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RESEARCH PROGRAMME OF THE DEPARTMENT FOR RADIATION AND RADIOBIOLOGICAL RESEARCH: ITS PERFORMANCE IN 2000 AND THE PROGRAMME FOR 2001

Report to the 89th Session
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Dubna 2000

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1. Scientific research programme for 2000

In 2000 the main directions of the programme were concentrated on:

- Neutron spectrometry and radiometry, radiation monitoring;
- Investigation of radiation fields around thick targets;
- Physical support of radiobiological experiments;
- Shielding calculations and design;
- 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 cell recovery;
- Investigation of ''methylene blue - ^{211}At'' complex therapy efficiency in melanoma cells.

In 2000 the theme "Radiation and Radiobiological Investigations at the JINR Basic Facilities and in Environment" was prolonged to 2003.

2. Execution of the 2000 programme

2.1. Radiation researches

Radiation fields' calculation. The study of reference neutron spectra of the polyethylene-moderated ^{252}Cf that simulate the spectra observed in the workplaces within nuclear reactors and accelerators was continued. The neutron spectra were calculated by Monte Carlo method without and with taking into account the calibration room scatter effect and compared with experimental data. Parameters needed to test dosimeters, such as ambient and personal dose equivalent per unit neutron fluence, averaged over the neutron spectra were defined.

Radiation shielding. All shields require holes or openings for cables, ventilation ducts, personnel access, etc. and considerable care has to be taken to ensure that radiation escaping through these holes does not seriously undermine the overall efficiency of the shield. The study of radiation scatter down holes in a shield was started by Monte Carlo method.

The programme of the experimental investigation of the characteristics of secondary particles around a thick lead target irradiated with 650 MeV protons was started at the JINR Phasotron. This work is carried out in the frame of the project of MOX subcrutical assembling on the accelerator and the target imitates the core of the subcritical assembling. This research programme is realized in collaboration with the LNP, FLNP and LIT. In the first stage of the programme the following characteristics were measured:

- the double differential (on angle and energy) distribution of the neutron around the target;
- the angle distributions of the hadrons (with different energy thresholds) around the target;
- the longitudinal distributions of the hadrons yield (with different energy thresholds) from the target;
- the total hadrons yield from the target.

The multisphere neutron spectrometer for measuring in the widest energy range and an activation detector technique were used. The results were applied for verification of the Monte-Carlo calculation of the internuclear cascade of the secondary particles generated by the primary protons within the target. The good agreement between the experimental data and the corresponding calculations was obtained. The work on the measurement of the activation rate of the radioactive nuclides generated within the target by the protons is now in process.

The work in collaboration with the LHE for estimation of radioactive waste transmutation cross-section was continued. The neutron yields from the thick lead target surrounded with the paraffin moderator irradiated by 1 and 1,5 GeV protons were studied.

The active neutron counter with activation indium detector was designed for thermonuclear neutrons' detection in specific conditions (very short duration of neutron pulse, the high level of the accompanied gamma radiation, the influence of the powerful electromagnetic field on electronic equipment and so on). The counter has a high sensitivity for fast neutrons.

The physics support of the biological experiment with the ^{12}C ions beam at the Nuclotron was carried out. The purpose of the experiment is the investigation of the ion beam characteristics for the radiobiological samples' irradiation.

Area and occupational personnel radiation monitoring in the field of nuclear installation was continued.

2.2. Radiobiological research

The obtained data on the frequencies of stable and unstable chromosomal aberrations in *human blood lymphocytes* were summed up after proton irradiation (1 GeV), γ-rays and nitrogen ions ^{14}N with energy 50 MeV/nucleon (LET ~ 77 keV/μm). During the investigation of their formation using conventional metaphase and FISH-methods, on the one hand, some earlier known regularities were confirmed. So, the frequency of

exchanged stable and unstable aberrations is a linear-quadratic function from doses, and for acentric fragments is linear after irradiation either electromagnetic (γ-rays) or corpuscular (protons with relativistic energy) types of ionizing radiation with low LET. After irradiation with densely ionizing nitrogen ions the linear function from doses was described for all types of aberrations. On the other hand, new quantitative and qualitative peculiarities of effects were revealed after action of radiation with different quality. They concern the spectrum of induced aberrations. So, the translocations prevailed in the total number of aberrations, and their frequencies had the inverse negative relationship from the LET radiation: their fractions were 40-45% after γ-and proton irradiation and ~25% - after nitrogen ions. In the last case high chromosome fragmentation manifested itself and, as a result, the relative decrease of stable chromosome aberration fraction was observed. Moreover, FISH-obtained data testified higher frequency of chromosomes 1 and 2 damage in the human genome after tested types of radiation. The data may confirm the supposition about different radiosensitivity of different human chromosomes. The obtained data on the induction of stable chromosome aberrations (translocations) were taken as principles of calibration curves and the basis for using such aberrations as bioindicators for estimation of absorbed doses of radiation with different quality. As the analysis shows, the exactness of γ-ray and proton dose estimation was 7-15% and it was ~ 20% for nitrogen ions. It improves when inducing doses increase.

