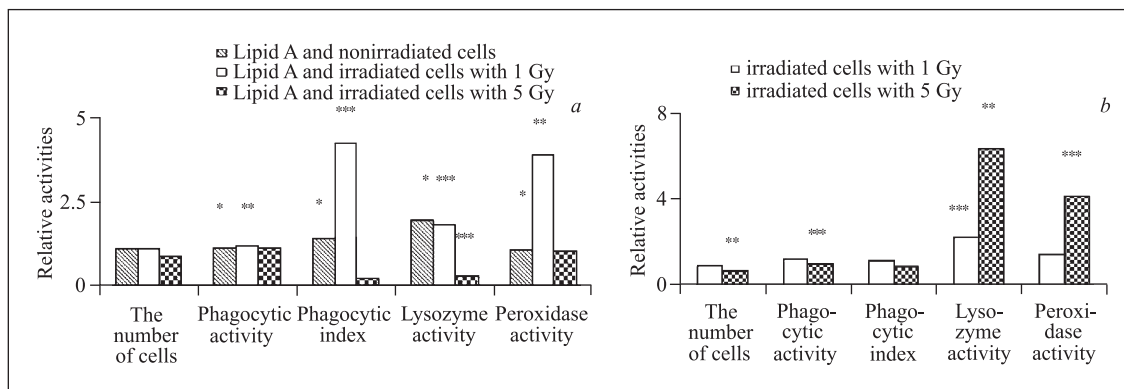


Fig. 4. Modifying effect of lipid A on the immune activity of human leukocytes irradiated with ^{60}Co γ quanta (* is $p < 0.05$, ** is $p < 0.01$, *** is $p < 0.001$)

Taking into account the performed work, further research on the genetic effect of high-energy heavy charged particles is planned, which will facilitate the solution of many practical issues challenging space radiobiology, radiation genetics, and medicine.

It was previously shown that low-dose γ irradiation induces complex nonlinear dependences of the number of aberrant cells on the dose. The region of high sensitivity to chromosome damage has been identified in mammalian cells at doses below 5–7 cGy, which precedes the occurrence of increased radioresistance (IRR). The nature of these phenomena is unknown. The observed hyperradiosensitivity (HRS) could be a consequence of a radiation-induced increase in reactive oxygen species (ROS) production in normal metabolism. This occurs, in particular, in the mitochondrial electron transport chain due to mitochondrial permeability transition (MPT). On the other hand, the radiation-induced amplification of ROS was shown to be a sensing mechanism for activation of cellular cytoprotective pathways. The extracellular signal-regulated kinase (ERK) seemed to be a better candidate for the role of the main protective protein in this case. Based on these findings, it was suggested that the activation of

pathways aimed at reducing the oxidative stress may cause a decrease in the number of aberrant cells observed in experiments after HRS. To investigate this hypothesis, it was examined whether substances affecting ROS, MPT, and ERK activity would prevent arising HRS and IRR modifying as a result of the dose–effect shape.

Human cal51 mammary carcinoma cells were exposed to 1–20 cGy γ irradiation in the presence of different modifiers: TPA (an ERK activator), PD98059 (an ERK inhibitor), and CsA (an MPT inhibitor); the ROS-scavenger DMSO was added immediately after irradiation so as not to affect radicals appearing due to water radiolysis. The number of aberrant anaphases was assessed after 8-hour incubation. The results are presented in Fig. 5.

DMSO prevents both HRS and IRR bringing the shape of dose–response curve closer to linear. The effect of the CsA is more pronounced: the aberrant cells yield drops to nearly the background level in the presence of this drug. There is a significant decrease in the number of aberrant cells treated with the ERK activator. This is most dramatic in the HRS region. Conversely, the cells pretreated with the ERK inhibitor lose IRR observed in untreated samples.

