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V. E. Aleinikov, E. A. Krasavin

RESEARCH PROGRAMME OF THE DEPARTMENT FOR RADIATION AND RADIOBIOLOGICAL RESEARCH: ITS PERFORMANCE IN 1999 AND THE PROGRAMME FOR 2000

Report to the 87th Session
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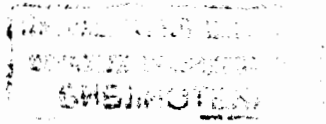

1. Scientific research programme for 1999

In 1999 the main lines of the plan were concentrated on:

- Neutron spectrometry and radiation monitoring;
- Physical support of radiobiological experiments;
- Theoretical modeling of ionizing radiation interaction with matter including interactions with biological structures and shielding calculations;
- Investigations of peculiarities and mechanisms of point and structural mutation induction in pro- and eukaryotic cells by radiation with different linear energy transfer (LET);
- Problem of low doses of radiation with different LET and sell recovery;
- Investigation of ''methylene blue - ^{211}At'' complex therapy efficiency in melanoma cells.

2. Execution of the 1999 programme

2.1. Radiation researches

Radiation protection conceptual framework of the Cylab cyclotron complex (Slovakia) was developed. The course of feasible study of major radiation protection issues of the complex comprised not only radiation protection of the cyclotron, but also problems related to the safe use of many nuclear and medical technologies. The study drawn upon information derived from extensive research and development work by the JINR, other scientific and engineering organisations and on techniques for the safe design and operation of such installations; and drawn upon experience in many countries in the use of radiation and nuclear techniques. Section of the request for proposal (RFP) of Cylab's design appertaining to radiation protection was prepared in the fullest detail. The radiation protection requirements needed for all nuclear and medical technologies to be used at the complex was specified. The following aspects of radiation protection were considered in all its bearings: criteria of radiation protection design, possible radiation sources, radiation shielding, radiation monitoring, waste management, possible radiation accidents and others.

The microdosimetric characteristics of the biological structures irradiated by radiation with different LET is a very important to study of peculiarities of mutagenic action of radiation. In the last years the codes for particles track structure simulation in biological tissue and modeling of the various damages of cells were developed. The application to practice and development of new theoretical methods were begun in 1999.

Attention was given to use of *Monte Carlo method* for shielding calculations, radiation detector responses study and dosimetric applications. In particular, the depth-dose distribution from a ^{166}Ho skin patch to skin tissue was estimated by the electron-gamma transport simulation. This work was done in frame of collaboration with the INP (Czech Republic) and connected with radionuclide therapy of skin cancers. The neutron spectra of JINR reference fields based on ^{252}Cf in polyethylene spherical moderators were calculated for real geometry. The calculated spectra was in a good agreement with experimental results.

The measurements of the neutron spectra generated by 1 GeV protons in the U+Pb+CH_2 assembling in a wide energy range were continued. This work was done in collaboration with the LHE for estimation of radioactive waste transmutation cross-section. The multisphere spectrometer and the code for neutron spectra unfolding by the statistical regularization method was used. Activation detector's technique for the estimation of the spatial neutron distribution around the assembling were applied. The data of the last experiments with proton energy of 1 and 1,5 GeV have been processed. As the results the neutron spectra under different angles and estimation of the total neutron yields (for various energy groups) were obtained.

The measurements of the radon-gas concentration in air and water in environment and JINR dwellings was completed. The measurements of radionuclides concentrations in soil near the Balakovo NPP as well as samples of soil and multiyear mosses from Yerevan environs were carried out with low-level activity gamma- spectrometer.

The experimental study of radiation detector characteristics were continued. The characteristics of the personnel dosimeters using by the JINR and Czech Republic were studied in the neutron reference fields. The microdosimetric characteristics of the track etch detectors and the thermoluminescent detector responses were also studied in the JINR neutron reference fields.

Area and occupational personnel radiation monitoring in the fields of nuclear installation were continued.

For precise dosimetry of a biological samples irradiated by particle beams it is needed to know the secondary particles dose in the sample. *The measurement of the secondary particles LET in CR-39 detectors* was carried out with 1 GeV proton beams. Results of the measurement permit to estimate the contribution of the secondary particles to the total dose of primary particles in the samples.