The obtained data were analyzed for spontaneous HPRT-mutants and radiation-induced ones by different doses of radiation with different quality: γ-rays, accelerated protons with energy of 1 GeV and nitrogen ions ^{14}N with LET ~ 77 keV/mkm. During revealing and selection of HPRT-mutants the arising of mutants with slow growth and increasing of such mutant fraction to 80-100% at high inducing doses of γ-rays (5-7 Gy) and at all used doses of nitrogen ions (up to 3 Gy) was observed. The cytogenetic analysis of spontaneous and radiation-induced mutant subclones revealed their heterogeneity on such tests as mitotic activity, aneuploidy, chromosomal aberration level. Chromosome instability of mutant subclones decreased with increasing of ionizing radiation LET. The fraction of mutants with higher chromosome aberration level as compared with intact control was: amongst spontaneous mutants – 71%, γ-induced ones – 47%, induced ones by protons – 33% and by nitrogen ions – 16%. The chromosome instability of spontaneous mutants was the highest. Among spontaneous

mutants the groups of mutants were revealed which did not differ from the intact control chromosome aberration level, with higher chromosome aberration level up to 2-4 times, with extremely high level of chromosome aberrations (more than 30% of aberrant cells) and tetraploid mutants. Also the appearance of mutants with lower chromosome aberrations level 2-4 times as compared with intact control was noted at radiation-induced mutagenesis. The fraction of such genetically stable clones with low chromosome aberration level was: amongst γ-induced mutants – 16%, induced ones by protons – 8 % and by nitrogen ions ^{14}N – 48%. Among spontaneous mutants they were not observed. These data may testify that the frequency of genetically stable mutants increases at high radiation LET. The heterogeneity of HPRT-mutants, revealed in our experiments, testifies that at mutagenesis a probability of infringement of chromosome integrity in mammalian cells rises and it may be regarded as a stage of corresponding genome reorganization that is adequate to changed vital conditions. Probably a degree of chromosome instability of the mutants may be conditioned by the differences of arising mutation types.

The study of the effect *of low dose irradiation* on mammalian cells was continued. One of the main aspects of this problem is the possibility of extrapolation of high dose effects to the low dose range. Non-linear dose-effect dependence with Chinese hamster and human melanoma cells was shown earlier when the anaphase method was used. This dose dependence was characterized by hypersensitivity at low doses (below 10-20 cGy), the reverse dose-effect dependence in the range 10-30 cGy and induced radioresistance at higher doses. These "anomalous" dose curves were confirmed by synchronized Chinese hamster cells, irradiated in G1-phase of the cell cycle. Metaphase analysis of chromosome aberrations in the first postradiation mitosis, both for the number of aberrant cells and for number of aberration per cell, showed the presence of early absorbed regularities. Analogous dose-effect curves were observed with human melanoma cell when micronuclear test was used, which integrates the reaction of the cell population for the whole cell cycle.

It was shown also that the adaptive response of human melanoma cells estimated with the micronuclear criterion was higher than that with the number of aberrant cells (0,55 and 0,8 respectively). The dose-effect dependence was changed when the preliminary irradiation at optimal doses was carried out five hours before the irradiation with doses of 0,1-2 Gy; the phase of high radiosensitivity was absent in this case and a number of

spontaneous aberrations were repaired. It can be concluded that the same inducible repair processes are analogous in mechanisms, and different in quantitative proportion for different cell type, underlying basically non-linearity dose-effect curves and induction of the adaptive response.

The investigations were continued in experimental approach of targeted radiotherapy of pigmented melanoma with radionuclide α-emitter ^{211}At and methylen blue (MTB). The selective action of ^{211}At-MTB on human melanoma cells was studied. The accumulation of radionuclide during the incubation of cells with ^{211}At-ion and ^{211}At-MTB was used for the evaluation of the degree of selectivity. 3-4 times more effective accumulation was shown of ^{211}At-MTB in pigmented melanoma cells than in Chinese hamster cells. ^{211}At-ion was accumulated by both types of cells equally and in very low quantity. These results correlate with our earlier data which demonstrate one order higher ^{211}At-MTB treatment efficiency on human melanoma cells in comparison with nonpigmented cells.