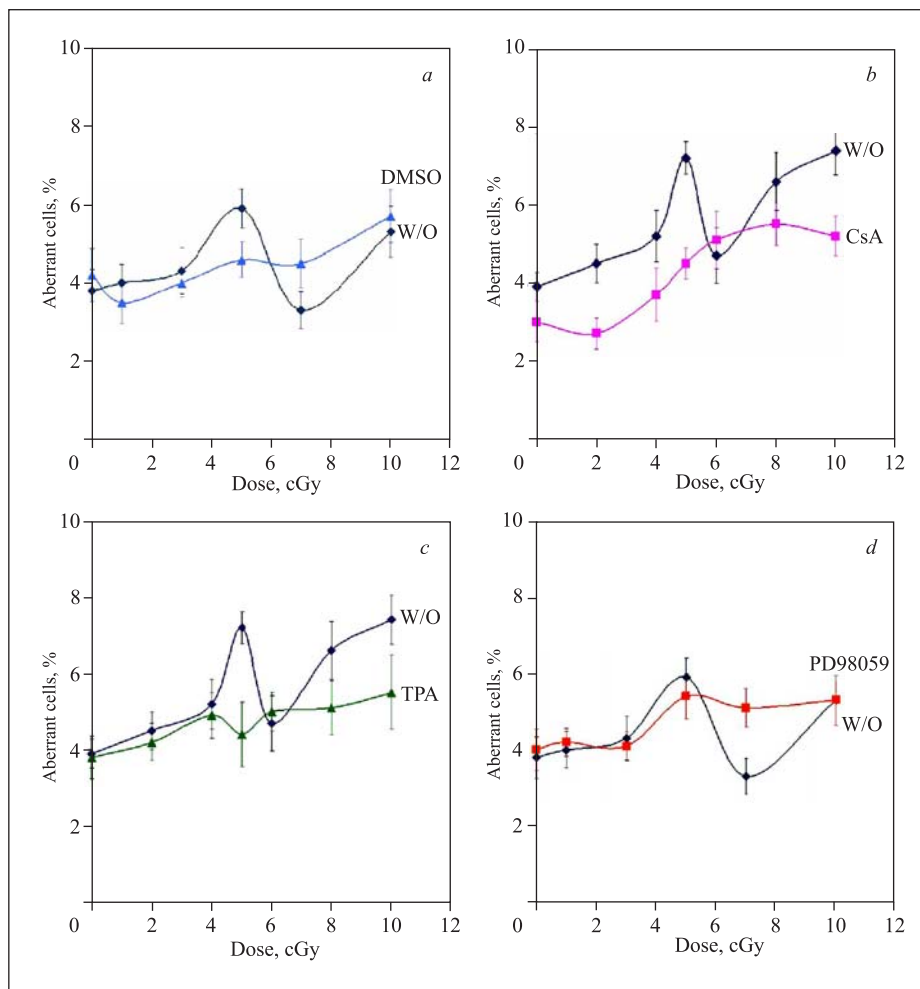


Fig. 5. Effect of modifiers on the frequency of aberrant cells induced by low-dose γ irradiation in cal51 mammary carcinoma cells

These results point strongly to ROS being a mediator of hyperradiosensitivity at low doses as well as signaling molecules of cytoprotective mechanism activation. As CsA-treatment shows, these ROS are mitochondrial in origin. The activation of ERK is required to protect cells against oxidative damage caused by ROS.

The investigation of adaptive response (AR) was continued on human lymphocytes. AR is the phenomenon by which cells exposed to low dose of radiation become less sensitive to subsequent high-dose exposure. It is assumed that AR could be a consequence of IRR as a result of protective mechanisms induction. No clear correlation between these phenomena was established due to high intra- and interdonor variability.

Mathematical Simulation of Radiation-Induced Mutagenesis

New mathematical approaches to the modeling of the mutation process induced by ultraviolet (UV) radiation in *Escherichia coli* (*E. coli*) bacterial cells were developed. A model was proposed that presents the

induced mutation process in terms of a detailed mathematical description of the key protein interactions in the course of the functioning of the *E. coli* SOS response system. For the first time, a connection is shown between the SOS response processes and translesion synthesis (TLS) efficiency; the whole chain of events from a damaging factor affecting the cell to the mutation formation in the DNA chain is analyzed in detail. Numerical solutions of the model's equations for each protein of the SOS system were obtained.

A mathematical model of TLS in *E. coli* bacterial cells was developed. A quantitative evaluation was performed of the probability of the occurrence of a certain number of errors during TLS. On the basis of the proposed model concepts, a quantitative estimation was performed of the gene mutation yield under UV irradiation. Using the *lacI* regulatory gene of *E. coli*, the dependence was calculated of the mutation frequency on the UV dose. For specific values of the model's free parameters, the modeling results match the experimental data [10–12].