2.2. Radiobiological research

- Radiobiological research in the frame of the program will be connected with:
- investigation of biological effect on mammalian and human cells under low doses of ionizing radiation;
- study of the induction of mutations and stable chromosomal aberrations in mammalian cells and human lymphocytes using FISH technique;
- study of the phenomenon of chromosomal instability in mutant clones of mammalian cells under the radiation with different LET;
- investigation of the induction of point and deletion mutations in yeast and bacterial cells after irradiation with different LET;
- the development of methods for target therapy of pigmented melanoma by using complex "methylthyonin chloride – Astatine-211".

The question about possibility of extrapolation the effects induced by high doses of irradiation to the range of *low doses* and also the problem of induction of adaptive response which is the increasing radioresistance of cells after irradiation with low doses to the following irradiation with higher dose, are the most important aspect of the biological effects of low doses of ionizing radiation.

The dose-effect dependence of cytogenetic damage in the dose range 0.1–2 Gy of the single-dose irradiation with Chinese hamster and human melanoma cells in culture had been studied. The dose-effect curves for both cell lines had been found to be having an analogical character. The non-linear dependencies are shown for the induction of chromosome aberration with dose. The radiosensitivity of cells was maximal at the doses lower than 10 and 20 cGy for melanoma and Chinese hamster cells respectively. This hypersensitivity was followed by increased radioresistance and the reverse dose-effect dependence had taken place at appropriate dose range. It was supposed that this phenomenon reflect an induced radioresistance mechanisms at some level of damage and that induced repair processes are more effective and operate at lower doses in melanoma than in Chinese hamster cells.

For verification this hypothesis, a quantitative comparison of induction of adaptive response after double-dose irradiation of these two cell lines was carried out. It was shown that maximal adaptive response was induced at 1 cGy for melanoma cells and 20 cGy for

Chinese hamster cells. It can be concluded that the same inducible repair processes are analogous in mechanisms and different in quantitative proportion for different cell types underlying on the base of non-linear dose-effect curves and induction of the adaptive response.

A data analysis has been conducted on *stable and unstable chromosomal aberrations* in human blood lymphocytes, induced by scarce and densely ionizing types of radiation (γ-rays, 1 GeV protons with LET ~0.218 keV/i m, nitrogen ions ^{14}N with LET ~77 keV/μm). Possible mathematical approach has been taken up to compare the data obtained by FISH and standard metaphase methods. Concluding from the supposition that the possibility of damage in single chromosomes at ionizing radiation action is proportional to their DNA contents, mathematical formulae are presented to recount stable aberrations frequencies for the whole genome (translocation) and frequencies of some unstable aberrations (dicentrics and fragments), detected by FISH-method in chromosomes 1 and 2 of human lymphocytes. The obtained data may serve as evidence for suppositions about various radiosensitivity of different human chromosomes. They testify on a higher frequency of damage of chromosomes 1 and 2 in the human genome.

The study of mutagenic action of ionizing radiation on mammalian cells was continued. Chinese hamster cells (line V79) were irradiated with 1 GeV protons at the LHE synchrophasotron at doses of 1; 1.5 and 2.5 Gy. HPRT-mutant subclones were revealed and separated from the irradiated cells' culture, their cytogenetic analysis was conducted. An increase of the growth duration of mutants is obtained in comparison to the intact control. The analysis showed that HPRT-mutants, induced by protons, were heterogeneous in a number of cytogenetical parameters. They were characterized by aneuploidy. Although the majority of mutants had the modal number of chromosome, equal to 22, the single mutants with the modal numbers of chromosome of 20 and 21 were discovered at the doses of 1 and 1.5 Gy.

In our earlier study of spontaneous and γ-induced mutants we distinguished the group I of mutants which did not differ essentially from the control in the chromosomal aberration level, and the group II, which had the level of chromosomal damages about 1.5 to 2 times higher. Besides, in contrast to spontaneous mutants among g-induced group I mutants those were obtained which had a chromosomal aberration level about 2 times lower than in the control. The frequency of cells with chromosome aberrations and the total number of chromosomal aberrations in the control were 8.7% and 9.9%, respectively. Group II mutants, induced by 1 GeV protons, had a higher level of such damages: the frequency of cells with chromosomal aberrations was from 11% to 18%, and total number of aberrations was from 11% to 28%. No difference was observed in the yield of various types of chromosomal aberrations, in comparison to g-induced mutants. The obtained data testify that irradiation of mammalian cells with γ-rays and high energy protons may induce stable on cytogenetical parameters types of radiation-induced mutants.