The induction of mutations of different nature after irradiation by ionizing radiation was studied in *yeast Saccharomyces cerevisiae* as a model system of eucaryotic cells. Mutagenic property of ionizing radiation was characterized by using three different mutator assays. They were a forward mutation rate assay that detects mutations inactivating the arginine permease gene (Can^r mutations) and reversion assays detecting mutations that revert a 4-base insertion in the LYS2 gene or that revert a +1T insertion in a streach of 6 T`s in the HOM3 gene. The reversion to Lys^+ and Hom^+ is due to deletion of a single nucleotide predominantly. The γ-ray induced forward and frameshift mutations efficiently. Frequency of direct mutations to canavanin resistance (Can^R) is 5.8×10^{-5}, frameshift mutations for reversion to Lys^+ is 1×10^{-6} and for reversion to Hom^+ is 1.6×10^{-7} for the dose of 100 Gy. Dose dependence of induction of forward and frameshift mutations is linear for dose 100-1000 Gy.

Induction of base-pair substitutions by γ-ray was studied earlier using special tester CYC1-system. Now we are studying induction of point mutations by heavy ions. Induction of AT – TA transversion in diploid yeast cells by ^4He-ions was tested. The shape dose curve is not linear for dose 100-1000 Gy. Efficiency of ^4He-ions with LET=80 keV/μm for induction of transversions is less than efficiencies of ^4He-ions with LET=20 keV/μm and γ-ray.

The study of genetic control of DNA damage-induced arrest of cell cycle progression, named checkpoint-control, was continued. We intend to

study interactions between the known checkpoint-genes RAD9, RAD24, RAD53 and our genes SRM5/CDC28, SRM8, SRM12 using such property as radiosensitivity. Genetically, CDC28 and RAD9 appear to form epistasis group one, but CDC28 and RAD53 define epistasis group two. So, CDC28 and RAD53 define two branches of the pathway controlling radiosensitivity. We demonstrate that RAD9 and RAD24 genes act in opposition in one pathway of controlling the radiosensitivity and they interact epistatically with RAD53 gene. RAD53 is believed to function further downstream. So, the control of radiosensitivity defined branch pathways.

Analysis of genetic characteristics of SRM1, SRM2, SRM5, SRM8, SRM12 genes was continued. We investigated effects of srm-mutations on lethal and mutagenic action of γ-ray and on cell cycle progression.

The study of point (col B, ton B) and deletion mutation induction in bacteria by γ-rays and heavy ions were continued. It was shown that the frequency of col B and ton B mutations as a function of the γ-ray and heavy ion dose (helium ions with LET = 20 and 78 keV/mm and carbon ions, LET = 200 keV/mm) is described by the linear-quadratic curves. The quadratic part of these curves is parallel shifted from the dependence with γ-irradiation. The relative biological effectiveness (RBE) depends on LET as a function with a local maximum. The maximal biological effect reveals after helium ion irradiation with LET = 20 keV/mm. The induction of deletion mutations by helium and carbon ions (LET = 78 and 200 keV/mm, respectively) is described by the linear function. The helium ions are more effective in induction of deletion mutations than the carbon ions.

The researches of *SOS response* in E.coli cells after irradiation by deuterons (LET = 5 and 9 keV/mm), helium ions (LET 20, 40 and 80 keV/mm), carbon ions (200 keV/mm) were continued. It was established that the relationship of SOS induction potency (SOSIP) on LET has a local maximum in region of 50–60 keV/mm.

The results that were obtained with bacterial cells indicate the important role of the cluster DNA damage in formation of gene mutations. On the other hand, the formation of deletion mutations is connected with induction of direct and enzymatic double strands breaks of DNA. The investigations of regularities and mechanisms of induction of precise excision of transposons by radiation with different characteristics have been started. The mobile elements are the discrete genetic structures (the segments of DNA) that are capable to be displaced from one to another position of bacterial genome.

The embedding of transposone starts with the covalent insertion of discrete genetic stable terminal sequences. Consequently, this process leads to translocation of all elements in a site of the gene-target as a result of both homologous rec A - dependent and nonhomologous recombination. A process of transposon elimination from genome can take place together with a translocation. A new system for monitoring and studying of the induced mutability – induction of precise excision of transposons by different types of ionizing radiation has been used in our experiments. The precision of transposon excision is the specific SOS dependent process that is connected with the formation of deletion mutations. The direct or inverted IS-sequences are involved in the formation of the deletions. The process can be used for revising the targeted and untargeted SOS-mutability and its relationship to the main genes for SOS-mutagenesis: the recA, lexA and umuCD. Differing drastically from point mutations in the nature of a cellular target for inducible SOS-mutability machinery, precise excision of transposons suggests a possibility to revisit the process of SOS-mutability via its functioning in the formation of genomic deletions.