A mathematical model was developed that describes the dynamics of the inducing signal of the SOS system

in *E. coli* bacterial cells under accelerated ions. Using the earlier developed model [13], the process of the formation of the main pre-mutation DNA lesions — base lesions (BL), single- and double-strand breaks (SSB and DSB), and clustered lesions (CL) — was described. In the used model, DNA is considered to be a linear target randomly positioned relative to the charged particle track. The proposed model takes into account the radial energy distribution in the particle tracks, which is very important for the assessment of the role of the delta electrons in the radiobiological effects of radiations with different LET.

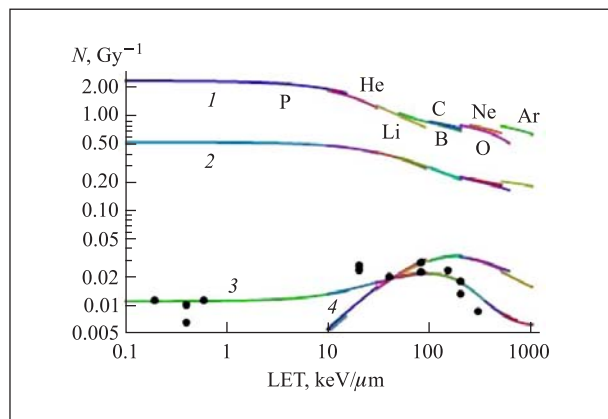


Fig. 6. The yield per one genome: base lesions (1), DNA single-strand breaks (2), clustered lesions (3), and DNA double-strand breaks (4) depending on the LET of particles with an energy of 3–10 MeV/nucleon (dots are the experimental data characterizing the dependence of the quantity SOSIP on the LET of radiations [13])

Figure 6 shows the comparative calculation of the BL, SSB, DSB, and CL yield depending on LET of different types of ions. The obtained results confirm that the character of the BL yield dependence on LET is similar to that of the DNA SSB yield dependence on LET, the DNA BL yield being four times higher than the SSB yield over the whole measured LET range due to an effective increase in the thickness of the linear DNA target. The DSB and CL yields are described by a curve with a maximum, after which any further increase in LET is inefficient. A comparison was made

PHOTORADIOBIOLOGICAL RESEARCH

A cataract, which is an opacity of the lens, is one of the main causes of eyesight deterioration. The initiation and development of a cataract is a disease that goes along with aging; moreover, it can be a marker of the organism aging. A lot of internal and external factors accelerate cataract development. For example, an increase in the blood sugar level makes for

between the calculation results describing the total yield of CL (independently of their type) and experimental data [13] characterizing the dependence of the quantity SOS induction potency (SOSIP), quantity measured by the SOS chromotest method, on LET of radiations. The calculation results were found to agree with the experimental data.

Models were developed of the main types of repair which lead to the formation of an inducing signal for the SOS system. It is the presence of single-strand parts of DNA that is the signal. As part of the research, the following types of repair were modeled: SSB repair involving DNA polymerase I, DSB repair by homologous recombination, modified base reconstruction by excision repair, and clustered lesion repair by SOS response. The models take into account the processes of clustered lesions transforming into DSBs and the transformation of DSBs into SSBs with the development of a temporary or permanent heteroduplex.

Results were obtained that characterize the inducing signal concentration dynamics under the influence of different types of ions in wild-type *E. coli* cells and in *umuDC* mutants.

A stochastic SOS response model was developed based on the Gillespie algorithm, which is widely used to model complex biological systems. This approach has the advantages of the correct description of the kinetics of the main protein interactions during SOS response in an isolated bacterial cell under low energy fluences of ultraviolet (UV) radiation ($< 1 \text{ J/m}^2$), and a possibility of the quantitative evaluation of the survival rate of cells under UV radiation. The proposed model allows the description of SOS response kinetics in *E. coli* bacterial cells for an arbitrary value of the energy fluence of UV radiation and, in perspective, can be extended to include other external damaging factors. In this work, the dynamics of primary and secondary DNA lesion formation and the main protein complexes in an isolated cell, and the survival rate of a cell population were calculated. Significant fluctuations of the concentration of the SOS system proteins were revealed in the case of a small number of molecules for the dimerized products of the *umuD* gene.