The experiments on the determination of survival and oncogenic transformation regularities of fibroblast cells at the 580 MeV proton irradiation have been started. We obtained the curves of survival and frequency of oncogenic transformation for these cells. At present, experiments are being conducted to obtain results of survival and oncogenic transformation frequency for these cells after γ-irradiation.

The study of genetic control of checkpoint-regulation in yeast Saccharomyces cerevisiae was continued. Checkpoint mechanisms cause the cell cycle to pause and allow DNA damage repair. In yeast several checkpoint-genes have been identified. We intend to study interactions between the known checkpoint-genes (RAD9, RAD17, RAD24, RAD53) and SRM genes, which affect both the maintenance of various genetic structures and their radiation sensitivity. Double- mutant strains have been constructed and their radiosensitivity

was studied. We suggest that srm mutations break the genes involved in the checkpoint controls that allow cell to delay progress through the division cycle if DNA-damage induced. Checkpoint- genes belong to branch pathway of checkpoint-regulations. These pathways differ from known epistasis groups of mutations affecting DNA repair after γ-irradiation.

Finally, we managed to directly demonstrate the effect of the srm5 (cdc28-srm) mutation on the specific delay of cell cycle progression of synchronized cultures irradiated with UV light. The post-irradiation delay appeared to be reduced in the case of the cdc28-srm mutant. This finding seems to support the idea of the CDC28 gene being a target of checkpoint controls. To further confirm this idea we continue studying genetic interactions between CDC28 and the known checkpoint genes.

We used special tester system for detection point mutations after radiation. Induction of base substitutions in haploid yeast strains by γ-irradiation were finished. The γ-irradiation induces efficiently all types of the base subsitutions. The dose dependence is linear, that differ from those of diploid strains that show linear-quadratic dependence. The base-substitutions spectrum in haploid yeast is the same as diploid cells.

Observation of base-substitution induction by heavy ion in yeast strain was begun. The first experiment with ^{4}He (LET=20 kev/μm) showed relative biology effectiveness about 2. The study of frameshift induction mutations in yeast was started by using a special test-system that is based on the reversion tests with a 4-base insertion in the LYS2 gene or a +1T insertion a stretch of 6T's in HOM3 gene. The optimal conditions for detecting a reversion mutation are chosen. We shall use both systems in our further investigations of regularity of mutagenesis after ionizing radiation with different LET.

Studies of regularities in the formation of spontaneous and induced deletion mutations in *E.coli* bacterial cells were continued. The applied test system based on the chromosomal *tonB* gene, which is situated in the distance of 4.6 kb from *trp*-operon, allows to take into consideration the *tonB-trp* deletion mutations that are determined by resistance to colicyne B, phage T1 and the presence of auxotrophity in tryptophane.

Dose dependence of formation frequency of *tonB-trp* deletion mutations at γ-irradiation is obtained. A series of preliminary experiments has been conducted on mastering the methods to study the induction process of deletion mutations with heavy ions. A selection is carried out of new *met* mutant clones to simplify the deletion mutant selection methods.

At the same time, dose dependence is obtained for *tonB*-mutants, which are selected according to resistance to colicyne B, and bacteriophage T1. The majority of these mutations are supposed to be short deletions localized in *tonB* gene. To verify this hypothesis, a checkout is being conducted on the ability of these mutants to reverse.

Studies on *the regularities of SOS-response in E.coli cells at the ultraviolet light action have been concluded.* In particular, the influence of visible light (photoreactivation) on the character of kinetic and dose curves of SOS-induction in *E.coli* cells (uvrA) was investigated. The analysis of these data along with the data obtained earlier concerning UV-induced SOS-response allowed to conclude that different molecular events lie in the basis of SOS-system induction

In the region of the 0-2 J/m^2 dose the SOS signal is most probably caused by gaps, generated in the process of the damaged DNA replication. In the region of 2-10 J/m^2 an interruption of DNA replication may be regarded as a SOS-inducing event. Studies of the influence of umuC-mutations on the SOS-response induced in E.coli cells with UV and γ- rays have been finished. It is shown that in both cases the presence of this mutation leads to 5 times growth of the SOS-induction level. Experiments on irradiated cells' exposure in the buffer allowed to make a conclusion that it is connected with more effective excision damage repair in

cells with the normal UmuC-protein, which may be a consequence of the UmuCD-dependent inhibition of DNA replication. The investigation of the SOS-response was continued on E.coli cells at the heavy ion action. In particular, kinetic curves of SOS-induction in irradiation of wild-type cells with different doses of γ-rays and ^{4}He ions with LET =20 keV/μm were obtained.