The exponential survival curves for E.coli wild type and repair deficient mutants (recA, recN) bearing the Tn5 and Tn10 transposons were obtained. The relationship of excision transposon frequency on the dose of γ-rays was determined. It was shown that the dependence for the wild type of cells bearing the Tn10 transposon is described by the curve with saturation that has a maximum. The recA mutation blocks the transposon excision completely and the recN mutation represses this process. These results enable to conclude that rec A and rec N genes are involved not only in DNA repair but also in the control of the induction of excision and insertion of Tn10 transposon in E.coli genome.

On the basis of the two-protection reaction model an analysis of *stochastic radiobiological effects of low-dose exposure* of different biological objects has been carried out. The stochastic effects are the results published in the last decade: epidemiological studies of human cancer mortality, the yield of thymocyte apoptosis of mice and different types of chromosomal aberrations. The results of the analysis show that as dependent upon the nature of a biological object spontanous effect, exposure conditions and radiation type with one or another form of the dose – effect relationship is realized: downwards concave, near to linear and upwards concave with the effect of hormesis included. This result testifies to the incomplete conformity of studied effects of 1990 ICRP recommendations

based on the linear non-threshold hypothesis about dose – effect relationship. Because of this the methodology of radiation risk estimation recommended by ICRP needs more precision and such quantity that the collective dose ought to be classified into the category of nonsence.

Low dose ionizing radiation action at laboratory experiments on peas and complex action of chemical and radionuclide pollution on plantain in Balakovo of Saratov region was studied. The changes of parameters of physical-chemical processes (antioxidant status of the seed), cytogenetic damages (chromosomal aberrations and mitotic activity of cells of seed roots) and adaptive response were studied at the investigation of chemical and radionuclide complex action. The changes in all samples were observed in comparison to the control lots.

3. Scientific programme for 2001

3.1. Radiation research in 2001 will be concentrated on 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. It is proposed also to continue the measurements of the neutron and hadrons yields from the thick targets at the Phasotron.
- *Physical Support of Radiobiological Experiments.* It is planned to ensure the dosimetric support of the radiobiological experiments at the Nuclotron ion beams.
- *Shielding Studies.* Application of Monte Carlo and engineering methods for shielding calculation will be continued.
- *Response Detectors Study.* The study of the response of different types of dosimeters and radiation detectors at the JINR basic facilities will be continued.

3.2. Radiobiological research in 2001 will be connected with:

- *Mutagenic Action of Radiation on microorganisms.* The study of deletion mutation induction in bacteria E.coli irradiated with helium ions will be continued for the next period. The investigations of regularities and mechanisms of induction of precise excision of transposons by densely ionizing radiation will be started. The study of gene and frame shift mutation induction in yeast exposed to radiation with broad region of LET will be continued.

- *Mutagenic Action on Mammalian Cells.* The regularities of induction of stable chromosomal aberrations in human lymphocytes after irradiation with ionizing radiation with different LET will be continued. The spectrum of induced aberrations in peripheral blood lymphocytes by heavy ions will be analyzed.
- *Biological Effects of Low-Dose Exposure.* Cytogenetic analysis in Chinese hamster cells after γ-irradiation with low doses by methaphase method will be performed. The connection between nonlinear form of the curve of chromosome aberrations induction and adaptive response after irradiation by sparsely and densely ionizing radiation will be established.
- *In the frame of the project "MITRA"* the methods of targeted radiotherapy with the complex 211-astatin-MTB will be developed in the experiments with mice. The study of selective "damage effect" of 211-At-MTB will be continued on human melanoma and normal cells.

4. Educational activity

The Timofeeff-Ressovsky Centennial International Conference "Modern problems of radiobiology, radioecology and evolution" has been held in JINR in September, 2000. The conference was supported by UNESCO, INTAS, Ministry of Industry, Science and Technology (Russia), Genetics Society of America, Soros foundation. More than 200 scientists from different countries took part in the conference. The memorial booklet had been published to the conference.

The education process at the chair "Biophysics" of the International University "Dubna" was continued. 10 new students were admitted in 2000 to the chair on specialty "Radiation protection of people and environment".

Administration activity

Personnel. The total personnel of the DRRR (with the Radiation Protection Division) was 84 including the Directorate staff 7.

Finance. Funding of research in the direction of radiation and radiobiological investigations in 2001 is shown in Table 1.

Table 1. Financing DRRR in 2001.

Area	Financing plan (k$US)
08-9-1015-96/2003 (1-st priority)	288,3
Infrastructure	66,7
	Total: 355,0

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