the diabetic cataract; ultraviolet (UV) irradiation leads to the UV cataract. A hypothesis has been around for more than 50 years that radiation accelerates aging. From this viewpoint, a radiation cataract can be considered to be a senile one. The lens opacity associated with a radiation cataract has a local character. In recent years, however, evidence appeared that cos-

monautes' cataract has a diffuse character. In this connection, it was suggested that this kind of cataract can be caused by the specifics of galactic radiation, which includes heavy charged particles (HCP). HCP damage crystallins (the lens proteins) causing internal breaks in molecules. After returning to the Earth, the lens is exposed to UV radiation, which reveals these «weakened» molecules; then the protein is denatured and aggregated. The short-range packing of molecules is thus disordered; local changes in protein concentration appear; and, as a result, the medium refraction coefficient locally changes; light scattering is enhanced; and an opacity develops.

To check this hypothesis, two series of experiments were performed: an in vitro study of HCP irradiation of crystallin solutions aimed at determining changes in their stability, and an in vivo study of cataract induction by radiation combined with UV rays.

An In Vitro Study of Changes in the Stability of Solutions of Crystallins under HCP Irradiation

Solutions of α and β_L crystallins of a bull lens obtained by gel filtration were irradiated with B^{11} , C^{12} and Li nuclei; then, structural and functional changes in the proteins were studied. The research focused on the stability of β_L crystallin against heating- and photo-induced denaturation, as well as the chaperone activity of α crystallin against the heating- and photo-induced denaturation of β_L crystallin. The structural changes in the proteins were characterized using tryptophan and nontryptophan fluorescence techniques, gel filtration, and electrophoresis in polyacrylamide gel (PAAG) in denaturing and reducing conditions. Irradiation with 32-MeV B^{11} nuclei did not lead to any evident changes in the stability of β_L crystallin against thermal denaturation, while a dose of 8 Gy resulted in a 30% shortening of the photo aggregation lag period under UV light. The electrophoresis data showed no irradiation-induced changes in the polypeptide composition of the samples. A study of fluorescence spectra indicates that there are certain conformational restructurings in β_L crystallin molecule already at a dose of 4 Gy. Gel filtration and small-angle X-ray scattering studies of the α crystallin showed that irradiation with B^{11} nuclei does not result in any significant changes either in the size or shape of the molecule. Also, no protein subunit linkages were observed (electrophoresis in PAAG). No changes in the chaperone activity against the thermal denaturation of β_L crystallin were also observed, but some decrease in the protein chaperone activity was observed against photo aggregation. Irradiation with 500-MeV C^{12} nuclei of the α and β_L crystallin was performed twice, but no significant changes were observed.

Irradiation with Li nuclei did not affect β_L crystallin stability against thermal denaturation. A study of the protein stability against photo aggregation un-

der UV light showed that irradiation shortens the lag phase and accelerates aggregation. However, the dose dependence turned out to be essentially nonlinear: the photo aggregation acceleration was maximal for a dose of 4 Gy, while a dose of 16 Gy did not lead to any deviation from the reference level. The electrophoresis data also showed no radiation-induced changes in the polypeptide composition of the samples. A study of fluorescence spectra showed that there are no evident changes in molecular spectra. A gel filtration study of α crystallin showed that irradiation does not cause significant changes in the size of the molecule; also, no protein subunit linkages were observed (electrophoresis in PAAG). No changes in the chaperone activity against thermal denaturation of β_L crystallin were observed. But, like under irradiation with B^{11} , some decrease was observed in the chaperone activity of the protein against photo aggregation. Thus, on the grounds of the obtained results, it is possible to conclude that high-energy nuclei (500 MeV) with a dose rate of 4 Gy/h do not affect crystallins, while nuclei with an energy of about 40 MeV with a dose rate of 4 Gy/min can damage both α and β_L crystallin molecules. The results, however, are rather contradictory and require further research.