The activities on computer simulation of genetic regulator system of SOS-response in E.coli bacteria have been concluded. They resulted in a model and corresponding differential equations which describe the dynamics of genetic regulation and inducing signal for the regulator SOS-response system after the UV action. It is shown that the excision repair influences the dynamics of SOS-response regulation and induction after UV-irradiation. Dynamic curves of the SOS-response regulator components after UV-irradiation, as well as dose dependence of maximum concentrations of genes recA and sulA production have been calculated and analyzed.

The combined effect of low-dose ionizing radiation and chemical agents on seeds of higher plants (plantain *Plantago Major*) in the area of the Balakovo Nuclear Power Plant (NPP), Saratov region was studied. The subjects of the studies were the antioxidant status, mitotic activity and chromosome material damage in meristem cells of plantain seedling apex. The data were analyzed with the account of results in radionuclide and chemical contamination determination, and simulation of the gaseous flux exhaust of inert gases from NPP.

The research demonstrated that the delay in sprouting and the number of not sprouted seeds in populations correlated with the chromosome damage yield in the first mitosis of meristem cells, in case the damage was large, both, at the radiation action and the effects of chemical agents. In these populations the level of chromosome aberrations was 3-4 times higher than the control level. Plants subjected to a weaker action should be studied more precisely.

The analysis of chromosome aberration yield and the quantity of proliferating cells in the apex in the first mitosis showed that the action of the ionizing radiation lead to classical dependence of these values on the fixation time, while the combined action with chemical agents detained and arrested the cell division. The decrease in the mean values on the quantity of dividing cells in the apex was observed in populations subjected to the effect of radioactive fallouts from NPP and chemical agents. These processes affected the normal growth of the plant root, studied in some populations. The study of the antioxidant status demonstrated its decrease 2-3 times in populations situated mostly on the wind direction from NPP. The further research will be aimed at the study of variations in specific values responsible for the influence of low doses of ionizing radiation, chemical agents and their combined action.

The analysis of the results in dose – effect relationship studies has been carried out on the cell and organism level, with the aim to obtain more precise data on the risk coefficient at low doses. The results are represented by two contrasting groups of dose dependence on effect: a downwards concave and a J-shaped curves. Both types of dependence are described by the equation solutions of an assumed unified protective mechanism, which comprises two components: constitutive and adaptive or inducible ones. The latest data analysis of the downwards concave dependence curves shows a considerable underestimation of radiation risk in all types of cancer, except leucaemia, for a number of critical groups in a population, at low doses comparing to the ICRP recommendations. With the dose increase, the decrease of the effect value per dose unit is observed. It may be possibly related to the switching of the activity of the adaptive protective mechanism, with some threshold dose values being exceeded.

The series of experiments had been finished for the purpose of quantitative comparison between degrees of damage of normal Chinese hamster cells and human melanoma tumor cells

in vitro treatment with free astatine-211 and ^{211}At-labelled methylene blue (MTB). The results of experiments confirmed our preliminary data that the efficiency of ^{211}At-labelled MTB on melanoma cells was one order higher than on Chinese hamster cells. Also it was shown the same efficiency of ^{211}At in ionic form for both cell lines.

This means that ^{211}At-MTB is selectively accumulated in pigmented tumor cell, which prove this compound to be clinically effective in radiotherapy of disseminated melanoma accompanied by minimal damage of normal tissues.

2.3. Collaboration with Russian institutes

The researches in the frame of the theme are performed in wide collaboration with different Russian Institutes. The specialists of the Center of Medical Radiology (Obninsk) take part in study of cytogenetic effects of heavy charged particles in human lymphocytes and mammalian cells. Mutagenic effects of ionizing radiation with different LET are investigated in collaboration with radiobiologists from Institute of Medico-Biological Problems. The combined effect of low-dose ionizing radiation and chemical agents on seeds of higher plants are studied by specialists of DRRR and Institute of Cell Biophysics (Puschino), Institute of Chemical Physics (Moscow), Saratov University. The quantitative comparison between degrees of damage of normal Chinese hamster cells and human melanoma tumor cells in vitro treatment with free astatine-211 and ^{211}At-labelled methylene blue was performed with Institute of Biophysics (Moscow).