Cataract Induction by γ Quanta Combined with UV Irradiation

Four randomized groups of F1 male mice (C57Black/CBA) were daily exposed to whole-body UV irradiation (280–380 nm, 15 min, $(5.5 \pm 0.8) \text{ W/m}^2$); once — to a whole-body γ quanta with a dose of 2 Gy; and to a combination of these factors (γ and UV rays). Group 4 was used as the reference for the age-related changes (R). The development of a cataract in the 7th and 10th months was evaluated by the peer review method using a 6-point scale. In the 7th month, the lens opacity was as follows. R: 0.0; UV: 2.50 ± 0.13 ; γ rays: 1.00 ± 0.28 ; γ rays + UV: 4.00 ± 0.45 (median $\pm \sigma$). The differences between the groups are reliable ($p < 0.001$ against the Mann–Whitney U-test). In the 10th month, the lens opacity significantly increased; its values were the following. R: 2.75 ± 0.17 ; UV: 5.75 ± 0.42 ; γ rays: 6.00 ± 0.29 ; γ rays + UV: 7.00 ± 0.38 (median $\pm \sigma$). The differences between the groups are also reliable ($p < 0.01$ against the Mann–Whitney U-test), excluding the «UV and γ rays» pair ($p = 0.69$). In the 10th month, the lens morphology and changes in its protein composition were studied. All the experimental groups showed morphological changes in the lens, but no changes were found that would be specific to any group. Though it had been shown earlier that the radiation cataract consists mainly in the formation of posterior cortical opacities, it was established in later research that the diffuse cataract is the most common in this case.

To study the histological changes in the lens, semi-fine sections and a spread epithelium preparation were used. Epithelium microscopy was done using a Technival (Carl Zeiss Jena) microscope equipped with a Nikon CoolPix P5000 digital photocopier. It should be noted that cortical parts of the lens were studied — the parts where opacities were mainly found. The general structure of the tissue, the mitotic index, and the presence of the following pathologic changes were examined: nuclei with vacuoles and macronuclei; cavities in the epithelium stratum; multilayer structures formed by transformed epithelial cells; desquamated cells; and cells with vacuoles in cytoplasm. A study of the lens epithelium also revealed an effect that is common to all exposures: a decrease in the density of cells. It agrees with the data showing that ionizing radiation, UV radiation, and aging decrease the density of lens epithelium cells. Thus, in the investigation of microscopic preparations, non-specific changes related to the aging of animals were found that are arranged in the order of increasing as follows: R, UV, gamma, gamma + UV. It should be noted that the lens opacity degree increased in the same order.

The lens proteome was studied using the differential electrophoresis technique. Lenses of animals in which the cataract matched the group median were used as samples; the opacity degree of the right and left lenses was considered to be equal. For an analysis, the following sample pairs were used: «R and γ rays», «R and UV», «R and γ rays + UV», « γ rays and UV», and « γ rays and γ rays + UV». One of the samples in a pair was stained with the Su-3 fluorescent dye ($\lambda_{\text{ex/em}} = 532/580$); the other with Su-5 ($\lambda_{\text{ex/em}} = 633/680$). After separation by equipment for two-dimensional electrophoresis (BioRad, the U.S.), the gel was scanned by the Typhoon 9410 (GE Healthcare) laser scanner. In the gel image, the proteins represented in both samples of a pair are painted yellow;

different ones — green (Su-3) or red (Su-5). The images were analyzed with the DeCyder (GE Healthcare) program. All the stains in the electrophoretic patterns of the pairs «R and γ rays», «R and UV», «R and γ rays + UV», « γ rays and UV», and « γ rays and γ rays + UV» — in both water-soluble and water-insoluble fraction of the proteins — turned out to be yellow. According to the differential electrophoresis principle, it means that these sample pairs do not differ in the protein composition. Thus, a long exposure to low UV doses, a γ irradiation with a dose of 2 Gy, and their joint effect do not influence the protein composition of both the soluble and insoluble fractions of the mouse lens. Research on changes in the protein composition of the lens developing with the organism aging and/or cataract formation clearly showed that protein molecules undergo post-translational modifications. It was expected that UV irradiation in the range of 280–390 nm should have a stronger damaging effect. But the integral method of differential electrophoresis showed that there is not any significant difference among aging, UV action, and radiation action. It can be attributed either to the actual absence of differences or to the sensitivity of fluorescent dyes being lower than that of staining with silver.

An idea was repeatedly expressed in literature that ionizing or UV radiation can be used as a tool to model senile cataract formation, but suggestions to the contrary were also made. In this work, a complex study of cataract development was performed for the first time based on using randomized experimental groups.

Thus, the results of this comparative research show that the aging process, UV and/or γ irradiation result in indistinguishable changes in the lens at all levels of its organization: molecular, cellular, and organ ones [14, 15].

COMPUTER MOLECULAR MODELING OF BIOPHYSICAL SYSTEMS

The research performed by the Computer Molecular Modeling Sector included molecular dynamics (MD) simulation of physical nanosystems and biological objects and was based on modern high-performance computational methods and computer farms. Molecular dynamics research has been performed in close collaboration with leading molecular simulation centres of Japan (RIKEN Genomic Science Centre in Yokohama and Keio University) and England (Daresbury Laboratory) [16].

MD simulation is an efficient method of studying the properties of nanosystems and biological (protein) macromolecules. Finding the relaxed conformational

states of mutant proteins based on traditional computational approaches can take years even for a single protein structure. In modern computational chemistry and nanobiotechnology research, efficient algorithms and different MD methods have been developed and introduced — from the classical to hybrid ones, for example, the classical one in combination with quantum chemistry. These methods allow the high-precision reproduction of the properties of molecular systems and the prediction of their nontrivial dynamics or new phenomena.

A comparative analysis of 3D simulations of different allele forms of the human kinase CDK2 was

performed [6]. The crystal structures of the human CDK2/cyclin A complex was modeled. The structure was modeled of the wild type and a mutant allele with a single substitution of glycine with serine in position 16 (G16S) in the conservative G-rich loop. It was shown that this substitution causes a serious modification in the protein structure. In yeast, such changes of the CDC28 homologous kinase have serious pleiotropic biological effects. To investigate the significance of the observed structural modifications, the structure was

studied of another mutant allele — R274Q — which has no biological effects in yeast at a permissive temperature. A comparison of simulated CDK2 structures of three alleles shows that the root mean square deviation of the kinase and kinase + cyclin does not change in the last allele of the kinase, although the structures of the T- and G-loops were modified. These results confirm the correlation between the observed changes in the kinase structure and the biological effect (Fig. 7).

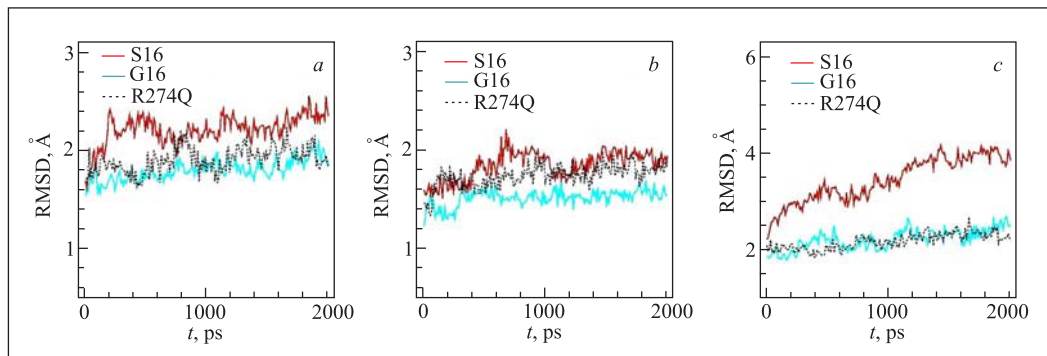


Fig. 7. Time dependence of configurations for different forms of the CDK2 kinase: the wild-type (G16 R274) and mutant forms (G16S and R274Q) (a), cyclin (b), kinase + cyclin (c)

MD simulations of the visual pigment rhodopsin with the E181K mutation, which is associated with retinitis pigmentosa, are performed (collaboration between JINR and Emanuel Institute of Biochemical Physics, RAS) [17]. Autosomal dominant retinitis pigmentosa leads to the photoreceptor cell death and retina degeneration. Approximately 25% of this pathology is associated with the rhodopsin gene mutation RP4(RHO)/Rhodopsin(3q). The amino acid substitution in the chromophore centre during rhodopsin biosynthesis leads to the most distinctive clinical pathology of this inherited disease. The consequence of mutations like these is protein misfolding. As a result, formation of a stable Schiff base linkage between the 11-*cis*-retinal chromophore and amino acid residue Lys296 is impossible. Using molecular simulation technique, the process of the 11-*cis*-retinal chromophore embedding into the chromophore centre of an opsin mutant form has been investigated. The comparative analysis of amino acid residues arrangement in the opsin chromophore centre and its interaction with 11-*cis*-retinal as in the wild (native) and in mutant opsins has been carried out. It was shown that there is no normal embedding of 11-*cis*-retinal into the chromophore centre of an opsin mutant form. As a result, the impairment of the conformation state of the opsin molecule takes place both in the chromophore centre and in the cytoplasmic domain. A stable covalent linkage of 11-*cis*-retinal with the protein part of the rhodopsin molecule is not formed; also, the active site in the cytoplasmic domain of the protein that is responsible for binding of the G-protein