3. Scientific programme for 2000

3.1. Radiation research in the frame of the programme will be connected with the following directions:

- *Neutron Spectrometry.* It is planned to prolong the participation with LHE in measuring of the neutron spectra generated in thick assembling by the high-energy protons. This work is performed for estimation of radioactive waste transmutation cross-section. It is proposed also to begin the measurements of the neutron spectra around the thick targets at LNP phasotron.
- *Physical Support of Radiobiological Experiments.* It is planned to concentrated the efforts for realization of radiobiological experiments at Nuclotron heavy ion beams with different LET. For this purpose it is intended to develop and design a system of monitoring and dosimetric measurements.
- *Physical Modeling of Radiation Induced Damage.* It is planned to continue the implementation of the track structure simulation code for microdosimetric modeling of biological effects.
- *Shielding Studies.* Application of Monte Carlo and engineering methods for shielding calculation will be continued.
- *Response Detectors Study.* The study of the responses of different type dosimeters and radiation detectors will be continued in reference neutron fields and with charged particle beams.

3.2. Radiobiological research

The main purposes on the 2000 in radiobiological research will be connected with:

- *Mutagenic Action on Mammalian Cells.* The regularities of induction of stable chromosomal aberrations in human lymphocytes after irradiation of ionizing radiation with different LET will be performed. The objects of investigations are peripheral blood lymphocytes of humans and V79 line, B11-d-ii-FaF28431 clone of Chinese hamsters cell cultures.
- *Mutagenic Action of Radiation on microorganisms.* The study of deletion mutant induction in bacteria irradiated with γ-rays and heavy ions will be continued for the next period. It is proposed to investigate also the interaction between checkpoint - genes and their action at various stages of cell cycle. To study of point mutation induction in yeast cells exposed to radiation with broad region of LET.
- *Biological Effects of Low-dose Exposure.* It is planned in 2000 to continue the research of low dose effect in mammalian cells. Cytogenetic damages after γ-irradiation with low doses in Chinese hamster cells will be performed. The connection between nonlinear form of the curve of chromosome aberrations induction and adaptive response will be established.
- *Radiobiological experiments with high energy ions.* In according with the agreement between DRRR and Radiation Biophysics Laboratory (RBL) NASA Johnson Space Center (Houston, USA) the radiobiological experiments in the study of cytogenetic damages in human lymphocytes after heavy ion irradiation will be commenced.
- *In the frame of the project "MITRA"* the in vitro and in vivo investigations of methods of targeted radiotherapy with the complex 211-astatin-MTB will be continued. The degree of damage effect" selectivity 211-At-MTB will be studied on human melanoma and normal cells.

4. Educational activity

The works for organization of the stable education process at the new chair "Biophysics" of the Dubna International Institute (established in 1998) were continued. It is intended to give the students the fundamental knowledge in radiobiology as well as nuclear physics and dosimetry fields. The first 11 students were admitted in autumn 1999 to the new chair on specialty "Radiation protection of people and environment". It was made the effort for organization of practices for students in the near future.

The second run of the 9-weeks IAEA Regional post-graduate educational course on radiation protection was held in JINR in autumn 1999. The course was opened to 25 young specialists from the IAEA Member States in East Europe and West Asia region. The course was organized on the basis of JINR DDDR and University Center. The course consisted of 125 lectures, 17 laboratory exercises, 10 scientific visits. The main part of the lectures and all practices were given by the specialists from DRRR. As the course result the listeners were awarded by the IAEA certificates. Taking into account the high level of the course organization and experience accumulated in the JINR it was proposed by the IAEA to prolong the effective cooperation in this field on the permanent basis.

5. Administration activity

Personnel. The total personnel of the DRRR (without of the Radiation Protection Division) was 81, including the Directorate staff 9.

Finance. Funding of research in the direction of radiation and radiobiological investigations in 2000 is shown in Table 1.

Table 1. Financing DRRR in 2000

Area	Lab	Financing plan (k$US)
08-9-1015-96/2000 (1-st priority)	DRRR	282,1
Infrastructure	DRRR	70,7
Total:		353,8

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