(so-called transducin) is not completely blocked. Based on the obtained data, a conclusion was drawn that the most efficient treatment of pigmented retinitis induced by a rhodopsin gene mutation may consist in using genetic engineering methods that could completely replace the genetic material in pathological cells.

MD simulation measurements of the partial molar volume limit of monocarboxylic acids in benzene were performed in collaboration among JINR, the Skobeltsyn Institute of Nuclear Physics (Lomonosov Moscow State University), and Taras Shevchenko National University (Kyiv, Ukraine). The limit thermodynamic molar characteristics of solutions contain information about the hypothetical state of the solute molecules at infinite dissolution. Thus, the limit partial volume reflects the compressibility of the structure packing of the solute molecule under the action of the solvent. Here, this volume is determined by means of MD simulation of limit solutions. For this purpose the integral analysis of the found radial distribution functions is used. The method is applied to study solutions of monocarboxylic acids (nonsaturated oleic and saturated stearic and myristic acids) in benzene, which are widely used in practice for stabilization of magnetic fluids (fine liquid dispersions of magnetic nanoparticles coated with surfactants). The found volume values are in agreement with experimental data of vibration densitometry and small-angle neutron scattering research. The structure organization of the solvent at the molecule interface is compared for the acids with respect to their different properties of stabilizing magnetic fluids [18].

In 2009, the work on the radiation situation prediction at the future NICA accelerator complex was continued. Different variants of the NICA elements shieldings were examined. The radiation source terms were simulated by the GEANT4 code. As an example, double differential neutron yields in the interaction of uranium nuclei of different energies with a thick target of different materials are shown in Fig.8 [19]. The calculations of both the dynamics of induced radioactivity accumulation in the Nuclotron and collider rings equipment and air activity within the ring's channels were started.

In cooperation with the Institute of Space Research and the Frank Laboratory of Neutron Physics, work was continued under the programme of research of planet surfaces with nuclear physics methods. The programme on the neutron detector LEND on board the spacecraft that was put into the Moon's orbit in summer 2009 was completed. Much work was done concerning the development, testing, and calibration of the time spectrometer of albedo neutrons for NASA's Mars rover with a neutron generator (the Russian project DAN), and the development of a neu-

tron and gamma spectrometer for the Russian «Phobos Soil» mission.

The reconstruction of the «Genom» automated irradiation device for radiobiological research at heavy-ion beams of the U400M accelerator was started in 2009 in cooperation with FLNR.

Much attention was paid to the development of the technique of neutron spectrometry in scattered radiation fields behind accelerator shielding and in the environment. In 2009, an autonomous portable netbook-based spectrometer was designed and produced for neutron measurements in the environment.

An algorithm was developed of the calculation of a ridge filter to form a carbon nuclei beam for the target therapy of tumors [20].

Under the Intergovernmental Scientific Agreement between Russia and India, experiments were carried out within the framework of Project P-2.53 PUC-12/JC-XII at the medical beam of the DLNP Phasotron to study performances of new nanocrystalline thermoluminescent detectors designed in India; also, properties of protective materials were investigated using isotope neutron sources.

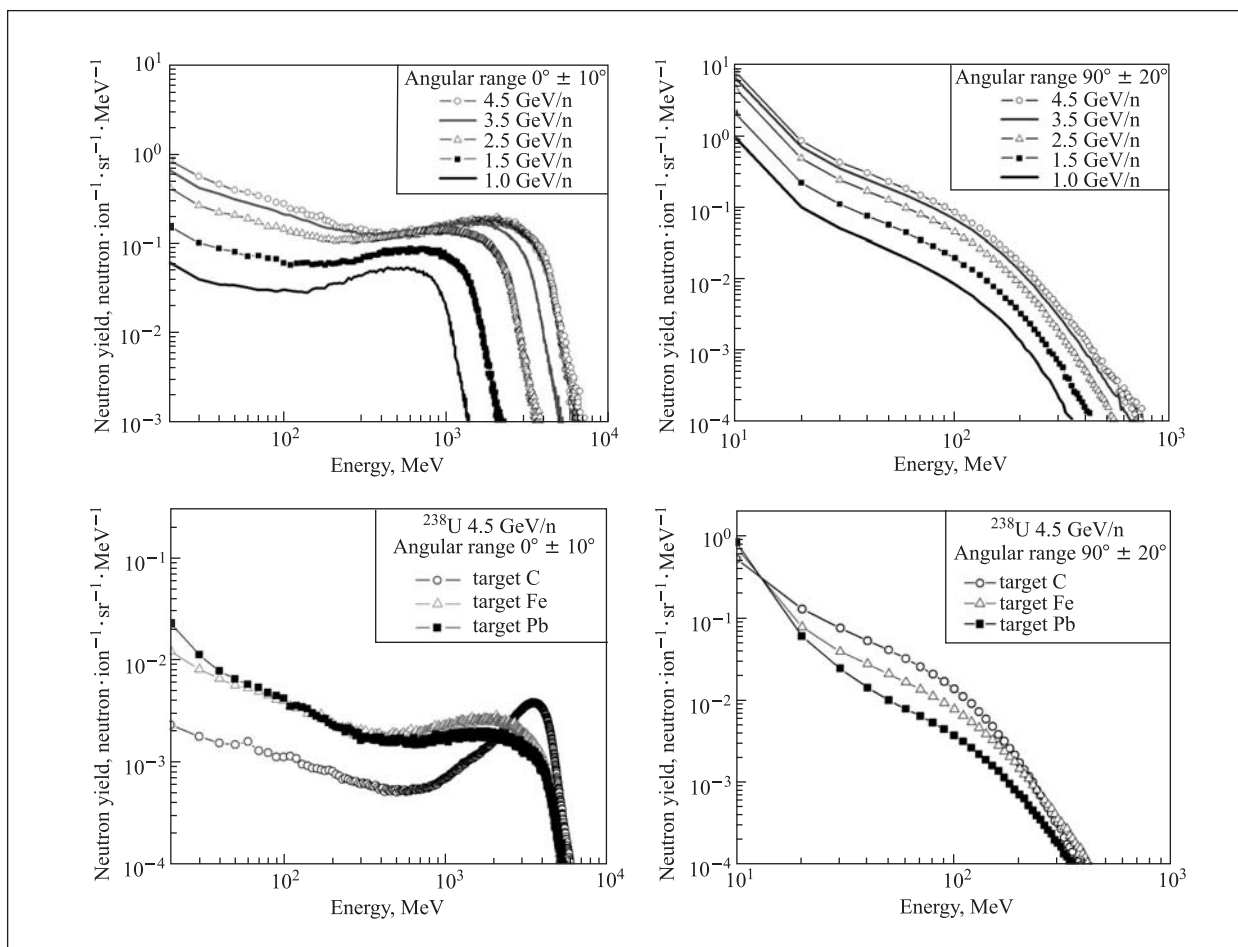


Fig. 8. Double differential neutron yields in the interaction of uranium nuclei of different energies with a thick target of different materials

During 2009, LRB scientists participated in more than 15 conferences in Russia and more than 6 international conferences in Europe and Asia.

The education process continued at the Department of Biophysics, Dubna University. The Department's total enrolment is 88 in the specialty «Human and Environmental Radiation Protection» and two postgraduate students in the specialty «Radiobiology». Fifteen students were admitted in 2009 to the Department. The fifth graduation (20 students) took place in 2009.

The education activity of the Computer Molecular Modeling Sector (CMM) staff included the supervision of diploma projects and teaching a course of molecular dynamics to students of Dubna University. A number of diploma projects on different aspects of homology modeling and molecular dynamics of nanosystems and proteins were successfully completed and defended. The courses have been offered at the Department of Biophysics and Department of Chemistry, Geochemistry, and Cosmic Chemistry. In 2009, the CMM head Dr Kh. T. Kholmurodov was appointed a Visiting Professor at the Faculty of Science and Technology of Keio University, one of the top private universities in Japan.

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